SUPPLEMENTAL MATERIAL

Supplementary Table 1. Frequency of consumption of food groups in MBD, MBD-T and PVD interventions

Food group	MBD and MBD-T	PVD
Cereals	26 servings/week	26 servings/week
Pasta	6 servings/week	6 servings/week
Rice	1 serving/week	1 serving/week
Pizza	1 serving/week	1 serving/week
Wholegrain bread	7 servings/week	7 servings/week
Breakfast cereals	4 servings/week	4 servings/week
Rusks	2 servings/week	2 servings/week
Biscuits	1 serving/week	3 serving/week
Crackers	4 servings/week	2 serving/week
Vegetables (without potatoes)	14 servings/week	14 servings/week
Fruit	21 servings/week	21 servings/week
Potatoes (including white and yellow potatoes)	1 serving/week	1 serving/week
Eggs	2 servings/week	2 serving/week
Milk	7 servings/week	7 servings/week
Yogurt	3 servings/week	3 servings/week
Cheese	2 servings/week	4 servings/week
Poultry	1 servings/week	-
Red meat	4 servings/week	-
Processed meat	3 servings/week	-
Fish	-	3 servings/week
Legumes	2 servings/week	4 servings/week
Sweets	3 servings/week	3 servings/week
Olive oil	14 servings/week	14 servings/week

MBD denotes meat-based diet; MBD-T meat-based diet supplemented with alpha-tocopherol: PVD pesco-vegetarian diet. Servings/week are calculated according to the portion sizes recommended by the Italian Recommended Dietary Allowances (pasta: 80 g; rice: 80 g; polenta: 80 g; pizza: 200 g; wholegrain bread: 50 g; breakfast cereals: 30 g; rusks: 30 g; biscuits: 50 g; croissant: 50 g; vegetables: 200 g; fruit: 150 g; nuts: 30 g; potatoes: 200 g; legumes: 150 g; eggs: 50 g; low fat milk: 125 ml; low-fat yogurt: 125 g; cheese: 75 g; poultry: 150 g; red meat: 150 g; processed meat: 50 g; fish: 150 g; sweets: 20 g; olive oil: 10 ml)

Supplementary Table 2. Baseline characteristics of the study population according to the completion of the study

Characteristic	Completers (n=103)	Non-completers (n=10)	p value°
Age, *years*	43 (25-47)	39 (20-50)	0.864
Female sex, *n (%)*	72 (69.9)	8 (80)	0.503
Body weight, *kg*	71.8 (14.4)	77.8 (24.3)	0.461
BMI, *kg/m²*	24.9 (4.1)	27.2 (7.3)	0.362
Fat mass, *%*	22.2 (8.6)	25.7 (10.2)	0.105
Risk factors			
Absent or light physical activity, *n (%)*	66 (64)	9 (90)	0.098
Total cholesterol, *mg/dL*	197.8 (35.4)	185.0 (23.0)	0.265
LDL-cholesterol, *mg/dL*	112.5 (32.3)	102.0 (24.8)	0.318
Glucose, *mg/dL*	84.0 (10.1)	87.3 (8.1)	0.318
Dietary profile			
Total energy, *kcal/day*	1774.4 (246.3)	1725.3 (172.8)	0.540
Carbohydrate, *% of energy*	45 (6.6)	46.8 (6.5)	0.422
Protein, *% of energy*	16.7 (3.4)	16.1 (2.2)	0.574
Total fat, *% of energy*	38.5 (5.9)	37.1 (5.8)	0.483
Total cholesterol, *mg/day*	194.4 (50.6)	182.5 (50.3)	0.481
Saturated fat, *mg/day*	18.5 (4.5)	18.2 (4.0)	0.845
Iron, *mg/day*	11.8 (2.3)	11.9 (1.9)	0.855
Fiber, *g/day*	17.9 (5.3)	17.4 (4.6)	0.769
Adherence to MD	9.3 (2.2)	10.5 (2.1)	0.105

Continuous variables are expressed as mean (SD) or as median (minimum, maximum). Categorical variables are expressed as number (percentage).

° p-values refer to the independent samples t-test for continuous variables and to chi-square tests for categorical variables

BMI denotes Body Mass Index; LDL Low-Density Lipoprotein; MD Mediterranean Diet

Supplementary Table 3. Changes in dietary intake according to interventions

	MBD (n=33)	**MBD-T** (n=33)	**PVD** (n=37)	**p-value°**
Total energy, kcal/day				
Change (after-before)	8.9 (142.1)	-2.2 (117.7)	12.8 (187.8)	0.916
Carbohydrate, % of energy				
Change (after-before)	8.2 (6.1)	7.2 (7.5)	8.7 (6.4)	0.649
Protein, % of energy				
Change (after-before)	0.4 (2.8)	0.4 (4.2)	0.9 (3.4)	0.849
Total fat, % of energy				
Change (after-before)	-8.9 (5.7)	-8.3 (6.3)	-9.4 (5.7)	0.726
Total cholesterol, mg/day				
Change (after-before)	2.1 (56.4)	-2.6 (46.9)	-32.9 (49.6) *^	0.009
Saturated fat, g/day				
Change (after-before)	3.5 (4.9)	1.0 (4.0)	-1.9 (3.2) *^	<0.001
Iron, mg/day				
Change (after-before)	6.9 (1.7)	5.7 (3.0)	1.6 (1.5) *^	<0.001
Fiber, g/day				
Change (after-before)	16.4 (4.4)	15.3 (6.1)	16.5 (5.2)	0.598

Data are reported as mean (SD)

° p-values refer to one-way ANOVA models

* $p < 0.05$ vs. MBD

^ $p < 0.05$ vs. MBD-T

MBD denotes meat-based diet, MBD-T meat-based diet supplemented with alpha-tocopherol, PVD pesco-vegetarian diet

Supplementary Methods

Genotoxic and cytotoxic activity of fecal water

Fecal water (FW) was prepared from each sample as representative of the mixture of all water-soluble harmful substances and metabolites in the feces. FWs were used to evaluate geno- and cytotoxicity induced on cultured HT-29 human cells. Genotoxic activity was determined by the Comet Assay that evaluated the induction of DNA strand breaks upon exposure of the cells to FW.

- FW preparation: Stool samples (300-600 mg) were diluted in sterile PBS (Dulbecco's Phosphate Buffered Saline) buffer in a 1:1 ratio (g/ml). The organic material was homogenized first by mixing and vortexing and then by means of an ultrasonic bath for 15 minutes. As a final step, centrifugation was carried out at 4°C at 16,000 x g for 90 minutes. The supernatant was collected, and FW samples thus obtained were aliquoted in 1.5 mL Eppendorf tubes and stored at -20°C until use.
- Cell Treatments: Colon cancer cells (HT-29 line) were grown in DMEM with 10% FBS. The treatment with FW or PBS (45 µL, final dilution 1:2) or NQO (final concentration 1 µM) was conducted for 30 min at 37°C, in tubes containing 45 µL of cell suspension (5x105 cells/mL) in medium, and stopped by immersion of the tubes in ice for 5 min.
- FW cytotoxicity: An aliquot (10 µL) of treated cells was taken and subjected to the Trypan Blue exclusion test, using a Burker chamber to count viable and dead cells. Cell viability was expressed as a fraction of control (PBS-treated cells) and indicated as “viability index”.
- FW genotoxicity: Gel preparation. At the end of the treatments, tubes were centrifuged (250 g, 5 min, RT), media discarded, and cells re-suspended in 60 µL of PBS. LMP agarose (140 µL per tube, final LMP concentration 0.7%) was then added and the suspension was distributed in 29 µL drops (about 3000 cells/gel) onto Gelbond films (100x85 mm). Each sample was run in duplicate. A duplicate of control cells treated with PBS was run in each experiment. Furthermore, two gels with an internal reference standard (K562 cells) were also run in each experiment to control for the electrophoretic run and inter-experimental variability. Aliquots of the reference cells (5x104 cells/aliquot) had been previously prepared from a single culture, frozen and stored at -80°C. On the day of the experiment, one aliquot was rapidly defrosted, added with LMP agarose and run through the comet assay with the experimental samples. The metabolic capacity of the cells was verified in each experiment by means of a further control: NQO (4-Nitroquinoline 1-oxide), a metabolically activated mutagen. One cell aliquot was incubated under the same conditions as FW samples with NQO, at 1 µM final concentration. These cells were then run through the comet assay with the other samples. NQO treatment induced an average damage of about 50% DNA in tail.
- DNA damage analysis: Each drop was rapidly covered with a 13 mm diameter round coverslip and gels allowed to solidify for 10 min on a cold plate. The films were then submerged in a freshly prepared cold lysis solution (NaCl 2.5 M, Na_2EDTA 100 mM, Tris HCl 10 mM, TritonX100 1%) and incubated for 1 h at 4°C. Alkaline unwinding was conducted in an alkaline buffer (NaOH 300 mM and EDTA 1 mM) by submerging the films in the cold electrophoresis apparatus for 40 min in cold room. Electrophoresis was subsequently initiated and continued for 30 min, in a cold room, with an applied voltage of 0.8 V/cm. At the end of this time, the films were washed in PBS (5 min twice), rinsed with water and finally let dry at least overnight at room temperature. SybrGold stain

was brought about by immersing the dried films in a solution of 1:10000 SybrGold in TE for 20 min at RT in the dark with gentle agitation. After a water wash (5 min in the dark with agitation) the films were stored in dark humidified boxes in the fridge for minimum 48 h and maximum 7 days. Image analysis was brought about with an epifluorescence Nikon microscope (Labophot-2) connected to the computer software Comet Assay IV (Perceptive Instruments). The images of 100 nuclei per sample (50 cells per duplicate gel) were randomly selected and analyzed in blind. Data were expressed as % DNA in tail, averaged over 100 cells per sample, and indicated as "genotoxicity index". For each FW sample, a genotoxicity index was produced (a single figure representing the mean DNA damage induced on HT-29 cells and expressed as % DNA in tail).

Lipid peroxidation

Given the suspected role of luminal heme iron-induced lipid peroxidation in colorectal cancer CRC development, heme iron, thiobarbituric acid reactive substances (TBARS) and specific peroxidation of omega 6 polyunsaturated fatty acids were measured in FW (28). The level of specific peroxidation of omega 6 polyunsaturated fatty acids peroxidation was explored with the assay of free 4-hydroxy-2-nonenal (4-HNE). After derivatization, 4-HNE determination in FW was achieved by a sensitive and validated Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry with Multiple Reaction Monitoring (UHPLC-MS/MS MRM) method using deuterated internal standards (29).

To complement FW measurements and strengthen evidence of lipid peroxidation, we quantified the urinary concentration of 1,4-dihydroxynonane mercapturic acid (DHN-MA), the major urinary metabolite of 4-HNE. DHN-MA is of the main toxic end-product of endogenous lipid peroxidation, alongside isoprostanes, which are chemically stable derivatives of polyunsaturated fatty acids peroxidation. Specifically, urinary 8-isoprostane was measured as a general marker of endogenous oxidative stress, rather than a specific indicator of luminal lipid peroxidation, since its precursor – arachidonic acid – is not abundant in typical diets. Both DHN-MA and 8-isoprostane were measured using a competitive enzyme immunoassay (Bertin Bioreagents and Cayman Chemical), as previously described (30). Urine samples were analyzed in simplicate.

Analysis of fecal SCFAs and bile acids

SCFAs and bile acids were measured by MS on fecal samples. SCFAs were analyzed using gas chromatography-mass spectrometry and bile acids by HPLC-MS/MS analysis based on retention times and accurate masses of metabolites.

Biochemical profile, iron metabolism and inflammatory markers

Venous blood samples were collected in evacuated plastic tubes (Vacutainer, Beckton Dickinson, Plymouth, UK) and centrifuged at 3,000 rpm for 15 minutes (4°C), aliquoted to yield serum, and then stored at -80°C until further analyses. Complete blood count, lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), fasting glucose, renal function tests (serum creatinine, uric acid), iron metabolism (iron, ferritin) and vitamin profile (vitamin B12, folic acid), were measured according to conventional laboratory standard methods. Pro- and anti-inflammatory cytokines [IL-1ra (interleukin-1ra), IL-2 (interleukin-2), IL-6 (interleukin-6), IL-8 (interleukin-8), MIP-1 α (macrophage inflammatory protein-1 α), VEGF (vascular endothelial growth factor), TNF-α (tumor necrosis factor α), VCAM-1 (vascular cell adhesion molecule-1), ICAM (intercellular

adhesion molecule)] were determined by a Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to the manufacturer's instructions.