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Gut Microbiome modulation in patients treated with an enriched nutraceutical composition: A multi target strategy

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ABSTRACT

Introduction: The contribution of gut microbiota to health and disease is now well accepted and under continuous investigation. It is the most representative in the body (about 70% of the total) with over 400 different species of microorganisms living there. It is exclusive to each individual and therefore represents a real biological imprint, capable of distinguishing humans from one another.

Gut microbiota works synergically with the host, promoting health by performing protective, metabolic and structural functions. Thus, when a modification of the personal normal gut microbiota composition occurs, a predisposition to develop different diseases may increase. Probiotics are functional food that may modulate gut microbiota stimulating protective bacterial properties and inhibiting detrimental activity. We have previously reported a beneficial effect from the administration of an enriched nutraceutical composition containing probiotics in reducing irritable bowel syndrome-related symptoms and improving metabolic profile by lowering triglycerides. However, if these effects are attributed to gut microbiota remain unknown.

Objective: This analysis aims at evaluating microbiota modulation in subjects before and after Triobiotix® administration using a metagenomic approach

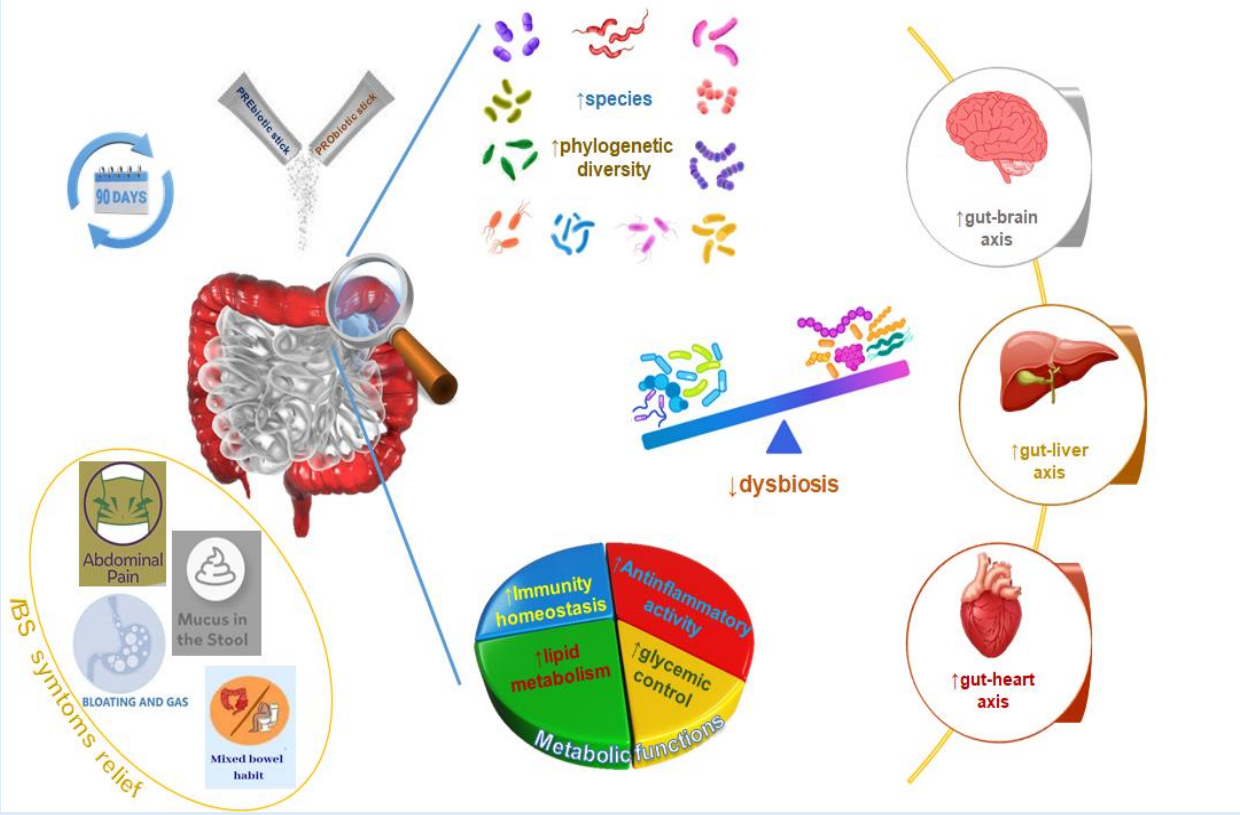
Methods: Triobiotix® is an enriched nutraceutical composition containing maltodextrin, hyaluroic acid and mineralized seaweed extract Lithothamnion Calcareum plus a mix of five different lactic bacterial species. A total of 46 subjects with

availability of sample test before and after treatment were included in the present analysis selected from 265 subjects. A fecal sample was collected before and up to 90 days after the administration of Triobiotix®. Feces were analyzed by Wellmicro® gut test that uses the next-generation sequencing of the 16S rRNA gene on Illumina MiSeq. Additionally, for 171 subjects a 90-day clinical evaluation was available.

Results: Treatment with this enriched nutraceutical for up to 3 months resulted in a positive modulation of the main indices of improved gut microbiota, such as biodiversity and dysbiosis. Improvement on metabolic functions, such as glycemic and lipid metabolisms, as well as on immunological and anti-inflammatory activities have been described, thus indicating that the observed clinical benefits associated with the administration of this nutraceutical formulation might be also attributed to a gut microbiota modulation. Improvement in main IBS-related symptoms and bloating was maintained up to 90 days as well as microbiota diversity.

Conclusions: Our analysis suggests that the gut microbiota of subjects taking Triobiotix® rapidly returns to a high diversity with reduced dysbiosis after treatment with associated improvement of main physiological functions linked to brain, heart and liver.

Keywords: gut microbiota; microbiome, metagenomic; Nutraceuticals



Graphical Abstract: Gut Microbiome modulation in patients treated with an enriched Nutraceutical composition: a multi target strategy.

INTRODUCTION

In the last two decades, the so-called "biology of complex systems" has developed [1]. It represents a transversal discipline based on a holistic and multidisciplinary approach, which assimilates the human body to a multisystem conditioned and regulated by a variety of factors (i.e genetic, nutritional, environmental, cultural and social). In this perspective, the characterization of a plurality of microbial ecological microenvironments in the gastrointestinal tract is suggestive and paradigmatic, representing a dynamic system interconnected with the host organism at the cellular, metabolic, immune, neurobehavioral and emotional levels [2]. A "cross talk" between the different microbial communities, located in the various "border" districts (intestinal, oropharyngeal, nasal, pulmonary, cutaneous and urogenital microbiota), is established since the early stages of the life [2], communicating and interacting each other and with the immune system through metabolites and cytokines [3]. The complexity of this scenario is further increased by two other elements: 1) each individual, despite the presence of a common core responsible for basal trophic-metabolic activities, is differentiated by a completely personal bacterial profile, rightly assimilated to a fingerprint, and 2) the composition of this microbiota may record variations in both physiological conditions (for example in relation to age, diet and lifestyle and living environment) and pathological conditions (exposure to antibiotics or radiation, obesity, psychophysical stress) [2,4,5]. Base on this background, it is of great importance the definition of a healthy microbiota (eubiosis), that should be linked to the concept of intestinal well-being, generically defined as the absence not only of disorders but also of unfavorable local conditions, such as for example an increase in permeability [6]. This concept could also be summarized as harmonious balance between structural, microbial and functional structure and that can find important support and maintenance tools in prebiotics and probiotics [7]. Hence, the altered composition of a microbial consortium, the so-called dysbiosis can be associated to gastrointestinal pathologies [8] but it can also affect other organs and districts (i.e. cardiovascular system [9,10], respiratory system [11], skin [12], joints [13], eyes [14], metabolic [15], immune [16] and central nervous systems [17]).

This ubiquitous involvement in health and diseases has reinforced current research in physiology to better understand the healthy role of human body microbiota, as well as a pharmacological development as a novel and promising therapeutic approach to human diseases [18,19]. Modulation of gut microbiota is frequently suggested in intestinal disorders [20] by using prebiotics and probiotics [21,22]. We have previously reported that a new enriched nutraceutical composition containing maltodextrin, hyaluroic acid and mineralized seaweed extract Lithothamnion Calcareum plus a mix of five different lactic bacterial species, is effective in the management of Irritable Bowel Syndrome (IBS). It reduces IBS-related symptoms such as bloating, abdominal pain, frequency and intensity of flatulence, evacuation urgency, tenesmus and, finally, positively modifying the sense of well-being perceived by the patient and evaluated by the doctor [21]. Intriguingly, an unexpected blood glucose and triglycerides levels reduction was also observed [21]. It has been shown that the composition of gut microbiota is able to influence lipids metabolism on host physiology [23]. The combination of housing conditions and the related changes in gut bacterial colonies may modulate gut barrier integrity for better or for worse [24]. It has been reported that high fat diet rapidly and reproducibly modifies human gut microbiome increasing bile-tolerant bacteria attributed to the high fat provision and reducing the levels of fermentable carbohydrates related bacteria [25]. Intestinal microbiota and triglycerides share the colonic tract. In the first step of their metabolism, triglycerides are breakdown into free fatty acid (FA) and

glycerol by lipases. In the human gut there are few variety of microorganisms possessing lipase activity [26,27]. Intestinal bacteria may react with FA, usually through hydration and biohydrogenation of the unsaturated bonds in the aliphatic chain of monounsaturated and/or polyunsaturated fatty acids [28]. A widespread activity in the gut microbiota is the formation of hydroxystearic acids from unsaturated FA [28]. Moreover, apart from the conversion of dietary lipids, the gut microbiota may also produce bioactive lipids that can pass the epithelial barrier, thus influencing host metabolism [28]. Considering the available evidence, it has been accepted that gut microbiota may regulate host cholesterol homeostasis [29]. On this regard, it has been reported that a low microbiota diversity is associated to higher triglycerides and lower high density lipoprotein-cholesterol (HDL-C) levels in humans [23,30]. Specifically, it has been estimated that up to 6% of triglycerides and up to 4% HDL variance is related to the microbiota composition [31]. In our previous report [21], we have found a significant reduction of triglycerides after one month administration of Triobiotix® that is a mix of five different bacterial strains of lactic ferments, thus increasing microbiota. To further expand how the administration of this enriched nutraceutical composition affects gut microbiota, we have used a metagenomics approach to better define its biodiversity and homeostatic function.

METHODS

Study design, patient selection and sample collection: A multicenter, retrospective observational study was conducted to evaluate gut microbiota modulation in subjects taking Triobiotix®. This study complies with the principle of Good Clinical Practice and the Declaration of Helsinki. Institutional Review Board/Ethics Committee approval is not required for retrospective analysis of database in Italy. Informed consent was obtained from all subjects involved in the study.

Study population was identified from the database of 50 family practitioners as previously reported [21] selecting subjects in whom analysis of feces was available. Fecal samples were self-collected by each subject. Fe-Col (Alpha Laboratories Ltd, Eastleigh, United Kingdom), a disposable paper device to prevent sample contamination, and SMART eNAT (Copan SpA, Brescia, Italy) for fecal sampling and preservation were used. The collected specimens were delivered to the Wellmicro® laboratory (Bologna, Italy) where they were stored at -20°C until processing. Samples were processed within 1 month of arrival.

DNA Extraction and Sequencing: Total microbial DNA was extracted from fecal samples using the DNeasy 96 PowerSoil Pro QIAcube HT Kit on a QIAcube HT instrument (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A bead-beating step with Lysing Matrix E (MP Biomedicals) was performed on a FastPrep24 bead-beater (MP Biomedicals, Irvine, CA) at 6.0 movements per second for 40 s, before total DNA extraction. Quantity and quality of the extracted DNA was assessed by spectrophotometric reading of the absorbance at 260 nm by the QIAExpert (Qiagen, Hilden, Germany) and electrophoretic run on the QIAdvanced (Qiagen, Hilden, Germany), respectively. As for standard procedure, the V3 and V4 regions of the 16S rRNA coding gene were amplified to profile the bacterial microbiota using the primer SD-Bact-0341-bS-17/SD-Bact-0785-aA-21 [32]. The mycobiome was profiled using Internal transcribed spacer 2 (ITS2) with the primer set ITS3: 5'-GCATCGATGAAGAACGCAGC-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3'[33]. Indexed libraries were prepared by limited-cycle PCR using Nextera technology (Illumina, San Diego, CA, USA) and further cleaned using VAHTS DNA Clean Beads (Vazyme, Red Maple Hi-tech Industry Park, Nanjing, PRC). Libraries were pooled at equimolar concentrations (4 nM), denatured, and diluted to 5 pM before loading onto a MiSeq Reagent Kit V3

(Illumina, San Diego, CA, USA). Sequencing on the MiSeq platform was performed using a 2 × 300 bp paired-end protocol according to the manufacturer's instructions (Illumina, San Diego, CA, USA).

Raw sequences were processed using a pipeline that combines PANDAseq [34] and QIIME [35]. The high-quality reads were grouped into Amplicon Sequence Variants (ASV) using DADA2 [36]. For the bacterial fraction, taxonomy was assigned using a custom database containing high quality 16S sequences retrieved from NCBI, Silva and Greengens (version May 2012). The fungal fraction was assigned using the UNITE ITS database [37].

The descriptive and functional data of the microbiota under examination are presented by comparing them with a reference database consisting of healthy adult volunteers of both sexes whose sequences were obtained from independent studies by adopting the same NGS methodology used to execute these reports, thus ensuring maximum reliability for the comparison of the analyzed sample with the reference dataset. Specifically, the gut microbiota report show the following parameters: the ratio between two bacterial phyla, Firmicutes and Bacteroidetes, that represent up to the 90% of the human intestine ecosystem and the ratio between Prevotella and Bacteroides, at both variations, increase and decrease, has been associated with several pathological conditions of a metabolic, cardiovascular and neurological nature [38]; dysbiosis index as a marker of alteration of the intestinal microbial ecosystem compared to what is considered a healthy profile; observed species and Phylogenetic Diversity Whole Tree provide diversity and variety of the gut microbiota; a metabolic and functional potential evaluating the production of specific metabolites (acetate, propionate and butyrate, lactate, sulphurated hydrogen, and lipopolysaccharide (LPS)); finally, the analysis microbiota/healthy status was performed associating the bacterial ecosystem profile with the main physiological functions in which the intestinal microbiota is involved.

Statistical Analysis: The IBM SPSS 26.0 software (https://www.ibm.com/analytics/us/en/technology/spss/) was used to perform the whole statistical analyses. Bonferroni corrected p-values < 0.05 were considered as statistically significant. QIIME by pairwise non-parametric t-test with 999 permutations and QIIME by PERMANOVA were used to elaborate significant differences in alpha and beta diversity, respectively. Additionally, permDISP was used to evaluate significant differences in dispersion [39,40]. Taxonomic comparisons were performed by Analysis of Composition of Microbiomes (ANCOM), which exploits compositional log-ratios to identify statistically significant taxa [41].

RESULTS

From the analysis of the database of 50 family practitioners 265 subjects were selected using the criteria already reported [21]. Among them, 88 patients underwent to fecal samples collection at T0 but only in 46 a T1 collection and metagenomic evaluation was available, thus the final analysis before and after Triobiotix® administration was performed in these 46 subjects. The mean age was 53 ± 15, men 19 and female 27. As specified in the inclusion criteria, the selected patients were not under lipid or glycemic lowering medications. The clinical benefits were in line with the previous report [21]. Additionally, for 171 subjects a 90 day evaluation was available. Of these 30% were reporting an improvement in main IBS-related symptoms, such as flatulence, tenesmus, evacuation urgency, incontinence, blood and mucus in the stool, and up to 39% improvement in bloating. However, the main goal was the evaluation of gut microbiome.

General microbiome composition: Several studies have consolidated the existence of 2 well-defined enterotypes in human intestine:

Enterotype 1 dominated by Bacteroides or Enterotype 2 dominated by Prevotella [42,43]. A third enterotype defined by a possible intermediate configuration is named mixed Enterotype. Enterotypes are associated with functional differences in terms of metabolic capacities [42,43]. The ratio between two bacterial phyla, Firmicutes and Bacteroidetes, that represent up to the 90% of the human intestine ecosystem and the ratio between Prevotella and Bacteroides, at both variations, increase and decrease, has been associated with several pathological conditions of a metabolic, cardiovascular and neurological nature [43-45]. In the present analysis, administration of Triobiotix® is not associated with a significant change in the Firmicutes/Bacteroidetes Ratio (1.98 ± 1.29 vs 2.09 ± 2.38; p=0.72, reference range 1.2 - 4.4), as well as Prevotella/Bacteroides Ratio (1.58 ± 1.99 vs 1.46 ± 2.01; p=0.71, reference range 0.01 - 0.2) as shown in Figure 1.

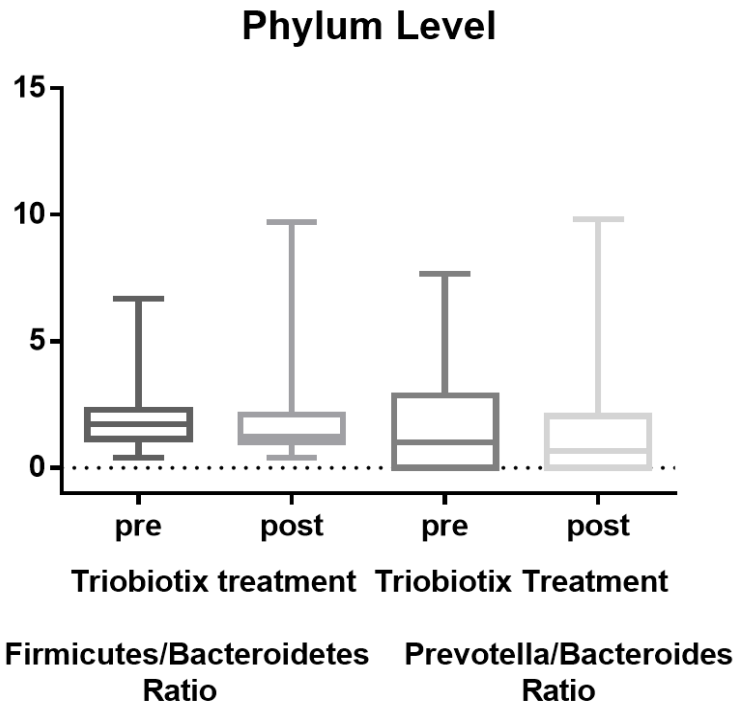


Figure 1. Phylum Level

It has been reported that the greater the number of bacterial species and their phylogenetic diversity, the higher the metabolic potential of the host [46-48]. Thus, to evaluate the modulation of gut microbiota composition, the number of observed species has been detected, the phylogenetic diversity has been evaluated and the biodiversity index has been calculated. As reported in Figure 2, we have found an increased number of observed bacterial species after Triobiotix® administration (107.5 ± 51 vs 127.2 ± 23; p<0.0001, reference range 90-230), a higher phylogenetic diversity (13.3 ± 5.3 vs 18.8 ± 5.9; p<0.0001, reference range 12-27) with subsequent biodiversity index (2.8 ± 1.55 vs 4.1 ± 1.9.; p<0.0001, reference range: very low 0-7.9, quite low 8-11.9, low 12-13.9, normal 14-17.9, high 18-20.9, very high ≥ 21)

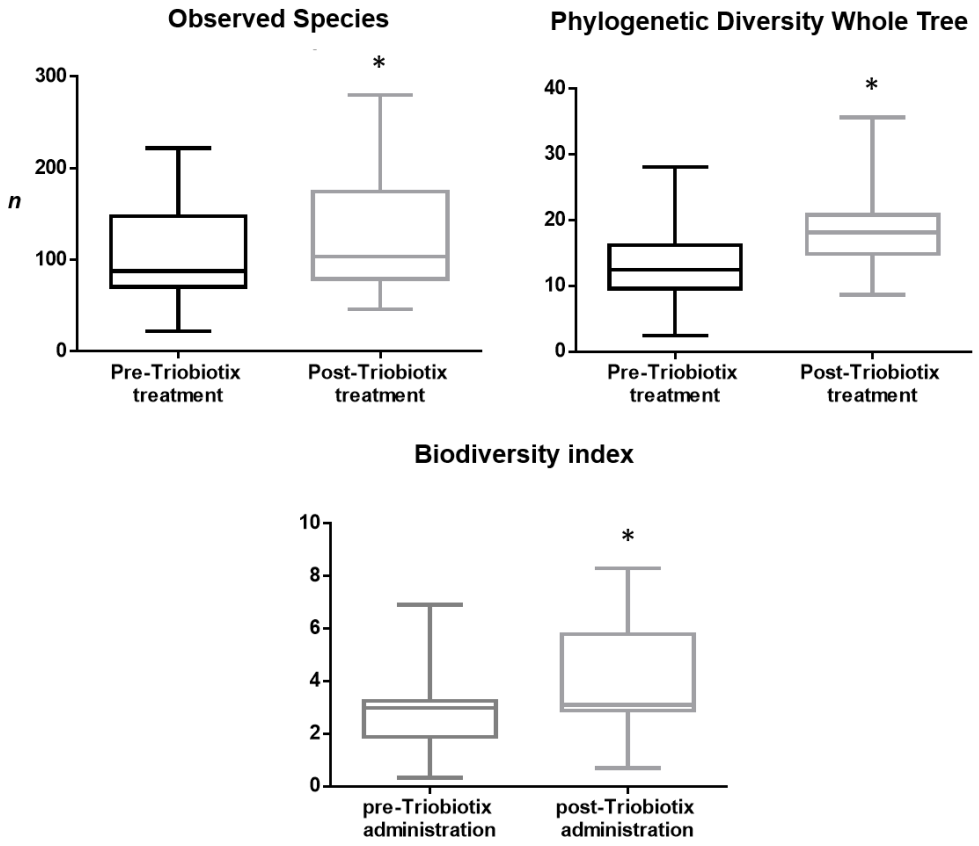


Figure 2. Gut microbiota composition

Finally, an improved dysbiosis index was also described (4.04 ± 2.99 vs 2 ± 0.82.; p<0.0001, reference range: normal 0-0.5, mild 0.6-3, high 3.1-6, very high 6.1-10) as shown in Figure 3

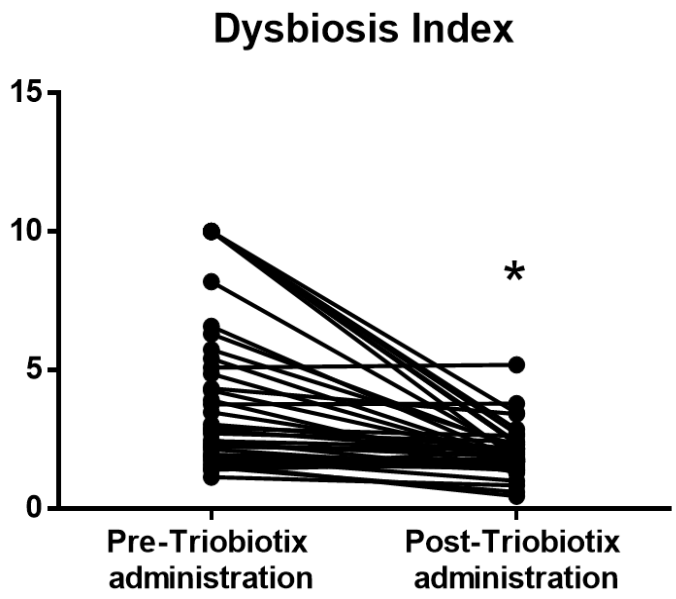


Figure 3. Dysbiosis index

Metabolic potential and functional analysis: A good homeostasis between host and microbiota is essential and is translated at metabolic functions. The Wellmicro® gut test by evaluating the level of short chain fatty acids (acetate, butirate and propionate) [49], lactate [50], sulphurated hydrogen [51], LPS [52], indolepropionic acid [53], mucolytic [54] and proteolytic [55] activity is able to define different metabolic potentials such as immunity, mucosal and glycemic homeostasis, lipid metabolisms, anti-inflammatory and antimicrobial activity. The results of this analysis are reported in table 1.

Table 1: Metabolic potential before and after Triobiotix® administration

	Pre-treatment	Post-treatment	*P value*
Immunity homeostasis	4.4 ± 1.2	5.2 ± 1.13	**0.0013**
Mucosal homeostasis	7.2 ± 1.3	7.5 ± 1.37	0.29
Glycemic homeostasis	6.3 ± 1.39	7.06 ± 1.2	**0.0069**
Lipid metabolism	5.3 ± 2.07	6.89 ± 1.38	**<0.0001**
Anti inflammatory activity	6.8 ± 1.22	7. 9 ± 1.31	**0.00012**
Antimicrobial activity	6.97 ± 1.09	6.91 ± 1.85	0.84

Reference range alteration: very high 0-1, high 2-3, mild 4-5, low 6-7, none 8-10

Of particular importance is indolepropionic acid (IPA), a small-molecule metabolite produced only by microbial degradation [56], that has been associated with host health [53]. Specifically, IPA has different protective effects [57], thus its variation might be an interesting marker of health and/or diseases [53]. IPA variation before and after treatment is reported in table 2.

Table 2. IPA variation before and after Triobiotix® administration

	Pre-treatment	Post-treatment	*P value*
IPA	- 2 ± 2	0 ± 2	**0.02**

Reference range alteration: from -4 (deficency) to +4 (excess). Zero is the optimal value

Finally, the contribution of gut microbiota with the main physiological functions in which it can be involved, has been evaluated. A significant improved contribution in the axis intestine-brain (5.19 ± 2.31 vs. 6.74 ± 2.74; p<0.001), intestine-cardiovascular system (5.95 ± 2.36 vs 7.6 ± 2.11.; p<0.001) and intestine-liver (5.3 ± 1.73 vs 6.84 ± 1.5.; p<0.001) has been found. No difference has been reported for the intestine-skin axis (4.34 ± 1.64 vs 4.43 ± 1.99.; p=0.77). These findings are shown in figure 4.

Clinical benefits in the long term: Of the 265 selected subjects, 171 had a 90-day clinical follow-up available with 30% reporting an improvement in main IBS-related symptoms, such as flatulence, tenesmus, evacuation urgency, incontinence, blood and mucus in the stool, and up to 39% improvement in bloating.

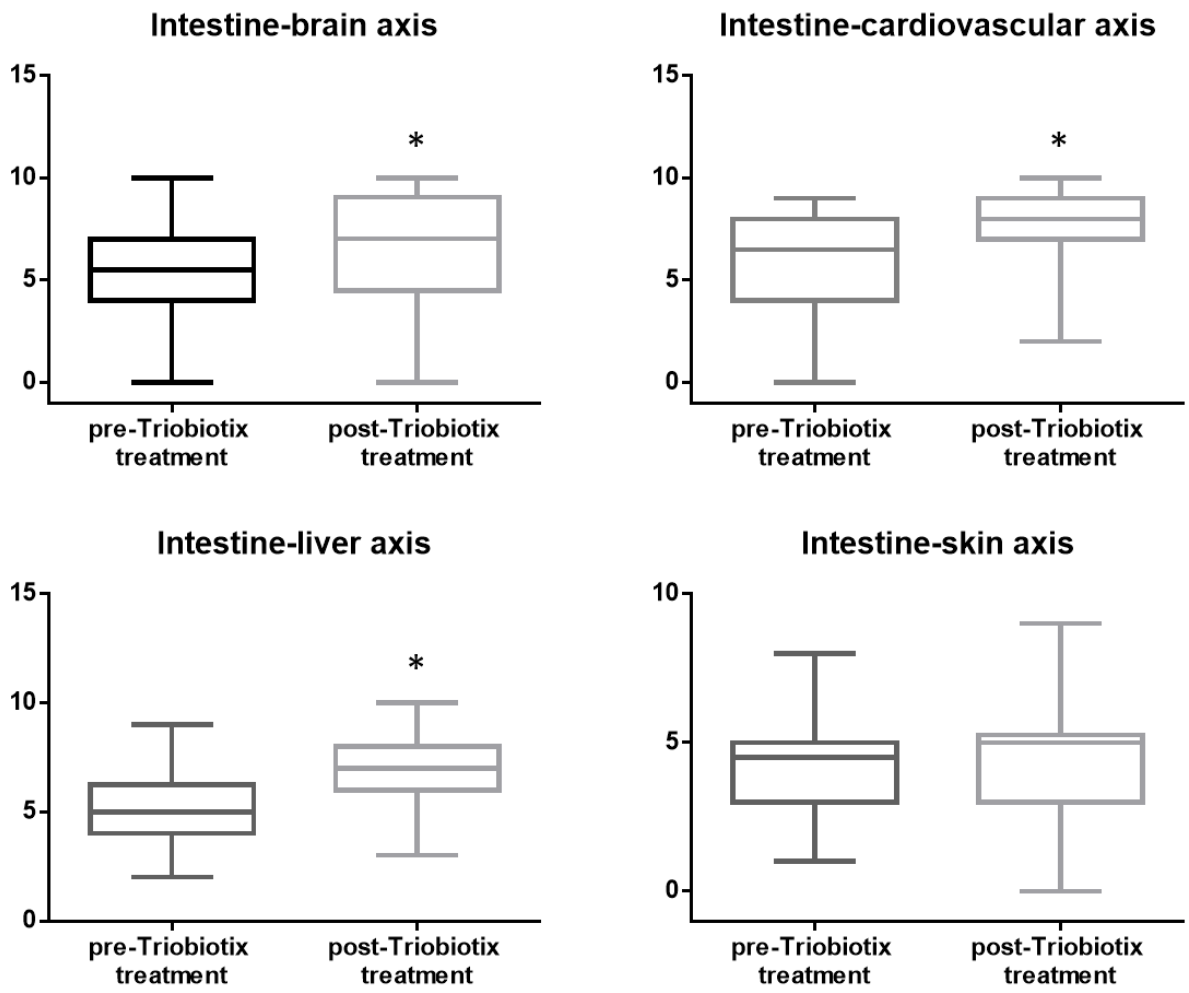


Figure 4. Microbiota contribution in physiological functions.

Reference range alteration: very high 0-1, high 2-3, mild 4-5, low 6-7, none 8-10

DISCUSSION

In the present descriptive report, with all the limitations of a retrospective study, we provide the microbiota description and analysis in patients taking an enriched nutraceutical composition already proved to be effective in reducing IBS related symptoms, as perceived by the patient and/or evaluated by the doctor, and providing a better glycemic and triglycerides profile [21]. Specifically, Triobiotix® administration up to 90-days results in gut microbiota modulation as follow: 1) increasing number of observed species and phylogenetic diversity thus improving biodiversity index; 2) reducing dysbiosis index; 3) improving metabolic potential at immunological, glycemic, lipid and anti-inflammatory level, also via IPA increasing concentrations that is important for mucosal intestinal homeostasis, oxidative stress prevention and inhibition of pro-inflammatory cytokines [53,58,59]; finally 4) positively increasing the microbiota contribution to intestine-brain axis, intestine-cardiovascular axis and intestine-liver axis.

In the last decades the correlation between gut microbiota and human diseases has been a matter of intense investigation thanks to the advancement of genomic, proteomic and metabolomic technologies [5]. Thus, our knowledge of the gut microbiota's physiology is greatly improved, becoming the new frontier for innovative therapeutic strategy [60]. The intestinal dysbiosis has been linked to gastroenteric functional disorders [61], metabolic diseases [15], inflammatory

bowel diseases [62,63], cardiovascular risk [10], neurological disorders [17] and many others [16,64,65]. The modulation of gut microbiota may be achieved by using prebiotics and probiotics alone or in combination [7]. Prebiotics, as non-digestible fibers, act as food for microorganisms, enhancing their growth and activity [7]. On the other hand, probiotics as living microorganisms may contribute to health benefits when consumed in adequate amounts [7]. Triobiotix® is a nutraceutical formula in two sticks: a PREbiotic stick pack containing Maltodextrin, Hyaluronic Acid [66] and mineralized seaweed extract Lithothamnion Calcareum, that as calcium source may contribute to the normal function of digestive enzymes [67]; a PRObiotic stick pack with a mix of ferment bacteria (corn starch, Bifidobacterium animalis ssp. Lactis BLC1 (DSM 17741), Lactobacillus acidophilus LA3 (DSM 17742), Lactobacillus rhamnosus IMC 501 (DSM 16104), Lactobacillus paracasei [68], Streptococcus thermophilus 501 IMC102 SP4 (DSM 19385). The use of separate sticks is an innovative technology to preserve bacteria vitality and maximize the product functionality. Beyond its already proved effect in managing IBS related symptoms and reducing glycemia and triglycerides [21], we provide a description of gut microbiota modulation after 90-days Triobiotix® administration.

Human microbiota composition is different according to the anatomical site/tract (respiratory, skin, vagina, oral, intestine) [2]. The gut microbiota is the most representative and significantly important factor in maintaining human health [4,18-20].Generally, it is composed of 6 phyla including Firmicutes and Bacteroidetes, which represent the major types accounting for up to 90% of the in loco species, with a small amount of Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia [42]. Alteration of the Firmicutes/Bacteroidetes (F/Bts) ratio (in up or down) has been associated to metabolic, cardiovascular and neurological disorders [15,17,69,70]. Bacteroides has high saccharolytic and proteolytic capacity and it is associated with frequent consumption of foods of animal origin while Prevotella exerts high fibrolytic capacity and is associated with frequent consumption of foods of plant origin. In the cohort of patients included in this analysis the F/Bts ratio was within the normal range without any change after nutraceutical administration. Conversely, the Prevotella/Bacteroides (P/B) ratio was out of reference range and increased without any change after treatment. This ratio has been shown to be a potential predictive marker for weight loss and glucose metabolism. It has been reported that in presence of high P/B ratio, individuals are more susceptible to weight loss, compared to those with a low P/B ratio, especially in response to a fiber enriched diet (and possibly carbohydrates), protein and low fat [71-74].

An important finding of the present analysis is the significant increase in the number of observed species, the phylogenetic diversity and the biodiversity index that finally converge in the improved dysbiosis index. Lower bacterial diversity has been reproducibly shown in different diseases [75] as well as dysbiosis [10,15,76,77]. For this reason, the presence of a gut ecosystem rich in bacterial variety may compensate for the function of other missing/lost species. Thus, enrichment of gut microbiota and increasing its biodiversity might be of great therapeutic importance since diversity appears to be a generally good marker of a “healthy gut” [75]. In our cohort of patients, the mean of observed species was within the lower range limit with a significant 20% increase, improving their phylogenetic diversity. Despite the biodiversity index significantly increasing, it remains within the lowest range. However, this data is promising and requires long-term confirmation. However, the enrichment of gut microbiota by increasing the observed species and the phygenetic diversity leads to significant dysbiosis improvement from high to mild alteration.

The improved eubiosis is also associated with more beneficial metabolic function. Specifically, in the cohort

of patients here analyzed, administration of Triobiotix® results in an improved immunological homeostasis, a better gylcemic and lipid metabolism and an increased antiflammatory activity. These findings might explain the clinical and metabolic benefits already described after Triobiotix® treatment [21]. Finally, the contribution of gut microbiota to the functionality of major organs has been also explored. Specifically, a better contribution to intestine-brain axis has been found. Brain and gut are inextricably linked. When microbiota is impaired, a dysregulation of neurological hormone may occur, leading to depression, impaired memory, learning, eating habits, moods and emotions [17]. Additionally, intestine-cardiovascular axis results also improved and this in line the better glycemic profile and lipid metabolism. It is well accepted that gut dysbiosis is linked to the development of cardiovascular diseases [10]. Also, a close relationship between intestine and liver through the gut microbiota and their metabolites has been reported [78]. Free FA includes short-chain fatty acids (SCFAs) and long-chain fatty acids (LCFAs). Acetate, propionate, and butyrate are the main SCFAs produced during the bacterial fermentation of dietary fibers [78]. By measuring these metabolites, the interaction between intestine and liver might be resumed. An improved contribution of gut microbiota to the liver has been postulated after Triobiotix® administration.

Additionally, the benefits of this treatment are confirmed at 90 days follow-up. A percentage of patients still reported a significant improvement in IBS-related symptoms and biodiversity of microbiota, measured as alpha diversity, was maintained. Alpha diversity is a key quantity in microbiome research [79]. The maintenance of abundance, richness and variety is of great importance for long-term benefits [80].

Limitations: Despite the major findings, for this descriptive study some limitations need to be stated: a) the retrospective analysis, b) the sample size that is relatively small, c) the unknown diet regimen for the selected subjects and d) the relatively short follow-up (up to 90 days). Additionally, the collection of data from the family practitioner's database might be another potential limitation since we cannot assure equity in database accuracy. Finally, a control group does not exist.

CONCLUSIONS

This analysis showed that this enriched nutraceutical formulation is able to positively modulate gut microbiota, resulting in reduced dysbiosis, improved metabolic potential and a better contribution from gut microbiota to the brain, heart and liver axis. However, further larger, well-designed and longer studies are needed to confirm the results of this analysis and the potential benefits exerted by this formulation on gut microbiota and it correlation with improved health status.

Abbreviations: FA, fatty acid; HDL-C, high density lipoprotein-cholesterol; LPS, lipopolysaccharide; OTUs, Operational Taxonomic Units; F/Bts, Firmicutes/Bacteroidetes ratio; P/B, Prevotella/Bacteroides ratio; SCFAs, short-chain fatty acids; LCFAs, long-chain fatty acids. IPA, indolepropionic acid

Competing interest: There are no conflicts of interest to declare.

Author contribution: FN, CF, ML, LC, RA, NM, RF and SDE analyzed data. FN, SDE and GC editing and revised the manuscript. FN, and GC designed the study. FN and GC analyzed data, wrote the manuscript, and provided overall supervision. All authors have read and approved the final manuscript.

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