

SUPPLEMENTARY METHODS

Subjects recruitment

The sample size to observe an effect of diet type on microbiota and metabolome was defined on the basis of previous studies. For the microbiota, abundance of *Prevotella* and *Bacteroides* in studies reporting differences between agrarian and Western diets [1, 2] indicated that 50 subjects per group were sufficient (error 0.05, 80% power, and 2-sided testing). A smaller size was needed for short chain fatty acids on the basis of butanoate and propanoate concentrations reported by De Filippo and collaborators.[3]

Four hundred and fifteen potentially eligible volunteers were contacted by the recruitment units, distributed across 4 geographically distant cities in Italy (Bari, Bologna, Parma and Torino). The VG and V volunteers were recruited with the collaboration of the Italian Society of Vegetarian Nutrition (<http://www.scienzavegetariana.it/>). Prospective participants were excluded according to the following criteria: V, VG and O dietary regime followed for less than 1 year, age under 18 or over 60 years, regular consumption of drugs, regular supplementation with prebiotics or probiotics, consumption of antibiotics in the previous 3 months, evidence of intestinal pathologies (Crohn's disease, chronic ulcerative colitis, bacterial overgrowth syndrome, constipation, celiac disease, Irritable Bowel Syndrome), and other pathologies (type I or type II diabetes, cardiovascular or cerebrovascular diseases, cancer, neurodegenerative disease, rheumatoid arthritis, allergies), pregnancy and lactation. All participants were asked questions about consumption of animal products in order to understand if their dietary habits in the last year diverged from the self-declared diet type. The 24% of volunteers potentially eligible were excluded because they did not meet all the inclusion criteria. The 24% of the remaining 317 individuals, did not accept to participate to the study for personal reasons. The 36% of the remaining (240) volunteers did not finish the study. Therefore, 153 individuals completed the study. The cohort comprised 51 vegetarians (18 males and 33 females, age 39 ± 9 years, body mass index (BMI) 21.9 ± 2.5 kg/m²), 51 vegans (23 males

and 28 females, age 37 ± 10 years, BMI 21.3 ± 2.2 kg/m²), and 51 omnivores (23 males and 28 females, age 37 ± 9 years, BMI 22.1 ± 2.0 kg/m²). All subjects were recruited between January and September 2013 following the guidelines of National Institute of Health. The analysis was performed between October 2013 and June 2014. Recruited subjects were encouraged to do not modify their habitual dietary pattern. The compliance to the declared diet type was verified by means of a 7-day weighed food diary, completed every day for a total of 7 days.

Each donor was provided with two sterile containers and polypropylene spoons and instructed to place about 15g of feces in each, one for DNA extraction and another for metabolome analysis. Moreover, they were provided with sterile urine containers for metabolome analyses. Urinary samples were collected in the morning (first void), discarding the first and the last part of the flow, for three consecutive weeks. The containers were immediately delivered at 4°C to the collection centres and stored at -80°C.

Microbiota diversity analysis

A two ml aliquot of a 1/10 dilution was centrifuged and the pellet was used for microbial DNA extraction using the PowerSoil DNA Isolation Kit (MoBIO Laboratories, Inc. Carlsbad, CA). The V1-V3 region of the 16S rRNA gene was amplified by using primers Gray27F 5'-TTTGATCNTGGCTCAG and Gray519r 5'-GTNTTACNGCGGCKGCTG (520 bp).[4] 454-adaptors were included in the forward primer followed by a 10 bp sample-specific Multiplex Identifier (MID). Each PCR mixture (final volume, 50 µl) contained 50 ng of template DNA, 0.4 µM of each primer, 0.50 mmol l⁻¹ of each deoxynucleoside triphosphate, 2.5 mmol l⁻¹ MgCl₂, 5 µl of 10 X PCR buffer and 2.5 U of native *Taq* polymerase (Invitrogen, Milano, Italy). The PCR conditions used were: 94°C for 2 min, 35 cycles of 95°C for 20 s, 56°C for 45 s and 72°C for 5 min, and a final extension at 72°C for 7 min. Each sample was amplified in duplicate and amplicons were pooled before purification. PCR products were purified twice by using the Agencourt AMPure kit (Beckman Coulter, Milano, Italy), quantified by using a QuantiFluor™ (Promega, Milano, Italy)

and an equimolar pool was obtained prior to further processing. The amplicons were sequenced on a GS Junior platform (454 Life Sciences, Roche Diagnostics, Italy) according to the manufacturer's instructions by using a Titanium chemistry. We failed to prepare the 16S rRNA gene libraries for 3 samples that were excluded for the microbiota analysis.

REFERENCES

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