BioPersMed Concept Paper

Supplements – Methods and baseline characteristics

Table of Contents

Haudum CW, *et al. BMJ Open* 2022; 12:e058890. doi: 10.1136/bmjopen-2021-058890

1. Echocardiography

For all cardiac ultrasound examinations, a GE vivid E9 device (GE Healthcare, United Kingdom) with a phased array transducer (GE M5s) was used. During the echocardiographic examination, patients were positioned in the left lateral decubitus position. An ECG tracing was recorded during the whole examination. Loops of 3 cardiac cycles of the parasternal long axis view, the parasternal short axis view (on the level of the aortic valve, the mitral valve, the papillary muscles, and the apex), the apical four chamber view, the apical two chamber view, the apical three chamber view, the apical five chamber view, and the subcostal view were recorded. Additionally, colour Doppler loops of all mentioned echocardiographic angulations were recorded for the semi-quantitative assessment of a regurgitation or stenosis. Doppler measurements of the transmitral flow, the transaortic flow, the tricuspid regurgitation flow, and the pulmonic venous flow were recorded as images. Cardiac chambers were quantified by the measurement with the M-Mode technique or as a measurement within the two-dimensional image. M-mode and Doppler images were recorded at a sweep speed of 100 mm/s. The colour Doppler measurements were recorded with a Nyquist limit of 65 ± 5.5 cm/s. For the calculation of the left ventricular mass, the modified Devereux formula [1] was used, for the calculation of the left atrial volume, the biplane formula was used. The body surface area was calculated from height and weight using the DuBois formula. Table S1 gives an overview of all assessed parameters of the baseline-examination.

Table S1: Echocardiographic parameters of the baseline cohort (N=1022)

	Method	Mean ± SD	Unit
Left Ventricle			
IVS *end diastolic* - PLAX	M-Mode	10.5 ± 1.9	mm
PW *end diastolic* - PLAX	M-Mode	10.6 ± 2.0	mm
LVD *end diastolic* - PLAX	M-Mode	47.4 ± 5.9	mm
LVM	Calculated (modified Devereux formula)	184.5 ± 61.3	g
LVMI	Calculated (LVM indexed to BSA)	96.8 ± 26.2	g/m²
LVD *end systolic* PLAX	M-Mode	29.8 ± 4.8	mm
LVD apical major axis *end diastolic* - A4CH	2D	76.1 ± 8.1	mm
LVD apical minor axis *end diastolic* - A4CH	2D	43.6 ± 5.5	mm
LVV *end diastolic* - A4CH + A2CH	2D (Simpson's biplane method)	91.4 ± 26.9	mL
LVV *end systolic* - A4CH + A2CH	2D (Simpson's biplane method)	34.0 ± 13.1	mL
EF	Calculated (Simpson's law)	63.8 ± 5.8	%
MAPSE medial annulus - A4CH	M-Mode	0.92 ± 0.16	cm
MAPSE lateral annulus - A4 CH	M-Mode	1.54 ± 0.26	cm
Right Ventricle			
TAPSE - A4CH	M-Mode	2.31 ± 0.09	cm
Left Atrium			
LA minor axis *end systolic* - PLAX	PLAX	38.4 ± 5.7	mm
LA major axis *end systolic* - A4CH	2D	48.0 ± 6.0	mm
LAA *end systolic* - A4CH	2D	17.6 ± 3.6	cm²
LAA *end systolic* - A2CH	2D	17.9 ± 3.6	cm²
LAV *end systolic* - A4CH + A2CH	Calculated (biplane method)	56.1 ± 16.6	mL
LAVI *end systolic* - A4CH + A2CH	Calculated (LAV indexed to BSA)	29.8 ± 7.9	mL/m²
Doppler Measurements			
AV-Vmax	CWD	1.2 ± 0.3	m/s
Peak E-wave velocity	PWD of transmitral flow	70.0 ± 16.1	cm/s
Peak A-wave velocity	PWD of transmitral flow	67.2 ± 18.1	cm/s
Mitral valve deceleration time	PWD of transmitral flow	221 ± 56	ms
E/A ratio	PWD of transmitral flow	1.10 ± 0.38	
Duration A-wave	PWD of transmitral flow	132 ± 37	ms
Duration IVRT	PWD of transmitral flow	115 ± 20	ms
Duration aortic flow	PWD of transmitral flow	276 ± 37	ms
Duration E-wave to A-wave	PWD of transmitral flow	447 ± 48	ms
Peak E-wave velocity *(valsalva)*	PWD of transmitral flow under valsalva	62.2 ± 16.0	m/s
Peak A-wave velocity *(valsalva)*	PWD of transmitral flow under valsalva	63.3 ± 18.6	m/s
Mitral walve deceleration time *(valsalva)*	PWD of transmitral flow under valsalva	241 ± 67	ms
e' velocity (medial annulus)	TDI-PWD	7.6 ± 2.2	cm/s
e' velocity (lateral annulus)	TDI-PWD	10.4 ± 3.0	cm/s
E/e' *mean*	Calculated	8.2 ± 2.4	
Tricuspid regurgitation systolic jet velocity	CWD	2.25 ± 0.27	m/s
Pulmonary vein systolic velocity	PWD of pulmonary venous flow	56.7 ± 12.5	cm/s
Pulmonary vein diastolic velocity	PWD of pulmonary venous flow	42.6 ± 10.1	cm/s
Pulmonary vein S/D ratio	PWD of pulmonary venous flow	1.37 ± 0.38	
Flow propagation time	CD M-Mode of transmitral flow	68.6 ± 31.0	cm/s
Valves			
Semiquantitative evaluation of regurgitation	CD		

Echocardiographic angulations and Doppler measurements - abbreviations:

PLAX = parasternal long axis view; A4CH = apical four chamber view; A2CH = apical two chamber view; CWD = continuous wave Doppler; PWD = pulsed wave Doppler; TDI = tissue Doppler imaging; CD = colour Doppler

BioPersMed Concept Paper

Cardiac structures - abbreviations

IVS = interventricular septum; PW = posterior wall; LVD = left ventricular diameter; LVM = left ventricular mass; LVMI = left ventricular mass index; LVV = left ventricular volume; EF = ejection fraction; MAPSE = mitral annulus plane systolic excursion; TAPSE= tricuspid annulus plane systolic excursion; LA = left atrium; LAA = left atrial area; LAV = left atrial volume; LAVI = left atrial volume index; IVRT = isovolumetric relaxation time

2. Physical functioning and exercise capacity spiroergometry

The whole study cohort underwent several examinations assessing the cardiac, pulmonary, and exercise capacity. Firstly, a blood pressure measurement with the Boso-medicus uno (Bosch + Sohn GmbH, Germany) was performed in the seating position after 10 minutes of rest. During the diagnostic workup, a spirometry was performed ahead of ergometry. Symptom limited cardiopulmonary exercise testing on a bicycle ergometer starts at a workload of 20W, followed by a stepwise 20W increment every 2 min. Criteria for discontinuation of the exercise test are defined as recommended by the European Society of Cardiology [2]. A standard 12-lead ECG continuously monitors heart rate, ST-segment changes, and arrhythmias. Blood pressure is recorded at rest and then every 2 min. Ventilatory exchange, oxygen uptake (VO_2), and other cardiopulmonary variables are acquired by averaging breath-by-breath measurements over 10 s intervals. Peak heart rate and workload is recorded immediately upon the end of exercise. Peak VO_2 is defined as the maximum value of the last three 10 s averages during exercise and anaerobic threshold is detected using the V-Slope method [3], based on standard operating procedures for spiroergometry. Finally, participants reported the degree of exhaustion expressed as the level of shortness of breath according to a Borg category-ratio scale (0 – 10 points)[4].

After a period of at least 15 minutes rest, a six-minute walk test was performed. Initially, resting blood pressure was assessed; then patients were instructed to walk for six minutes as far as possible without running or jogging. The Borg category-ratio scale (0 – 10 points) was assessed after the six-minute walking test too.

Finally, a handgrip test using a Jamar® Hydraulic Hand Dynamometer (Performance Health UK, United Kingdom) was performed to measure grip strength of both hands. The participant was tested in the seated position with elbow flexed at 90 degrees without touching the trunk.

The handle position was adjusted to fit the participant's hand. The participant was asked to squeeze the dynamometer at maximal effort for three trials, with a 30-second break between each trial. The average of three trials was calculated for data analysis. Table S2 gives an overview on the single assessed physical functioning and exercise capacity related biomarkers.

Table S2: Baseline parameters of spirometry, spiroergometry, 6-minute walking test and hand-grip strength test

	Male	Female	Unit
Blood pressure measurement (N=1022)	**Mean ± SD**	**Mean ± SD**	
SBP – seating position	141 ± 19	133 ± 20	mmHg
DBP – seating position	88 ± 11	85 ± 11	mmHg
Spirometry (N=1022)			
FVC	4.1 ± 0.9	3.0 ± 0.6	L
PNV (FVC)	4.6 ± 0.5	3.1 ± 0.4	L
FVC / PNV	90.3 ± 16.1	97.1 ± 17.9	%
FEV1	3.3 ± 0.8	2.4 ± 0.5	L/s
PNV (FEV1)	3.5 ± 0.4	2.5 ± 0.4	L/s
FEV1 / PNV	93.2 ± 20.0	94.3 ± 19.7	%
FEV1 / FVC	102.3 ± 17.6	102.3 ± 19.8	%
Spiroergometry (N=1022)			
Wmax	158 ± 36	111 ± 23	W
SBP – resting	125 ± 19	118 ± 19	mmHg
DBP – resting	83 ± 12	78 ± 11	mmHg
SBP - maximal	206 ± 23	189 ± 25	mmHg
DBP - maximal	90 ± 16	89 ± 18	mmHg
HR – resting	77 ± 13	79 ± 13	/min
HR - maximal	143 ± 20	147 ± 19	/min
Peak VO_2	23.9 ± 7.0	20.5 ± 6.4	mL/kg/min
Anaerobic threshold	107 ± 41	79 ± 25	W
Borg-CR10	3.9 ± 2.0	3.9 ± 2.0	
Six-minute walk test (N=980)			
SBP – resting	139 ± 18	133 ± 20	mmHg
DBP – resting	88 ± 11	85 ± 11	mmHg
SBP - maximal	147 ± 22	141 ± 22	mmHg
DBP - maximal	89 ± 11	88 ± 12	mmHg
HR – resting	73 ± 14	73 ± 11	/min
HR - maximal	80 ± 16	82 ± 14	/min
Distance walked	521 ± 90	501 ± 91	m
Prematurely terminated	3 / 446	4 / 559	N / total
Borg-CR10	0.4 ± 0,8	0.6 ± 1.2	
Hand-grip test (N=680)			
Right arm	96 ± 20	59 ± 13	lbs
Left arm	90 ± 20	54 ± 13	lbs

spiroergometry, six-minute walk test & hand-grip test - abbreviations

FVC = forced vital capacity; PNV = predicted normal value; FEV1 = forced expiratory volume (1 second); Wmax = maximal workload; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; VO_2 = oxygen uptake; Borg-CR10 = Borg category-ratio scale

3. Pulse wave analysis and ECG

Measurements of pulse wave analysis (PWA) and pulse wave velocities (PWV) were performed with the SphygmoCor© device (Atcor Medical, Australia). The measurement takes place in supine position after a minimum of 10 to 15 min rest in a quiet, temperature-controlled room after measuring the blood pressure on the right and left side [5]. Central BP measurements were recorded noninvasively by applanation tonometry.

PWV (carotid–femoral), which reflects arterial stiffness, were measured by using the foot-to-foot velocity method. The waveforms were recorded transcutaneuosly at the right common carotid artery and the right femoral artery. In addition, the augmentation index was determined by applanation tonometry on the central pressure waves measured in the right radial artery according to previous recommendations [6]. The SphygmoCor device quality index (QI), which represents waveform reproducibility, was checked after each pulse wave analysis (PWA) and the measurements are repeated in case of an QI<80. As an instant quality check for the measurement of the PWV, the time difference between the ECG-signal and the signal from the recording sites was suggested to have a SD ≤10% of the mean value. All measurements of PWA and PWV were performed in duplicate.

Briefly after the pulse wave analysis, a 12-lead resting ECG was performed. An overview of the ECG and PWA related biomarkers is shown in table S3.

Table S3: Baseline parameters of 12-lead ECG and pulse wave velocity/analysis		
	Mean ± SD	**Unit**
12-lead ECG (N=1019)		
HR	64.4 ± 11.1	/min
PR-Interval	159.3 ± 24.9	ms
QRS-duration	95.8 ± 15.9	ms
QTc-time	418.6 ± 27.6	ms
PWA (N=993)		
SBP	126 ± 18	mmHg
DBP	86 ± 11	mmHg
AP	12.2 ± 7.6	mmHg
AIx	28.2 ± 11.4	%
AIx(HR75)	23.9 ± 11.3	%
PP	39.9 ± 13.1	mmHg
PWV (N=963)		
Carotid-femoral PWV	7.4 ± 2.1	m/s

12-lead-ECG and pulse wave analysis – abbreviations

HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; AP = augmentation pressure; AIx = augmentation index; AIx(HR75) = augmentation index normalized to the heart rate of 75 beats per minute; PP = pulse pressure

4. Ultrasound of abdomen

An ultrasound of the abdomen was performed using the GE vivid E9 ultrasound device with a curved array transducer (GE CI5). The dimensions of the spleen were assessed, and the echogenicity of the liver was evaluated in order to identify signs of liver-steatosis. The findings of the abdominal ultrasound are summarized in table S4.

Table S4: Baseline parameters of ultrasound of the abdomen

	Number (N total = 982)	**%**
Ultrasound of abdomen		
Normal	626	64
Blunted liver edge	32	3
Irregular liver surface	2	< 1
Ascites	1	< 1
Signs of steatosis	321	33
Dimension of spleen	**Mean ± SD**	**Unit**
Longitudinal	101.8 ± 14.4	mm
Transversal	36.6 ± 7.7	mm

5. Carotid ultrasound examinations

Carotid ultrasound examination was performed with a GE vivid E9 ultrasound device with a linear array transducer (GE 9L). Each measurement includes both the near-wall and far-wall data respectively for the right and left carotid arteries [7]. The common carotid intima-media thickness (CIMT) was calculated by an automated measurement program by GE at more than 200 points distal from the bulbus. Sonographers are taking images showing the maximum thickness of a particular site. Detected plaques at specific sites are included in the maximum CIMT measurement. Table S5 gives an overview on the biomarkers of the carotid ultrasound examination.

Table S5: Baseline parameters of carotid ultrasound

	Mean ± SD	**Unit**
CIMT		
Right side	0.73 ± 0.16	mm
Left side	0.73 ± 0.17	mm
Presence of calcified plaques or stenosis	**Number (N=1021)**	**%**
Right side	117	11
Left side	106	10

Carotid ultrasound – abbreviation:

CIMT = carotid intima-media thickness

6. Bone density and body composition

Bone imaging via 2D bone density measurements and body composition measurements were performed using Lunar iDXA (General Electrics, USA) at each visit including measurements at the lumbar spine, the hip and whole-body sites. Thorough body composition, automated comparison to prior measurements and an additional trabecular bone score (TBS, as a measure of bone texture correlated with bone microarchitecture, software by iDEXA solutions, Switzerland [8]) has been documented. Table S6 shows an overview on the parameters of the bone density measurements.

Table S6: Baseline parameters of bone density and body composition

	Male (N=341)	Female (N=448)	%
Bone mineral density			
normal BMD	325	412	93
Osteopenia or osteoporosis	16	36	7
Body Composition	**Mean ± SD**	**Mean ± SD**	**Unit**
Total BMD	1.28 ± 0.11	1.13 ± 0.12	g/cm²
Total BMC	3132 ± 392	2293 ± 336	g
Total T-Score	0.78 ± 1.09	0.48 ± 1.14	STD
Total Z-Score	0.63 ± 1.04	1.01 ± 0.94	STD
Total Tissue mass	84397 ± 13301	68502 ± 13681	g
Total Bone mass	3194 ± 243	2180 ± 181	g
L1-L4 BMD	1.25 ± 0.19	1.14 ± 0.17	g/cm²
L1-L4 BMC	82.2 ± 16.4	62.4 ± 12.5	g
L1-L4 T-Score	0.09 ± 1.52	-0.43 ± 1.36	STD
L1-L4 Z-Score	0.14 ± 1.51	0.29 ± 1.19	STD

Bone density measurements – abbreviations:

BMD = Bone mineral density in [g/cm²], BMC = Bone mineral content [g], L1-L4 = region vertebrae L1-L4, T-Score= standard deviations from mean BMD level of young adults, Z- or T-Score STD = standard deviations from age adjusted mean BMD level

7. Laboratory assessment and measurements

Routine clinical laboratory parameters such as blood count and coagulation, serum electrolytes, kidney and liver function, HbA1c, fasting c-peptide, insulin as well as fasting blood glucose followed by a standardized oral glucose tolerance test (oGTT, at 30, 60 and 120 minutes after 75mg glucose load) with consecutive insulin and c-peptide measurements, high-sensitive c-reactive protein (hsCRP), lipids (total cholesterol, high-density and low-density lipoproteins (HDL and LDL), lipoprotein a (LP(a), triglycerides (TG), high-sensitive troponin T (hsTropT) and cardiac parameters such as creatine kinase (CK, CK-MB), as well as urinary proteins, electrolytes, cell count and c-peptide are measured in all participants at each study visit. The baseline measurements are presented in table S7.

In addition to the oGTT and glucose metabolism monitoring, a large panel of hormonal and metabolic analyses are performed in women as well as in men. These biomarkers include estrogen, testosterone and free testosterone, androstenedione, dehydroepiandrosterone (DHEAS), 17(OH)progesterone, and anti-Müllerian hormone (AMH), saliva hormones (e.g. cortisol), bone metabolism including formation and resorption parameters (osteocalcin, N-terminal propeptide of type 1 procollagen (P1NP), serum-crosslaps (CTX)), calciotropic hormones such as 25(OH)vitamin D and parathyroid hormone (PTH), pituitary hormones (LH, FSH, ACTH) and others.

Routine biochemical parameters (liver, kidney, lipid, electrolyte and other profiles) were determined using a Cobas® Analyzer (Roche Diagnostics, Germany), complete blood counts by Beckmann-Coulter, Germany. Hormonal measurements such as insulin and c-peptide were measured by chemiluminescence on an Advia Centaur system (Siemens Healthcare Diagnostics, Germany). Automated analysers were used to measure hormones: anti-Mullerian hormone (AMH) (Beckmann-Coulter, Germany); testosterone, cortisol, thyrotropin,

triiodothyronine and thyroxine (Siemens Advia Centaur, Germany); sexual hormone-binding globulin (SHBG) (Roche Diagnostics, Germany); estrogen, luteotropic hormone (LH) and follicle-stimulating hormone (FSH) (Triturus, Biomedical Diagnostics, Antwerp, Belgium); 25(OH)vitamin D and bone metabolism parameters such as bone-specific alkaline phosphatase (bALP), osteocalcin (OC), C-terminal telopeptide (CTX) and procollagen type 1 N-terminal propeptide (P1NP) by iSYS, (IDS, UK, or Roche Diagnostics, Germany, respectively). Endocrine and bone biomarkers are shown in table S8, all of them collected in the morning after an overnight fast.

Table S7: Baseline parameters of general, metabolic and cardiovascular laboratory assessment

	Male (N=444)	Female (N=556)	Unit
Liver	**Mean ± SD**	**Mean ± SD**	
ALP	93 ± 32	109 ± 11	U/L
ALT	24.7 ± 31.3	16 ± 7.3	U/L
AST	20.9 ± 17.1	18.1 ± 7.4	U/L
CHE	11059 ± 928	11161 ± 1153	U/L
GGT	44.8 ± 54.2	18 ± 20.1	U/L
LDH	161 ± 19	166 ± 19	U/L
Kidney			
Creatinine	0.98 ± 0.14	0.8 ± 0.13	mg/dL
Creatinine/urine	142 ± 35	130 ± 45	mmol/L
Urea	18.8 ± 1.5	18.1 ± 2.2	mg/dL
Uric Acid	6.3 ± 1.2	4.8 ± 1.2	mg/dl
Proteins			
Albumin	4.6 ± 0.3	4.6 ± 0.3	g/dL
CRP	2.3 ± 4.1	2.5 ± 3.7	pg/mL
Ferritin	158± 173	123 ± 47	ng/mL
Total Protein	7.4 ± 0.4	7.4 ± 0.4	g/dL
Transferrin	2.5 ± 0.3	2.5 ± 0.3	g/L
Electrolytes & Iron			
Calcium/urine	2.41 ± 1.88	2.21 ± 1.69	mmol/L
Chloride	103 ± 2	104 ± 2	mmol/L
Chloride/urine	149 ± 33	135 ± 41	mmol/L
Iron	132 ± 24	126± 22	µg/dL
Magnesium	0.85 ± 0.06	0.85 ± 0.06	mmol/L
Phosphate	2.61 ± 0.75	2.96 ± 0.49	mg/dL
Phosphate/urine	18.6 ± 14.5	15.1 ± 10.8	mg/dl
Potassium	4.1 ± 0.5	4.0 ± 0.3	mmol/L
Total Calcium	2.39 ± 0.09	2.39 ± 0.1	mmol/L
Haematopoiesis			
Haemoglobin	15.1 ± 1.0	13.6 ± 0.9	g/dL
Platelets	174 ± 20	180 ± 18	10^9/L
TLC	5.77 ± 1.44	5.76 ± 1.76	10^9/L
Glucose metabolism			
Glucose fasting	99 ± 20	91 ± 17	mg/dL

Glucose 30 min	158 ± 38	142 ± 36	mg/dL
Glucose 60 min	158 ± 62	128 ± 52	mg/dL
Glucose 120 min	115 ± 57	100 ± 46	mg/dL
Insulin fasting	13 ± 17	10 ± 8	µU/mL
Insulin 30 min	64 ± 54	56 ± 48	µU/mL
Insulin 60 min	94 ± 87	73 ± 68	µU/mL
Insulin 120 min	62 ± 72	51 ± 62	µU/mL
HbA1c	38.6 ± 7.1	37.7 ± 5.8	mmol/mol
Heart			
CK	151 ± 132	130 ± 25	IU
NT-Pro-BNP	79 ± 18	122.66 ± 105.7	pg/mL
PT	105.7 ± 5.0	106.8 ± 5.2	%
Lipid parameters			
Cholesterol	174.8 ± 18.9	181.6 ± 16.6	mg/dL
LDL	130.9 ± 36.5	132.1 ± 33.6	mg/dL
VLDL	19.6 ± 21.9	15.7 ± 2.8	mg/dL
HDL	48.5 ± 16.3	65.1 ± 20.2	mg/dL
LPA	1.37 ± 3.47	1.81 ± 4.16	mg/dL
Triglycerides	144 ± 80	133 ± 26	mg/dL

Laboratory measurements – abbreviations:

ALP = Alkaline Phosphatase; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; CHE = Cholinesterase; CRP = C-Reactive Protein; GGT = Gamma Glutamyl Transferase; LDH = Lactate Dehydrogenase; HB = Haemoglobin; PLT = Platelets; TLC = Total leucocyte count; HbA1c = glycated haemoglobin; CK = Creatine Kinase; NT-Pro-BNP = N-terminal pro brain natriuretic peptide; PT = Prothrombin Time; LDL = Low Density Lipoprotein; VLDL = Very low Density Lipoprotein; HDL = High Density Lipoprotein; LPA = Lipoprotein A

Table S8: Baseline parameters of endocrine laboratory assessment

	Male (N=446)	**Female (N=557)**	**Unit**
Endocrine parameters	**Mean ± SD**	**Mean ± SD**	
17(OH)Progesterone	0.9 ± 0.4	0.4 ± 0.3	ng/ml
ACTH	21.3 ± 11.4	14.7 ± 8.1	pg/ml
Aldosterone	8.7 ± 7.6	9.0 ± 8.1	ng/dl
Androstenedione	2.6 ± 1	1.9 ± 0.9	ng/ml
Cortisol	123.2 ± 36.5	111.9 ± 38.13	ng/ml
Cortisol/saliva	7 ± 4.4	6.4 ± 4.2	ng/ml
DHEAS	1.1 ± 0.7	0.7 ± 0.5	µg/ml
Estradiol	58.4 ± 280.8	49.4± 63.8	pg/ml
Free Thyroxine	15.1 ± 2.2	15.1 ± 2.7	pmol/l
Free Triiodothyronine	5 ± 0.5	4.6 ± 0.6	pmol/l
FSH	9.34 ± 8.7	67.2 ± 30.9	mU/ml
Insulin	13.4 ± 16.9	9.9 ± 7.7	mU/l
LH	6.0 ± 5.7	14.2 ± 6.4	mU/ml
Progesterone	0.51 ± 1.18	0.9 ± 4.06	ng/ml
Renin	58.3 ± 106.5	30.9 ± 74.4	µU/ml
SHBG	41.1 ± 42.1	119.5 ± 51.8	nmol / L
Total Testosterone	11.0 ± 3.9	1.4 ± 0.7	pg/ml
TSH	2.0 ± 1.1	2.2 ± 6.3	µU/ml
Bone parameters			
25(OH)Vitamin D	32.4 ± 11.3	33.5 ± 11.9	ng/ml
Crosslaps/serum	0.3 ± 0.12	0.37 ± 0.17	ng/ml

Osteocalcin	18.8 ± 5.6	23.2 ± 8.7	ng/ml
P1NP	40.4 ± 13.6	49.8 ± 20.4	ng/ml

Laboratory measurement: endocrinology & bone metabolism - abbreviations:

ACTH = Adrenocorticotropic hormone DHEAS = dehydroepiandrosterone sulfate, FSH = Follicle-stimulating hormone, LH = Luteinizing hormone, SHBG = Sex hormone-binding globulin, TSH = Thyroid-stimulating hormone, thyrotropin, P1NP = procollagen type 1 N-terminal propeptide

8. Autoantibody phenotyping

Data are available for routine thyroid and gluten autoantibodies, further autoimmune parameters for glucose and bone metabolism, body growth, and cardiovascular risk were analysed using a non-automated bridge assay protocol based on the method from EU patent 20170276675 together with Charité – Universitätsmedizin Berlin, Germany. Gliadin and tissue transglutaminase autoantibodies were measured by an automated Chemiluminescence Immunoassay (CLIA) from IDS (Immunodiagnostic Sytems, Boldon, UK), see table S9. More autoantibodies are currently measured for endocrine and potentially cardiovascular receptors and targets of interest.

Table S9: Baseline parameters of autoantibody phenotyping

	Male (N=377)	Female (N=484)	Unit
Laboratory assessment	**Mean ± SD**	**Mean ± SD**	
Gliadin IgA	2.2 ±4.9	0.7 ± 2.1	ng/ml
Gliadin IgG	2.0 ± 11.2	1.6 ± 11.4	ng/ml
Tissue transglutaminase IgA	2.5 ± 0.8	2.2 ± 0.5	ng/ml
Tissue transglutaminase IgG	0.3 ± 0.9	0.3 ± 1.1	ng/ml

Laboratory measurement: autoantibody phenotyping - abbreviations:

IgA = Immunoglobulin A, IgG = Immunoglobulin G

9. Metabolic phenotyping by Nuclear Magnetic Resonance (NMR)

Untargeted metabolomics was done in 1012 serum and urine samples using NMR-based metabolic phenotyping by NMR spectroscopy. Technical measurements were performed on a 600 MHz Avance Neo NMR spectrometer and using 1D CPMG (Carr−Purcell−Meiboom−Gill). NOESY and 2D J-resolved pulse sequences. Data processing: Principle Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (O-PLS-DA)] were performed. Metabolite reference chemical shifts from databases and metabolites were cross-checked using reference compounds and Chenomx software package if necessary. Metabolite concentrations were determined using internal/external standards and using the Eretic 2 approach implemented in Topspin [9,10].

10.Genome-wide characterization of cohort

More than 650,000 single nucleotide polymorphisms (SNPs) have been measured in a whole genome global screening array (GSA, Illumina bead chip (Infinium Global Screening Array-24 V2; Illumina Inc, USA)) in all cohort patients in cooperation with the Human Genotyping Facility (Genetic Lab at the Erasmus University Rotterdam, Netherlands) based on Illumina technologies in 2017. These genetic data, as well as specific genotyping e.g. for primary hypolactasia, are available for genome-wide association analyses and the inclusion in subsequent meta- and mega-analyses. As the results are stored in database, bioinformatical approaches such as diverse R-scripts allow for a fast gene-, pathway- or function-specific selection and export of SNP data. Epigenetic phenotyping is ongoing. The potential of various circulating non-coding RNAs for the diagnosis and prediction of (subclinical) diseases is currently evaluated for diabetes, as well as osteological, metabolic and CVD conditions.

11.Optometric phenotyping

For the ophthalmologic examinations, a Canon CR-2 AF (Canon Europa, Amstelveen, Netherlands) and for the optical coherence tomography (OCT) an OCT Spectralis (Heidelberg Engineering, Heidelberg, Germany) was used. Retinal vessel diameters were assessed with Integrative Vessel Analysis (IVAN software, N. Ferrier, University of Wisconsin, USA) and with the OCT device's inbuilt calliper tool from 12° peripapillary circle scans. Additional OCT scans included a macular volume scan and a high-resolution single scan through the fovea. Measured parameters are described in Table S10.

Table S10: Baseline parameters of optometric phenotyping

	Mean ± SD	**Unit**
Right eye	Number (N=349)	
CRAE6	147.5 ± 12.5	µm
CRVE6	212.2 ± 17.7	µm
AVR	0.70 ± 0.06	
Left eye	Number (N=344)	
CRAE6	148.3 ± 13.6	µm
CRVE6	212.1 ± 18.1	µm
AVR	0.70 ± 0.06	

Optometric phenotyping – abbreviations:

CRAE6: mean diameter of the six biggest arterioles; CRVE6: mean diameter of the six biggest venules; AVR: arterioles-venules ratio

12.Biobank Storage

Until 2020, samples (Preservative: EDTA, Sodium-citrate and Lithium-heparin) were transferred immediately after sampling to an adjacent laboratory run by technicians from the local biobank. Sodium citrate samples were centrifuged at 2880g for 15min and the rest of the samples at 3360g for 10min at 4°C. The centrifuged biospecimen were then aliquoted manually. Since November 2013 samples were automatically aliquoted using a pipetting robot (Hamilton Microlab STARlet). After aliquoting, samples were temporally stored at -20°C. The transport to the final -80°C Biobank Graz storage unit was performed under ambient temperature. Since March 2019 the transport was performed under dry-ice conditions. Since 2020 these processes took place in another laboratory and technicians from the biobank picked up the samples at latest within one hour after sampling. Additionally, timepoints of sampling and execution of single work steps were digitally documented.

13.Bibliography

1 Lang RM, Badano LP, Victor MA, *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* 2015;**28**:1-39.e14. doi:10.1016/j.echo.2014.10.003

2 Björnstad H, Cohen Solal A, Dubach P, *et al.* Recommendations for exercise testing in chronic heart failure patients. *European Heart Journal* Published Online First: 2001. doi:10.1053/euhj.2000.2388

3 Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *Journal of Applied Physiology* 1986.

4 Borg G. Psychophysical scaling with applications in physical work and the perception of exertion. *Scandinavian Journal of Work, Environment and Health* 1990;**16**:55–8. doi:10.5271/sjweh.1815

5 Laurent S, Cockcroft J, van Bortel L, *et al.* Expert consensus document on arterial stiffness: Methodological issues and clinical applications. European Heart Journal. 2006. doi:10.1093/eurheartj/ehl254

6 Nichols WW, O'Rourke MF, Vlachopoulos C, *et al. McDonald's blood flow in arteries theoretical, experimental and clinical principles*. 2011. doi:10.1111/j.1540-8175.1991.tb01207.x

7 Wohlin M, Sundström J, Andrén B, *et al.* An echolucent carotid artery intima-media complex is a new and independent predictor of mortality in an elderly male cohort. *Atherosclerosis* Published Online First: 2009. doi:10.1016/j.atherosclerosis.2009.01.032

8 Leslie WD, Aubry-Rozier B, Lamy O, *et al.* TBS (trabecular bone score) and diabetes-related fracture risk. *Journal of Clinical Endocrinology and Metabolism* Published Online First: 2013. doi:10.1210/jc.2012-3118

9 Maher AD, Crockford D, Toft H, *et al.* Optimization of human plasma 1H NMR spectroscopic data processing for high-throughput metabolic phenotyping studies and detection of insulin resistance related to type 2 diabetes. *Analytical Chemistry* Published Online First: 2008. doi:10.1021/ac801053g

10 Cloarec O, Dumas ME, Craig A, *et al.* Statistical total correlation spectroscopy: An exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. *Analytical Chemistry* Published Online First: 2005. doi:10.1021/ac048630x