Appendix S1: Detailed description of the experimental procedure, the assessment of parameters, and the laboratory analyses

Assessment of vascular function: arterial stiffness, retinal vessel diameters

Before and after the intervention period, participants were admitted to the Department of Sport, Exercise and Health of the University of Basel to assess arterial stiffness and retinal vessel diameters. To prepare for the measurements, participants were instructed to abstain from caffeine, alcohol and heavy physical activity, and to fast at least four hours.

Arterial stiffness was measured oscillometrically using a VaSera VS-1500 vascular screening system (Fukuda Denshi Co. Ltd, Tokyo, Japan). Triple measurements were taken in a supine position after resting for at least 10 minutes and 5 minutes between the measurements in a quiet room with a controlled temperature between 22-24 °C. Standard blood pressure cuffs were placed at each upper arm and above each ankle. Electrocardiogram leads were attached at each wrist, and a phonocardiogram was placed on the sternal border in the second intercostal space. The heart-ankle pulse wave velocity (PWV) was measured based on the vessel length between the heart valve and the ankle artery divided by the time taken for the pulse wave to propagate from the aortic valve to the ankle. ARCSolver algorithm was applied to pulse wave signals acquired at the left upper arm to estimate central PWV (1). The average of the three PWV-measurements was taken for statistical analysis.

A fundus camera (Topcon TRC NW) and the analyzing software Visualis (Visualis 2.80, Imedos Systems UG) were used for retinal image acquisition. Three crisp images of each eye were taken at an angle of 45 degrees with the optic disc at the center. All arterioles and venules 0.5–1 disk diameter away from the optic disk margin were marked. Retinal arteriolar and venular diameter segments were semi-automatically marked using an analysis software (Vesselmap 2®; IMEDOS Systems GmbH, Jena, Germany). Arteriolar and venular diameters

from the three images of each eye were averaged to Central Retinal Arteriolar Diameter Equivalents (CRAE) and Central Retinal Venular Diameter Equivalents (CRVE) by use of the Parr–Hubbard formula (2). The arteriolar-to-venular diameter ratio (AVR) was calculated from the CRAE and CRVE. The values of each eye were again averaged. To guarantee optimal standardization, the same vessel segments were chosen at both time points using the pre-intervention assessment as a reference.

Assessment of abdominal fat: quantification and distribution

Before and after the intervention period, participants were admitted to the Department of Radiology and Nuclear Medicine of the University Hospital of Basel for an abdominal MRI. Data for the evaluation of percentage liver fat (%-LF), total liver volume (TLV), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) were acquired using a MAGNETOM Prisma 3T scanner (Siemens Healthineers, Erlangen, Germany). A transversal T2*-IDEAL (3D spoiled gradient echo with 6 echoes) sequence was used for the liver fat measurement, and a two-point Dixon (3D spoiled gradient echo with two echoes) sequence in coronal orientation for the SAT and VAT quantification. Both scans were performed in breath holding (up to 20 seconds each). Details on data acquisition and evaluation are described elsewhere (3). In short, the T2*-IDEAL scan covered the whole liver. Those images were segmented manually to derive %-LF and TLV. The two-point Dixon scan was used for the automatic SAT and VAT volume segmentation which was subsequently manually corrected, and spatially confined to the upper end of the femoral head and the lower end of the ninth thoracic vertebra.

Assessment of glucose tolerance, blood lipids, uric acid, hepatic enzymes, and creatinine

Before and after the intervention period, participants were admitted to the St. Clara Research

Ltd. in the morning after an overnight fast for an oral glucose tolerance test (OGTT). For this purpose, they were instructed to abstain from heavy physical activity within the two days before the test, to eat meals containing carbohydrates, and to come on an empty stomach, i.e., not eat or drink (water allowed until two hours before the test) nor consume alcohol within ten hours before the test.

An antecubital catheter was inserted into a forearm vein for blood collection. After taking a fasting blood sample (t = -15 min) for assessment of fasting glucose and insulin concentrations (ethylenediaminetetraacetic acid (EDTA) tubes), blood lipids, uric acid, hepatic enzymes, creatinine concentrations (lithium heparin tubes), participants received a standardized solution containing 75 g of glucose.

Blood samples were taken at regular time intervals after administration of the solution (t = 30, 60, 90, and 120 min) for assessment of glucose and insulin concentrations. The samples were collected on ice into tubes (EDTA, 6 µmol/L blood). After centrifugation (4 °C at 3000 rpm for 10 min), plasma samples were immediately processed into different aliquots and stored at -80 °C until analysis.

Assessment of gastrointestinal tolerance and dietary patterns

Gastrointestinal tolerance was assessed using the Gastrointestinal Symptom Rating Scale (GSRS) (4) before and during the second and fourth week of intervention. The 15 items of this scale combine into five symptom clusters: reflux, abdominal pain, indigestion, diarrhea and constipation.

To assess changes in dietary patterns, participants completed dietary records seven days before the intervention and during the second and the fourth week of the intervention. The dietary records were analyzed qualitatively by categorizing the foods into 11 different groups (vegetables, fruits, cereals/bread, other carbohydrates, meat/fish/other protein sources, dairy

products, fats/nuts/seeds, beverages with added sugar (e.g., coffee with sugar), beverages with sweeteners, sugar-sweetened beverages, and sweets) and comparing the number of servings per food group.

Laboratory analysis

Plasma glucose was measured by a glucose oxidase method (Rothen Medizinische Laboratorien AG, Basel, Switzerland; range of assay, 0.6 to 45.0 mmol/L). Plasma insulin was quantified using an electro-chemiluminescent immunoassay (Rothen Medizinische Laboratorien AG, Basel, Switzerland; range of assay, 4.0 to 1000.0 mIU/L; intra- and inter-assay variability below 4.3% and 5.3%).

The laboratory of the St. Clara Hospital in Basel assessed fasting blood lipids, uric acid, hepatic enzymes, and creatinine. Serum blood lipids (serum triglyceride, cholesterol, and high density lipoprotein (HDL)) were measured with enzymatic colorimetric tests. The intra- and inter-assay variability are below 2.1% and 2.3% (triglyceride), 2.1% and 7.4% (cholesterol), and 1.1% and 1.5% (HDL). Low density lipoprotein (LDL) was calculated with the Friedewald-Formula (5). Serum uric acid was measured with an enzymatic colorimetric test with an intra- and inter-assay variability below 1.7% and 2.0%. Hepatic enzymes alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were measured with enzymatic colorimetric tests. The intra- and inter-assay variability are below 1.8% and 1.8% (ALAT), and 1.6% and 1.8% (ASAT). Creatinine was assessed with a kinetic colorimetric test based on the Jaffe method (6) with an intra- and inter-assay variability below 2.9% and 3.4%.

References

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