**Supplementary methods:**

*Daily macronutrient intake*

Daily macronutrient intake was calculated utilising a validated food frequency questionnaire, designed to capture dietary intake for the preceding 12 months (Dietary Questionnaire for Epidemiological studies v2).[1](#_ENREF_1) Macronutrient composition was expressed as a percentage of total energy intake.[2](#_ENREF_2)

*Anthropometric measurements*.

Height, weight, waist circumference, and blood pressure were obtained, and BMI was calculated. Peripheral venous blood samples were obtained after an overnight fast and serum alanine aminotransferase, bilirubin, albumin, creatinine, fasting triglyceride and high density lipoprotein (UniCel® DxC 800 Biochemical Analyser, Beckman Coulter), prothrombin time (ACL TOP 700 Haemostasis Testing System, Instrumentation Laboratory) and platelet count (XE-5000 Automated Haematology System, Sysmex) were measured. HCV RNA levels (COBAS TaqMan HCV quantitative assay, Version 2, Roche) were measured from peripheral blood samples collected within 3 months. The presence of the MetS was evaluated as per IDF/AHA/NHLBI 2009 consensus criteria.[3](#_ENREF_3)

*Hepatic stiffness*

Liver stiffness measurement (LSM) was determined by transient elastography (Fibroscan® 502 Touch, Echosens, Paris, France) performed after a three hour fast and in the recumbent position, by trained operators (A.S.R. and M.B.). The measurement reported was the median of at least 10 readings. Criteria for acceptability were 10 valid readings, at least 60% success rate, with an interquartile/median LSM<30.[4](#_ENREF_4) The cut-off for cirrhosis was defined as LSM≥12.5 kPa.[5](#_ENREF_5)

*Small intestinal paracellular permeability*

Small intestinal paracellular permeability was measured within six weeks of transient elastography by a dual sugar enteral challenge. Subjects avoided alcohol for 48 hours prior to testing, and after an overnight fast drank a solution of 5g (14607mol) lactulose (AlphaPharm, Millers Point, Australia) and 1g (5488mol) rhamnose (Sigma Aldrich, Castle Hill, Australia) in 100mL water. Ninety minutes post-ingestion, peripheral venous blood was drawn and the concentration of lactulose and rhamnose were measured in plasma by HPLC with pulsed amperometric detection (Dionex, Sunnyvale, CA) and Chromeleon® Chromatography Management System (Version 6.80, Dionex), as per the method of Haase et al.[6](#_ENREF_6) Results were considered acceptable if the coefficient of variation (CV) of the calibration standards was <15% for each HPLC run, and the sample values fell within the range obtained for the standards. Small intestinal permeability was measured as the ratio of Lactulose: Rhamnose (L: R) x 100.

*Peripheral blood endotoxemia*

Plasma endotoxin concentrations were measured as a marker for bacterial translocation, using a kinetic chromogenic limulus amebocyte lysate (LAL) assay (Endosafe® Endochrome-K™, Charles River, SC, USA). Plasma was diluted 1:20 with endotoxin free water (LAL reagent water, Charles River) and heated for a 20 mins at 75°C, and the assay performed as per the manufacturer. Optical Density measurements to derive the endotoxin concentration were performed using the Tecan Sunrise™ plate reader and Endoscan™ Version V software (Charles River, SC, USA), using the kinetic chromogenic function. A reference standard curve was generated by polynomial regression. Results were accepted based on the following criteria as per the manufacturer: correlation coefficient of standard curve ≥0.98; CV of standards <15%; CV of duplicates <20%; endotoxin recovery in samples of 50–200%. The lowest limit of endotoxin detection in the sample was 0.02 EU/mL.

*Mucosa-associated Microbiota assessment*

Duodenal tissue collected from endoscopy was snap frozen on dry ice. Genomic DNA (gDNA) was isolated from tissue specimens using a modification of the “repeated bead beating plus column purification” method described by Yu and Morrison.[7](#_ENREF_7) Following bead-based cell lysis, the gDNA was purified using the Maxwell MDx AS1000 automated DNA purification system (Promega, Madison, WI) and Maxwell Tissue DNA Purification Kit (Promega), according to the manufacturer’s protocol. All steps were performed in a biosafety laminar flow hood, and tissue was handled with sterile techniques. Bacterial DNA was selectively amplified from total gDNA by PCR with primers 917F (GAATTGRCGGGGRCC; bacterial domain specific) and 1392R (ACGGGCGGTGWGTRC; universal) (Integrated DNA Technologies, Singapore), that target the V6-V8 hypervariable region of the bacterial 16S rRNA gene and also contained Illumina adapter sequences. Subsequent barcoding of the PCR amplicons and downstream manipulations and preparation for amplicon sequencing using the Illumina MiSeq platform were performed as previously described.[8](#_ENREF_8) To control for ubiquitous environmental DNA, negative controls (DNA extraction reagents only, with no added tissue/DNA) were also sequenced.

The sequence data was processed using the Quantitative Insights into Microbial Ecology (QIIME, Version 1) workflow.[9](#_ENREF_9) Operational Taxonomic Units (OTU) were assigned using the Greengenes database (version 13.8) with a sequence similarity of 97% applied.[10](#_ENREF_10) OTUs with an abundance of less than 0.1%, and those present in the negative controls were removed from the overall analysis to generate a list of OTUs considered representative of gut MAM. The absolute number of OTUs present in each sample was normalised using cumulative sum scaling (CSS) to account for variations in sampling depth.[11](#_ENREF_11) Total sum scaling was used for relative abundance measurements.

*Statistical analysis of microbiota data*

Alpha diversity (derived from CSS data) was assessed using the Shannon index (to account for both the number of species, as well as their relative abundance).[12](#_ENREF_12) Differential abundance of microbial taxa (derived from raw data, to reduce potential biases of transformed data) was analysed by both differential expression analysis based on the negative binomial distribution (DESeq2) and analysis of composition of microbiomes (ANCOM)[13](#_ENREF_13) statistical tests. Analyses were adjusted for multiple comparisons testing. Significance was defined as p<0.05 with a False Discovery Rate q value (FDRq) <0.1.

Beta diversity was assessed by Principal Co-ordinate Analysis (PCoA), to show separation of individual patient profiles based on microbial composition. Plots were generated (from CSS OTU counts), using both the Bray-Curtis distance metrics. To ascertain which taxa contributed to the profiles of CLD and control subjects, both linear discriminant analysis effect size (LEfSe, using the CSS normalised data) and the constrained multivariate Mixomics method, sparse partial least squares discriminant analysis (sPLS-DA), which identifies OTU features discriminating multiple groups of samples[14](#_ENREF_14) were used. Predicted function of the metagenome based on the 16S rRNA sequence data was determined using the software programme PICRUSt, to assign metabolic pathways derived from KEGG (Kyoto Encyclopedia of Genes and Genomes).

**References**

1. Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2). User Guide. Nutrition Assessment Office, Cancer Council Victoria, 2015.

2. Nutrient Reference Values for Australia and New Zealand, Australian National Health and Medical Research Council and Ministry of Health New Zealand, 2015.

3. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640-5.

4. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960-74.

5. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835-47.

6. Haase AM, Kukuruzovic RH, Dunn K, et al. Dual sugar permeability testing in diarrheal disease. J Pediatr 2000;136:232-7.

7. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 2004;36:808-12.

8. Zhong L, Shanahan ER, Raj A, et al. Dyspepsia and the microbiome: time to focus on the small intestine. Gut 2016.

9. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335-6.

10. McDonald D, Price MN, Goodrich J, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 2012;6:610-8.

11. Paulson JN, Stine OC, Bravo HC, et al. Differential abundance analysis for microbial marker-gene surveys. Nat Methods 2013;10:1200-2.

12. Spellerberg IF, Fedor PJ. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the ‘Shannon–Wiener’ Index. Global Ecology & Biogeography 2003;2003:177-179.

13. Mandal S, Van Treuren W, White RA, et al. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microb Ecol Health Dis 2015;26:27663.

14. Le Cao KA, Costello ME, Lakis VA, et al. MixMC: A Multivariate Statistical Framework to Gain Insight into Microbial Communities. PLoS One 2016;11:e0160169.