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Differential Modulation of Human Intestinal *Bifidobacterium* Populations after Consumption of a Wild Blueberry (*Vaccinium angustifolium*) Drink

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ABSTRACT: Bifidobacteria are gaining increasing interest as health-promoting bacteria. Nonetheless, the genus comprises several species, which can exert different effects on human host. Previous studies showed that wild blueberry drink consumption could selectively increase intestinal bifidobacteria, suggesting an important role for the polyphenols and fiber present in wild blueberries. This study evaluated the modulation of the most common and abundant bifidobacterial taxonomic groups inhabiting the human gut in the same fecal samples. The analyses carried out showed that *B. adolescentis*, *B. breve*, *B. catenulatum*/*pseudocatelulatum*, and *B. longum* subsp. *longum* were always present in the group of subjects enrolled, whereas *B. bifidum* and *B. longum* subsp. *infantis* were not. Furthermore, it was found that the most predominant bifidobacterial species were *B. longum* subsp. *longum* and *B. adolescentis*. The results obtained revealed a high interindividual variability; however, a significant increase of *B. longum* subsp. *infantis* cell concentration was observed in the feces of volunteers after the wild blueberry drink treatment. This bifidobacterial group was shown to possess immunomodulatory abilities and to relieve symptoms and promote the regression of several gastrointestinal disorders. Thus, an increased cell concentration of *B. longum* subsp. *infantis* in the human gut could be considered of potential health benefit. In conclusion, wild blueberry consumption resulted in a specific bifidogenic effect that could positively affect certain populations of bifidobacteria with demonstrated health-promoting properties.

KEYWORDS: *bifidobacteria, Bifidobacterium longum subsp. infantis, wild blueberry, human study, microbiota, diet, prebiotic*

INTRODUCTION

The human intestinal microbiota is strongly emerging as a main protagonist in the maintenance of host homeostasis.[1] Particularly, specific bacterial populations in the human gut, such as bifidobacteria and lactobacilli, are classically recognized as beneficial for human health, and their reduction has been associated with several definite host dysfunctions.[2]

Intestinal microbiota markedly depends on diet,[3] and numerous nutritional interventions have been shown to selectively modify specific bacterial groups, including bifidobacteria. For instance, certain dietary compounds, named prebiotics, have been reported to selectively increase the beneficial components of microbiota, such as bifidobacteria.[4] According to the Food and Agriculture Organization of the United Nations (FAO), a prebiotic is "*a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota*".[5]

Although most studies have been conducted on the effect of isolated prebiotic molecules or prebiotic-fortified food products, the ability to stimulate the growth of bifidobacteria in the human gut (conventionally referred as bifidogenic effect) has been also recognized for conventional foods that are naturally rich in prebiotics, such as banana,[6] apple,[7] whole grain breakfast cereals,[8] red wine,[9] and coffee.[10] Interestingly, polyphenolic food components including anthocyanins (ACNs) have been documented to have possible effects on intestinal microorganisms,[1,9] suggesting that also ACNs could potentially be prebiotic molecules.

Berries are rich sources of ACNs, which together with flavonoids, phenolic acids, folate, minerals, and fiber are important contributors to the in vivo biological activities of these fruits.[12] It is well-known that absorption of ACNs is quite limited, so that most of the compounds reach the colon, where they are widely metabolized by the intestinal microbiota.[13] Thus, the regular intake of ACN-rich products, such as berries, may affect the different bacterial groups with potential, yet undetermined, benefits.[11] In this context, we recently reported that intestinal bifidobacteria significantly increased in healthy subjects following a 6 week consumption of a wild blueberry (*Vaccinium angustifolium*) drink.[14]

Bifidobacteria are among the very first colonizers of the human intestine immediately after birth, soon becoming the predominant component of the microbiota in breast-fed infant intestine, where they have been purported to play a key role in the maturation of the host's immune system.[15] Bifidobacteria are also abundant in the adult human population, comprising up to 15% of the normal intestinal microbiota.[16] Additionally, the reduction of intestinal bifidobacteria that occurs in the

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elderly has been correlated to enhanced susceptibility to infections and improper bowel functionality.[17] The genus *Bifidobacterium* comprises several species, which can differ from each other in several features, such as surface properties,[18] adhesion to enterocytes,[19] mucin degradation,[20,21] and carbohydrate metabolism.[22] Such phenotypic variances affect the ability of the different bifidobacterial species to interact with the host.[23] In particular, the *Bifidobacterium*'s ability to positively modulate the immune system has been shown to be species specific.[24] For instance, it was pointed out that allergic infants have not only lower counts of bifidobacteria than healthy control subjects but also differences in species. Precisely, *B. adolescentis* and *B. longum* subsp. *longum* were isolated from allergic infants as the predominant bifidobacteria, whereas the predominant ones isolated from age-matched healthy infants were *B. breve*, *B. longum* subspecies *infantis*, and *B. bifidum*.[25] Similarly, an association between reductions in total *Bifidobacterium* and *B. longum* populations and celiac disease in children was reported.[26] This literature suggests that a plausible link between the different bifidobacterial species and the host's immune atopy or tolerance exists.

In light of the above stated considerations, in the present study, the fecal samples obtained in the previously designed repeated-measures, crossover dietary intervention on human volunteers[14] were used to further assess the effect of the wild blueberry drink on seven different bifidobacterial taxonomic groups, including most of the species normally colonizing the human gut. To our knowledge, this is the first study that evaluates the bifidogenic prebiotic activity of wild blueberries in humans by considering intragenus groups of intestinal bifidobacteria.

EXPERIMENTAL METHODS

Experimental Design. The full experimental design was previously reported.[14,27] In brief, 20 healthy male volunteers, with at least one risk factor for cardiovascular diseases, were recruited from the staff of the University of Milan.

Volunteers had homogeneous eating habits, especially for fruit and vegetable consumption, as verified by an interview. Subjects who followed a specific diet, such as vegetarian, vegan, or macrobiotic, and those who had a specific aversion to blueberries or their products were excluded. Subjects were specifically excluded from the study if they were taking drugs, supplements, specific prebiotics, or medications during the last month before the beginning of the experiment. Moreover, subjects were excluded for clinical diseases such as diabetes, renal insufficiency, known food allergic reactions, chronic constipation, diarrhea, or any other gastrointestinal problem or disease.

The dietary intervention was performed using a crossover design. Participants were randomly divided into two groups. Subjects of the first group consumed a drink (250 mL) of wild blueberry (WB) every day for 6 weeks (WB treatment). After a 6 week wash-out period, these volunteers consumed for 6 weeks a placebo drink "control treatment". Subjects of the second group followed the sequence: control treatment−wash-out−WB treatment. For the duration of the experiment, volunteers were instructed to maintain their normal dietary and lifestyle habits (as assessed before enrollment) and to abstain from consuming ACN-rich foods (a list of prohibited foods was provided). The volunteers were also asked to keep a food record during the experimental period, to monitor compliance with the given instructions. At the beginning and at the end of each experimental period, fecal samples were collected and stored at −80 °C until analysis.

The study was approved by the Local Ethics Committee and in accordance with the Declaration of Helsinki. All participants gave informed consent to the study.

Five volunteers of the 20 originally involved in the study did not collect fecal samples for the complete duration of the experiment and were excluded from the study.

In Table 1 the baseline characteristics of the subjects are reported. Most parameters were within the range of normality. However, according to the guidelines of the American Heart Association,[28] six subjects were classified as overweight (BMI > 25 kg m^{-2}), five had high levels of total cholesterol (≥6.2 mmol/L), and seven borderline high levels of cholesterol (5.17−6.18 mmol/L). Four subjects had high levels of LDL-cholesterol (≥4.13 mmol/L), and seven were borderline high (3.36−4.11 mmol/L). Nine subjects were prehypertensive (systolic pressure 120−139 mmHg and diastolic pressure between 80 and 89 mmHg). Seven subjects were smokers/ex-smokers.

Table 1. Subject Characteristics at the Beginning of the Study (n = 15)[1a]

variable (unit)	value
age (years)	47.1 ± 9.2
body weight (kg)	75.5 ± 8.7
BMI (kg m^{-2})	24.8 ± 3.0
systolic blood pressure (mmHg)	120 ± 12
diastolic blood pressure (mmHg)	79.4 ± 7.4
glucose (mmol/L)	5.0 ± 0.4
triglycerides (mmol/L)	1.2 ± 0.5
total cholesterol (mmol/L)	5.7 ± 1.1
LDL-cholesterol (mmol/L)	3.6 ± 0.9
HDL-cholesterol (mmol/L)	1.4 ± 0.2
S-aspartate aminotransferase (U/L)	22.9 ± 5.1
S-alanine aminotransferase (U/L)	27.2 ± 12.1
S-γ-glutamyltransferase (U/L)	25.9 ± 10.4
creatinine (mmol/L)	79.5 ± 9.7
anthocyanins (μmol/L)	nd
vitamin C (μmol/L)	62.4 ± 17.8
folate (nmol/L)	7.4 ± 2.0
vitamin B_{12} (pmol/L)	415 ± 157
GSH (μmol/g Hb)	5.7 ± 1.0
GSSG (μmol/g Hb)	0.67 ± 0.44
GSH/GSSG ratio	10.9 ± 4.7

[a]Data are expressed as the mean ± SD. BMI, body mass index; nd, not detectable; GSH, reduced glutathione; GSSG, oxidized glutathione.

Wild Blueberry and Placebo Drink Preparation. The WB drink was prepared by suspending 25 g of WB freeze-dried powder, that is, a composite from Wayman's (Cherryfield, ME, USA), standardized at 1.5% total ACNs by FutureCeuticals, (Momence, IL, USA) in 250 mL of water to deliver an amount of WB equivalent to 1 cup of raw fruits (148 g, providing approximately 375 mg of ACNs and 127.5 mg of chlorogenic acid). Details about the nutritional composition of the soluble WB powder used in the study were previously reported.[27] The placebo drink did not contain polyphenols and consisted of 250 mL of water, 7.5 g of fructose, 7 g of glucose, and 0.5 g of citric acid. To have sensory and color characteristics similar to the WB drink, 0.03 g of blueberry flavor (Kerry Ingredients & Flavours Italia S.p.A., Bergamo, Italy) and colorants (280 μL of allura red AC 1%, 70 μL of brilliant blue FCF 1%, typically used by the food industry for the production of sweet soft drinks) were added to the placebo drink. Drinks were freshly prepared each morning and were provided to the subjects in ice boxes. Participants kept the drinks in the dark, under refrigeration, and they consumed the drink within the morning. Every Friday, subjects received the drinks for the weekend and kept them refrigerated. The microbiological and chemical stability of the two drinks was ensured respectively until 72 and 48 h, as previously reported.[27]

DNA Extraction and Quantification. DNA was extracted from 230 mg of feces using the QIAamp DNA stool Mini kit (Quiagen, Hilden, Germany) following the manufacturer's instructions. The

Table 2. Primers Used for the qPCR Analyses,[1] Including Species *B. catenulatum* and *B. pseudocatenulatum*[a]

target	primer	sequence (5′−3′)	product size (bp)
Bifidobacterium spp.	g-Bifid-F	CTCCTGGAAACGGGTGG	∼550
	g-Bifid-R	GGTGTTCTTCCCGATATCTACA	
B. adolescentis genotype A	BiADOg-1a	CTCCAGTTGGATGCATGTC	279
	BiADOg-2	CGAAGGCTTGCTCCCAGT	
B. adolescentis genotype B	BiADOg-1b	TCCAGTTGACCGCATGGT	279
	BiADOg-2	CGAAGGCTTGCTCCCAGT	
B. bifidum	BiBIF-1	CCACATGATCGCATGTGATTG	278
	BiBIF-2	CCGAAGGCTTGCTCCCAAA	
B. breve	BiBRE-1	CCGGATGCTCCATCACAC	288
	BiBRE-2	ACAAAGTGCCTTGCTCCCT	
B. longum subsp. *infantis*	BiINF-1	TTCCAGTTGATCGCATGGTC	828
	BiINF-2	GGAAACCCCATCTCTGGGAT	
B. catenulatum group[1]	BiCATg-1	CGGATGCTCCGACTCCT	285
	BiCATg-2	CGAAGGCTTGCTCCCGAT	
B. longum subsp. *longum*	BiLON-1	TTCCAGTTGATCGCATGGTC	831
	BiLON-2	GGGAAGCCGTATCTCTACGA	

[a]All pairs of primers were according to Matsuki et al.[31]

DNA was then quantified using a SmartSpec Plus spectrophotometer (Bio-Rad Laboratories S.r.l., Segrate, Italy). Possible major contaminations in the DNA preparations were evaluated through 260/280 and 260/230 absorbance ratios. After quantification, DNA samples were diluted with nuclease-free water to reach a DNA concentration of 5 ng/μL and stored at −20 °C.

Quantification of the Bifidobacterial Cell Concentrations by Real-Time Quantitative PCR. Bifidobacterial cell concentration in fecal samples was carried out by quantitative real-time polymerase chain reaction (qPCR) using a SsoFast EvaGreen Supermix (Bio-Rad Laboratories) on a CFX96 thermocycler (Bio-Rad Laboratories). The analyses were performed in duplicate using eight pairs of primers targeting different bifidobacterial groups (Table 2). In brief, qPCR reactions were carried out in a final volume of 15 μL containing 7.5 μL of EvaGreen Supermix (Bio-Rad Laboratories), 0.3 μM of each primer, and 30 ng of template DNA. The following PCR protocol was used: initial denaturation at 95 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, 58 °C for 20 s, and 72 °C for 20 s. Melting curve analyses were finally performed to confirm the specificity of the amplification products. To determine bifidobacterial cell concentrations, calibration curves were generated with the genomic DNA, isolated from the following bifidobacterial pure cultures: *B. adolescentis* DSM 20083^T (for BiADO genotype A primers); *B. adolescentis* MIMBa24 (for BiADO genotype B primers); *B. bifidum* DSM 20456^T and *B. bifidum* MIMBb75 (BiBIF primers); *B. breve* DSM 20213^T and *B. breve* BBR02 (BiBRE primers); *B. catenulatum* ATCC 27539^T, *B. catenulatum* MIMBc15, *B. pseudocatenulatum* DSM20438^T, and *B. pseudocatenulatum* MIMBp287 (BiCAT primers); *B. longum* subsp. *longum* DSM 20219^T and *B. longum* subsp. *longum* MIMBl8 (BiLON primers); *B. longum* subsp. *infantis* ATCC 15697^T and *B. longum* subsp. *infantis* BNF01 (BiINF primers). The calibration curve with g-Bifid primers was prepared with all of the above bacterial strains mixed in equal proportion. All strains indicated with the suffix "MIM" belong to the culture collection of the Division of Food Microbiology and Bioprocessing, Department of Food, Environmental and Nutritional Sciences (DeFENS, Università degli Studi di Milano). In detail, a total of 10^9 bacterial cells was counted by means of a Neubauer-improved counting chamber (Marienfeld GmbH, Lauda-Königshofen, Germany), and DNA was extracted with a QIAamp DNA stool Mini kit (Quiagen); DNAs from pure culture mixes were subsequently subjected to six 10-fold serial dilutions and used as the template in qPCR. Finally, standard curves were generated by plotting the log(10) of the bacterial cell numbers against the corresponding Ct values obtained from the amplification of diluted DNAs. Calibration curves showed good correlation between Ct and the number of cells over the considered range (regression coefficients r^2 between 0.964 and 0.999). The resulting detection limits were betweem 1.1 and 1.9 log(10) bacterial cells per reaction mix.

Statistical Analysis. Statistical analyses were performed by means of Statistica software (Statsoft Inc., Tulsa, OK, USA). The presence of a significant carry-over effect was evaluated by a repeated-measure analysis of variance (ANOVA) with the sequence of treatments (WB then placebo or placebo then WB) as the independent factor. As no carry-over effect was observed, data were matched and analyzed with ANOVA considering treatments (WB and placebo) and time (before and after treatments) as dependent factors. A similar analysis was also performed considering as treatments WB (or placebo) and the wash-out period. Results were considered significant at $p \leq 0.05$. Differences between means were further evaluated by the least significant difference test (LSD).

RESULTS AND DISCUSSION

In a recent repeated-measure, crossover intervention study in our laboratory, the consumption of a wild blueberry drink was shown to selectively increase bifidobacteria in the feces of healthy volunteers.[14] Bifidobacteria are commonly recognized as health-promoting microorganisms so that an increase in bifidobacterial cell number in the intestine has been used as an end-point in intervention studies with intestinal health-targeted products. In fact, several studies demonstrated that dietary interventions, particularly with prebiotic molecules such as fructo-oligosaccharides, galacto-oligosaccharides, or inulin, can raise the overall number of intestinal bifidobacteria in humans.[29] However, although several clinical/intervention studies have monitored total numbers of bifidobacteria, the

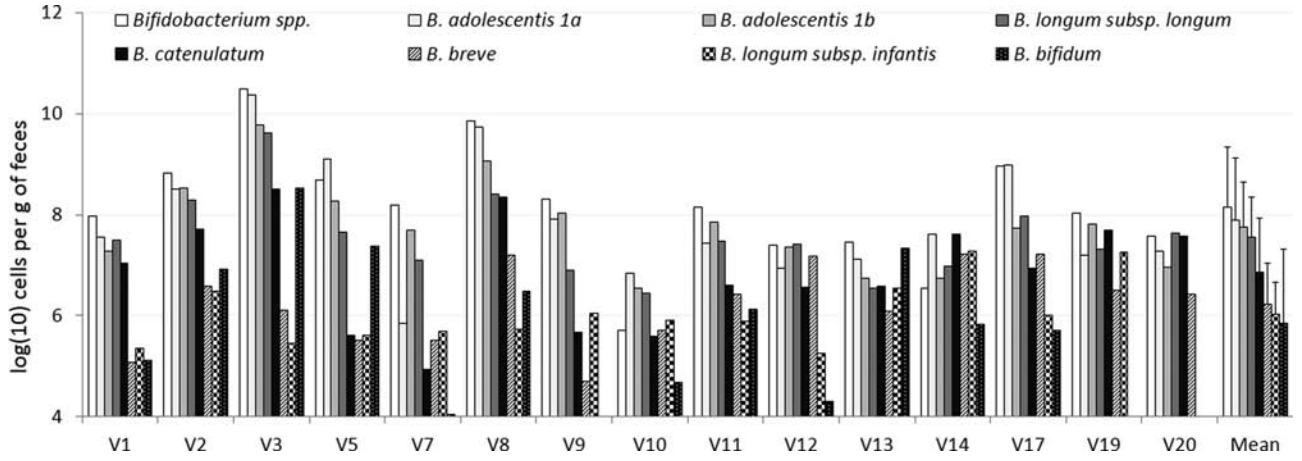

Figure 1. Cell concentration of bifidobacterial groups in the feces of volunteers at recruitment (n = 15). Data are expressed as logarithm-transformed numbers of cells. Standard deviations (calculated on two replicates) are always below 15%, and they are not shown in the histogram.[1] Mean values (± standard deviation) were calculated by considering the data of all volunteers for a specific microbial group. V1−V20, volunteers' codes.

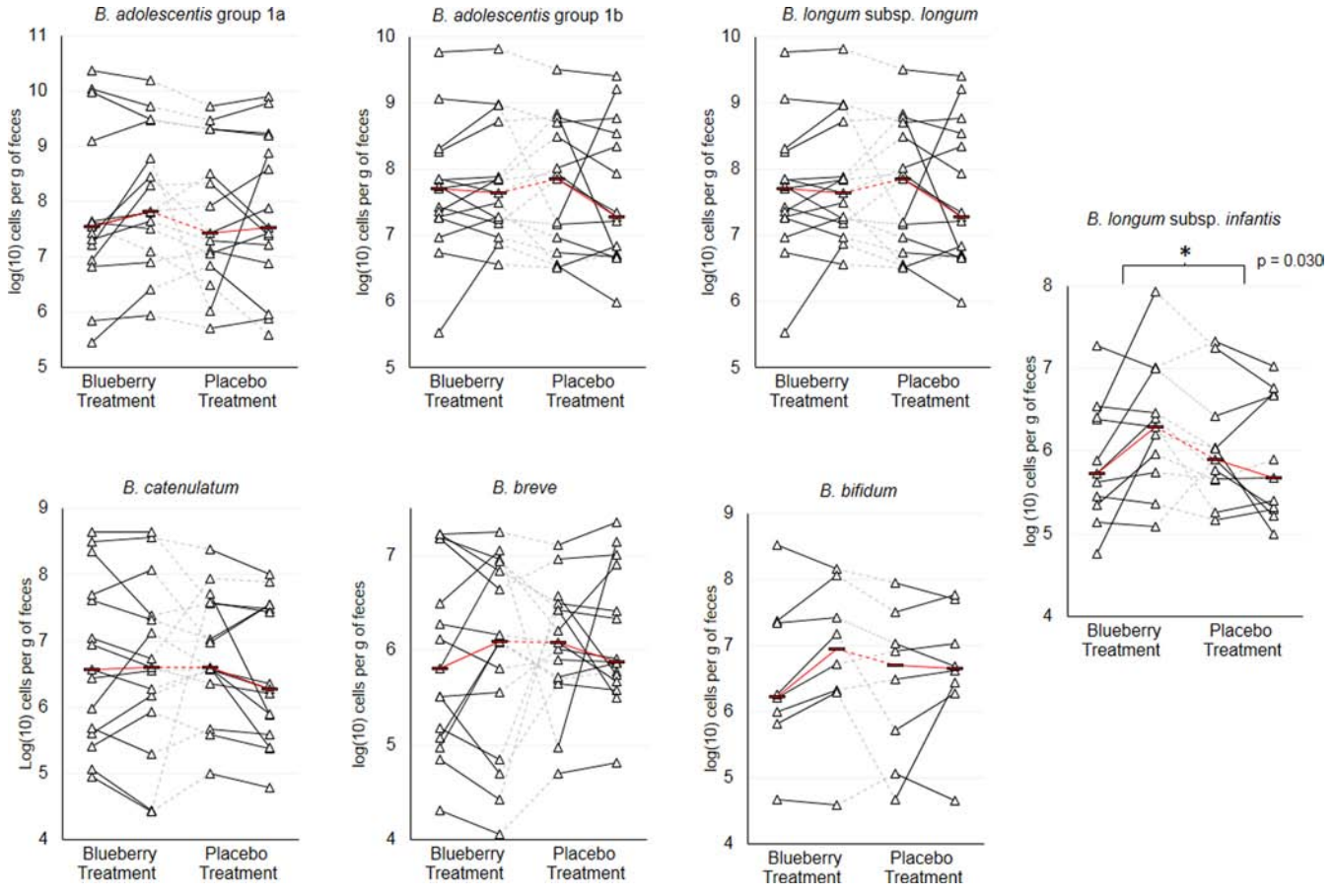

Figure 2. Variations of cell concentration in different bifidobacterial groups evaluated in fecal samples of each volunteer before and after blueberry and placebo treatments (n = 15; *B. longum* subsp. *infantis*, n = 11; *B. bifidum*, n = 8). ∗, statistically significant difference as calculated through repeated-measure ANOVA.

individual species of the genus have been considered only very rarely.[30]

In this study, we used real-time quantitative PCR technology to enumerate the cell concentration of seven different intragenus bifidobacterial taxonomic clusters in the same fecal samples collected during the study by Vendrame et al.[14] The microbial groups that we selected are among the most common and abundant bifidobacterial species inhabiting the human gut. Specifically, we analyzed *B. longum* subsp. *longum*, *B. adolescentis* (genotypes 1a and 1b),[31] and *B. catenulatum*/*pseudocatenulatum*, which are usually found in the adult intestine,[16,32] as well as *B. longum* subsp. *infantis*, *B. breve*, and *B. bifidum*, which are the most prevalent species in infants[33] but can also inhabit the intestinal tract of adults.[18,32]

The analyses carried out document that *B. adolescentis* (both genotypes), *B. breve*, *B. catenulatum*/*pseudocatelulatum*, and *B.*

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longum subsp. *longum* were present at all four time points (i.e., before and after blueberry and placebo treatments) in the whole group of subjects enrolled (n = 15), whereas *B. bifidum* and *B. longum* subsp. *infantis* were not detected in the fecal samples of two subjects (who, therefore, were not included in the analysis for this group of bifidobacteria) (data not shown; Figure 1). Furthermore, in accordance with the literature,[16] we found that the most predominant bifidobacterial species were *B. longum* subsp. *longum* (population levels of log(10) 7.5 ± 0.8 per gram of feces expressed as mean ± standard deviation) and *B. adolescentis* (7.9 ± 1.3 for subgroup 1a and 7.7 ± 1.0 for subgroup 1b). On the contrary, *B. bifidum*, *B. breve*, and *B. longum* subsp. *infantis* were less represented, with an average concentration that was approximately 2 orders of magnitude lower than that of *B. longum* subsp. *longum* and *B. adolescentis* (Figure 1).

Previous investigations revealed that Finnish adult subjects' intestines are typically colonized with one to four bifidobacterial species,[34] whereas the intestines of Japanese adults harbor between one and six bifidobacterial species.[32] Moreover, Matsuki et al.[34] analyzed the distribution of *Bifidobacterium* species in 48 Japanese adults, reporting that *B. adolescentis* was present in 60% of the subjects, *B. bifidum* in 38%, *B. breve* in 13%, *B. catenulatum* group in 92%, and *B. longum* subsp. *longum* in 65%, whereas *B. longum* subsp. *infantis* was never detected. In the present study, we found a wider distribution of bifidobacterial species compared to the cited literature, which can be explained by the high sensitivity of the qPCR protocol employed. Plausibly, the larger dissemination of bifidobacterial species in our samples could be also related to the abundance of bifidobacteria in the Italian population, which were shown to be 2−3-fold higher than in other European populations, independent of age.[16]

Additionally, we performed statistical analyses designed to assess the effect of wild blueberries on the different bifidobacterial targets. The large variability observed among different bifidobacterial cell concentration groups resulted in nonstatistical significance. Nonetheless, our analyses revealed significant variations for *B. longum* subsp. *infantis* (p = 0.030, Figure 2). Specifically, *B. longum* subsp. *infantis* changed in the feces of volunteers from 5.9 ± 0.7 to 6.3 ± 0.8 log(10) cells/g after the WB drink treatment ($p \leq 0.05$) and from 6.00 ± 0.7 to 5.9 ± 0.7 log(10) cells/g after the placebo drink treatment. It should be also mentioned that about half (i.e., 5 of 11) of the subjects had a high increase (i.e., >0.6 log(10)) of *B. longum* subsp. *infantis* following the blueberry drink cunsumption, and this was not dependent on initial concentrations of these bacteria. On the contrary, nonstatistical significant changes in cell concentration were detected in the other bifidobacterial groups (p > 0.25).

The positive impact of wild blueberries on different gut bifidobacterial targets was also confirmed through analysis of the effect of WB treatment on the wash-out period. Specifically, the effect of the WB or placebo drink consumption was compared to the modification in the number of the microbial cells after the 4 week washout period. Analysis of variance revealed significant differences exclusively for *Bifidobacterium* spp. (i.e., the whole genus; p = 0.016) and, again, *B. longum* subsp. *infantis* (p = 0.029) (Figure 3). Additionally, we found a numerical but not statistically significant increase of bifidobacterial cell concentration increase for *B. adolescentis* group 1a (p = 0.059), *B. adolescentis* group 1b (p = 0.121), and *B. bifidum* (p = 0.148) (data not shown).

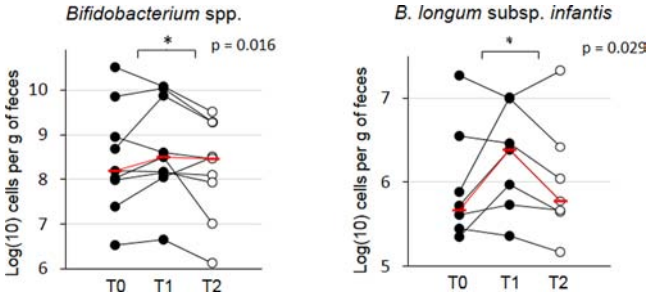

Figure 3. Cell concentration variations in genus *Bifidobacterium* (n = 9) and in the subspecies *B. longum* subsp. *infantis* (n = 7) before (T0) and after (T1) the blueberry treatment and following the wash-out period (T2). ∗, statistically significant difference as calculated through repeated-measure ANOVA.

The in silico analysis of bifidobacterial genomes revealed that >8% of the genes are putatively involved in carbohydrate metabolism, with many of them devoted to the hydrolysis of glycosidic bonds. As previously proposed,[14] such a wide presence of carbohydrate hydrolytic enzymes could enable bifidobacteria to predominate over the other intestinal bacteria from the intake of the blueberry fiber and ACN fractions. Particularly, ACNs could have significantly affected bifidobacteria in the large intestine because they are present in blueberry as glycosylated molecules. Once in the distal tract of the gut, the sugar moiety of ACNs could be used as a carbon and energy source by bifidobacteria.[14,35,36]

The ACNs of the WB powder employed in this study were mainly bound to glucose (34.6% w/w), galactose (31.2%), and arabinose (16.8%).[13] In a recent study, the prebiotic and bifidogenic potential of two low molecular weight polysaccharidic fractions obtained from the partial hydrolysis of gum tragacanth were tested.[37] Interestingly, the tragacanth fractions were shown to be particularly rich in arabinose and galactose and were demonstrated to selectively stimulate the growth of *B. longum* subsp. *infantis* in vitro over that of the other bifidobacterial strains tested.[37]

Genome analysis of strains belonging to *B. longum* subsp. *infantis* revealed the presence of many genes putatively coding for enzymes involved in the hydrolysis of different types of glycosidic linkages, including five genes encoding β-galactosidases.[38,39] Notably, the genome of *B. longum* subsp. *infantis* was shown to possess a 43 kb gene cluster comprising several glycosyl hydrolase encoding genes.[38] It was proposed that these genes could significantly contribute to the ability of *B. longum* subsp. *infantis* to metabolize human milk oligosaccharides (HMO), which constitute a structurally diverse family of lactose-based short-chain carbohydrates containing different types of glycosidic bonds.[38] Furthermore, it was reported that enzymes involved in the metabolism of lactose, glucose, galacto-oligosaccharides, fructo-oligosaccharides, and HMO are constitutively expressed by *B. longum* subsp. *infantis*.[40] Similarly, we hypothesize that *B. infantis*, thanks to its glycosyl hydrolases, can benefit from the utilization of glycosylated ACNs, resulting in their selective proliferation over the other intestinal bifidobacteria.

Certain strains of *B. longum* subsp. *infantis* have been associated with a wide spectrum of effects on the host's physiological processes, which principally involve immunomodulation,[41−44] and with positive effects on several pathologies or dysfunctional conditions. Recent studies reported, for instance, reduction of necrotizing enterocolitis incidence,[45]

normalization of sensitivity to colorectal distension in a rat model of postinflammatory colonic hypersensitivity,[46] inhibition of rotavirus infection,[47] attenuation of the effect of *Salmonella* infection on brush border enzyme activity and weight loss in mice,[48] improvement of distension scores and flatulence in irritable bowel syndrome,[49] and alleviation of symptoms in untreated celiac disease. [50] In addition, *B. longum* subsp. *infantis* and human intestinal bifidobacteria in general are commonly considered safe; a correlation between infections and bifidobacteria has rarely been documented in clinical literature and exclusively in patients with underlying severe medical conditions.[51,52] Therefore, according to the state of knowledge concerning the interaction between *B. longum* subsp. *infantis* and the host, an increase of the cell concentration of this group of bacteria in the human gut could be quite univocally considered a potential health benefit.

In conclusion, this study revealed the ability of blueberry food components to selectively modulate intestinal bifidobacterial populations. A positive role on host health is commonly attributed to all intestinal members of the genus *Bifidobacterium*. Nonetheless, it appears evident from the literature that diverse bifidobacterial species can differently influence host physiology, with certain species (such as *B. bifidum* and *B. longum* subsp. *infantis*) being more commonly associated with specific positive effects[53,54] than others. In this context, we suggest blueberries as a source of prebiotic (bifidogenic) molecules (possibly, fibers and glycosylated ACNs) that can selectively increase certain populations of bifidobacteria (viz., *B. longum* subsp. *infantis*) with demonstrated health-promoting properties.

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Notes

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