SUPPLEMENTARY DATA

Supplemental Research Design and Methods

Anthropometric and clinical evaluations

Weight, height, waist circumference and four skinfolds (biceps, triceps, subscapular and ileocrestal) were measured with international techniques after training and standardizing evaluators. Weight was measured with Cardinal Detecto DR400C digital scales (St. Webb City, MO), height with Seca portable measuring rods (Hamburg, Germany), waist circumference with Mabis measuring tapes (Waukegan, IL) and skinfolds with Guide Slim adipometers (Plymouth, MI). Each anthropometric measure was evaluated twice and the average of the two measures was reported. Skinfolds served to compute the percentage of body fat using the logarithm of the sum of the four folds to estimate body density (1) and calculate the fat percentage (2).

To measure clinical parameters in blood, we collected peripheral venous blood from all the participants and isolated the serum using standard protocols. HDLcholesterol, LDLcholesterol, VLDL cholesterol, total cholesterol and triglycerides were measured by colorimetric enzymatic assay (cobas 701; Roche, Mannheim, Germany); apolipoprotein B by immune-nephelometry (BN II system; Siemens, Malvern, USA); fasting glucose by ultraviolet irradiation assay (cobas 701; Roche, Mannheim, Germany); glycated hemoglobin (HbA1C) by high-performance liquid chromatography (Premier Hb9210; Lab Care, England); fasting insulin by chemiluminescence immunoassay (cobas E411; Roche, Mannheim, Germany); leptin by micro ELISA (DSX-ELISA Processing System; Dynex, Louvain-la Neuve, Belgium); adiponectin by lanthanide chelate excite ultra assay (LANCE; Perkin Elmer, Waltham, USA); and high sensitivity CRPby particle-enhanced immune-turbidimetric assay (cobas 502; Roche, Mannheim, Germany). Blood insulin served to calculate the insulin resistance index using the homeostasis model assessment HOMA2 v2.2.3 (3).

Dietary intake assessment

Food intake was evaluated through 24-hour dietary recalls(4). Briefly, each participant was personally interviewed by a standardized member of the research team whom captured detailed information about all foods and beverages consumed by the respondent in the past 24 hours. Interviews were randomly distributed in the different days of the week. Food models, geometric figures and pictures were used by the interviewers to assess portion sizes and improve accuracy. 10% of the participants were interviewed a second time a different day of the week to take intra-subject variability into account. Estimation of energy intake, macro- and micronutrients was obtained for each participant using EVINDI 4.0 (5) and PC-SIDE 1.0 (6).

SUPPLEMENTARY DATA

Microbial DNA analysis

We extracted DNA sequences and quality scores from the paired fastq files and assembled the reads into contigs. We eliminated sequences containing bases with a quality score below 20, ambiguous bases, or shorter than 275 base pairs. Next, we aligned the sequences using the SILVA reference alignment v.123 (7), removed sequences with a homopolymer run ≥8 nucleotides and sequences that did not overlap the region of the alignment spanning the V4 region of the 16S rRNA gene. Then, we carried a preclustering step in which sequences with an identity ≥99% (*i.e.*, sequences differing in two nucleotides or less) were merged. The chimeric sequences were detected and discarded with UCHIME (8). After that, we assigned the taxonomic classification to sequences using the Ribosomal Database Project PDS database v.14 (9) and removed sequences classified as mitochondria, eukaryota or unknown. Finally, using the average neighbor algorithm we generated operational taxonomic units (OTUs) delimited at 97% identity, which we then taxonomically classified by consensus using Greengenes 13_8_99 (10). We tested for potential contaminating sequences by comparing the relative abundances of unique OTUs detected in actual samples and negative controls. Parallel sequencing of a mock community revealed a mean error rate of 0.12% (min = 0.015%, max = 0.233%, median = 0.108%).

SUPPLEMENTARY DATA

Supplemental References

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SUPPLEMENTARY DATA

Supplementary Table S1. Drugs taken on a regular basis within the last three months by participants of the study. T2D-met^{+} = diabetic participants taking metformin; T2D-met^{-} = diabetic participants not taking metformin; ND = non-diabetic participants. NSAID = non-steroidal anti-inflammatory drug. *Patients unaware of their diabetes status at the beginning of the study.

Patient ID	Group	Drugs
MI-013	T2D-met^{+}	Lovastatin, Metformin
MI-025	T2D-met^{+}	Atorvastatin, Eutirox, Metformin, Sertraline
MI-027	T2D-met^{+}	Insulin, Metformin
MI-040	T2D-met^{+}	Fluoxetine, Metformin
MI-091	T2D-met^{+}	Metformin, Omeprazole
MI-126	T2D-met^{+}	Acetaminophen, Losartan, Metformin, Metoprolol
MI-135	T2D-met^{+}	Biperiden, Carbamazepine, Clonazepam, Insulin, Levomepromazine, Metformin, Tramadol
MI-137	T2D-met^{+}	Losartan, Metformin
MI-219	T2D-met^{+}	Insulin, Metformin
MI-254	T2D-met^{+}	Aspirin, Insulin, Losartan, Lovastatin, Metformin, Omeprazole, Thioctacid
MI-403	T2D-met^{+}	Amlodipine, Atorvastatin, Enalapril, Glibenclamide, Losartan, Metformin, NSAID
MI-412	T2D-met^{+}	Atorvastatin, Loratadine, Losartan, Metformin
MI-425	T2D-met^{+}	Atorvastatin, Esomeprazole, Losartan, Metformin
MI-450	T2D-met^{+}	Fluoxetine, Loratadine, Metformin
MI-010	T2D-met^{-}	Atorvastatin, Aspirin, Enalapril, Glibenclamide, Omeprazole
MI-017*	T2D-met^{-}	Enalapril, Gemfibrozil
MI-043*	T2D-met^{-}	Enalapril, Verapamil
MI-072	T2D-met^{-}	Atorvastatin, Hydrochlorothiazide, Losartan, Verapamil
MI-182	T2D-met^{-}	Fluconazole
MI-213	T2D-met^{-}	Losartan
MI-236	T2D-met^{-}	Glibenclamide
MI-289	T2D-met^{-}	None
MI-301	T2D-met^{-}	None
MI-315	T2D-met^{-}	Insulin, Thyroxine
MI-344	T2D-met^{-}	Losartan, Metoprolol
MI-420	T2D-met^{-}	Aspirin, Cabergoline, Diovan, Esomeprazole, Inhaler (unknown), Levothyroxine, Montelukast, Tolterodine
MI-428	T2D-met^{-}	None
MI-449	T2D-met^{-}	None
MI-004	ND	Atorvastatin, Esomeprazole, Losartan
MI-009	ND	None
MI-018	ND	None
MI-020	ND	Enalapril, Lovastatin, Verapamil
MI-023	ND	Beclometasone, Salbutamol, Esomeprazole, Losartan, Ketotifen, Atorvastatin
MI-030	ND	Enalapril, Lovastatin, NSAID
MI-035	ND	Levothyroxine
MI-037	ND	None
MI-038	ND	None
MI-041	ND	Folic acid, Losartan, Omeprazole
MI-046	ND	Lovastatin, Thyroxine
MI-052	ND	None
MI-055	ND	None
MI-064	ND	None
MI-065	ND	Amlodipine, Coumadin, Losartan, Metildigoxin, Metoprolol, Spironolactone, Warfarin
MI-069	ND	None
MI-070	ND	Losartan, Metoprolol, Pregabalin, Sertraline

MI-074	ND	Atorvastatin
MI-075	ND	Acetaminophen, Loratadine, Losartan, Lovastatin
MI-076	ND	None
MI-079	ND	Hydrochlorothiazide, Losartan
MI-088	ND	Eutirox, Gemfibrozil
MI-104	ND	None
MI-112	ND	None
MI-115	ND	None
MI-119	ND	None
MI-120	ND	None
MI-123	ND	None
MI-138	ND	Losartan, Lovastatin, Omeprazole
MI-150	ND	Aspirin, Losartan
MI-156	ND	None
MI-159	ND	None
MI-169	ND	None
MI-172	ND	None
MI-174	ND	None
MI-175	ND	None
MI-188	ND	None
MI-189	ND	Losartan
MI-210	ND	None
MI-215	ND	None
MI-220	ND	None
MI-225	ND	None
MI-226	ND	None
MI-248	ND	Statins (unknown)
MI-256	ND	Acetaminophen
MI-260	ND	Losartan, Lovastatin, Metoprolol, Pregabalin, Verapamil
MI-261	ND	None
MI-262	ND	Amlodipine, Aspirin, Losartan
MI-266	ND	None
MI-269	ND	None
MI-271	ND	None
MI-274	ND	Losartan
MI-275	ND	None
MI-276	ND	None
MI-278	ND	None
MI-284	ND	Blood pressure drug (unknown), Cholesterol drug (unknown), Thyroid drug (unknown)
MI-306	ND	None
MI-307	ND	None
MI-308	ND	Analgesics (unknown), Methocarbamol
MI-316	ND	None
MI-317	ND	None
MI-320	ND	None
MI-332	ND	None
MI-336	ND	None
MI-338	ND	None
MI-356	ND	None
MI-363	ND	None
MI-372	ND	Losartan
MI-374	ND	None
MI-384	ND	Loratadine
MI-389	ND	None

MI-393	ND	None
MI-394	ND	None
MI-396	ND	None
MI-397	ND	None
MI-399	ND	Enalapril, Eutirox, Fluoxetine, Imipramine
MI-404	ND	None
MI-411	ND	None
MI-416	ND	None
MI-417	ND	None
MI-430	ND	None
MI-440	ND	None
MI-451	ND	Losartan
MI-458	ND	None

SUPPLEMENTARY DATA

Supplementary Figure S1. Rarefaction curves based on the number of observed operational taxonomic units (OTUs) in the three groups of participants. Yellow = diabetic participants taking metformin (T2D-met+); red = diabetic participants not taking metformin (T2D-met-); blue = non-diabetic participants (ND).