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Effect of probiotics on the intestinal microbiota of healthy adults

Submission date 11/03/2014	**Recruitment status** No longer recruiting	[_] Prospectively registered [_] Protocol
Registration date 29/07/2014	**Overall study status** Completed	[_] Statistical analysis plan [X] Results
Last Edited 10/03/2016	**Condition category** Other	[_] Individual participant data

Plain English summary of protocol

Background and study aims
Probiotics, or friendly bacteria, are live bacteria and yeasts that some consider to have a number of various health benefits. They are often eaten in yoghurts or taken as food supplements. They have been taken by people on antibiotics in order to prevent antibiotic-associated diarrhoea. Antibiotics can destroy the protective bacteria in the gut, leading to the aforementioned diarrhoea; high doses of certain probiotics can help restore the balance of gut bacteria and prevent the condition. However, the benefits to healthy people taking probiotics is difficult to measure. The aim of the PROBIOTA study is to assess the effects of taking a probiotic on the balance of gut bacteria in healthy people.

Who can participate?
Healthy adults, aged between 18 and 55.

What does the study involve?
Participants are initially asked to follow their normal diet for four weeks, with the only restriction being to not eat food containing probiotics (such as probiotic yoghurts). After this, each participant is randomly allocated to one of two groups. Those in group 1 are given probiotic capsules. Group 2 are given a placebo. The capsules are taken, preferably with water, twice a day for another four weeks. This is followed by a four week washout period, where all participants resume their normal diet (excluding probiotics). The participants in group 2 are then given the probiotic for four weeks, and group 1 the placebo. Fecal samples are collected from each participant before and after each treatment to test for gut bacteria.

What are the possible benefits and risks of participating?
The benefits and risks of this trial are those conventionally associated with eating commercial probiotic products and are considered low.

Where is the study run from?
The Department of Food, Environmental and Nutritional Sciences, University of Milan (Italy)

When is the study starting and how long is it expected to run for?
February 2013 to May 2014

Who is funding the study?
The study is partially financed by a research grant from the Cariplo Foundation (Milano, Italy) and partly by a research collaboration fund stipulated between Università degli Studi di Milano and Sofar S.p.A (Italy)

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers
N/A

Study information

Scientific Title
Effect of the consumption of probiotic microorganisms on the composition and activity of the fecal microbiota of healthy adults

Acronym
PROBIOTA

Study objectives

The intestinal microbiota exerts a number of activities on human physiology. The modulation of the intestinal microbiota is therefore proposed to have effects on human health. Probiotics are believed to affect the composition of the intestinal microbiota, but such modifications have been never carefully defined for the healthy population. The use of high throughput sequencing technology in metagenomics may serve to properly describe the potential modifications induced by a probiotic dietary intervention of intestinal microbiota.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Ethics Committee of the University of Milan, 19/12/2012, ref: 37/12

Study design
Randomized double-blind placebo-controlled crossover study

Primary study design
Interventional

Secondary study design
Randomised controlled trial

Study setting(s)
Other

Study type(s)
Other

Participant information sheet
Not available in web format, please use the contact details below to request a patient information sheet

Health condition(s) or problem(s) studied
N/A

Interventions
Current interventions as of 30/10/2015:
The trial consists of four phases:
1. Pretreatment (4 weeks)
2. First treatment (4 weeks)
3. Washout (4 weeks)
4. Second treatment (4 weeks)

The trial includes five visits per participant:
1. Before pretreatment (visit V0)
2. Before the first treatment (i.e. after the pretreatment) (V1)
3. After the first treatment (i.e. before the washout) (V2)
4. Before the second treatment (i.e. after the washout) (V3)
5. After the second treatment (V4).

During a pre-recruitment phase (4 weeks), volunteers will follow their conventional diet with the only prohibition to consume: probiotic fermented milk (traditional yogurt is allowed), probiotic food formulas, foods enriched in prebiotic molecules, prebiotic food formulas. At the end of the pre-recruitment stage, volunteers will be randomized to receive to receive the one probiotic formulation or placebo for 4 weeks. Probiotic formulations are two: (i) Enterolactis® Plus constituted by capsules containing at least 24 billion CFU (colony forming units) of freeze-dried Lactobacillus paracasei DG; (ii) Bb probiotic, constituted by capsules containing at least 1 billion CFU (colony forming units) of freeze-dried Bifidobacterium bifidum Bb. The placebo will be constituted by capsules with identical dimensions, color and taste to the probiotic product. The capsules will be consumed with water in the morning (preferably) at least 10 minutes before breakfast or in the evening at least 2 hours after the last meal of the day. After the 4-week treatment, a 4-week wash-out period will follow. After the wash-out, volunteers will receive the probiotic or placebo capsules for 4 weeks, according to the cross-over design.

One fecal sample is collected from each participant at V1, V2, V3, and V4. Every fecal samples is prepared for analysis of the microbiota and the quantification of microbial metabolites. Specifically, the microbiota of fecal samples will be characterized by Ion Torrent PGM sequencing of 16S rRNA-based amplicons obtained using the primer pair Probio_Uni and /Probio_Rev, which encompass the V3 hypervariable region of the 16s rRNA gene. Analyses of microbial metabolites in feces will be performed by high performance liquid chromatography (HPLC; for the quantification of short chain fatty acids) and ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS; for the quantification of primary and secondary bile salts).

Previous interventions:
The trial consists of four phases:
1. Pretreatment (4 weeks)
2. First treatment (4 weeks)
3. Washout (4 weeks)
4. Second treatment (4 weeks)

The trial includes five visits per participant:
1. Before pretreatment (visit V0)
2. Before the first treatment (i.e. after the pretreatment) (V1)
3. After the first treatment (i.e. before the washout) (V2)
4. Before the second treatment (i.e. after the washout) (V3)
5. After the second treatment (V4).

During a pre-recruitment phase (4 weeks), volunteers will follow their conventional diet with the only prohibition to consume: probiotic fermented milk (traditional yogurt is allowed), probiotic food formulas, foods enriched in prebiotic molecules, prebiotic food formulas. At the end of the pre-recruitment stage, volunteers will be randomized to receive to receive the probiotic formulation or placebo for 4 weeks. Enterolactis® Plus will be constituted by capsules containing at least 24 billion CFU (colony forming units) of freeze-dried Lactobacillus paracasei DG. The placebo will be constituted by capsules with identical dimensions, color and taste to the probiotic product. The capsules will be consumed with water in the morning (preferably) at least 10 minutes before breakfast or in the evening at least 2 hours after the last meal of the day. After the 4-week treatment, a 4-week wash-out period will follow. After the wash-out, volunteers will receive the probiotic or placebo capsules for 4 weeks, according to the cross-over design.

One fecal sample is collected from each participant at V1, V2, V3, and V4. Every fecal samples is prepared for analysis of the microbiota and the quantification of microbial metabolites. Specifically, the microbiota of fecal samples will be characterized by Ion Torrent PGM sequencing of 16S rRNA-based amplicons obtained using the primer pair Probio_Uni and /Probio_Rev, which encompass the V3 hypervariable region of the 16s rRNA gene. Analyses of microbial metabolites in feces will be performed by high performance liquid chromatography (HPLC; for the quantification of short chain fatty acids) and ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS; for the quantification of primary and secondary bile salts).

Intervention Type
Other

Phase
Not Applicable

Primary outcome measure
Assessment of the ability of the probiotic to modify the fecal microbiota in healthy adults (as determined by 16S rRNA gene metagenomic profiling). All outcomes are assessed at V1, V2, V3, and V4.

Secondary outcome measures
1. Change in bowel habit
2. Change in fecal metabolite concentration (e.g., short chain fatty acids, bile salts)
3. Persistence of probiotic microorganism in the intestine of volunteers
All outcomes are assessed at V1, V2, V3, and V4.

Overall study start date
01/02/2013

Completion date
01/05/2014

Eligibility

Key inclusion criteria
1. Healthy male or female subjects, aged between 18 and 55 years
2. Signed study-specific informed consent

Participant type(s)
Patient

Age group
Adult

Lower age limit
18 Years

Sex

Both

Target number of participants
At least 20 subjects

Key exclusion criteria
1. Antibiotic therapy during one month before the first visit
2. Viral or bacterial enteritis during the two months before the first visit
3. Gastric or duodenal ulcers during the five years before the first visit
4. Pregnant or breastfeeding women
5. Recent or presumed episodes of alcoholism or drug addiction
6. Presence of conditions determining a non-conformity of the volunteer to the study protocol

Date of first enrolment
01/02/2013

Date of final enrolment
01/05/2014

Locations

Countries of recruitment
Italy

Study participating centre
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Organisation
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Sponsor type
University/education

Funder(s)

Funder type
Research organisation

Funder Name
The Cariplo Foundation (Italy), research grant 2010-0678

Funder Name
Università degli Studi di Milano (Italy)

Funder Name
Sofar S.p.A (Italy)

Results and Publications

Publication and dissemination plan
A first paper concerning the intervention trial with strain L. paracasei DG has been published in 2014 (see below). A second manuscript including the data of the trial with the probiotic strain B. bifidum Bb is intended to be published in 2016.

Microbiomics data (sequence reads) are publicly available in the European Nucleotide Archive of the European Bioinformatics Institute under accession codes PRJEB5801 and PRJEB11694.

Intention to publish date

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Other

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	results	01/11/2014		Yes	No