SUPPLEMENTARY METHODS

Metagenomic analysis

Bacterial DNA extraction

DNA is extracted as described in [1] using a combination of physical cell-disruption (Bead-beater) and silica column purification using QIAamp DNA Stool Mini Kit (QIAGEN, Missisauga, ON, Canada) [1]. Briefly, approximately 100 mg of feces are manually crushed in ASL buffer (QIAGEN) and mixed with 0.4 g of 0.1 mm zirconium beads. Homogenisation is carried out in a MiniBeadBeater (Biospec Products, Bartlesville, OK, USA) to break bacterial cells. After centrifugation (1 min, 20 000 *g*), supernatant is treated with an InhibitEx reagent (QIAGEN) in order to remove PCR inhibitors present in large quantities in the feces. RNA and proteins are degraded using RNAse (Roche Diagnostics, Indianapolis, IN, USA) and proteinase K (QIAGEN). DNA Purification is then carried out using QIAamp DNA Stool Mini Kit (QIAGEN) according to supplier's recommendations. Total extracted DNA is quantified with a ND-1000 Nanodrop (Nanodrop Technologies, Wilmington, DE, USA). All extracted DNA samples were pooled according to dietary/treatment conditions (Chow, HFHS, and CE) and time of sampling (weeks 0, 1, 5 and 9) which resulted in a total of 12 DNA samples.

16S rRNA gene amplification and purification

For each pooled DNA sample, a fragment of the V6-V8 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR with bar-coded primers adapted from existing rRNA primers as described in Comeau et al. [2]. The PCR reaction mixture contained 1 µL of template DNA, 200 µM of each dNTP (Feldan Bio), 1X HF polymerase buffer (NEB), 0.2 µM of each 454 primer (Invitrogen), 0.4 mg/mL BSA (Fermentas), 1 U of Phusion High-Fidelity DNA polymerase (NEB). The PCR program was as follows: an initial cycle at 98°C during 30 s, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, with a final extension of 72°C during 5 min. PCR products were purified using the QIAquick PCR purification kit (QIAGEN). The concentration of the samples was evaluated

spectrophotometrically using a NanoDrop ND-1000 (Thermo scientific). Final amplicon mean length varied between 497-507 bp for all samples.

454 pyrosequencing

Sequencing of the 12 bar-coded amplicons was conducted using 1/8th plate on a single run of Roche 454 GS-FLX Titanium platform at the IBIS (Institut de Biologie Intégrative et des Systèmes - Université Laval). The pyrosequencing run yielded 132611 sequences with numbers of reads varying between 4560 and 8745 per sample.

Raw sequence pre-processing quality control and re-sampling

After de-multiplexing of the raw 454 reads by the Roche MID (multiplex identifier) script, a series of sequence processing was carried out with Mothur [3] to remove low quality reads. Briefly, sequences were filtered using the following restrictions: presence of one or more uncertain bases (N); too short (<150 bp after adaptor and bar-code removal) or too long reads (greater than expected amplicon size); sequences that begin with low quality (incorrect F primer) or containing homopolymers longer than 8 nucleotides. All bases beyond the R primer were trimmed. According to Huse et al., those quality control steps have shown to reduce 454 sequencing reads error rates <0.2% [4]. Mothur was used to identify and eliminate chimeric reads and sequences considered as contaminants (with mitochondrial or chloroplastidial origin). Reads that passed the above quality filter were then aligned against SILVA reference alignments using the k size = 9 parameter. Then, aligned reads were manually screened to remove all misaligned sequences. All aligned reads were randomly re-sampled according to their respective bar-code to equalize the number of reads between all samples. After re-sampling, each of the 12 samples contained 2566 reads which was equal to the smallest amount of sequences originally found in the bar-coded samples. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra/) under the Bioproject ID PRJNA239219.

OTU detection

Sequences that successfully passed pre-processing and filtering steps were used in Mothur to generate a pair wise distance matrix from which reads were then clustered into OTUs (Operational Taxonomic Units) using the furthest-neighbor algorithm and specifying a distance cutoff value of 0.03. The sequence identity threshold of 97% allows reasonable discrimination of sequences at the species- or at least genus-level [5, 6, 7]. The choice of a clustering distance of 0.03 is also judicious considering that the intragenomic distance between 16S rRNA gene copies is typically less than 0.03, and thereby avoiding classifying replicate 16S rRNA gene sequences from the same genome into different OTUs [8]. Singletons (OTUs with a unique sequence occurring only once among all reads) were discarded at this step.

OTU identification

Taxonomical classification of OTUs was carried out in Mothur with the Wang method [9] and a 50% bootstrap cut-off value. This strategy has been shown to be ≥95% accurate at the genus level on real and simulated pyrosequencing reads [4, 10, 11], even for much shorter sequences (around 80 pb) than those produced in this study [10]. As described in Comeau *et* al., representative sequences of each OTU were taxonomically assigned comparing them to a customized collection of reference sequences based on the Greengenes [2, 12] and the RDP databases [13], and trimmed to the V6-V8 region.

Statistical comparisons of metagenomic samples

We employed the Statistical Analysis of Metagenomic Profiles (STAMP; version 2.0.0) statistical probability model to identify biologically relevant differences between metagenomic communities [14]. This model allows choosing appropriate statistical methods to evaluate differences in the proportions of sequences assigned to different taxonomic groups between metagenomes, while considering effect sizes and confidence intervals in assessing biologically relevant differences. Statistically significant differences between taxonomic profiles (at the genus-level) of multiple metagenomic samples obtained after different dietary and temporal conditions were calculated using Principal Component Analysis (PCA), using ANOVA model combined with the Tukey-

Kramer method as Post-hoc test, and the Storey's false-discovery-rate (FDR) or Benjamini-Hochberg FDR method as a multiple-hypothesis test correction. Two-way comparisons of taxonomic distributions (at the phylum- or genus-level) between metagenomic samples were tested within STAMP, using the Fisher's exact test associated with the Newcombe-Wilson method for calculating confidence intervals (nominal coverage of 95%). To indicate the percentages of false positives (reported by q values) that should be expected among all significant taxonomic units illustrated on extended error bar plots, a Storey or Benjamini-Hochberg FDR approach was employed. Two filters were applied to all analyses performed with STAMP to remove features with a q value of >0.05 or an effect size <1.

Real Time PCR

Total RNA was extracted from jejunum and proximal colon and purified using the RNeasy Microarray Tissue Mini Kit (Qiagen). Purified RNA was used for cDNA synthesis using a reverse transcription PCR kit (Applied Biosystems). Real-time PCR was performed using the SYBR Green Jump-Start Gene Expression Kit (Sigma) with 1:10 diluted cDNA product from the reverse transcription. Hprt (hypoxanthine guanine phosphoribosyl transferase) was used as the housekeeping gene. Data were calculated according to the $2^{-\Delta\Delta Ct}$ method. Primer sequences for targeted mouse gene are available in Table S3.

Cranberry extract and phenolic characterization

Plant material and chemicals

Standardized cranberry (*Vaccinium macrocarpon* Aiton) extract was provided by Nutra Canada (Quebec, Canada). The phenolic standards were obtained from Sigma-Aldrich (MO, USA) except for cyanidin 3-glucoside, which was purchased from Extrasynthèse (France).

Total phenolic content determination

The total phenolic content of cranberry extract was determined using Folin-Ciocalteu method, using gallic acid as standard. In a 96-well plate, 100 µl of a water-diluted Folin-

Ciocalteu reagent (1/10) and 80 µl of sodium carbonate solution (75 g/l) were added to 20 µl of a 20% MeOH 0.1% TFA solution of the extract. After incubation for 1h at room temperature, the absorbance was measured at 765 nm using a BMG Labtech Fluostar Omega microplate reader.

Characterization of anthocyanins and procyanidins

Anthocyanins were characterized by reverse-phase analytical HPLC using an Agilent 1100 series system equipped with a diode array detector. The separation was performed on a Phenomenex Develosil C18 reverse-phase column (250 mm x 4 mm, 5 µm particle size), protected with an Ultrasep C18 guard column, using a previously described methodology [15]. Anthocyanins were quantified using cyanidin 3-glucoside as standard. Procyanidins were characterized by normal-phase analytical HPLC using an Agilent 1260/1290 infinity system equipped with a fluorescence detector. The separation was performed at 35°C on a Phenomenex Develosil Diol column (250 mm x 4.6 mm, 5 µm particle size), protected with a Cyano SecurityGuard column, using a published methodology [16]. The fluorescence was monitored at excitation and emission wavelengths of 230 and 321 nm. Procyanidins with degrees of polymerization (DP) from 1 to >10 were quantified using external calibration curve of epicatechin, taking into account their relative response factors in fluorescence [17].

Characterization of phenolic acids and flavonoids

Phenolic acids and flavonoids were analyzed using a Waters Acquity UHPLC-MS/MS equipped with an H-Class quaternary pump system, a flow through needle (FTN) sample manager system, a column manager and a TQD mass spectrometer equipped with a Z-spray electrospray interface. The separation was achieved at 40°C on an Agilent Plus C18 column (2.1 mm x 100 mm, 1.8 µm) with a flow rate of 0.4 ml/min. The mobile phase consisted of 0.1% formic acid in ultrapure water and acetonitrile (solvent A and B respectively) was used with following gradient conditions: 0-4.5 min, 5-20% B; 4.5-6.45 min, 20% B; 6.45-13.5 min, 20-45% B; 13.5-16.5 min, 45-100% B; 16.5-19.5 min, 100% B; 19.5-19.52 min, 100-5% B; 19.52-22.5 min, 5% B. The MS/MS analyses were carried out in negative mode using following electrospray source parameters: electrospray

capillary voltage: 2.5 kV, source temperature: 140°C, desolvation temperature: 350°C, cone and desolvation gas flows: 80 l/h and 900 l/h respectively. Data were acquired through multiple reaction monitoring (MRM) using Waters Masslynx V4.1 software. Phenolic standards were analyzed using the same parameters and used for the quantification.

Characterization of simple sugars and fibers

Sugars were extracted from cranberry extract using 80% ethanol. After heating at 70°C for 10 minutes, the solution was centrifuged (5 minutes, 3000 rpm, 4°C), evaporated and resuspended in ultrapure water. The characterization was achieved by HPLC using a Waters 600 system equipped with a LKB Bromma 2142 refractometer. The separation was performed on a Waters Sugar Pak column (300 mm x 6.5 mm, 10 µm particle size) with an isocratic elution with EDTA 5 mg/l at a flow rate of 0.5 ml/min. Total dietary fibers were determined using the AOAC 985.29 procedure.

Data analysis

All phenolic characterizations were carried out in triplicate and results were expressed as mean ± standard deviation (SD).

Immunoblot Analysis

Tissues were frozen with liquid nitrogen and then crushed in order to obtain homogeneous powdered samples. Proteins were denatured in a buffer containing SDS and ß-mercaptoethanol, separated on a 7.5% SDS-PAGE and electroblotted onto Hybond nitrocellulose membranes (Amersham, Canada). Bradford assay (Bio-Rad, Ontario, Canada) was used to determine the protein concentration of each sample. Signals were detected with an enhanced chemiluminescence system for antigen-antibody complexes. Unspecific binding sites in the membranes were blocked by defatted milk proteins. Membranes were then incubated with the following primary antibodies: 1:1000 polyclonal anti-COX-2 (70 kDa; Novus, Canada); 1:10000 polyclonal anti-NF-kB (65 kDa; Santa Cruz Biotechnology, USA); 1:5000 polyclonal anti-IkB (39 kDa; Cell Signaling, USA); 1/5000 polyclonal anti-tumor necrosis factor (TNF)-α (26 kDa; R&D,

Canada) and 1:40000 monoclonal anti-β-actin (42 kDa; Sigma, USA). The relative amount of primary antibody was detected with specie-specific horseradish peroxidase-conjugated secondary antibody (Jackson, USA). Blots were developed and the protein mass was quantitated by densitometry using an HP Scanjet scanner equipped with a transparency adapter and the UN-SCAN-IT gel 6.1 software.

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