Supplemental material 4—Detailed description of applied health examinations

Cardiac Computed Tomography (CT) scans
Cardiac CT scans will include a non-contrast CT scan to evaluate CAC score, aortic valve calcifications, lung density analysis, and bone mineral density (BMD). BMD is most commonly assessed by Dual-energy X-ray absorptiometry (DXA) but can also be assessed by CT [1]. Furthermore, a CT angiography is applied to evaluate cardiovascular and heart structures and subclinical obstructive coronary atherosclerosis. CT imaging will be performed using a 320-multidetector scanner (Aquilion One, Canon Medical Systems).

Participants are instructed to abstain from coffee, tea, cocoa, and chocolate from 4 p.m. the day before the CT scan. Prior to the CT scan, a cardio-selective beta-blocker (metoprolol 25–150 mg) may be administered orally in participants with a heart rate of >60 bpm and no contraindications prior to scanning. Intravenous contrast media (Visipaque) is given after assessment of kidney function (estimated Glomerular Filtration Rate (eGFR) >60 ml/min/1.73m²). A fixed-target protocol using one rotation acquisition with a prospective exposure window fixed at 350 ms centred at the 75 % phase of the RR cycle will be used to restrict radiation dose.

Arterial stiffness
Arterial stiffness will be assessed based on carotid-femoral Pulse Wave Velocity (cfPWV), a non-invasive measure considered the gold standard method of assessing direct arterial stiffness [2]. The SphygmoCor XCEL instrument (AtCor Medical, Sydney, Australia) will be applied to measure cfPWV. cfPWV measurements are performed under standardised conditions according to guidelines [2] and follow the quality demands suggested by the manufacturer. Calibration is performed yearly by using the XCEL Calibration Kit.

Prior to the measurement, the participant must be fasting for 3 hours (including the absence of coffee, tea, smoking, and alcohol) and resting in a lying position for 10 minutes in a quiet room. Blood pressure is measured three times with a Microlife BP A6 PC blood pressure device, and the mean blood pressure is used. cfPWV is defined as the distance between the two recording sites divided by the difference in pulse wave travel time and expressed in meters per second. Distance is directly measured as a straight line by a calliper from the recording sites at the carotid to the femoral artery, and the total distance is multiplied by 0.8 [2]. The transit time is based on measurements of pulse waves assessed by use of an applanation tonometer at the carotid artery on the neck and from a blood pressure cuff on the thigh. cfPWV measurements will be performed twice, and if these vary by more than 0.5 m/s, a third measurement will be performed.

Blood pressure
Blood pressure (mmHg) is measured using a Microlife BP A6 PC blood pressure device in a sitting position after 5 minutes of rest. Three repeated measurements with 1-minute intervals will be conducted, and a mean of the 2 last measurements will be applied.
Anthropometrics and body composition
Height is measured without shoes and socks with a Holtain Harpenden Stadiometer (model: 602VR). Weight and body fat percentage are measured by the bioelectrical impedance device InBody770 wearing light clothes. Waist circumference is measured at the midpoint between the lowest point of the lowest rib and the highest point of the iliac crest. Hip circumference is measured at the point of the greater femoral trochanter.

Spirometry
Lung function will be measured through spirometry performed with Vyntus SPIRO (Vyaire Medical), disposable MicroGard pulmonary function filters with nose clips (V-892391) and Sentrysuite software (V3.20.3). The examinations will be performed according to the 2005 American Thoracic Society and the European Respiratory Society (ATS/ERS) spirometry standard [3] after a daily calibration with a 3-litre calibrated syringe. The spirometer calibration syringe will be calibrated yearly to comply with the international standard [4]. Body weight is measured using a digital scale (Tanita, BC 420), and 1 kg is automatically subtracted to account for the weight of the participant’s clothes. Height is measured without shoes with a Holtain Harpenden Stadiometer (model: 602VR). Respiratory function measurements, i.e., expiratory forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), will be conducted.

Handgrip strength
Handgrip strength will be performed with a Jamar Hydraulic Hand dynamometer to assess maximum hand muscle strength. Measurements are performed three times in a sitting position with the participant’s dominant hand.

Physical performance
A 30-second sit-to-stand test will be performed to examine participants’ physical performance according to the number of times participants get to a full stand in 30 seconds [5].

Blood sampling and biochemical analyses
After a minimum of 6 hours of fasting, blood samples will be drawn for biochemical analyses. Up to 100 mL of venous blood from each participant are drawn at the enrolment and follow-up visits. The samples will be centrifuged and analysed. Remaining blood will be centrifuged and stored in the biobank for future research. Biochemical analyses include dp-ucMGP, MK-7, PIVKAII, lipid profile (total cholesterol, TG, LDL, and HDL), glucose-metabolism (e.g., fasting glucose, insulin, C-peptide and HbA1c), biomarkers of bone metabolism (FGF23, osteoprotegerin, C-terminal telopeptide of type I collagen CTX, and P1NP etc.), biomarkers of liver function (Alanine Aminotransferase (ALAT)), kidney function (creatinine), and vitamin D status (25-OH-vitamin-D).
Urine samples
A single urine spot sample will be collected at home and analysed for creatinine and albumin. Remaining urine will be stored in the biobank for future research.

References