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Cohort profile: 'Biomarkers of Personalised Medicine' (BioPersMed): a single-centre prospective observational cohort study in Graz/Austria to evaluate novel biomarkers in cardiovascular and metabolic diseases

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ABSTRACT

Purpose Accumulating evidence points towards a close relationship between cardiovascular, endocrine and metabolic diseases. The BioPersMed Study (Biomarkers of Personalised Medicine) is a single-centre prospective observational cohort study with repetitive examination of participants in 2-year intervals. The aim is to evaluate the predictive impact of various traditional and novel biomarkers of cardiovascular, endocrine and metabolic pathways in asymptomatic individuals at risk for cardiovascular and/or metabolic disease.

Participants Between 2010 and 2016, we recruited 1022 regional individuals into the study. Subjects aged 45 years or older presenting with at least one traditional cardiovascular risk factor or manifest type 2 diabetes mellitus (T2DM) were enrolled. The mean age of the participants was 57±8 years, 55% were female, 18% had T2DM, 33% suffered from arterial hypertension, 15% were smokers, 42% had hyperlipidaemia, and only 26% were at low cardiovascular risk according to the Framingham 'Systematic COronary Risk Evaluation'.

Findings to date Study procedures during screening and follow-up visits included a physical examination and comprehensive cardiovascular, endocrine, metabolic, ocular and laboratory workup with biobanking of blood and urine samples. The variety of assessed biomarkers allows a full phenotyping of individuals at cardiovascular and metabolic risk. Preliminary data from the cohort and relevant biomarker analyses were already used as control population for genomic studies in local and international research cooperation.

Future plans Participants will undergo comprehensive cardiovascular, endocrine and metabolic examinations for the next decades and clinical outcomes will be adjudicated prospectively.

Strengths and limitations of this study

- The main strength of the BioPersMed cohort is the joint evaluation of cardiovascular, endocrine and metabolic phenotyping including a broad spectrum of highly innovative diagnostic, imaging and functional tools.
- Biobanking with a large number of samples aliquoted and stored at each visit enables a prospective view on candidate biomarkers in the context of a large longitudinal cohort, where specific approaches can be predefined.
- A specifically adapted large electronic data capture system (OpenClinica; www.openclinica.com) and iterated monitoring assures the quality of data entry and delivery as well as the validity and reliability of biomarkers analyses.
- A potential weakness of this study is the wide time range of recruitment due to logistic reasons between 2010 and 2016, with a prolonged follow-up period of study participants to date between 4 and 10 years.
- Some biomarkers are not available for the complete duration of the entire cohort.

INTRODUCTION

Cardiovascular (CVD) and metabolic diseases (MD) are globally representing the most important cause of disability and premature

death.[1] Next to our genetic programming, modern lifestyle, including the use of tobacco, unhealthy nutritional habits, physical inactivity, and psychosocial stress are major risk factors of CVD and MD within different age groups not only promoting excess cardiovascular (CV) and metabolic morbidity but ultimately triggering excess mortality.[2 3] In turn, primary prevention of these diseases has the potential to avoid many of related deaths.[4] However, the initial euphoria about a decline of CVD prevalence at the beginning of this century[5] gradually gives way to a sense of frustration. This comes in the light of increasing numbers of type 2 diabetes mellitus (T2DM),[6] a very high lifetime-risk for the development of heart failure with stable incidence over the last decades,[7] and a persistent high stroke mortality.[8] In addition, the relevant underdiagnosing and/or undertreatment of patients at high risk for CVD related risk factors remains of utmost important.[9] Therefore, early detection of asymptomatic CV and/or metabolic risk remains a crucial challenge in the prevention of both, onset and progression of CVD as well as of related complications.[10]

Considering the multiplicity of risk pathophysiology, an integrative approach is needed to identify novel and to validate established CV and metabolic biomarkers for their scientific and clinical utility. Practical biomarkers are required to facilitate (1) the understanding of underlying mechanisms of disease development, (2) the detection of potential targets for specific preventive therapies, and finally (3) the precise estimation of individual risk. For this purpose, there is an unmet need for a cohort studies recruiting individuals at risk for CVD and/or MD well before clinical manifestation of the diseases.

In the BioPersMed cohort (Biomarkers of Personalised Medicine), we enrolled community dwelling and asymptomatic individuals from the regional communities who were at risk for CVD or MD in order to evaluate the predictive value of various traditional and novel biomarkers and to observe disease courses starting in the preclinical, asymptomatic phase. The latter shed light on different pathways of CVD and MD development by use of cutting edge laboratory measurements, advanced imaging techniques, comprehensive genetic investigations, and state of the art functional tests. The BioPersMed cohort is located at the Medical University of Graz (Austria) in a dedicated clinical outpatient research centre and biobank (www.biobank.medunigraz.at). The aim of the study is to evaluate large-scale screening tools for the improvement of (1) CVand metabolic risk stratification, (2) early diagnosis of CVD and/or MD (3), individual prediction of clinical outcomes, (4) and long-term monitoring of risk and/or early CV and/or metabolic changes in an apparently healthy but representative at-risk population at high CV and/or metabolic risk in a prospective manner. Ultimately, the data obtained from this cohort aims to facilitate the implementation of risk-adapted personalised interventions in both primary and secondary prevention of CVD and MD.

COHORT DESCRIPTION

The BioPersMed project is designed as a single-centre, prospective, observational study. Only asymptomatic subjects without diagnosed CVD but with at least one traditional CV risk factor were eligible to participate. According to the published European Guidelines on CVD prevention in clinical practice, traditional CV risk factors besides of age and gender comprise (1) smoking, (2) elevated total cholesterol levels and (3) arterial hypertension.[11] Moreover, sedentary lifestyle, obesity, social environment, T1DM or T2DM, low High-density lipoprotein (HDL) cholesterol, increased triglyceride levels, elevated fibrinogen, apolipoprotein B, lipoprotein(a), familial hypercholesterinaemia, increased high sensitivity C reactive protein, preclinical evidence of atherosclerosis and chronic kidney disease (glomerular filtration rate ≤60 mL/min/1.73 m^2) were regarded as additional potential CV and MD risk factors. From October 2010 (first patient in) to February 2016 (last patient in), we enrolled a total of 1022 community dwelling adult men and women who live in the greater Graz area via an established recruitment network, consisting of general practitioners, peripheral hospitals, and in most cases through the outpatient clinics of the Departments of Cardiology as well as Endocrinology and Diabetology, respectively.

Subjects presenting with significant non-CVD or who were expected not to be able to complete study specific examinations, were excluded from participation. The BioPersMed study was conducted in compliance with the laws and guidelines of the Medical University of Graz and complies with the Declaration of Helsinki and the Austrian laws.[12 13] All participants in the BioPersMed cohort were thoroughly checked for inclusion and exclusion criteria before the first phenotyping at baseline examination in order to avoid screening failures.

The baseline examinations have been repeated every 2 years in addition to interim telephone visits, which take place between the on-site visits. A summary of all examinations is shown in figure 1, and a more detailed description can be found in online supplemental table S1–S10.

According to the presented scheme, participants will be followed for the next decades and clinical outcomes are adjudicated prospectively. A total of 169 (17%) participants dropped-out for various reasons. Causes for premature unintended termination of the study ranged from a change in the place of residence (n=5) to limited personal time or lacking will for continuous study visits (n=136) or new onset of non-CV-related diseases (cancer: 4, accident: 3, other: 9). Twelve people have died so far (cancer: 7, sepsis: 3, CVD: 2). In summary, 1022 persons were included in the baseline examination and 799 persons attended the first follow-up 2 years after baseline examination. With 1 September 2021, 628 persons have completed the second follow-up at 4 years after baseline visit, 531 persons have completed the third follow-up at 6 years after the baseline visit, and 225 persons have completed the follow-up of 8 years after the baseline visit. A small number of participants skipped one follow-up

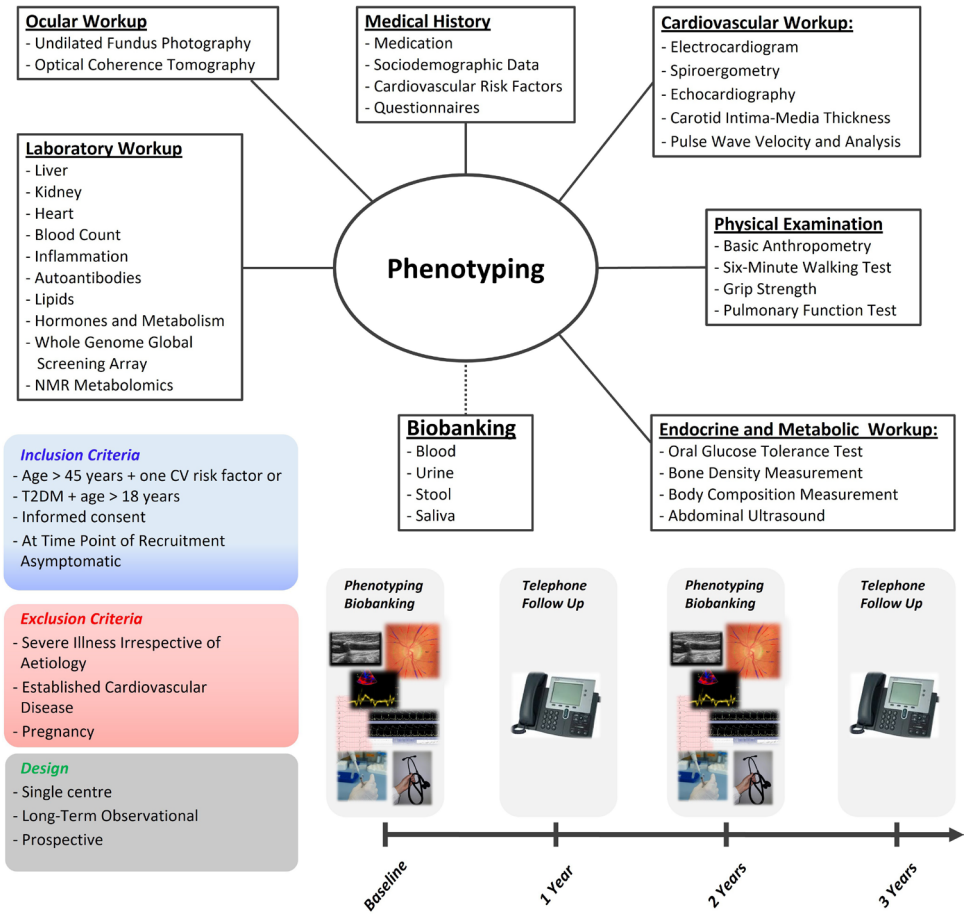

Figure 1 Illustration of the comprehensive phenotyping and biosampling of the BioPersMed cohort. Follow-up visits are performed according to a tight preplanned schedule, including reminder-phone calls by study nurses with phenotyping and biosampling every second year and a follow-up telephone visit in the years between. At baseline, every participant received a patient's diary for documentation of medical events (source data). CV, cardiovascular; T2DM, type 2 diabetes mellitus.

but decided to continue to participate in the study. This issue explains the discrepancy between the number of drop-outs and the number of missing follow-up visits. A detailed overview of the recruitment process and study protocol is presented in figure 2.

At baseline and at regular 2 years-follow-ups, an in-depth diagnostic CV and MD work-up was carried out, laying emphasis on standardisation and reproducibility of history taking, questionnaires for health, psychological and sleep issues, physical examination, ECG, laboratory/blood sampling with biobanking, exercise testing (6 min walking test, grip strength, spiroergometry), echocardiographic analysis of cardiac structure and function, pulmonary function testing, carotid intima/media-thickness measurement, pulse-wave analysis (PWA), and ophthalmologic examinations as well as body composition, bone density including bone and hormonal biomarkers, and oral glucose tolerance testing (OGTT). The number of examinations performed at each visit increased over time, due to additional new diagnostic tools (eg, non-mydriatic funduscopy). A detailed description of all methods used, as well as a concise overview of the assessed data can be found in the online supplemental file 1. Statistics have been calculated using RStudio V.1.2.5033 (RStudio, USA).[14] Besides the already included packages others, namely 'hmisc'(version : 4.6), 'ggplot2'(3.3), 'tidyr'(1.12), 'readxl'(1.3), 'MatchIt'(4.1), 'data.table'(1.13), 'dplyr'(1.0) and 'lubridate'(1.7), were needed for structuring of data, analysis and visual representation. Normal distribution of data was tested and, if positive, Pearson-correlation calculated. In case of a violation of normal distribution, a non-parametric equivalent was used. A description of the data is given in the corresponding tables. GCP trained and authorised independent persons from the Clinical Trials Unit at the Medical University Graz have carefully evaluated and monitored the data and relevant procedures around the cohort study. Based on the steering committees agreement, risk based monitoring of 100% of the baseline dataset and 80% of the follow-up dataset was performed. This was done to generate an adequate security of the structure and verification of the assessed data.

Biological and technical outliner were manually identified and corrected. This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)-ME recommendations for reporting cohort studies.[15]

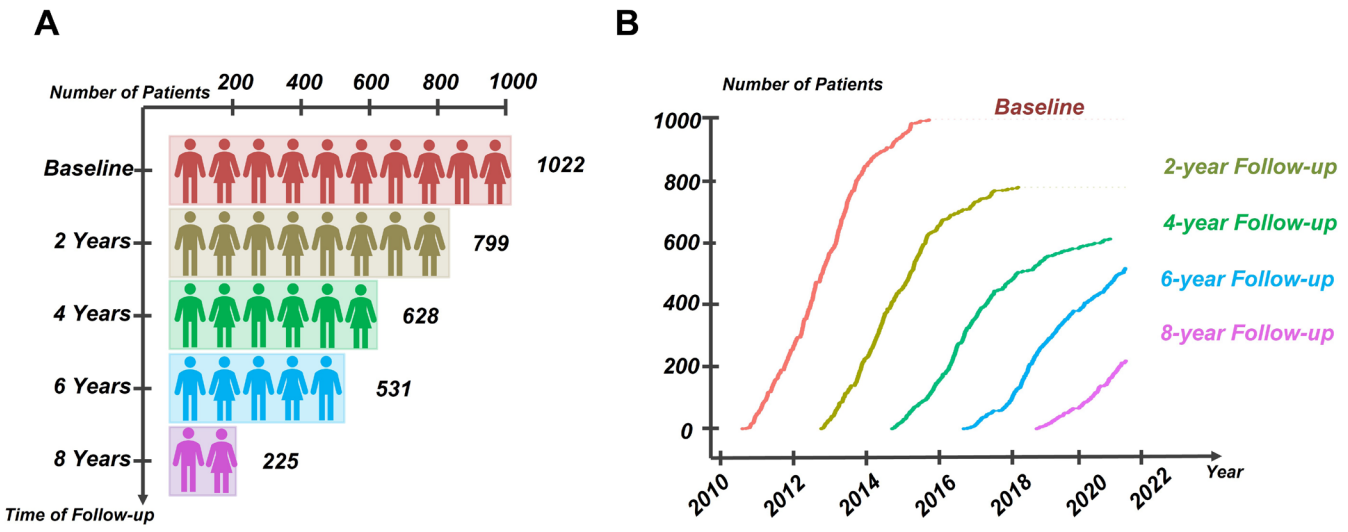

Figure 2 The recruitment status over time and the follow-up of the participants' phenotyping in the BioPersMed cohort. (A) Absolute number of participants who have completed various on-site follow-ups until first September 2021. (B) Timeline of recruitment and follow-up processes.

Health status

At each visit, a careful assessment of the participants' health status was performed. Data collection documents anthropometric, biochemical, metabolic, hormonal, dietary, physical activity, socioeconomic, medical and other variables.

Questionnaires

Questionnaires at all clinical visits include the Short-Form 36 Health Survey and the Hospital Anxiety and Depression Scale, as well as other questionnaires to assess depressive or disease-related symptoms and sleep qualities.[16–21] Raw data of these questionnaires have been collected and are available for further analysis.

Echocardiography

Cardiac chamber geometry and function were assessed via state-of-the-art transthoracic echocardiography. Two-dimensional, Doppler-mode and M-mode echocardiography were performed following standardised protocols according to current guidelines and are digitally archived.

Physical functioning and exercise capacity spiroergometry

For the assessment and interaction of vascular disease, CV risk and biomarkers, an assessment of physical functioning/exercise capacity was essential. Complementary to questionnaires on physical activity, physical fitness and symptom-limited cardiopulmonary exercise capacity was assessed by spiroergometry, 6 min walk test[22] and handgrip measurements.[23 24]

Pulse wave analysis and ECG

PWA and pulse wave velocity measurements were performed according to the expert consensus published by Laurent *et al*.[25] Additionally, a 12-lead ECG was performed in every patient.

Ultrasound of the abdomen

Routine abdominal ultrasound examinations with special attention to liver and spleen characteristics (dimensions, texture, abnormalities) were carried out at each visit.

Carotid ultrasound examinations

Carotid ultrasound examinations were performed in supine position on the right and left side. The intima/media thickness of the left and right common carotid artery is measured.[26]

Bone density and body composition measurements

Regular dual energy X-ray absorptiometry measurements of bone density at the spine, hip and whole-body density include body composition and Trabecular Bone Score.[27]

Laboratory and functional metabolic measurements

Sample acquisition includes serum, plasma, saliva and urinary as well as stool samples. Routine tests include liver and kidney function and electrolytes, blood counts, hormonal and metabolic data including lipid profiles and urinary analyses. A standardised OGTT (including insulin and c-peptide) was performed. These materials were collected at each visit for immediate analysis as well as biobanking (including samples of function tests).

Biobanking

Serum, EDTA and citrate plasma, whole blood and cell pellets, spot urine, and saliva were collected at each study visit. They were immediately aliquoted and stored at the biobank of the Medical University of Graz (Austria) at –80°C.[28] Biobanking guarantees an accurate description of the sample collection and sample handling according to the STROBE-ME recommendations.[15] Biospecimen-derived measurements adhere to the European guidelines.[29]

Autoantibody phenotyping

Routine measurement of thyroid autoantibodies and autoimmune parameters for coeliac disease were performed in all participants. Further exploratory autoantibody detection (endocrine receptors, various epitopes) was done using newly developed luciferase-based fusion protein assays together with the Charité-Universitätsmedizin Berlin, Germany.[30]

Metabolic phenotyping by nuclear MR

(Un)targeted metabolomic analysis of 1012 baseline serum and urine samples was performed by nuclear MR spectroscopy and state-of-the art chemoinformatics.[31–33] Metabolite and lipoprotein concentrations were determined using Topspin and the Pre-Clinical Screening and In Vitro Diagnostics Research package of Bruker.[34–36]

Genome-wide characterisation of cohort

A whole genome global screening array (Illumina bead chip (Infinium Global Screening Array-24 V.2; Illumina, USA)) with nearly 700 k specific single nucleotide polymorphisms (Illumina, in cooperation with the Human Genotyping Facility at the Erasmus University Rotterdam, NL) is available from all cohort participants.

Optometric phenotyping

In addition, a large part of the cohort has been assessed by non-mydriatic retinal photography since 2015. Ophthalmologic examinations comprised undiluted fundus photography and optical coherence tomography including the assessment of retinal vessel diameters.

Data monitoring and quality assurance

All incoming data are checked by the study staff for completeness and plausibility and are entered into an electronic data capture system (OpenClinica; www.openclinica.com), specifically adapted for this project. Additional validation processes such as cross-validation with that is, external independent validation in samples were regularly performed. External monitoring by certified clinical monitors has been done in 100% of the baseline study records. Adequate external monitoring of the follow-up data is regularly ongoing.

Patient and public involvement

No patient involved.

FINDINGS TO DATE

A descriptive overview of the cohort including the CV, endocrine and metabolic risk profile is given in table 1. More female (55%) than male (45%) persons were included in the study. The mean age of the participants is 57±8 years and the mean body mass index (BMI) is 26.5±4.5 kg/m². A majority of 59% of the examined persons has a BMI greater than 25 kg/m². Although asymptomatic, only 26% of the study population is considered to be at low CV risk according to the Framingham 'Systematic COronary Risk Evaluation',[37] while 38% show

Table 1 Cardiovascular, endocrine, and metabolic risk profile of the BioPersMed cohort (N=1022)

	No (N=1022)	%
Sex		
Men	455	45
Women	567	55
Smoking		
Active smoker	154	15
Former smoker	326	32
Non-smoker	506	49
Unknown	36	4
Framingham SCORE*		
Low risk (<3 %)	263	26
Intermediate risk (3%–4%)	390	38
High risk (5%–9%)	267	26
Very high risk (≥10%)	9	1
Unknown	93	9
Diabetes status†		
NG	390	38
PreD	315	31
T2DM	181	18
Unknown	136	13
Medical history‡		
Arterial hypertension	341	33
Hyperlipidaemia	434	42
Stroke/TIA	7	1
Antihypertensive drugs per patient		
No antihypertensive drug	670	65
One antihypertensive drug	154	15
Two antihypertensive drugs	118	12
More than two antihypertensive drugs	80	8
Lipid-lowering drugs per patient		
No lipid-lowering drug	876	85
One lipid-lowering drug	139	14
More than one lipid-lowering drugs	7	1
Antidiabetic drugs per patient		
No antidiabetic drug	844	82
Dietetic treatment	119	12
One antidiabetic drug	54	5
More than one antidiabetic drugs	5	1
Age		
<55 years	437	43
55–65 years	382	38

Continued

Table 1 Continued

	No (N=1022)	%
66–75 years	178	17
>75 years	25	2
Body mass index		
<18.5 kg/m²	6	1
18.5–25.0 kg/m²	408	40
>25.0 kg/m²	608	59

Health status—abbreviations.
*Whenever present, a direct categorisation due to comorbidities was performed.
†Diabetes status based on oGTT results.
‡Hyperlipidaemia was assumed based on total cholesterol >200 mg/dL or the use of a lipid-lowering drug.
.NG, normoglycaemia; OGTT, oral glucose tolerance tests; PreD, pre-diabetes ; SCORE, Systematic COronary Risk Evaluation; T2DM, type 2 diabetes mellitus; TIA, transient ischaemic attack .

intermediate risk, 26% show high risk and 1% show very high risk. Due to some missing biomarkers at the baseline visit, we were not able to calculate the CV risk in 9% of the study population using the Framingham Score. The most common risk factor is hyperlipidaemia (42% of the population), followed by arterial hypertension (33% of the population), T2DM (18% of the population) and active smoking (15% of the population). A rather high number, nearly a third of the study population, has been identified as pre-diabetics based on oGTT data.

The purpose of this manuscript is to describe the study cohort and to give an overview of the baseline characteristics. These are reported in detail including additional information on used materials and methods in online supplemental tables S1–S10. The central figure 3 shows an R-correlation plot of most measured biomarkers at baseline. In this plot, biomarkers are not grouped (eg, in organ systems); instead, clusters of high correlations were formed (blue indicates a positive correlation, red indicates a negative correlation and white indicates no correlation).[38] Thereby, associations between different biomarkers of different organ systems can be revealed which may further serve as a basis for a multidisciplinary in-depth analysis.

Such analyses already identified the so far unknown correlation between IGF1 receptor autoantibodies with body composition and height as presented at the European Congress of Endocrinology in Barcelona, Spain 2018.[39] Another preliminary finding revealed a correlation between diabetes status and echocardiographic parameters of the diastolic heart function as presented at the Congress of the European Association for the Study of Diabetes in Barcelona, Spain 2019.[40] These observations were in line with previous findings of another research group.[41] Furthermore, genetic data from the genome-wide association study and optometric phenotyping of the

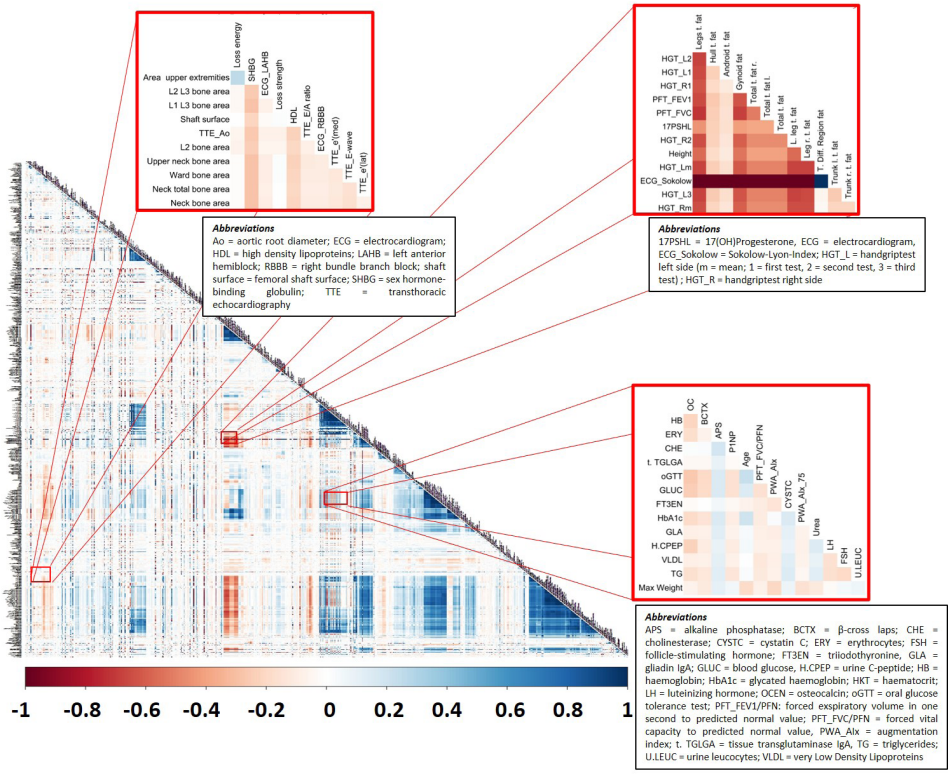

Figure 3 R-plot of the analysed data set. Potential biomarkers are not grouped; instead, random clusters of correlations are formed. blue zones indicate positive correlations, red zones indicate negative correlations. As an example, zones of strong correlations are zoomed and potential biomarkers are depicted.

BioPersMed cohort were already used as control data for a large keratoconus genomic study in cooperation with researchers from the UK and the Netherlands,[42] for the analysis of allelic determinants with a reported association to 25(OH)D levels and their influence on vitamin D,[43] and to identify novel biomarkers for non-alcoholic fatty liver disease[44] in cooperation with local researchers. Data on bone morphology from the BioPersMed cohort were used to link Sarcopenia with increased risk of falls, osteoporosis and mortality.[45] In addition, miRNA profiles were linked to Hashimoto's Disease and Thyroid Antibodies.[46]

Strengths and limitations

The main strength of the BioPersMed cohort is the joint evaluation of both CV and metabolic phenotyping including a broad spectrum of diagnostic, imaging, and functional tools. This assures comprehensive biomedical and scientific dimensions of the project within high-end diagnostic and analytical parameters and biomarkers. Second, biobanking with a large number of samples including serum/plasma and blood cells, urine, saliva and stool at each visit that have been aliquoted and stored on high-quality certification rules enabling a prospective view on candidate biomarker in the context of a large longitudinal cohort, where specific approaches can be predefined.[47 48] In addition, a specifically adapted large electronic database (OpenClinica; www.openclinica.com) assures the quality of data entry and delivery as well as the validity and reliability of biomarkers analyses in the BioPersMed cohort together with a continuous data monitoring.

A potential weakness of this study is the wide time range of a prolonged recruitment due to logistic reasons between 2010 and 2016. This results in a prolonged follow-up period of study participants between 4 and 10 years. Second, after a thorough standardised baseline phenotyping, of the BioPersMed cohort this phenotyping has been expanded by additional diagnostic parameters at either later points in time (eg, non-mydriatic funduscopy) or in subpopulations. Although some of these biomarkers are not available for the complete duration of the entire cohort, cross-sectional analysis of a considerable number of participants can already be performed with these data sets and will be available for longitudinal comparison of follow-up visits thereafter.

Collaboration

The design of the BioPersMed study, data management, biobanking and data analyses are compliant to the STROBE, STROBE-ME and STREGA recommendations. Collaboration in data analysis and publications will be welcome through specific research proposals sent to the BioPersMed investigators listed as corresponding authors of this manuscript. If desired, retrospective analyses can be performed because all data are recorded as raw data and more than 300 000 samples of blood, serum and urine are stored in the biobank.

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Contributors BP, TRP, AS and BO-P designed and supervised the study. AS and BO-P administrated the study. CWH, EK, NV, BO-P and AS wrote the manuscript. EK, CC, IM, MU-M, TG, BH, AL, CR, NV, KA, MW, EP-K, NJT, GS and AS investigated participants and collected data. CWH and EK calculated the statistics performed principal data analysis. BO-P and AS acted as guarantor. AT, NS, NV, TM, AS, AW, AZ, TK and RS contributed with data analysis and the allocation of resources. All authors reviewed the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval Ethical approval for the BioPersMed cohort study has been granted by the Ethics Committee of the Medical University of Graz, Austria and is renewed every year (EC Nr. 24-224 ex 11/12).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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Supplements – Methods and baseline characteristics

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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

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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.

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