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Endocrine-metabolic markers and subclinical cardiovascular disorders

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List of Abbreviations

ATC	Anatomic, therapeutic and chemical (code)
BMI	Body mass index
CI	Confidence Interval
CVD	Cardiovascular diseases
EBP	Exercise blood pressure
EDBP	Exercise diastolic blood pressure
ESBP	Exercise systolic blood pressure
FMD	Flow-mediated dilation
HbA_{1c}	Hemoglobin A_{1c}
HDL-C	High-density lipoprotein cholesterol
IGF-I	Insulin-like growth factor-I
IGFBP-3	Insulin-like growth factor binding protein-3
LDL-C	Low-density lipoprotein cholesterol
OR	Odds ratio
SHIP	Study of Health in Pomerania
SD	Standard deviation
TSH	Thyroid-stimulating hormone, Thyrotropin
WC	Waist circumference

Abstract

Background: Cardiovascular diseases are the leading cause of death worldwide. Subclinical alterations of the cardiovascular system, such as increased exercise blood pressure or an endothelial dysfunction confer a higher risk of manifest cardiovascular diseases and incident events. Detecting associations between circulating markers of the endocrine-metabolic system and the subclinical cardiovascular phenotypes could be useful to better understand cardiovascular disease progression and to improve risk prediction for manifest cardiovascular diseases.

Methods: The associations between (a) serum thyroid-stimulating hormone and increased exercise blood pressure, (b) serum hemoglobin A_{1c} and endothelial dysfunction as well as (c) serum insulin-like growth factor I and endothelial dysfunction were studied using cross-sectional data from around 1400 subjects aged 25 to 85 years collected during the 5-year follow-up of the population-based Study of Health in Pomerania (SHIP-1). Increased exercise blood pressure was defined as a value above the sex- and age-specific 80^{th} percentile measured at the 100 W stage of a symptom-limited bicycle ergometry test. Endothelial dysfunction was defined as an impaired flow-mediated dilation measured as a continuous decrease or below the median of sex-specific distribution. Non-fasting blood samples were drawn from the cubital vein in the supine position.

Results: The odds for increased systolic exercise blood pressure (odds ratio 1.24, 95% confidence interval 0.88; 1.76) and diastolic exercise blood pressure (odds ratio 0.98, 95% confidence interval 0.70; 1.39) as well as for exercise-induced increase of systolic and diastolic blood pressure were not significantly different between subjects with high and low serum thyroid-stimulating hormone levels within the reference range. In women without current use of antihypertensive medication, increasing serum hemoglobin A_{1c} levels were associated with decreasing flow-mediated dilation levels ($\beta = -1.17$, 95% confidence interval -2.03; -0.30). Such an association was not found in men. In men, logistic regression analysis revealed an odds ratio of 1.27 (95% confidence interval 1.07; 1.51) for decreased flow-mediated dilation for each decrement of serum insulin-like growth factor I standard deviation. In women, no significant association between serum insulin-like growth factor I levels and flow-mediated dilation was observed (odds ratio 0.88, 95% confidence interval 0.74; 1.05).

Conclusions: Based on the presented results it is concluded that (a) serum thyroid-stimulating hormone levels are not associated with exercise blood pressure in the general population,

(b) higher serum hemoglobin A_{1c} levels in non-diabetic subjects are inversely associated with flow-mediated dilation in women without antihypertensive medication, but not in men, and (c) lower serum insulin-like growth factor I levels are associated with impaired endothelial function in men, but not in women. Therefore the metabolic marker hemoglobin A_{1c} and the endocrine marker insulin-like growth factor I might be markers facilitating the identification of subjects at high risk of subclinical cardiovascular alterations.

1. Introduction

In 2008, a total of 57 million people (out of the world population of 6.7 billion people) died with 36 million deaths being attributable to non-communicable diseases and 17 million of them to cardiovascular causes [1, 2]. Therefore, cardiovascular diseases (CVD) are the leading cause of death worldwide [1]. CVD are defined as diseases of the heart and/or the circulatory system with coronary heart disease and stroke being the main forms [3]. Myocardial infarction and stroke are acute cardiovascular events with high mortality [4, 5].

Subclinical cardiovascular status is defined as alterations in the cardiovascular system (that are e.g. detectable by functional measures or imaging technologies) without overt clinical symptoms. Examples for subclinical cardiovascular disorders are increased exercise blood pressure (EBP) and endothelial dysfunction. Importantly, subclinical diseases predispose to overt events [6, 7]. Therefore, early detection of subclinical cardiovascular disorders and a deeper understanding of the underlying risk factors that contribute to subclinical alterations may facilitate an earlier identification of subjects at high cardiovascular risk and may finally help prevent manifest CVD and cardiovascular events.

Established risk factors for CVD are poverty, stress, social, economic and cultural change including globalization, urbanization, and population ageing [8]. Behavioral risk factors that are responsible for about 80% of CVD are unhealthy diet, physical inactivity and tobacco use [8]. Unhealthy diet and physical inactivity may affect blood pressure, blood glucose, blood lipids, body weight [8] and therefore the endocrine system and the metabolic status.

The endocrine system consists of endocrine glands that produce and control hormones that regulate several physiological body functions such as growth and metabolism. Examples for endocrine hormones are the thyroid-stimulating hormone (TSH) and the insulin-like growth factor I (IGF-I). TSH is synthesized and secreted in the anterior pituitary and it stimulates the thyroid to produce hormones that are regulating the energy balance of the body and act on the cardiovascular system [9]. IGF-I is produced mainly in the liver and mediates the effects of growth hormone by stimulating growth of almost every cell in the body. IGF-I is suggested to regulate normal cardiovascular physiological responses and may increase the risk for hypertension and atherosclerosis in the abnormal status [10].

A classic marker of the metabolic status is hemoglobin A_{1c} (HbA_{1c}). HbA_{1c} is glycated hemoglobin which reflects the average blood glucose level of several weeks prior to the

measurement and therefore, allows the diagnosis and reflects the degree of control of type 2 diabetes [11], a major cardiovascular risk factor.

The overall goal of the present research aims the investigation how the markers of the endocrine system serum TSH and serum IGF-I and the marker of the metabolic status serum HbA_{1c} are associated with subclinical cardiovascular disorders as reflected by increased EBP and endothelial dysfunction. All analyses were performed using data from the population-based Study of Health in Pomerania (SHIP).

1.1. Subclinical cardiovascular phenotypes

1.1.1. Exercise blood pressure

Exaggerated blood pressure response to exercise is a risk factor for cardiovascular morbidity [12] and mortality [13] and may provide additional information for the evaluation of the risk of hypertension and cardiovascular events beyond information provided by resting blood pressure alone [14].

The factors predicting blood pressure response to exercise are currently not well substantiated but may include high total cholesterol levels, high fasting triglyceride levels, high body mass index (BMI) and glucose intolerance [15], as well as endothelial dysfunction, increased left ventricular mass and increased peripheral resistance [14].

1.1.2. Endothelial function

The endothelium plays a major role in the regulation of vascular tone. The most extensively used non-invasive technique for the assessment of endothelial function is the determination of flow-mediated dilation (FMD) of the brachial artery by ultrasound [16]. Endothelial dysfunction precedes the development of clinically apparent atherosclerosis in humans [17]. Furthermore, low FMD values predict cardiovascular events in patients independently of established atherosclerosis [18]. The cardiovascular risk factors associated with endothelial dysfunction include hypertension, hypercholesterolemia and smoking [19, 20]. Furthermore, type 2 diabetes mellitus is under discussion to be associated with endothelial dysfunction [21].

1.2. Endocrine-metabolic markers

1.2.1. Thyroid-stimulating hormone

The thyroid gland produces the hormones thyroxine and triiodothyronine. These hormones regulate the activity of the metabolism. TSH controls the release of thyroid hormones. Hormone levels are regulated by a feedback loop at which the amount of TSH is controlled by the amount of free thyroid hormone in the circulation [22]. A low concentration of thyroid hormones due to under-activity of the thyroid gland stimulates the release of excess amounts of TSH, while a high concentration of thyroid hormones due to over-activity of the thyroid gland suppresses the release of TSH by the thyrotropin-releasing hormone, produced in the hypothalamus [22]. According to circulating TSH levels, the health status can be defined as normal, hypothyroid (indicated by increased TSH levels) or hyperthyroid (indicated by decreased TSH levels) [23]. While there is good evidence that overt thyroid dysfunction including goiter and autoimmune thyroid diseases is associated with cardiovascular morbidity, it is under debate whether subclinical dysfunction may lead to similar alterations as well [24, 25].

1.2.2. Hemoglobin A_{1c}

HbA_{1c} was recently recommended as an alternative method to diagnose type 2 diabetes mellitus instead of standard measurement of fasting glucose [26]. Since serum HbA_{1c} levels correlate with micro- and macrovascular complications in patients with type 2 diabetes mellitus, serum HbA_{1c} target levels are used to monitor antihyperglycemic therapy [27]. In individuals without type 2 diabetes mellitus, high serum HbA_{1c} levels are associated with future diagnosis of type 2 diabetes mellitus, future cardiovascular disease, cardiovascular and all-cause mortality [28]. These associations are similar or even stronger compared to fasting glucose levels [28].

1.2.3. Insulin-like growth factor-I

IGF-I represents an essential growth factor for the regulation of cell proliferation and differentiation [29]. Many cell types, including cardiac myocytes [10], secrete IGF-I and they are sensitive to its trophic action. IGF-I significantly interacts with endothelial physiology.

Human endothelial cells express more IGF-I receptors than insulin receptors [30], and IGF-I stimulates the nitric oxide production by endothelial cells, contributing to the regulation of vascular tone and other anti-atherosclerotic properties [31]. Circulating IGF-I is predominantly bound to insulin-like growth factor-binding proteins (IGFBP). Most of IGF-I is carried by IGFBP-3 [32]. Consequently, the IGF-I/IGFBP-3 ratio might mirror the biological impact of total IGF-I levels. Circulating IGF-I levels are inversely related to ultrasound measures of atherosclerosis [33], the risk of ischemic heart disease [34], stroke, coronary events [35] and cardiovascular mortality [36].

1.3. Hypotheses

1.3.1. Thyroid-stimulating hormone and increased exercise blood pressure

Although both, overt hyper- and hypothyroidism, are associated with increased resting blood pressure [37, 38], and the association between subclinical thyroid dysfunction and resting blood pressure is seen to be controversial [39], there is little information about the association between thyroid dysfunction and EBP. Clinical studies [40-42] usually investigated relatively small sample sizes, had limited statistical power and yielded conflicting results. Thus, one study [40] reported increased blood pressure responses to exercise in ten patients with long-term TSH-suppressive therapy, whereas two other studies [41, 42] found no association between thyroid function and EBP in twelve [42] and 42 patients [41] with hyperthyroidism. Information regarding the potential association between thyroid function and blood pressure responses to exercise from population-based studies is currently not available. Based on available evidence that subclinical hypothyroidism has a stronger impact on the risk of CVD than subclinical hyperthyroidism [43], the following was hypothesized: High serum TSH levels within and above the upper reference range are related to increased exercise systolic and diastolic blood pressures.

1.3.2. Hemoglobin A_{1c} and endothelial dysfunction

Only few observational studies [44, 45] investigated the association between parameters of glucose metabolism with endothelial function in non-diabetic individuals. One study [44] found an inverse association between fasting blood glucose levels and FMD in Chinese.

Another, more recent study [45] observed similar results: an inverse association was identified between serum HbA_{1c} levels and FMD in non-diabetic individuals with a BMI <26.1 kg/m^2, however, no such association was observed in subjects with higher BMI values. Importantly, the association between serum HbA_{1c} and FMD has not been addressed in a population-based sample so far. To investigate the potential association between glucose homeostasis and endothelial function in an adult non-diabetic population the following was hypothesized: High serum HbA_{1c} levels are associated with an impaired FMD.

1.3.3. Insulin-like growth factor-I and endothelial dysfunction

Studies designed to elucidate the association between serum IGF-l levels and FMD are scarce and mostly confined to patients with disorders in growth hormone metabolism. In patients with low serum IGF-I levels due to growth hormone deficiency, substitution of growth hormones leads to normalization of a previously reduced FMD [46]. On the other hand, in patients with acromegaly having high serum IGF-I levels, surgical resection of the growth hormone-producing tumor was followed by an improvement of previously reduced FMD [47]. More recently, an association between serum IGF-I levels and the acetylcholine-stimulated increase of forearm blood flow as measured by plethysmography was reported in a sample of 100 untreated hypertensive patients [48]. There are no epidemiological data available investigating the potential association of serum IGF-I or IBFBP-3 levels with endothelial function. The hypothesis of this substudy was as follows: Low serum IGF-I and IGFBP-3 levels are associated with an impaired FMD.

2. Methods

2.1. Study of Health in Pomerania

SHIP is a population-based study in the northeast area of Germany (Figure 1), which included 4308 subjects at baseline examination, and it took place between 1997 and 2001 (SHIP-0). The first follow-up examination (SHIP-1) was conducted five years (mean 5.2±0.5 years) after the baseline examination between 2002 and 2006, and it was attended by 3300 subjects (83.5% of the eligible sample) [49]. All participants gave informed written consent. The study followed the recommendations of the Declaration of Helsinki and was approved by the ethics committee of the University of Greifswald. For the present work data from SHIP-1 was analyzed.

Figure 1 Study region of the Study of Health in Pomerania

2.2. Study sample

A total of 1708 subjects (874 women) volunteered for a standardized progressive incremental exercise test on a cycle ergometer. Subjects who did not reach the 100 W stage of the exercise test (N=145), persons with missing blood pressure data during the 100 W stage (N=32) and participants with missing serum TSH data (N=4) were excluded. Furthermore, 36 subjects with previous myocardial infarction, 23 subjects with previous stroke and 34 subjects with impaired left ventricular function defined by a fractional shortening < 22% in women and < 20% in men [50] were excluded. Thus, the final study sample comprised 1438 subjects (711 women) aged 25 to 83 years that were available for the analysis of the association between serum TSH and EBP (Figure 2).

A total of 1788 subjects (913 women) volunteered for measuring FMD of the brachial artery. Exclusion criteria for FMD measurements were equipment malfunction (N=36) and any medical contraindication (N=19), mostly hypotension with systolic blood pressure below 100 mmHg (N=15). Image quality of 215 FMD examinations was insufficient for valid readings.

From the analysis of the association between serum HbA_{1c} and FMD, subjects without data on serum HbA_{1c} levels (N=13) and subjects with self reported physician's diagnosis of diabetes mellitus or use of antidiabetic medication (N=121) were also excluded. This resulted in a final study sample of 1384 subjects (696 women) aged 25 to 85 years (Figure 2).

For the analysis of the association between serum IGF-I and FMD, 36 subjects with missing serum IGF-I levels were not available. This resulted in a final study sample of 1482 subjects (736 women) aged 25 to 85 years (Figure 2).

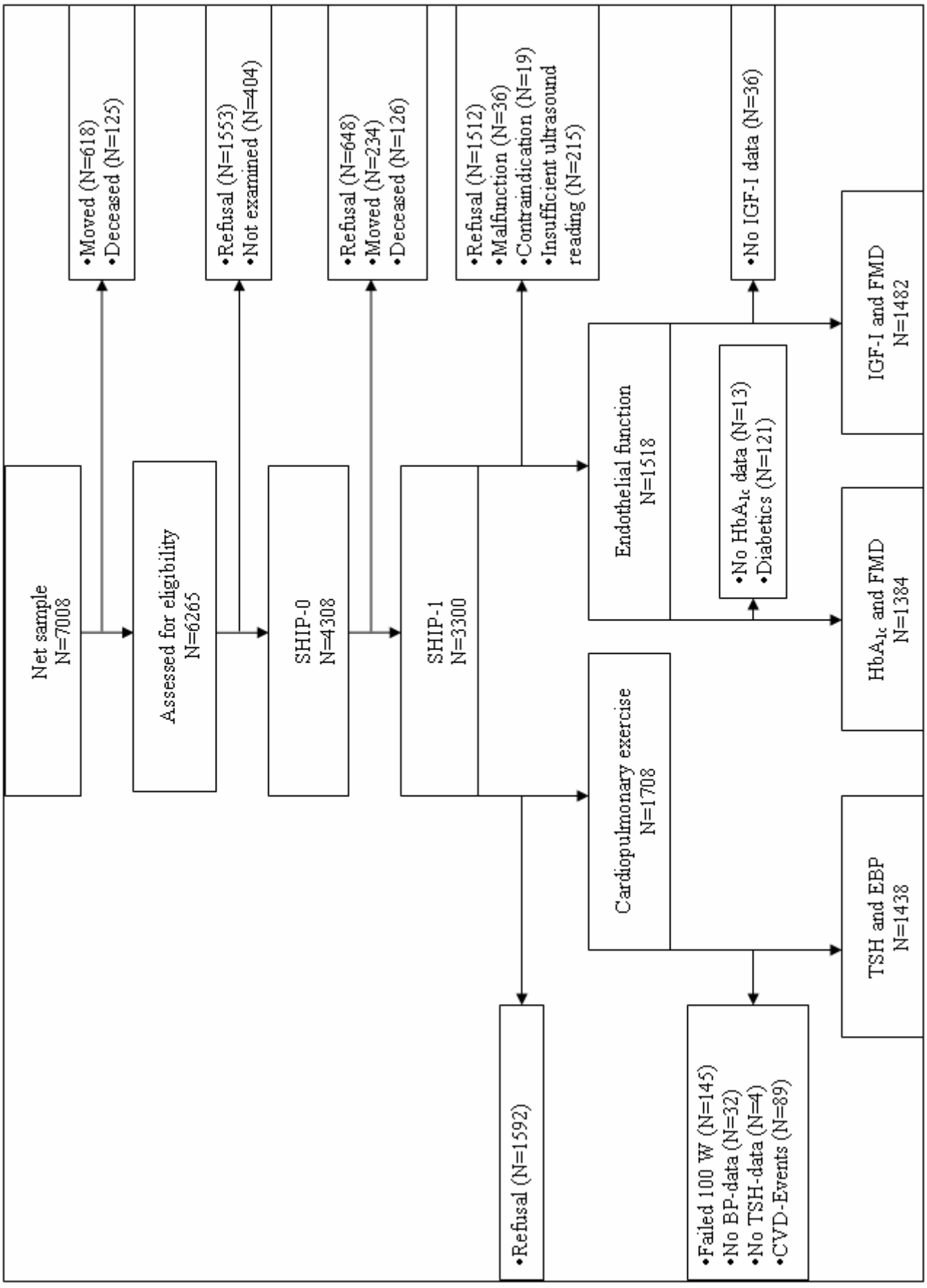

Figure 2 Flow chart of the sample recruitment of the three SHIP subsamples

2.3. Measurement of subclinical cardiovascular phenotypes

2.3.1. Exercise blood pressure

A symptom-limited exercise test using calibrated electromagnetically braked cycle ergometer with an electrical seat height adjustment (Ergoselect 100; Ergoline, Bitz, Germany) was performed according to a protocol modified from Jones et al. [51] (stepwise increase in work load of 16 W after every minute, starting with 20 W, Figure 3). In the absence of chest pain and electrocardiographic abnormalities, all tests were continued as symptom-limited (volitional exertion, dyspnea or fatigue), while patients were encouraged to reach maximal exhaustion. Systolic and diastolic blood pressure were measured once before the start of exercise testing and then monitored continuously at each level of work load.

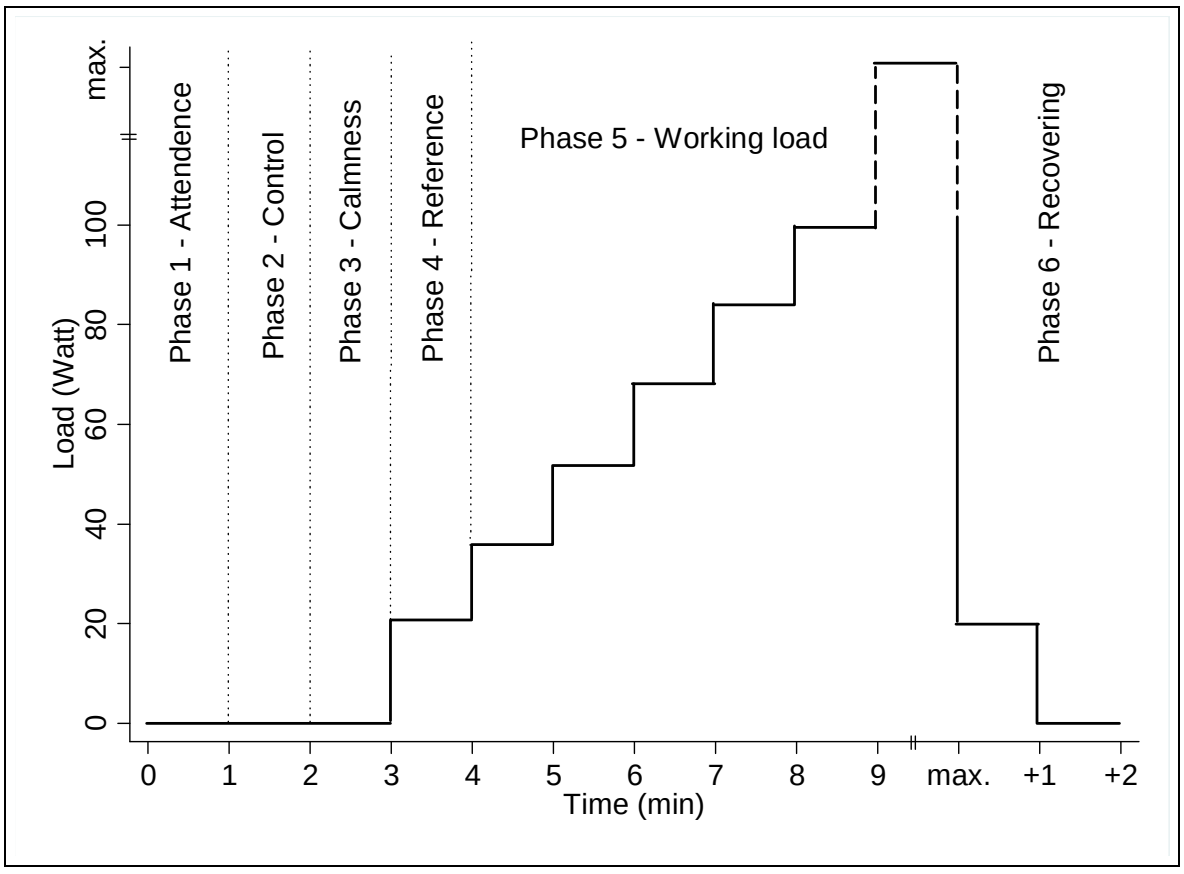

Figure 3 Protocol of symptom-limited exercise testing

Increased values for EBP were defined as values above the 80th sex- and 10-year-age-group percentile during 100 W stage of exercise testing of participants with serum TSH levels within the reference range [52]. The following exercise-associated variables were analyzed at

the 100 W stage: increased exercise systolic blood pressure (ESBP), increased exercise diastolic blood pressure (EDBP) and increased exercise systolic or diastolic blood pressure (ESBP|EDBP). Additionally, increased differences between EBP and baseline resting blood pressure were considered as outcome variables.

2.3.2. Endothelial function

FMD of the right brachial artery was assessed by measuring the increase of the brachial artery diameter during reactive hyperemia after transient forearm-ischemia. The brachial artery was visualized using a 10 MHz linear array transducer (Cypress, Siemens, Erlangen, Germany). The participants had lain down quietly for 10 minutes before measurements. A blood pressure cuff was placed around the right forearm 5 cm distally from the right antecubital fossa. B-mode longitudinal images of the brachial artery were obtained at the level of the antecubital fossa. After marking the optimal position of the transducer, baseline images of the brachial artery were digitally stored. Arterial flow of the forearm was interrupted by insufflation of the forearm cuff for 5 minutes by 200 mmHg or 50 mmHg above systolic blood pressure, whichever was highest. Exactly one minute after cuff deflation, B-mode longitudinal images of the brachial artery were obtained for FMD measurements (Figure 4).
Enddiastolic vessel diameters were measured from the anterior to the posterior M-line (i.e. the interface between the media and adventitia) of the vessel wall.

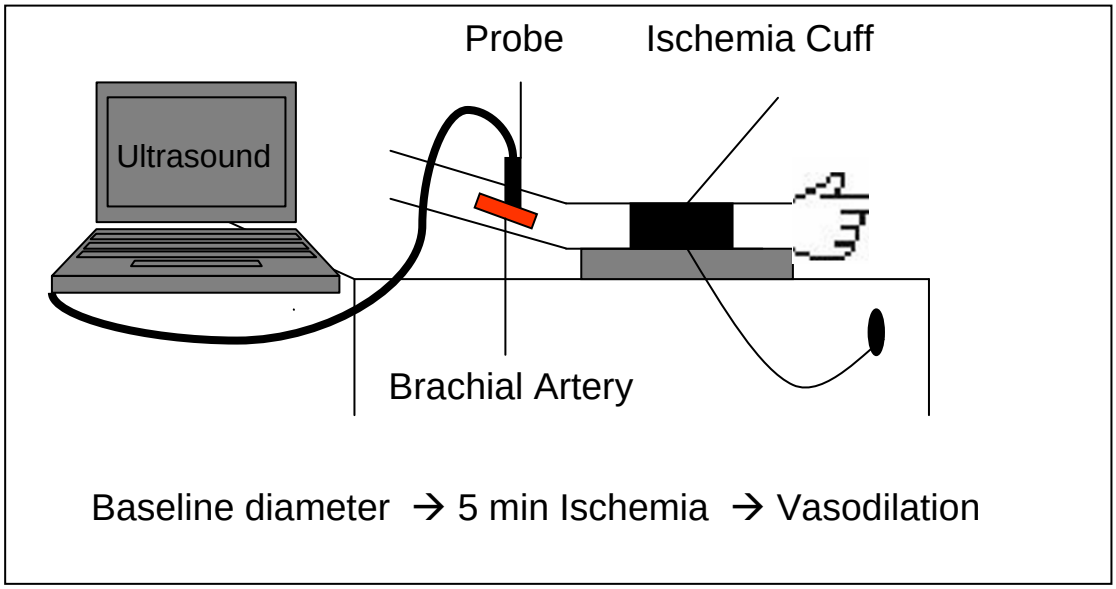

Figure 4 Set-up for the measurement of flow-mediated dilation

Diameters were calculated from the average of three measurements of four consecutive cardiac cycles. Absolute FMD was calculated by subtracting baseline vessel diameters from post-ischemia vessel diameters. Relative changes were expressed as percentage of absolute FMD to baseline diameters [16]:

$$\text{FMD (\%)} = ((\text{FMD diameter} - \text{Baseline diameter}) / \text{Baseline diameter}) \times 100$$

FMD was modeled as a binary trait considered as decreased when the value was below the sex-specific median for the analysis of the association between serum IGF-I levels and FMD. For the analysis of the association between serum HbA_{1c} levels and FMD, FMD was modeled as a continuous variable.

2.4. Measurement of endocrine-metabolic markers

Non-fasting blood samples were drawn between 07:00 a.m. and 04:00 p.m. from the cubital vein in the supine position. The samples were analyzed immediately for all parameters except serum IGF-I and IGFBP-3, for which the serum was stored at -80°C. Internal quality controls were performed at least once per day.

2.4.1. Thyroid-stimulating hormone

Serum TSH levels were analyzed by immunochemiluminescent procedures [Immulite 2000, Third generation, Diagnostic Products Corporation (DPC), Los Angeles, CA, USA]. According to the recently established serum TSH reference range (0.25 – 2.12 mIU/L) in the region [52], participants were divided into five groups as follows: group 1: < 0.25 mIU/L; group 2: 0.25 - 0.65 mIU/L; group 3: 0.66-0.99 mIU/L; group 4: 1.00-2.12 mIU/L; group 5: >2.12 mIU/L. By definition, subjects in group 1 had decreased serum TSH levels (N_1=67), subjects in groups 2 to 4 had serum TSH levels within the reference range according to tertiles (N_2=454; N_3=426; N_4=421), and subjects in group 5 had increased serum TSH levels (N_5=70).

2.4.2. Hemoglobin A_{1c}

Serum HbA_{1c} levels were measured by high performance liquid chromatography (HPLC) (Bio-Rad DiamatTM Analyzer, ClinRep® kit, RECIPE Chemicals + Instruments GmbH, Munich, Germany). For the statistical analysis, serum HbA_{1c} levels were modeled as a continuous variable.

2.4.3. Insulin-like growth factor-I

Serum IGF-I and IGFBP-3 levels were measured using a chemiluminescent immunometric assay on an Immulite 2500 analyzer (Siemens Immulite 2500; Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). Measurements were carried out from April to May 2008. An aliquot of two levels of the manufacturer's control material (IGF-Control-Module, ref. LGCOC, lot 022, Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany) was included within each series in single determination. For the statistical analysis, serum IGF-I and IGFBP-3 levels were divided by their negative standard deviation (SD) to demonstrate a level decrease.

2.5. Measurement of confounders and covariates

A computer-assisted personal interview was used to collect information on sociodemographic characteristics and medical conditions. Regarding smoking, subjects were classified as never-, ex-, or current smokers. Diabetes mellitus, liver disease, previous history of myocardial infarction and stroke were defined as self-reported physician’s diagnosis. Subjects who participated in physical training for at least one hour a week for at least four month a year were classified as being physically active.

Current medication was recorded by a computer-aided method using the anatomic, therapeutic and chemical (ATC) code. The following drugs were considered as antihypertensive medications: vasodilators used in cardiac diseases (ATC C01D), antihypertensives (ATC C02), diuretics (ATC C03), peripheral vasodilators (ATC C04), beta-blockers (ATC C07), calcium antagonists (ATC C08), angiotensin I-converting enzyme inhibitors and angiotensin II receptor blockers (ATC C09). For women, the use of sex hormones (ATC G03) was considered.

During SHIP core examinations after a five minute rest period, systolic and diastolic blood pressure were measured three times at the right arm of seated subjects using a digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan) with each reading being followed by a further rest period of three minutes. The mean of the second and third measurements was calculated and used for evaluation of resting blood pressure. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of antihypertensive medication. Waist circumference (WC) was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet. BMI was calculated as weight in kilograms divided by the square of height in meters.

Serum creatinine levels were measured with a modified Jaffé method, total serum cholesterol was measured photometrically (both Dimension RxL HM Max, Siemens Healthcare Diagnostics, Germany). The definition of renal disease was based on self-report or estimated creatinine clearance values less than 50 ml/min as estimated by the Cockcroft–Gault formula [53].

Low- and high-density-lipoprotein cholesterol (LDL-C and HDL-C) levels were quantified by lipid electrophoresis (HELENA SAS-3 system, Helena 7 BioSciences Europe, Tyne & Wear, UK). Triglycerides and glucose were determined enzymatically using reagents from Nobis and Roche Diagnostics, respectively (Hitachi 717, Roche Diagnostics, Mannheim, Germany).

2.6. Statistical analyses

For the description of the study sample, data on quantitative characteristics are expressed as mean and SD and data on qualitative characteristics are expressed as absolute numbers and percent values. Bivariate comparisons were made using Mann-Whitney`s U-test for continuous data and χ^2-test for nominal data.

Different multivariable statistical analyses were performed including linear and logistic regression analysis. Adjusted ß-coefficients or odds ratios (OR) and their 95% Wald confidence intervals (CI) are provided.

All analyses were adjusted for important confounders including sex, age, WC or BMI, diabetes mellitus, smoking status, hypertension or antihypertensive medication, liver and

renal diseases, serum HDL-C and LDL-C levels. For women, regression models were further adjusted for use of sex hormones (ATC G03). If necessary, analyses were performed in women and men separately.

Different sensitivity analyses were performed to confirm the observed findings, e.g. in the subsample of participants without antihypertensive medication according to ATC code or restricted to participants without previous CVD. For the analysis of the association between EBP and serum TSH levels, linear regression models with EBP and serum TSH levels on the continuous scale were conducted. Multivariable fractional polynomial models were used to explore possible non-linear associations between serum HbA_{1c} and FMD by testing if any other power transformation of the independent variables than the linear one improved the fit of the model. Interactions of the independent variables with different confounder including age, smoking and BMI were also tested. To control for possible selection bias introduced by exclusion of subjects (i.e. non-participants of FMD examination), inverse probability weighting according to major variables of difference between study sample and non-participants was used in further sensitivity analysis.

A value of $p<0.05$ was considered statistically significant. Statistical analyses were performed using Stata 10 (Stata Corporation, College Station, TX, USA).

3. Results

To show exemplarily differences in potential confounders between the subsample of the SHIP side projects (including cardiopulmonary stress testing and FMD measurement) and non-participants the largest substudy population of the analysis of the association between serum IGF-I levels and FMD was chosen.

Compared to non-participants, the study sample with comprehensive CVD phenotyping was more often male, younger, less often suffered from diabetes mellitus, renal diseases and hypertension, used less frequently antihypertensive medication and had lower glucose levels.

Table 1 Selected characteristics of the study subsample and non-participants

	Study population N=1482	Non-participants N= 1818	p-value*
Age, yr	52.1 (13.5)	56.4 (16.4)	<0.001
Sex (male)	746 (50.3)	843 (46.4)	0.023
Smoking status			0.308
Never-smoker	634 (42.8)	744 (41.1)	
Ex-smoker	487 (32.9)	584 (32.3)	
Current smoker	361 (24.4)	483 (26.7)	
Body mass index, kg/m^2	27.8 (4.7)	28.1 (5.1)	0.493
Waist circumference, cm	92.4 (13.5)	92.9 (14.4)	0.443
Diabetes mellitus	118 (8.0)	252 (13.9)	<0.001
Liver diseases	27 (1.8)	34 (1.9)	0.918
Renal diseases	67 (4.5)	205 (11.3)	<0.001
Systolic blood pressure, mmHg	130.7 (18.1)	134.1 (21.3)	<0.001
Diastolic blood pressure, mmHg	81.7 (10.1)	81.1 (11.0)	0.007
Hypertension	715 (48.3)	998 (54.9)	<0.001
Use of antihypertensive medication	513 (34.6)	853 (46.9)	<0.001
HDL-C, mmol/l	1.18 (0.42)	1.17 (0.43)	0.578
LDL-C, mmol/l	3.52 (1.02)	3.53 (1.00)	0.832
Triglycerides, mmol/l	1.85 (1.94)	1.83 (1.51)	0.622
Glucose, mmol/l	5.35 (1.27)	5.66 (1.84)	<0.001
Use of sex hormones	127 (8.6)	156 (8.6)	0.991

Data are given as number (percentage) or mean (standard deviation), * χ^2-test (nominal data) or Mann-Whitney-U-test (interval data), HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol

There were no differences in smoking habits, BMI, WC, liver disease, HDL-C and LDL-C levels and triglyceride values or in the use of sex-hormone medication between the study sample with comprehensive CVD phenotyping and non-participants (Table 1).

3.1. Association between thyroid-stimulating hormone and exercise blood pressure

In men and women, the unadjusted means of ESBP, EDBP and the differences between EBP and resting blood pressure did not differ significantly between serum TSH level groups compared to the reference group of subjects with serum TSH levels in the lower reference range.

For those individuals with serum TSH values within the reference range (0.25 – 2.12 mIU/L), the 80th percentile values for ESBP were higher in women (overall 192.4 mmHg) than in men (182.0 mmHg) and increased over the age groups in both sexes. The 80th percentile values for EDBP were almost constant across the age groups in both sexes and only slightly higher in women (overall 100.0 mmHg) than in men (95.0 mmHg). The increase in blood pressure from rest to exercise was also higher in women (overall 80th percentile systolic difference=69.4 mmHg; diastolic difference=12.0 mmHg) than in men (54.0 mmHg; 8.0 mmHg).

The fully adjusted multivariable logistic regression models did not reveal a significant and consistent association between serum TSH levels and any EBP variable (ESBP, EDBP and the difference between EBP and baseline blood pressure) when subjects with decreased or increased serum TSH as well as with higher serum TSH levels within the reference range were compared to those with serum TSH levels in the lower reference range (Table 2). Analyses adjusting for the interaction between gender and serum TSH level groups did not show different associations of serum TSH levels with EBP variables between men and women. Additional sensitivity analysis excluding subjects with current antihypertensive medication did not change the main findings substantially. Further multivariable linear regression models with EBP and serum TSH levels on the continuous scale including resting blood pressure as an additional covariate were assessed. No association was found between serum TSH levels and ESBP (ß=0.23, 95% CI -0.79; 1.26) or EDBP (ß=0.04, 95% CI -0.52; 0.61) in the whole sample.

Table 2 The association between serum TSH levels and exercise blood pressure

Exercise blood pressure	Serum TSH levels (mIU/L)				
	<0.25	0.25-0.65	0.66-0.99	1.00-2.12	>2.12
	(N=67)	(N=454)	(N=426)	(N=421)	(N=70)
Increased (>80th percentile)					
Systolic EBP	0.79 (0.37; 1.66)	1 (Ref.)	1.23 (0.87; 1.73)	1.24 (0.88; 1.76)	1.28 (0.67; 2.42)
Diastolic EBP	0.62 (0.29; 1.30)	1 (Ref.)	0.82 (0.58; 1.16)	0.98 (0.70; 1.39)	1.13 (0.60; 2.12)
Diastolic or systolic EBP	0.79 (0.43; 1.46)	1 (Ref.)	1.06 (0.78; 1.42)	1.30 (0.96; 1.75)	1.29 (0.74; 2.25)
Systolic EBP difference	0.50 (0.22; 1.14)	1 (Ref.)	1.07 (0.76; 1.49)	0.94 (0.67; 1.33)	1.01 (0.52; 1.93)
Diastolic EBP difference	0.75 (0.36; 1.53)	1 (Ref.)	0.92 (0.65; 1.29)	0.84 (0.59; 1.20)	0.89 (0.45; 1.74)
Diastolic or systolic EBP difference	0.70 (0.38; 1.28)	1 (Ref.)	1.04 (0.78; 1.39)	0.91 (0.68; 1.23)	0.97 (0.55; 1.70)

Data are OR (95% confidence interval). * $p<0.05$ logistic regression, model adjusted for sex, age, waist circumference, smoking status, diabetes mellitus, antihypertensive medication. TSH, thyrotropin; EBP, exercise blood pressure; Ref., reference group

3.2. Association between hemoglobin A_{1c} and endothelial function

In women, linear regression models revealed an age-adjusted, non-significant association between serum HbA_{1c} levels and FMD (β = -0.60; 95% CI -1.26; 0.05, p = 0.070). After further adjustment for BMI, smoking status, hypertension, LDL-C, and sex-hormone medication, an inverse association between serum HbA_{1c} levels and FMD was observed (β = -0.76; 95% CI -1.46; -0.06, p = 0.033; Table 3). Analyses in women without current use of antihypertensive medication disclosed decreasing FMD values for increasing serum HbA_{1c} levels for the age-adjusted model (β = -0.96; 95% CI -1.78; -0.13, p = 0.023) and the fully adjusted model (β = -1.17; 95% CI -2.03; -0.30, p = 0.009).

In men, there was an unadjusted inverse association between serum HbA_{1c} levels and FMD (β = -0.72; 95% CI -1.13; -0.31, p = 0.001). However, after adjustment for major confounders, the full model revealed no association between serum HbA_{1c} levels and FMD (β = 0.05; 95% CI -0.34; 0.45, p = 0.787). Analyses in men without current use of antihypertensive medication substantiated no association between serum HbA_{1c} levels and FMD in the age-adjusted and fully adjusted model (Table 3).

The association between serum HbA_{1c} levels and FMD did not differ substantially between non-smoking and currently smoking subjects (serum HbA_{1c}*smoking interaction, p = 0.344 for women; p = 0.693 for men) and between subjects divided by the female median BMI of 26.3 kg/m^2 (serum HbA_{1c}*BMI interaction, p = 0.559 for women; p = 0.311 for men) without current use of antihypertensive medication in the fully adjusted model. Furthermore, a serum HbA_{1c}*age interaction effect was present in women (p < 0.001), but not in men (p = 0.823). The association between serum HbA_{1c} levels and FMD was observed in women younger than 55 years (β = -1.44; 95% CI -2.49; -0.40, p = 0.007), but not in women equal or older than 55 years (β = 0.11; 95% CI -1.47; 1.68, p = 0.892).

Table 3 The association between serum HbA_{1c} levels and FMD

	FMD (%)			
	Whole study population		Study population without antihypertensive medication	
Serum HbA_{1c} (%)	β (95 % CI)	p-value	β (95 % CI)	p-value
Women	(*n* = 696)		(*n* = 489)	
Model 1	-1.61 (-2.24; -0.98)	<0.001	-1.65 (-2.45; -0.85)	<0.001
Model 2	-0.60 (-1.26; 0.05)	0.070	-0.96 (-1.78; -0.13)	0.023
Model 3	-0.74 (-1.44; -0.05)	0.035	-1.14 (-2.00; -0.27)	0.010
Model 4	-0.76 (-1.46; -0.06)	0.033	-1.17 (-2.03; -0.30)	0.009
Men	(*n* = 688)		(*n* = 465)	
Model 1	-0.72 (-1.13; -0.31)	0.001	-0.51 (-1.08; 0.06)	0.079
Model 2	-0.08 (-0.46; 0.30)	0.692	0.01 (-0.56; 0.58)	0.975
Model 3	0.05 (-0.34; 0.45)	0.787	0.20 (-0.37; 0.78)	0.482

Data are β-coefficients of linear regression (95 % confidence interval); Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age, body mass index, smoking, hypertension, and low-density lipoprotein cholesterol; Model 4: Model 3 + adjusted for sex-hormone medication. FMD, flow-mediated dilatation

3.3. Association between insulin-like growth factor-I and endothelial function

In multivariable logistic regression analyses, there was an age-adjusted OR of 1.28 (CI 1.08-1.53; p=0.005) for decreased FMD in males with each decrement of serum IGF-I SD (SD=51.9 ng/ml). Adjustment for further potential confounders including WC, hypertension, diabetes mellitus, liver and renal disease, smoking status, serum HDL-C and LDL-C did not influence the results substantially. In women no association between serum IGF-I levels and FMD was identified after adjustment for age and further confounders. In both men and women, no association between serum IGFBP-3 levels and FMD was present. Only in men

there was a higher full adjusted OR of 1.24 (CI 1.06; 1.46; p=0.009) for decreased FMD with each decrement of serum IGF-I/IGFBP-3 ratio SD (SD=0.0098).

Additional analyses were performed after excluding subjects with antihypertensive medication. In the fully adjusted model in men, there was an OR of 1.40 (CI 1.12; 1.75; p=0.003) and an OR of 1.38 (CI 1.11; 1.71; p=0.003) for decreased FMD with each decrement of serum IGF-I SD (SD=48.1 ng/ml) and serum IGF-I/IGFBP-3 ratio SD (SD=0.0093), respectively. The associations of serum IGF-I levels and serum IGF-I/IGFBP-3 ratio with FMD in women and serum IGFBP-3 levels and FMD in women and men remained non-significant after this change (Table 4).

Table 4 The association between serum IGF-I / IGFBP-3 levels and decreased FMD

	Decreased FMDa			
	Women		Men	
Models*	OR (95% CI)	p-value	OR (95% CI)	p-value
Serum IGF-I (SD decrement)				
Model 1	0.96 (0.78-1.18)	0.695	1.35 (1.09-1.68)	0.005
Model 2	0.95 (0.77-1.16)	0.595	1.40 (1.12-1.75)	0.003
Serum IGFBP-3 (SD decrement)				
Model 1	1.04 (0.86-1.26)	0.677	1.09 (0.89-1.32)	0.410
Model 2	0.99 (0.81-1.21)	0.926	1.09 (0.88-1.34)	0.450
Serum IGF-I/IGFBP-3 ratio (SD decrement)				
Model 1	0.93 (0.76-1.13)	0.440	1.29 (1.06-1.58)	0.011
Model 2	0.94 (0.76-1.15)	0.542	1.38 (1.11-1.71)	0.003

* for subjects without antihypertensive medication

[a] <Median (<5.50% for women; <4.42% for men), logistic regression, Model: 1 (adjusted for age); 2 (+ adjusted for waist circumference, diabetes mellitus, systolic and diastolic blood pressure, liver and renal diseases, smoking status, HDL-C and LDL-C), IGF-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein-3; FMD, flow-mediated dilation; OR, odds ratio; CI, confidence interval; SD, standard deviation

4. Discussion

4.1. Thyroid-stimulating hormone and exercise blood pressure

In the present study, serum TSH levels were not related to EBP after adjustment for major confounders. In particular, the hypothesis that high serum TSH levels might be related to increased ESBP and EDBP was not confirmed, even after exclusion of subjects with current antihypertensive medication. This is the first population-based study that investigated the association between thyroid function and EBP.

Although the present study is the largest investigation on this issue to date, some smaller studies [41, 42] found similar results. In a case-control study [41], 42 patients with untreated overt hyperthyroidism had similar systolic and diastolic blood pressures during maximal exercise as 22 healthy controls. Moreover, no changes in systolic and diastolic blood pressure responses to exercise were observed in these patients after restoration of euthyroidism during six months follow-up. Likewise, no effects of antithyroid drugs on EBP were found in another, small interventional study [42] of twelve hyperthyroid patients after a treatment period of ten months. In contrast, in another study [40], ten patients with long-term TSH-suppressive therapy with levothyroxine had a similar systolic blood pressure during maximal exercise but a higher systolic blood pressure during a submaximal exercise workload of 75 W compared to a control group of 10 euthyroid subjects. These contrasting findings might be due to small sample sizes and limited control for potential confounders such as age, sex, body habitus, physical activity and cardiac disease.

While two previous population-based studies [54, 55] reported a modest association between high-normal serum TSH levels and resting blood pressure, the present results are in agreement with other studies [56, 57] that did not find an association between subclinical hypothyroidism and blood pressure at rest. In a cross-sectional Chinese study [56] including 806 subjects with subclinical hypothyroidism and 5669 euthyroid controls, subclinical hypothyroidism was not associated with increased resting blood pressure. Likewise, in the cross-sectional Busselton Thyroid Study [57] including 105 subjects with subclinical hypothyroidism and 1859 euthyroid controls from Western Australia, subclinical hypothyroidism was not associated with hypertension. It is possible that a modest association reported previously by the two large population-based studies [54, 55] with 5872 and 30728 subjects, respectively, was not detected in the present analysis because of the smaller study

sample. The clinical relevance of weak but statistical significant associations detected in very large studies, however, may be questionable.

In designing the present study and data analyses several decisions were made that could have affected the results. First, cycle ergometry was opted as a means of physical exercise for the subjects since bicycling is common in Germany. Furthermore, the EBP data obtained during the 100 W stage of exercise testing was uniformly analyzed assuming similar fitness levels of subjects in all five serum TSH groups. This decision was based on similar levels of self-reported physical activity in all groups. Sex differences in biological work power were considered by using sex-specific definitions of increased EBP and by conducting additional sensitivity analysis for men at higher workloads. For reasons of statistical power, sex- and 10-years-age-specific 80^{th} percentile values for increased EBP variables were used. In sensitivity analysis, 90^{th} percentiles for cut-offs for increased EBP were also considered. Because the value of EBP depends on the blood pressure at rest, blood pressure increase, defined as the difference between blood pressure at rest and EBP, was also considered as dependent variable. None of these additional sensitivity analyses detected an association between serum TSH levels and EBP.

4.2. Hemoglobin A_{1c} and endothelial function

This study was further performed to elucidate the association of serum HbA_{1c} levels as a long-term marker of glucose metabolism with endothelial function in non-diabetic subjects. After adjustment for a broad range of confounders, the hypothesis of an inverse association between serum HbA_{1c} levels and FMD was partly confirmed in the overall female population, especially in women without antihypertensive medication.

In partial concordance with the present results, a previous study by Voidonikola et al. [45] found an inverse association of serum HbA_{1c} levels and FMD in subjects with a BMI below the median, whereas in overweight subjects with a BMI above the median, this association was not apparent. In the present analysis, we found no effect modification by BMI. Since the authors of the latter study did not present sex-stratified analysis, it cannot be ruled out that the observed findings in that study were primarily driven by an associations between serum HbA_{1c} and FMD in women, particularly since the study by Voidonikola et al. was predominantly female (61%). Such a gender-specific association is consistent with the present

study. Moreover, subjects with lower BMI in the latter study were more often females (69 %) and had a lower proportion of hypertension (11 %) with less-likely use of antihypertensive drugs than did subjects with higher BMI.

In general, various factors may potentially influence the association between glucose hemostasis and endothelial function. Antihypertensive drugs differ in their impact on glucose metabolism. For example, calcium channel blockers and angiotensin-converting enzyme inhibitors do not influence glucose or serum HbA_{1c} levels substantially, whereas beta-blockers and diuretics lead to impaired glucose homoeostasis [58]. On a parallel note, antihypertensive therapy may have significant effects on the endothelium, which may impede detection of an association between serum HbA_{1c} levels and FMD [59] in participants receiving antihypertensive treatment. Given the conflicting data on the association between antihypertensive therapy and glucose metabolism on the one hand and endothelial dysfunction on the other, participants taking antihypertensive medication were excluded from the present analysis on serum HbA_{1c} and endothelial function.

There is evidence that the production of nitric oxide is greater in premenopausal women than in men: which could partially explain the observed differences in vascular function between men and women [60]. Furthermore, there is evidence that female sex hormones have an influence on vascular endothelial function [61] that may also affect the association between serum HbA_{1c} levels and FMD in women. A similar gender difference was also detected in the association between increased fasting glucose and coronary artery calcification, which revealed stronger associations in women than in men [62]. Further hypotheses how hyperglycaemia may adversely affect endothelial function have until now described by multiple mechanisms and include the following: increased polyol pathway flux, augmented advanced glycation end-product formation, activation of protein kinase C isoforms, and increased hexosamine pathway flux [63].

4.3. Insulin-like growth factor-I and endothelial function

In the present study, an association between low serum IGF-I levels and decreased FMD in men after adjustment for age was detected. Adjustment for additional confounding cardiovascular risk factors including waist circumference, diabetes mellitus, hypertension,

liver and renal diseases, smoking status as well as HDL-C and LDL-C levels did not have a significant impact on the main findings of the present analysis.

Various antihypertensive medications such as beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers modulate endothelial function [59]. Exclusion of participants with current use of antihypertensive medication from the analysis revealed no significant impact on the association between serum IGF-I and FMD in the present study.

Since circulating IGF-I is predominantly bound to IGFBP-3 [32], separate analysis to detect a possibly differing association between IGF-I/IGFBP-3 ratio and FMD was performed. The results of the presented analyses were essentially unchanged when the serum IGF-I/IGFBP-3 ratio was used as exposure variable instead of serum IGF-I levels. This fact indicates the biological relevance of the association between serum IGF-I levels and FMD in men.

An association between low serum IGF-I levels and impaired endothelium mediated vasodilation has been described previously in a population of 100 untreated, hypertensive individuals [48]. Since the authors of the latter study did not present sex-stratified analysis (men (N=65) and women (N=35)), it cannot be ruled out that the reported association of serum IGF-I levels and acetylcholine-induced forearm blood flow in the entire population was mainly driven by a strong association in men in that study [48]. Other studies focusing on the association between serum IGF-I levels and endothelial function were even smaller and mostly confined to patients with disturbed growth hormone secretion like acromegaly or growth hormone deficiency [46, 47]. In patients with low and high preinterventional serum IGF-I levels, respectively, normalization of serum IGF-I levels led to an improvement of previously reduced FMD [46, 47]. The present study sample comprised 1482 subjects with a broad age range (25 to 85 years) and serum IGF-I levels largely within the reference range which points towards a relevant impact of serum IGF-I on the cardiovascular system in male subjects under physiological circumstances.

The association between serum IGF-I and decreased FMD as a marker of preclinical atherosclerosis in men might represent a possible link to cardiovascular morbidity and mortality. Indeed, SHIP data have recently demonstrated that men with low serum IGF-I levels had an almost two-fold higher risk of cardiovascular disease mortality compared to men with normal serum IGF-I levels [64].

In contrast to men, serum IGF-I levels did not reveal any relevant association with FMD in women. In concordance with the present observations with respect to subclinical CVD phenotype of FMD, no association between serum IGF-I levels and mortality [64] and risk of myocardial infarction [65] was found in women. Few studies have addressed possible sex

differences in cardiovascular risk factors in relation to the GH/IGF system. Sexual dimorphism exists in growth hormone secretion: women in their fertile age have higher serum growth hormone levels and lower serum IGF-I levels than men [66]. Also in patients with growth hormone deficiency receiving growth hormone replacement therapy, sex-specific effects have been described [67]. Men require lower growth hormone doses than women [68], and have apparently greater benefits from the substitution with regard to improvement in body composition, lipid profile and bone mass [69]. While these findings are in good agreement with the sex-specific associations found in the present study, the specific mechanisms underlying this sex-specificity remain to be investigated.

4.4. Strength and limitations

The present studies have several strengths and potential limitations that should be considered. All analyses are limited by their cross-sectional design that precludes any causal inference.
Furthermore, the definition of the history of diabetes mellitus is based on a self-reported physician's diagnosis but not on biochemical measurements such as fasting glucose levels or serum HbA_{1c}. Therefore, a certain proportion of participants might have had undiagnosed type 2 diabetes mellitus. Moreover, physical exercise could potentially have affected the results of FMD measurements. Thus, endurance exercise on the day preceding FMD measurements (that was not assessed in the study protocol or interview) could have led not only to increased insulin sensitivity but also to improvement of postprandial FMD in healthy humans [70]. However, it seems unlikely that the results were substantially influenced by recent physical exercise, since only exhausting physical exercise is known to influence endothelial function [70]. Exclusion of several subjects (i.e., non-participants in FMD examination) could, moreover, have led to selection bias. Inverse probability weighting according to age, WC, and hypertension confirmed, nevertheless, that the present findings were not biased by the differences among these variables between study participants with and without FMD measurements.
Major strengths of the present studies include the population-based design, the comprehensive assessment of metabolic and cardiovascular risk factors and potential confounders [49], the size of the study sample and the accurate FMD measurements under strict quality management using standardized protocols and certified staff [25]. The method of forearm

ischemia induction of FMD precludes potential contribution by ischemia of the brachial artery itself – in contrast to upper-arm ischemia-induced FMD, which is known to induce greater vasodilation [16].

4.5. Conclusions

The main conclusions of the present analyses are:

(1) Serum TSH levels are not associated with EBP in the general population.

(2) Higher serum HbA_{1c} levels in non-diabetic subjects are inversely associated with FMD in women not on antihypertensive medication. In men without diabetes mellitus, higher serum HbA_{1c} levels are not associated with FMD.

(3) Low serum IGF-I levels are associated with impaired endothelial function in men, which might represent a possible link to increased cardiovascular morbidity and mortality. In women, serum IGF-I levels are not associated with endothelial function.

4.6. Perspectives

Identification of markers for CVD, especially for subclinical CVD, supports reducing a main global health burden and the main cause of death by understanding the associated risk factors and by evaluating a better risk profile for CVD that could be the basis for health actions of different health system institutions. To be considered as a high quality marker, a risk marker has to be consistently associated with comparable CVD phenotypes in different studies of similar size using a similar mode of examinations. In the present work, serum HbA_{1c} and IGF-I levels are associated with FMD, and therefore, emerge as potential candidate markers for endothelial dysfunction. However, both findings as well as the result of the lacking association between serum TSH levels and EBP need further confirmation by other longitudinal observational and clinical intervention studies.

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6. Appendix

Original articles

Serum Thyrotropin Levels and Blood Pressure Response to Exercise in a Population-based Study

Roberto Lorbeer[1], Marcus Dörr[2], Till Ittermann[1], Beate Koch[2], Ralf Ewert[2], Rainer Rettig[3], Matthias Nauck[4], Stephan B. Felix[2], Henri Wallaschofski[4], Henry Völzke[1]

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Serum Thyrotropin Levels and Blood Pressure Response to Exercise in a Population-Based Study

Roberto Lorbeer,[1] Marcus Dörr,[2] Till Ittermann,[1] Beate Koch,[2] Ralf Ewert,[2] Rainer Rettig,[3] Matthias Nauck,[4] Stephan B. Felix,[2] Henri Wallaschofski,[4] and Henry Völzke[1]

Background: Studies on the relation between thyroid function and exercise blood pressure (EBP) are rare and not population-based, and have yielded inconsistent results. The aim of this study was to investigate whether serum thyrotropin (TSH) levels are related to increased EBP.
Methods: Cross-sectional data from 1438 subjects (711 women) aged 25–83 years without histories of cardiovascular diseases from the 5-year follow-up of the population-based Study of Health in Pomerania (SHIP-1) were analyzed. Blood pressure was measured at the 100 W stage of a symptom-limited bicycle ergometry test. Increased EBP was defined as a value above the sex- and age-specific 80th percentile of participants with serum TSH levels within the reference range (0.25–2.12 mIU/L).
Results: There was no association between serum TSH levels and EBP after adjusting for sex, age, waist circumference, diabetes mellitus, smoking status, and antihypertensive medication. The odds for increased systolic EBP (odds ratio 1.24, 95% confidence interval 0.88; 1.76) and diastolic EBP (odds ratios 0.98, 95% confidence interval 0.70; 1.39) as well as for exercise-induced increase of systolic and diastolic blood pressure were not significantly different between subjects with high and low serum TSH levels within the reference range. Similar findings were found for both subjects with TSH levels below and above the reference range, respectively.
Conclusions: We conclude that serum TSH levels are not associated with exercise-related blood pressure response.

Introduction

High resting blood pressure, even within the normal range, is associated with an increased risk of cardiovascular diseases (CVD), including atherosclerosis (1), stroke, and myocardial infarction (2,3). Further, exaggerated blood pressure responses to exercise are a risk factor for cardiovascular morbidity (4–7) and mortality (8,9) and may provide more information for the evaluation of the risk of hypertension and cardiovascular events than resting blood pressure (10,11).

The factors predicting blood pressure responses to exercise are currently not well understood but may include high total cholesterol levels, high fasting triglyceride levels, high body mass index, and glucose intolerance (12), as well as endothelial dysfunction, increased left ventricular mass, and increased peripheral resistance (11). Although both overt hyper- and hypothyroidism are often associated with increased resting blood pressure (13,14), there is little information about the relation between thyroid dysfunction and exercise blood pressure (EBP). Available clinical studies (15–17) are small, have limited statistical power, and yielded conflicting results. Thus, one study (17) reported increased blood pressure responses to exercise in 10 patients with long-term thyrotropin (TSH)-suppressive therapy, whereas two other studies (15,16) found no association between thyroid function and EBP in 12 (16) and 42 patients (15), respectively, with hyperthyroidism. Information regarding the potential relation between thyroid function and blood pressure responses to exercise from population-based studies is currently not available.

We performed a population-based study including more than 1400 subjects covering a wide range of serum TSH levels to investigate the relation between thyroid function and EBP. On the basis of available evidence that subclinical hypothyroidism has a stronger impact on the risk of CVD than subclinical hyperthyroidism (18–20), we hypothesized that high serum TSH levels within and above the upper reference range are related to increased exercise systolic and diastolic blood pressures (ESBP/EDBP). The present investigation is a substudy of the Study of Health in Pomerania (SHIP).

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Materials and Methods

Study population

The design of SHIP has been published previously (21,22). In brief, SHIP is a population-based study in the northeast area of Germany, which included 4308 subjects at baseline between 1997 and 2001. The first follow-up examination (SHIP-1) was conducted 5 years (mean 5.2 ± 0.5 years) after baseline, between 2002 and 2006, and comprised 3300 subjects (83.5% of the still eligible population) (23). All participants gave informed written consent. The study followed the recommendations of the Declaration of Helsinki and was approved by the ethics committee of the University of Greifswald.

A total of 1708 subjects (874 females) volunteered for a standardized progressive incremental exercise test on a cycle ergometer. We excluded 145 individuals who did not reach the 100 W stage of the exercise test, 32 persons with missing blood pressure data during the 100 W stage, and 4 participants with missing serum TSH data. Furthermore, participants with a previous history of CVD events (36 myocardial infarctions and 23 strokes) were not considered. In addition, we excluded 34 subjects with impaired left ventricular function defined by a fractional shortening <22% in women and <20% in men (24). Some subjects were excluded because of more than one CVD event. Thus, the final study population comprised 1438 subjects (711 women) aged 25–83 years who were available for the present analyses.

Computer-assisted interview

A computer-assisted personal interview was used to collect information on sociodemographic characteristics and medical conditions. Cigarette smokers were divided into never, ex-, and current smokers. Diabetes mellitus, history of myocardial infarction, and stroke were defined as self-reported physician's diagnosis. Subjects who participated in physical training for at least 1 hour a week for at least 4 months a year were classified as being physically active.

Present medication was recorded by a computer-aided method using the anatomic, therapeutic, and chemical (ATC) code. The following drugs were considered as antihypertensive medications: vasodilators used in cardiac diseases (ATC C01D), antihypertensives (ATC C02), diuretics (ATC C03), peripheral vasodilators (ATC C04), beta-blockers (ATC C07), calcium antagonists (ATC C08), angiotensin I-converting enzyme inhibitors, and angiotensin II receptor blockers (ATC C09).

Blood pressure and waist circumference

During SHIP core examinations after a 5-minute rest period, systolic and diastolic blood pressure were measured three times at the right arm of seated subjects using a digital blood pressure monitor (HEM-705CP; Omron Corporation, Tokyo, Japan) with each reading being followed by a further rest period of 3 minutes. The mean of the second and third measurements was calculated and used for evaluation of resting blood pressure. Hypertension was defined as a resting systolic blood pressure ≥140 mmHg, a resting diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication. Waist circumference was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet.

Serum TSH

Nonfasting blood samples were drawn from the cubital vein in the supine position. The samples were taken between 07:00 a.m. and 04:00 p.m. and analyzed immediately for all parameters. Serum TSH levels were analyzed by immunochemiluminescent procedures (Immulite 2000, Third generation; Diagnostic Products Corporation, Los Angeles, IL). According to the recently established TSH reference range (0.25–2.12 mIU/L) in the region (25), participants were divided into five groups as follows—group 1: <0.25 mIU/L; group 2: 0.25–0.65 mIU/L; group 3: 0.66–0.99 mIU/L; group 4: 1.00–2.12 mIU/L; group 5: >2.12 mIU/L. By definition, subjects in group 1 had decreased TSH levels, subjects in groups 2–4 had TSH levels within the reference range according to tertiles, and subjects in group 5 had increased TSH levels.

Exercise test

A symptom-limited exercise test using one calibrated electromagnetically braked cycle ergometer with an electrical seat height adjustment (Ergoselect 100; Ergoline, Bitz, Germany) was performed according to a protocol modified from Jones *et al.* (26) (stepwise increase in work load of 16 W after every minute, starting with 20 W). The procedure was continuously monitored by a physician. In the absence of chest pain and ECG abnormalities, all tests were continued as symptom-limited (volitional exertion, dyspnea, or fatigue), while patients were encouraged to reach maximal exhaustion. All tests were performed in room air according to current guidelines for exercise testing (27,28). Systolic and diastolic blood pressure were measured once before the start of exercise testing and then monitored continuously at each level of work load. Increased values for EBP were defined by sex and 10-year age-specific 80th percentile cut-off values during 100 W stage of exercise testing of participants with serum TSH levels within the reference range. The following exercise-associated variables were analyzed at the 100 W stage: increased ESBP, increased EDBP, and increased ESBP or EDBP. Additionally, increased differences between EBP and baseline resting blood pressure were considered as outcome variables.

Statistics

Data on quantitative characteristics are expressed as mean and standard deviation. Data on qualitative characteristics are expressed as absolute numbers and percent values. Bivariate comparisons between serum TSH level groups were made using Student's *t*-test (continuous data) or χ^2-test (nominal data). Comparisons were performed separately against the group with low serum TSH (first tertile) within the reference range (0.25–0.65 mIU/L). Multivariable statistical analyses were performed using logistic regression analysis. Multivariable comparisons were made between all defined serum TSH groups relative to the second TSH group. Adjusted odds ratios (OR) and their 95% confidence intervals (CI) are provided. In sensitivity analyses linear regression models with EBP and serum TSH levels on the continuous scale were

Table 1. Selected Characteristics of the Study Population

Characteristics	Serum TSH levels (mIU/L)				
	<0.25 (n = 67)	0.25–0.65 (n = 454)	0.66–0.99 (n = 426)	1.00–2.12 (n = 421)	>2.12 (n = 70)
Sex (male)	32 (47.8%)	241 (53.1%)	215 (50.5%)	213 (50.6%)	26 (37.1%)[a]
Age (year)	53.8 ± 11.3	52.5 ± 13.4	49.8 ± 12.3[a]	48.3 ± 12.9[a]	49.8 ± 13.5
Smoking status					
Never smoker	21 (31.3%)	189 (41.6%)	194 (45.5%)	184 (43.7%)	33 (47.1%)
Ex-smoker	32 (47.8%)[a]	133 (29.3%)	122 (28.6%)	135 (32.1%)	25 (35.7%)
Current smoker	14 (20.9%)	132 (29.1%)	110 (25.8%)	102 (24.2%)	12 (17.1%)
Waist circumference (mm)	919 ± 137	920 ± 132	913 ± 136	916 ± 140	899 ± 144
Physical activity	22 (32.8%)	168 (37.0%)	155 (36.4%)	177 (42.0%)	34 (48.6%)
Resting systolic blood pressure (mmHg)	130.7 ± 16.9	129.6 ± 18.3	129.2 ± 17.5	130.3 ± 17.4	130.1 ± 17.7
Resting diastolic blood pressure (mmHg)	81.7 ± 10.3	81.3 ± 9.4	81.7 ± 10.1	82.4 ± 9.9	82.0 ± 9.8
Resting hypertension	36 (53.7%)	192 (42.3%)	194 (45.5%)	177 (42.2%)	31 (44.3%)
Use of antihypertensive medication	24 (35.8%)	148 (32.6%)	120 (28.2%)	110 (26.1%)[a]	26 (37.1%)
Diabetes mellitus	4 (6.0%)	29 (6.4%)	24 (5.6%)	27 (6.4%)	7 (10.0%)

Groups according to different serum TSH levels. Data are given as number (percentage) or mean ± standard deviation.
[a]$p < 0.05$; χ^2-test (nominal data) or Student's t-test (interval data). Comparisons were performed separately against the group with low serum TSH within the reference range (0.25–0.65 mIU/L).
TSH, thyrotropin.

conducted. All analyses were controlled for sex, age, waist circumference, diabetes mellitus, smoking status, and antihypertensive medication. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using Stata 10 (Stata Corporation, College Station, TX).

Results

Baseline characteristics of the serum TSH level groups are presented in Table 1. Compared with the reference group of subjects with serum TSH levels in the lower reference range, subjects with increased serum TSH levels were more often women. Subjects with serum TSH levels in the higher reference range were younger and used antihypertensive medications less often relative to the reference group. Subjects with decreased serum TSH levels were more often ex-smokers than subjects of the reference group. Serum TSH level groups did not differ with respect to waist circumference, physical activity, systolic and diastolic blood pressure at rest, as well as hypertension and diabetes mellitus (Table 1).

Table 2. Serum Thyrotropin Levels (mIU/L) and Exercise Blood Pressure (100 W) in Women and Men

	Serum TSH levels (mIU/L)				
	<0.25	0.25–0.65	0.66–0.99	1.00–2.12	>2.12
Women	n = 35	n = 213	n = 211	n = 208	n = 44
Exercise					
Systolic BP (mmHg)	172.8 ± 24.3	169.7 ± 27.1	169.4 ± 24.5	171.0 ± 27.3	172.5 ± 23.1
Diastolic BP (mmHg)	90.4 ± 11.9	90.2 ± 11.8	89.7 ± 12.7	88.9 ± 13.7	92.3 ± 11.9
Difference (exercise-rest)					
Systolic BP (mmHg)	48.3 ± 20.3	53.0 ± 21.2	51.6 ± 19.6	51.0 ± 20.7	52.3 ± 21.7
Diastolic BP (mmHg)	4.3 ± 7.9	4.8 ± 10.4	3.9 ± 11.5	3.1 ± 11.5	5.2 ± 9.2
Men	n = 32	n = 241	n = 215	n = 213	n = 26
Exercise					
Systolic BP (mmHg)	164.7 ± 22.7	164.0 ± 21.1	163.7 ± 24.2	161.8 ± 21.7	164.5 ± 27.5
Diastolic BP (mmHg)	84.9 ± 11.4	84.9 ± 11.9	86.4 ± 12.3	85.6 ± 12.4	85.3 ± 13.6
Difference (exercise-rest)					
Systolic BP (mmHg)	42.3 ± 15.0	41.8 ± 17.2	41.2 ± 19.0	38.7 ± 17.3	38.1 ± 19.2
Diastolic BP (mmHg)	−0.5 ± 9.0	0.4 ± 11.2	−0.1 ± 9.0	−0.8 ± 9.0	−0.4 ± 13.7

Data are given as mean ± standard deviation. No result shows a significant $p < 0.05$; Student's t-test (interval data). Comparisons were performed separately against the group with low serum TSH within the reference range (0.25–0.65 mIU/L).
BP, blood pressure.

Table 3. The Relation Between Thyrotropin Levels and Exercise Blood Pressure (100 W) in the Total Sample

	Serum TSH levels (mIU/L)				
Exercise BP	*<0.25 (n = 67)*	*0.25–0.65 (n = 454)*	*0.66–0.99 (n = 426)*	*1.00–2.12 (n = 421)*	*>2.12 (n = 70)*
Increased (>80th percentile)					
Systolic BP	0.79 (0.37; 1.66)	1 (Ref.)	1.23 (0.87; 1.73)	1.24 (0.88; 1.76)	1.28 (0.67; 2.42)
Diastolic BP	0.62 (0.29; 1.30)	1 (Ref.)	0.82 (0.58; 1.16)	0.98 (0.70; 1.39)	1.13 (0.60; 2.12)
Diastolic or systolic BP	0.79 (0.43; 1.46)	1 (Ref.)	1.06 (0.78; 1.42)	1.30 (0.96; 1.75)	1.29 (0.74; 2.25)
Systolic BP difference	0.50 (0.22; 1.14)	1 (Ref.)	1.07 (0.76; 1.49)	0.94 (0.67; 1.33)	1.01 (0.52; 1.93)
Diastolic BP difference	0.75 (0.36; 1.53)	1 (Ref.)	0.92 (0.65; 1.29)	0.84 (0.59; 1.20)	0.89 (0.45; 1.74)
Diastolic or systolic BP difference	0.70 (0.38; 1.28)	1 (Ref.)	1.04 (0.78; 1.39)	0.91 (0.68; 1.23)	0.97 (0.55; 1.70)

Data are OR (95% confidence interval). No result shows a significant $p < 0.05$ logistic regression, model adjusted for sex, age, waist circumference, smoking status, diabetes mellitus, and antihypertensive medication.
Ref., reference group; OR, odds ratio.

In both sexes the unadjusted means of ESBP, EDBP, and the differences between EBP and resting blood pressure did not differ significantly between serum TSH level groups compared with the reference group of subjects with serum TSH levels in the lower reference range (Table 2).

The 80th percentile values for ESBP according to age within the population of TSH reference range were higher in women (overall 192.4 mmHg) than in men (182.0 mmHg) and increased over the age groups in both sexes. The 80th percentile values for EDBP were almost constant over the age groups in both sexes and only slightly higher in women (overall 100.0 mmHg) than in men (95.0 mmHg). The increase in blood pressure from rest to exercise was also higher in women (overall 80th percentile systolic difference = 69.4 mmHg; diastolic difference = 12.0 mmHg) than in men (54.0 mmHg; 8.0 mmHg).

The fully adjusted multivariable logistic regression models did not reveal a significant and consistent relation between serum TSH levels and any EBP variable (ESBP, EDBP, and the difference between EBP and baseline blood pressure) when subjects with decreased or increased TSH as well as with higher serum TSH levels within the reference range were compared to those with serum TSH levels in the lower reference range (Table 3). Analyses adjusting for the interaction between gender and serum TSH level groups did not show different associations of serum TSH levels with EBP variables between men and women. Additional sensitivity analysis excluding subjects taken antihypertensive medications did not change the main findings substantially (Table 4).

In further sensitivity analyses we varied the definitions of ESBP and EDBP stepwise. Applying the definition of blood pressure values >80th percentile for higher exercise stages (116, 132, and 148 W) or using the 90th percentiles as cut-offs did also not reveal a statistically significant association between serum TSH levels and EBP. Likewise, analyses excluding subjects with a 5-year history of thyroid disease or current use of thyroid therapy drugs (ATC H03) did not significantly change our main results. In multivariable linear regression models with EBP and serum TSH levels on the continuous scale including resting blood pressure as an additional covariate, we also found no association between serum TSH levels and systolic EBP ($\beta = 0.23$, 95% CI −0.79; 1.26) or diastolic EBP ($\beta = 0.04$, 95% CI −0.52; 0.61) in the whole sample.

Discussion

In the present study, serum TSH levels were not related to EBP after consideration of major confounders. In particular, our hypothesis that high serum TSH levels might be related to

Table 4. The Relation Between Thyrotropin Levels and Exercise Blood Pressure (100 W) Among Subjects Without Antihypertensive Medication

	Serum TSH levels (mIU/L)				
Exercise BP	*<0.25 (n = 43)*	*0.25–0.65 (n = 306)*	*0.66–0.99 (n = 306)*	*1.00–2.12 (n = 311)*	*>2.12 (n = 44)*
Increased (>80th percentile)					
Systolic BP	1.62 (0.74; 3.52)	1 (Ref.)	1.28 (0.84; 1.95)	1.45 (0.96; 2.20)	1.08 (0.45; 2.59)
Diastolic BP	0.54 (0.20; 1.46)	1 (Ref.)	0.98 (0.65; 1.49)	1.06 (0.71; 1.60)	1.18 (0.53; 2.62)
Diastolic or systolic BP	1.09 (0.53; 2.24)	1 (Ref.)	1.09 (0.76; 1.56)	1.33 (0.93; 1.90)	1.14 (0.56; 2.34)
Systolic BP difference	0.72 (0.29; 1.81)	1 (Ref.)	1.11 (0.74; 1.68)	1.14 (0.76; 1.72)	0.75 (0.30; 1.88)
Diastolic BP difference	0.74 (0.29; 1.84)	1 (Ref.)	1.00 (0.67; 1.52)	0.87 (0.57; 1.33)	1.09 (0.49; 2.43)
Diastolic or systolic BP difference	0.80 (0.38; 1.71)	1 (Ref.)	1.15 (0.81; 1.63)	1.06 (0.74; 1.51)	0.99 (0.49; 2.02)

Data are OR (95% confidence interval). No result shows a significant $p < 0.05$ logistic regression; model adjusted for sex, age, waist circumference, smoking status, and diabetes mellitus.

increased ESBP and EDBP was not confirmed, even after exclusion of subjects with current antihypertensive medication. This is the first population-based study that investigated the association between thyroid function and EBP.

Although the present study is the largest investigation on this issue to date, some smaller studies (15,16) found similar results. In a case–control study (15), 42 patients with untreated overt hyperthyroidism had similar systolic and diastolic blood pressures during maximal exercise as 22 healthy controls. Moreover, no changes in systolic and diastolic blood pressure responses to exercise were observed in these patients after restoration of euthyroidism during 6 months' follow-up. Likewise, no effects on EBP were found in another, small interventional study (16) of 12 hyperthyroid patients after a treatment period of 10 months. In contrast, in a further study (17), 10 patients with long-term TSH-suppressive therapy with levothyroxine had a similar systolic blood pressure during maximal exercise but a higher systolic blood pressure during a submaximal exercise workload of 75 watt compared to a control group of 10 euthyroid subjects. These contrasting findings might be due to small sample sizes and limited control for potential confounders such as age, sex, body habitus, physical activity, and cardiac disease. Our design with a large sample size including more than 400 subjects in each of the three groups within the reference range of serum TSH levels (groups 2–4) would have allowed us to detect a relevant OR of 1.7 for increased EBP with a statistical power of 80% ($p < 0.05$). The groups with serum TSH levels below (group 1) and above the reference range (group 5) were substantially smaller resulting in a detectable OR of 2.6 for increased EBP with a statistical power of 80% ($p < 0.05$).

While two population-based studies (29,30) reported a modest association between high-normal serum TSH levels and resting blood pressure, our results are in agreement with other studies (31–33) that did not find an association between subclinical hypothyroidism and blood pressure at rest. In a cross-sectional Chinese study (31) including 806 subjects with subclinical hypothyroidism and 5669 euthyroid controls, subclinical hypothyroidism was not associated with increased resting blood pressure. Likewise, in the cross-sectional Busselton Thyroid Study (32) including 105 subjects with subclinical hypothyroidism and 1859 euthyroid controls from Western Australia, subclinical hypothyroidism was not associated with hypertension. Two review articles (34,35) concluded that there is no conclusive evidence for a higher risk of hypertension in subjects with subclinical hypothyroidism. It is possible that we did not detect a modest association reported by the two large population-based studies (29,30) with 5872 and 30,728 subjects, respectively, because of our smaller study population. The clinical relevance of weak associations detected as statistically significant in very large studies, however, may be questionable.

Our findings also support other studies (31,33) that reported no association between subclinical hyperthyroidism and blood pressure at rest. In line with these findings, previous investigations of the SHIP population in a cross-sectional (36) and a longitudinal approach (37) with a median follow-up of 5 years revealed no association between subclinical hyperthyroidism and hypertension.

In designing our study and data analyses we had to make several decisions that may have affected our results. Thus, we opted for cycle ergometry as a means of physical exercise for our subjects since bicycling is common in Germany with 80% of all households owning at least one bicycle (38). Further, we chose to uniformly analyze the EBP data obtained during the 100 W stage of exercise testing assuming similar fitness levels of subjects in all five TSH groups. This decision was based on similar levels of self-reported physical activity in all groups (Table 1). Sex differences in biological work power were considered by using sex-specific definitions of increased EBP and by conducting additional sensitivity analyses for men at higher workloads. For reasons of statistical power, we used sex- and 10-year age-specific 80th percentile values for increased EBP variables. In sensitivity analyses we also considered 90th percentiles for cut-offs for increased EBP. As the value of EBP depends on the blood pressure at rest we considered blood pressure increase defined as the difference between blood pressure at rest and EBP as dependent variable. None of these additional analyses detected an association between serum TSH levels and EBP.

We used the TSH reference range of 0.25–2.12 mIU/L that was recently established for the study region—a previously iodine-deficient area (25). The distribution of serum TSH levels within a population strongly depends on the iodine supply (39). While the distribution curve is skewed toward lower levels in iodine-deficient regions, it is shifted toward higher levels in iodine-replete areas. Consequently, both lower and upper TSH reference values are lower in iodine-deficient areas than in regions with sufficient iodine supply (25,40,41). Thus, the reference values presented herein may be representative for populations from currently or previously iodine-deficient areas, but may be less generalizable for populations from iodine-replete regions.

Our study is limited by its cross-sectional design that precludes conclusions as to possible cause-and-effect relations. The strengths of our study include the population-based approach and the assessment of a large variety of potential confounders. Nevertheless, our results need confirmation by additional studies of similar size using a similar mode of exercise testing.

We conclude that serum TSH levels are not associated with EBP in the general population.

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Disclosure Statement

The authors declare that no competing financial interests exist.

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Association between glycosylated hemoglobin A_{1c} and endothelial function in an adult non-diabetic population

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ABSTRACT

Objective: Endothelial dysfunction precedes apparent atherosclerosis in humans and is associated with a number of cardiovascular risk factors, including Type 2 diabetes. To investigate the impact of long-term glucose homeostasis on endothelial function in an adult non-diabetic population, we analysed the association of serum HbA_{1c} levels with endothelial function.
Methods: We studied cross-sectional data from 1384 subjects (696 women), aged 25–85, without diabetes, from the population-based Study of Health in Pomerania (SHIP-1). Flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD) measurements of the brachial artery were performed using standardised ultrasound techniques. Linear regression models were carried out to assess the association between serum HbA_{1c} levels and FMD/NMD.
Results: Multivariable analyses disclosed an inverse association between serum HbA_{1c} levels and FMD in women, but not in men. In women without current use of antihypertensive medication, increasing serum HbA_{1c} levels were associated with decreasing FMD levels after adjustment for age, body mass index, smoking status, hypertension, low-density lipoprotein cholesterol, and sex-hormone medication ($\beta = -1.17$; 95% CI −2.03; −0.30, $p = 0.009$). There was an inverse association between serum HbA_{1c} levels and NMD in men ($\beta = -1.68$; 95% CI −2.83; −0.52, $p = 0.005$), but not in women.
Conclusion: We conclude that higher serum HbA_{1c} levels in non-diabetic subjects are inversely associated with FMD in women without antihypertensive medication, but not in men. The gender-specific aspects concerning the association of HbA_{1c} levels and NMD in this population should be investigated in further studies. Our results support current considerations that subclinical disorders of glucose metabolism measured by serum HbA_{1c} are associated with subclinical cardiovascular diseases detected by FMD, especially in women.

1. Introduction

The endothelium plays a major role in the regulation of vascular tone. Endothelial function can be evaluated through a number of invasive and non-invasive methods. Today, the most extensively used non-invasive technique for assessment of endothelial function is sonographic determination of flow-mediated dilation (FMD) of the brachial artery [1]. FMD is induced by and consequently measured after an ischemic episode; its pathophysiology is not yet completely understood. It is currently believed that vasodilation is induced by the resulting increase of blood flow velocity and consecutive shear stress, which in turn leads to activation of endothelial cells and increased production of nitric oxide (NO) [2]. In contrast to FMD, it is considered that nitroglycerin-mediated dilation (NMD) represents endothelium-*independent* vasodilation, and that NMD is induced pharmacologically via direct effects of NO on the musculature of the vessel wall [1]. By comparison of FMD with NMD, it is possible to elucidate changes in smooth muscle function and arterial compliance that may affect the observed changes in FMD [1,2]. A lack of association between an FMD risk factor and NMD would emphasize that the risk factor is exclusively related to endothelium-dependent vasodilation.

Endothelial dysfunction precedes apparent atherosclerosis in humans [1,3]. Low FMD values predict cardiovascular events in patients independently of established atherosclerosis [4]. The

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cardiovascular risk factors associated with endothelial dysfunction include hypertension [5], hypercholesterolaemia [6], and smoking [7]. Furthermore, Type 2 diabetes is associated with endothelial dysfunction [8].

HbA_{1c} has recently been recommended for diagnosis of Type 2 diabetes, as alternative to standard measurement of fasting glucose [9]. Serum HbA_{1c} levels mirror average blood glucose levels for a period of up to 8–10 weeks prior to blood drawing [10]. Since serum HbA_{1c} levels correlate with micro- and macrovascular complications in patients with Type 2 diabetes, serum HbA_{1c} target levels have been defined for the guidance of antihyperglycaemic therapy [11]. In individuals without Type 2 diabetes, high serum HbA_{1c} levels are associated with future diagnosis of Type 2 diabetes, future cardiovascular disease, and cardiovascular and all-cause mortality [12]. These associations could be similar or even more pronounced than those of fasting glucose levels [12].

Only few observational studies [13,14] have investigated the association between parameters of glucose metabolism and endothelial function in non-diabetic individuals. One study [13] found an inverse association between fasting blood-glucose levels and FMD in Chinese. Another, more recent study [14] observed similar results between serum HbA_{1c} levels and FMD in non-diabetic individuals with a BMI < 26.1 kg/m^2, but no such association in subjects with higher BMI values. However, this issue has not until now been addressed by population-based studies.

To investigate the potential association between glucose homeostasis and endothelial function in an adult non-diabetic population, we analysed the association of serum HbA_{1c} levels with FMD. To emphasize the expected association with FMD, we also analysed the association of serum HbA_{1c} levels with NMD to eliminate possible association with endothelium-*independent* vasodilation measured by FMD. Owing to various potential effects of antihypertensive therapy on glucose homoeostasis and endothelial function, we performed additional analyses after exclusion of subjects with current antihypertensive medication. Since previous studies [13,14] on this issue were small in scope with inclusion of fewer than 350 subjects, and since they studied selected subjects who included outpatients and hospital staff [14], we employed a large-scale, cross-sectional, population-based study, the Study of Health in Pomerania (SHIP). Our objective here was to generalise previous findings on the one hand and to investigate various subgroups on the other.

2. Methods

2.1. Study population

The design of SHIP has been published previously [15]. Briefly, SHIP is a population-based project in northeast Germany that included 4308 subjects at baseline between 1997 and 2001. The first follow-up examination (SHIP-1) was conducted five years (mean 5.2 ± 0.5 years) after baseline and comprised 3300 subjects (83.5% of still eligible subjects). The study was approved by the Ethics Committee of the University of Greifswald. All participants gave informed written consent.

Between March of 2003 and October of 2006, 1788 subjects volunteered for measurement of the FMD of the brachial artery. Exclusion criteria for FMD measurements were equipment malfunction (n = 36), hypotension with systolic blood pressure < 100 mmHg (n = 15), and the presence of any other medical contraindication (n = 4). The image quality of 215 FMD examinations was insufficient for appropriate readings. From data analysis, we further excluded participants without data for serum HbA_{1c} levels (n = 13), subjects with a self-reported physician's diagnosis of diabetes mellitus, and patients using antidiabetic medication (n = 121). This resulted in a final study population of 1384 subjects (696 women).

2.2. Measurements

Sociodemographic characteristics and previous history of diseases were collected by computer-assisted personal interviews. In the present study we included age, gender, smoking status (never a smoker, ex-smoker, or current smoker), school education (<10 years, 10 years, >10 years), and self-reported use of medication. Current use of medication was recorded by a computer-aided method using the anatomic, therapeutic, and chemical (ATC) code [16]. The following drugs were considered as antihypertensive medications: vasodilators used in cardiac diseases (ATC C01D), antihypertensives (ATC C02), diuretics (ATC C03), peripheral vasodilators (ATC C04), beta-blockers (ATC C07), calcium antagonists (ATC C08), angiotensin I-converting enzyme inhibitors, and angiotensin II receptor blockers (ATC C09). For women, the use of sex hormones (ATC G03) was taken into consideration for analyses.

After a 5-min rest period, we measured systolic and diastolic blood pressure three times at the right arm of seated subjects using a digital blood-pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan), with each reading followed by a rest period of 3 min. The mean of the second and third measurements was calculated and used for the present analyses. Systolic and diastolic blood pressures ≥140 mmHg and ≥90 mmHg, respectively, were considered as elevated. Hypertension was defined as increased systolic or diastolic blood pressure or self-reported use of antihypertensive medication. Waist circumference (WC) was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet. BMI was calculated as weight in kilograms divided by the square of height in meters.

Non-fasting blood samples were drawn from the cubital vein in the supine position. The samples were taken between 07.00 a.m. and 04.00 p.m. and were analysed immediately. Serum HbA_{1c} levels were measured by high-performance liquid chromatography (HPLC) (Bio-Rad DiamatTM Analyzer, ClinRep® kit, RECIPE Chemicals + Instruments GmbH, Munich, Germany). Low- and high-density lipoprotein cholesterol (LDL-C and HDL-C) levels were quantified by lipid electrophoresis (HELENA SAS-3 system, Helena 7 BioSciences Europe, Tyne & Wear, UK). Triglycerides and glucose were determined enzymatically using reagents from Nobis and Roche Diagnostics, respectively (Hitachi 717, Roche Diagnostics, Mannheim, Germany) [17].

2.3. Endothelial function

The FMD examinations were described previously [18]. In brief, FMD of the brachial artery was assessed by measuring the increase of the brachial artery diameter during reactive hyperaemia after transient forearm ischaemia. The brachial artery was visualised using a 10-MHz linear array transducer (Cypress, Siemens AG, Erlangen, Germany). A blood pressure cuff was placed around the right forearm 5 cm distally from the right antecubital crease. B-mode longitudinal images of the brachial artery were obtained at the level of the antecubital fossa. After marking of the optimal position of the transducer, baseline images of the brachial artery were digitally stored. Arterial flow to the forearm was interrupted by insufflation of the forearm cuff for 5 min by 200 mmHg, or by 50 mmHg above systolic blood pressure, whichever was higher. Exactly 1 min after cuff deflation, B-mode longitudinal images of the brachial artery were obtained for FMD measurements. Additionally, we measured NMD 3 min after sublingual administration of nitroglycerin (400 mg) in 1062 subjects (456 women). Absolute

FMD and NMD were calculated by subtracting baseline vessel diameters from post-ischaemia and post-nitroglycerin vessel diameters. Relative changes were expressed as percentage of absolute FMD and NMD to baseline diameters.

2.4. Statistical analyses

Data on quantitative characteristics are expressed as median and interquartile range. Data on qualitative characteristics are expressed as absolute numbers and per cent values. Comparisons between women and men as well as between study population and non-participants were made using the Mann–Whitney *U*-test (continuous data) and χ^2-test (nominal data). Multivariable analyses were performed separately in women and men. Linear regression models with robust variance estimates were applied to assess the association between serum HbA_{1c} levels and FMD. Adjusted β-coefficients and 95% confidence intervals (CI) were calculated. The full model included age, BMI, smoking status, hypertension, and LDL-C as confounders. For women, regression models were further adjusted for use of sex hormones (ATC code G03). Further analyses were performed in women and men without antihypertensive medication, in accordance with the ATC code, to exclude the possibility that antihypertensive medication affects the association of serum HbA_{1c} levels with FMD and NMD. Multivariable fractional polynomial models were employed to explore possible nonlinear associations by testing whether any other than linear power transformations of the independent variables enhanced the fit of the model. To check for possible selection bias introduced by exclusion of subjects (i.e., non-participants in the FMD examination), we applied inverse probability weighting according to major variables of difference between the study population and non-participants in further sensitivity analyses. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using Stata 11 (Stata Corporation, College Station, TX, USA).

3. Results

Females had smaller baseline diameters of the brachial artery and higher FMD and NMD values than did males (Table 1). They were younger and were less commonly current smokers or ex-smokers, and had smaller BMI and WC than did males. Females exhibited lower serum levels of non-fasting-glucose, serum HbA_{1c} levels, LDL-C, and TG – but showed higher HDL-C levels compared to males. Female subjects had lower systolic and diastolic blood pressure and, consequently, less often hypertension – but used similarly often antihypertensive medication relative to male subjects. The proportion of sex-hormone use was 18.3% ($n = 127$) in females (Table 1).

Compared to non-participants, the study population was younger, had lower BMI and WC, demonstrated lower systolic and higher diastolic blood pressure, less often suffered from hypertension, and less frequently used antihypertensive medication. Furthermore, the study population had higher HDL-C and TG levels and lower non-fasting-glucose and serum HbA_{1c} levels relative to non-participants. There were no differences in gender, smoking habits, LDL-C levels, or use of sex-hormone medication between the study population and non-participants (Table 1).

In female subjects, linear regression revealed an age-adjusted, non-significant association ($\beta = -0.60$; 95% CI −1.26; 0.05, $p = 0.070$) between serum HbA_{1c} levels and FMD. After further adjustments for BMI, smoking status, hypertension, LDL-C, and sex-hormone medication, we determined an inverse relation ($\beta = -0.76$; 95% CI −1.46; −0.06, $p = 0.033$) between serum HbA_{1c} levels and FMD (Table 2). Analyses in female subjects without current use of antihypertensive medication disclosed decreasing FMD values for increasing serum HbA_{1c} levels for the age-adjusted model ($\beta = -0.96$; 95% CI −1.78; −0.13, $p = 0.023$) and the fully adjusted model ($\beta = -1.17$; 95% CI −2.03; −0.30, $p = 0.009$; Fig. 1). This finding persisted after further adjustment for school education ($\beta = -1.17$; 95% CI −2.05; −0.29, $p = 0.009$) and after exclusion of subjects with history of myocardial infarction or stroke ($\beta = -1.22$; 95% CI −2.10; −0.34, $p = 0.007$).

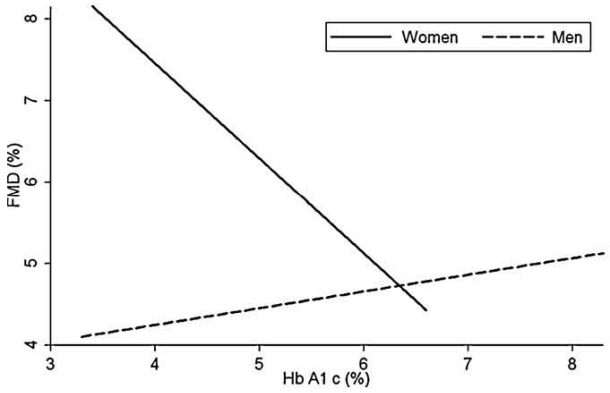

Fig. 1. Association between Hba1c and FMD for non-diabetic women (solid line) and men (dashed line) without antihypertensive medication. Example is for subjects that are non-hypertensive, never-smokers, 51 years old, have a BMI of 26.3 kg/m^2 and LDL-C of 3.35 mmol/l. Hba1c × sex interaction was significant, with $p < 0.001$.

In male subjects, there was an unadjusted inverse relation between serum HbA_{1c} levels and FMD ($\beta = -0.72$; 95% CI −1.13; −0.31, $p = 0.001$). However, after adjustment for major confounders, the full model revealed no association between serum HbA_{1c} levels and FMD ($\beta = 0.05$; 95% CI −0.34; 0.45, $p = 0.787$). Analyses in male subjects without current use of antihypertensive medication substantiated no association between serum HbA_{1c} levels and FMD in the age-adjusted and fully adjusted model (Table 2).

The association between serum HbA_{1c} levels and FMD did not differ substantially between non-smoking and currently smoking subjects (HbA_{1c} × smoking interaction, $p = 0.344$ for women; $p = 0.693$ for men) and between subjects divided by a BMI of 26.3 kg/m^2 (HbA_{1c} × BMI interaction, $p = 0.559$ for women; $p = 0.311$ for men) without current use of antihypertensive medication in the fully adjusted model. Furthermore an interaction effect of age was present in women ($p < 0.001$), but not in men ($p = 0.823$). The association between serum HbA_{1c} levels and FMD was observed in women younger than 55 years ($\beta = -1.44$; 95% CI −2.49; −0.40, p = 0.007), but not in women older than 55 years ($\beta = 0.11$; 95% CI −1.47; 1.68, $p = 0.892$).

In females, there were no significant associations between serum HbA_{1c} levels and NMD in any statistical model (Table 3). However, in male subjects multivariable analyses disclosed decreasing NMD values for increasing serum HbA_{1c} levels in the fully adjusted model for both the whole study population ($\beta = -1.24$; 95% CI −1.96; −0.51, $p = 0.001$) and the study population without antihypertensive medication ($\beta = -1.68$; 95% CI −2.83; −0.52, $p = 0.005$). This finding persisted after exclusion of two males with high serum HbA1c levels (7% and 8.3%) and without physician's diagnosis of diabetes ($\beta = -1.52$; 95% CI −2.82; −0.23, $p = 0.021$). No findings changed substantially after employment of multivariable fractional polynomial models and inverse probability weighting according to age, WC, or hypertension.

4. Discussion

This study was performed to elucidate the association of serum HbA_{1c} levels as a long-term marker of glucose metabolism with

Table 1
Selected characteristics of the study population and non-participants.

	Study population					
	Women $n = 696$	Men $n = 688$	p-value[a]	All participants $n = 1384$	Non-participants $n = 1916$	p-value[a]
Age (years)	51 (40; 60)	52 (40; 63)	0.026	51 (40; 62)	59 (43; 70)	<0.001
Sex (male)	–	–		688 (49.7)	901 (47.0)	0.128
Smoking status			<0.001			0.432
Never-smoker	388 (55.8)	208 (30.3)		596 (43.1)	782 (40.9)	
Ex-smoker	140 (20.1)	296 (43.1)		436 (31.5)	635 (33.3)	
Current smoker	168 (24.1)	183 (26.6)		351 (25.4)	493 (25.8)	
School education			0.008			<0.001
<10 years	199 (28.6)	237 (34.5)		436 (31.5)	899 (47.1)	
10 years	377 (54.2)	315 (45.9)		692 (50.0)	757 (39.6)	
>10 years	120 (17.2)	135 (19.7)		255 (18.4)	254 (13.3)	
Body mass index (kg/m^2)	26.3 (23.3; 30.3)	27.5 (25.3; 30.1)	<0.001	27.1 (24.3; 30.2)	27.7 (24.5; 31.3)	<0.001
Waist circumference (cm)	85.0 (76.1; 93.0)	96.9 (90.1; 104.0)	<0.001	91.5 (82.0; 100.1)	93.2 (83.2; 103.2)	<0.001
Systolic blood pressure (mmHg)	123 (113; 137)	134 (124; 146)	<0.001	129 (118; 141)	133 (120; 146)	<0.001
Diastolic blood pressure (mmHg)	80 (73; 86)	84 (78; 91)	<0.001	82 (75; 89)	80 (74; 88)	<0.001
Hypertension	266 (38.3)	370 (53.9)	<0.001	636 (46.1)	1068 (55.9)	<0.001
Use of antihypertensive medication	207 (29.7)	223 (32.4)	0.283	430 (31.1)	936 (48.9)	<0.001
HDL-C (mmol/l)	1.33 (1.07; 1.58)	0.98 (0.79; 1.21)	<0.001	1.13 (0.89; 1.44)	1.10 (0.85; 1.40)	0.008
LDL-C (mmol/l)	3.35 (2.74; 4.18)	3.52 (2.93; 4.25)	0.033	3.46 (2.83; 4.22)	3.42 (2.81; 4.16)	0.474
TG (mmol/l)	1.20 (0.81; 1.77)	1.70 (1.14; 2.45)	<0.001	1.43 (0.94; 2.13)	1.52 (1.02; 2.24)	0.009
Use of sex-hormone medication	127 (18.3)	–		127 (9.2)	156 (8.1)	0.295
Glucose (mmol/l)	5.00 (4.70; 5.36)	5.20 (4.82; 5.60)	<0.001	5.10 (4.76; 5.50)	5.23 (4.80; 5.90)	<0.001
HbA_{1c} (%)	5.2 (4.8; 5.5)	5.3 (4.9; 5.6)	<0.001	5.2 (4.9; 5.5)	5.4 (5.0; 6.0)	<0.001
Baseline diameter A. brachialis (mm)	3.37 (3.06; 3.63)	4.38 (4.02; 4.72)	<0.001	3.85 (3.36; 4.41)	–	
FMD (%)	5.18 (2.56; 8.45)	3.97 (2.07; 6.26)	<0.001	4.49 (2.29; 7.18)	–	
NMD (%)	15.63 (9.93; 21.31)	12.48 (8.76; 16.81)	<0.001	13.43 (9.23; 18.68)	–	

Data are given as number (percentage) or median (25th and 75th percentile). HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides; FMD, flow-mediated dilatation; NMD, nitrate-mediated dilatation.

[a] χ^2-test (nominal data) or Mann–Whitney U-test (interval data).

Table 2
Association between serum HbA_{1c} levels and FMD in women and men without diabetes mellitus.

	FMD (%)			
	Whole study population		Study population without antihypertensive medication	
HbA_{1c} (%)	β (95% CI)	p-value	β (95% CI)	p-value
Women	($n = 696$)		($n = 489$)	
Model 1	−1.61 (−2.24; −0.98)	<0.001	−1.65 (−2.45; −0.85)	<0.001
Model 2	−0.60 (−1.26; 0.05)	0.070	−0.96 (−1.78; −0.13)	0.023
Model 3	−0.74 (−1.44; −0.05)	0.035	−1.14 (−2.00; −0.27)	0.010
Model 4	−0.76 (−1.46; −0.06)	0.033	−1.17 (−2.03; −0.30)	0.009
Men	($n = 688$)		($n = 465$)	
Model 1	−0.72 (−1.13; −0.31)	0.001	−0.51 (−1.08; 0.06)	0.079
Model 2	−0.08 (−0.46; 0.30)	0.692	0.01 (−0.56; 0.58)	0.975
Model 3	0.05 (−0.34; 0.45)	0.787	0.20 (−0.37; 0.78)	0.482

Data are β-coefficients of linear regression (95% confidence interval). Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age, body mass index, smoking, hypertension, and low-density lipoprotein cholesterol; Model 4: Model 3 + adjusted for sex-hormone medication. FMD, flow-mediated dilatation

Table 3
Association between serum HbA_{1c} levels and NMD in women and men without diabetes mellitus.

	NMD (%)			
	Whole study population		Study population without antihypertensive medication	
HbA_{1c} (%)	β (95% CI)	p-value	β (95% CI)	p-value
Women	($n = 456$)		($n = 279$)	
Model 1	−2.71 (−4.03; −1.39)	<0.001	−1.66 (−3.43; 0.11)	0.066
Model 2	−0.69 (−2.01; 0.63)	0.306	0.06 (−1.71; 1.84)	0.945
Model 3	− 0.18 (−1.52; 1.17)	0.799	0.39 (−1.45; 2.24)	0.674
Model 4	−0.19 (−1.55; 1.16)	0.778	0.38 (−1.48; 2.24)	0.686
Men	($n = 606$)		($n = 415$)	
Model 1	−2.67 (−3.46; −1.89)	<0.001	−2.67 (−3.79; −1.55)	<0.001
Model 2	−1.32 (−2.07; −0.58)	<0.001	−1.71 (−2.87; −0.55)	0.004
Model 3	−1.24 (−1.96; −0.51)	0.001	−1.68 (−2.83; −0.52)	0.005

Data are β-coefficients of linear regression (95% confidence interval). Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age, body mass index, smoking, hypertension, and low-density lipoprotein cholesterol; Model 4: Model 3 + adjusted for sex-hormone medication. NMD, nitrate-mediated dilatation.

endothelial function in non-diabetic subjects. After adjustment for a broad range of confounders, our hypothesis of an inverse association between serum HbA_{1c} levels and FMD was partly confirmed in the overall female population, especially in females without antihypertensive medication.

In partial concordance with our results, a previous study by Voidonikola et al. [14] found an inverse association of serum HbA_{1c} levels and FMD in subjects with a BMI below the median, whereas in overweight subjects with a BMI above the median, this association was not apparent. In our analysis this difference was not significant. Since the authors did not present analysis separately for men and women, it cannot be ruled out that the observed findings in the whole population (61% females) were primarily due to associations only in women similar to the gender-specific associations found in the present study. Moreover, subjects with lower BMI in this study were more often females (69%) and had a lower proportion of hypertension (11%) with less-likely use of antihypertensive drugs than did subjects with higher BMI.

In general, various factors may potentially influence the relation between glucose haemostasis and endothelial function. Accordingly, antihypertensive drugs differ in their impact on glucose metabolism. For example, calcium channel blockers and angiotensin-converting enzyme inhibitors do not influence glucose or HbA_{1c} levels substantially, whereas beta blockers and diuretics lead to impairment of glucose homoeostasis [19]. On the other hand, antihypertensive therapy may have significant effects on the endothelium, which may impede detection of an association between serum HbA_{1c} levels and FMD [20]. Until now, it remains unclear whether these effects are drug-specific or inherent to a class of antihypertensives. For example, perindopril improves FMD in patients with hypertension [21], but this finding was not replicated for other angiotensin-converting enzyme inhibitors [22]. For further antihypertensives, findings of various studies have likewise proved inconsistent. For example, treatment of hypertensive patients with atenolol had no effect on FMD in one study [21], but it increased FMD in another [23]. Given the conflicting data on the association between antihypertensive therapy and glucose metabolism on the one hand and endothelial dysfunction on the other, we excluded subjects taking antihypertensive medication from the whole study population.

In addition to the study of Voidonikola et al. [14], we also analysed the association of serum HbA_{1c} levels with NMD. We observed an inverse association between serum HbA_{1c} levels and NMD only in men without Type 2 diabetes. This finding persisted after exclusion of individuals with current antihypertensive medication and may point toward vascular smooth muscle cell dysfunction independent of the endothelium [1]. However, the mechanisms by which high serum HbA_{1c} levels are linked to low NMD values in men cannot be derived from our data. In another study [8], there was no association between HbA_{1c} levels and NMD in patients with Type 2 diabetes. Since there are no other data from non-diabetic populations available, this relation needs to be replicated in independent research.

Although there was an association between serum HbA_{1c} levels and endothelium-*dependent* vasodilation (FMD) in women, we did not find such an association with endothelium-*independent* vasodilation (NMD). This strengthens the hypothesis that FMD measures solely endothelium-*dependent* vasodilation and that hyperglycaemia affects endothelial function via the NO system, hypothetically by blocking NO production [1,2] in women. There is evidence that the production of NO is greater in premenopausal women than in men: which could underlie differences in vascular function between men and women [24]. Furthermore, there is evidence that female sex hormones have an influence on vascular endothelial function [25] that may also effect the association between serum HbA_{1c} levels and FMD in women. An analogous gender difference was also detected in the association between increased fasting glucose and coronary artery calcification, which revealed stronger associations in women than in men [26]. Further hypotheses that have treated the manner in which hyperglycaemia may adversely affect endothelial function have until now described multiple mechanisms and include the following: increased polyol pathway flux, augmented advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux [27]. However, our study is limited by its cross-sectional design, which renders speculative any interpretation on causal inferences in the association of serum HbA_{1c} levels and FMD.

A further limitation of the present study is our definition of the history of diabetes mellitus as based on a self-reported physician's diagnosis. It cannot therefore be excluded that a certain proportion of volunteers had undiagnosed Type 2 diabetes. Moreover, physical exercise could potentially have affected our results. Thus, endurance exercise on the day preceding FMD measurements that was not recorded in our study could have led not only to increased insulin sensitivity but also to improvement of postprandial FMD in healthy humans [28]. However, it seems unlikely that our results were substantially influenced by recent physical exercise, since only exhausting physical exercise is known to influence endothelial function [28]. Exclusion of several individuals (i.e., non-participants in FMD examination) could, moreover, have led to selection bias. Inverse probability weighting according to age, WC, and hypertension confirmed, nevertheless, that our findings were not biased by the differences among these variables between study population and non-participants.

Major strengths of our study are population-based design, comprehensive and detailed assessment of metabolic and cardiovascular confounding factors [15], size of the study population, and accurate FMD measurement under strict quality management by standardised protocol and certified staff [18]. Our method of forearm ischaemia induction of FMD precludes potential contribution by ischaemia of the brachial artery itself – in contrast to upper-arm ischaemia-induced FMD, which is known to induce greater vasodilation [1].

In conclusion, higher serum HbA_{1c} levels in non-diabetic subjects are inversely associated with FMD but not with NMD in women not on antihypertensive medication. In men without diabetes mellitus, higher serum HbA_{1c} levels are not associated with FMD, but with NMD. These gender specific findings should be confirmed by further studies. Our results support current considerations that subclinical disorders of glucose metabolism measured by serum HbA_{1c} are associated with subclinical cardiovascular diseases detected by FMD, especially in women.

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Conflicts of interest

Nothing to declare.

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Association of serum IGF1 with endothelial function: results from the population-based study of health in Pomerania

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CLINICAL STUDY

Association of serum IGF1 with endothelial function: results from the population-based study of health in Pomerania

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Abstract

Objective: IGF1 mediates multiple physiological and pathophysiological responses in the cardiovascular system. The aim of this study was to analyze the association between serum IGF1 as well as IGF-binding protein 3 (IGFBP3) levels and endothelial function measured by flow-mediated dilation (FMD).
Design: Cross-sectional population-based observational study.
Methods: The study population comprised 1482 subjects (736 women) aged 25–85 years from the Study of Health in Pomerania. Serum IGF1 and IGFBP3 levels were determined by chemiluminescence immunoassays. FMD measurements were performed using standardized ultrasound techniques. FMD values below the sex-specific median were considered low.
Results: In males, logistic regression analyses revealed an odds ratio (OR) of 1.27 (95% confidence interval (CI) 1.07–1.51; $P=0.008$) for decreased FMD for each decrement of IGF1 s.d. after adjustment for major cardiovascular confounders. In females, no significant relationship between serum IGF1 and FMD was found (OR 0.88, CI 0.74–1.05; $P=0.147$). After exclusion of subjects with the current use of antihypertensive medication, these findings were similar (males: OR 1.40, CI 1.12–1.75; $P=0.003$; females: OR 0.95, CI 0.77–1.16; $P=0.595$). There was no association between serum IGFBP3 levels and FMD in both sexes.
Conclusions: Low serum IGF1 levels are associated with impaired endothelial function in males. In women, serum IGF1 is not associated with endothelial function.

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Introduction

Insulin-like growth factor-1 (IGF1), a circulating peptide hormone, is structurally and functionally related to insulin (1). IGF1 represents an essential growth factor for the regulation of cell proliferation and differentiation (2). IGF1 is synthesized under the control of GH mainly in hepatic cells (3). However, many other cell types, including cardiac myocytes (4), also secrete IGF1 and are sensitive to its tropic action. IGF1 significantly interacts with endothelial physiology. Human endothelial cells express more IGF1 receptors than insulin receptors (5), and IGF1 stimulates nitric oxide production by endothelial cells, contributing to the regulation of vascular tone and other anti-atherosclerotic properties (6, 7). Circulating IGF1 is predominantly bound to IGF-binding proteins (IGFBP). Most of IGF1 is carried by IGFBP3 (8). Consequently, the IGF1/IGFBP3 ratio might mirror the biological impact of total IGF1 levels.

Impaired flow-mediated dilation (FMD) represents endothelial dysfunction and precedes the development of clinically apparent atherosclerosis in humans with cardiovascular risk factors, such as diabetes mellitus, arterial hypertension, hypercholesterolemia, or smoking (9). Circulating IGF1 levels are inversely related to ultrasound measures of atherosclerosis (10), the risk of ischemic heart disease (11), stroke (12, 13), coronary events (13), and cardiovascular mortality (14).

Studies designed to elucidate the relationship between serum IGF1 levels and FMD are scarce and primarily confined to patients with disorders in GH metabolism. In patients with low serum IGF1 levels due to GH deficiency, substitution of GH leads to normalization of a previously reduced FMD (15, 16). On the other hand, in acromegaly patients with high IGF1 levels, surgical resection of the GH-producing tumor was followed by an improvement of previously reduced FMD (17). More recently, an association between serum IGF1 levels and the acetylcholine-stimulated increase of forearm blood flow, as measured by plethysmography, was described in a population of 100 untreated hypertensive patients (18).

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There are no epidemiological data available investigating the potential association of IGF1 or IGFBP3 levels with endothelial function. The objective of this study was to analyze the association of serum IGF1 and IGFBP3 levels with endothelial function as measured by FMD in an adult population of a large-scale, cross-sectional, population-based study – the Study of Health in Pomerania (SHIP).

Methods

Study population

The design of SHIP has been published previously (19, 20). Briefly, SHIP is a population-based study in the northeast area of Germany. Baseline examinations were performed in 4308 subjects between 1997 and 2001. The first follow-up examination (SHIP-1) was conducted 5 years (mean 5.2 ± 0.5 years) after baseline and comprised 3300 subjects (83.5% of the still eligible population). The study was approved by the ethics committee of the University of Greifswald. All participants gave informed written consent.

Between March 2003 and October 2006, 1788 subjects (54%) of the SHIP-1 population volunteered for measuring FMD of the brachial artery. Exclusion criteria for FMD measurements were equipment malfunction ($n=36$), any medical contraindication ($n=19$), and primarily hypotension with systolic blood pressure (BP) below 100 mmHg ($n=15$). Image quality of 215 FMD examinations was insufficient for correct readings. In 36 subjects, serum IGF1 levels were not available. This resulted in a final study population of 1482 persons (736 women) with both complete examinations of FMD of at least sufficient image quality and available IGF1 and IGFBP3 datasets. All data and analyses in this report refer to the SHIP-1 population. There was no subject suffering from acromegaly or GH deficiency.

Measurements

Sociodemographic characteristics and medical histories were assessed by computer-assisted personal interviews. As for smoking status, participants were classified as never-smokers, ex-smokers, or current smokers. The definition of diabetes mellitus was based on self-reported physician's diagnosis or use of antidiabetic medication. Liver disease was defined as self-reported physician's diagnosis. The definition of renal disease was based on self-report or creatinine clearance values <50 ml/min as estimated by the Cockcroft–Gault formula (21).

Waist circumference (WC) was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the subject standing comfortably with weight distributed evenly on both feet. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. After a 5 min rest period, systolic and diastolic BP were measured three times at the right arm of seated subjects using a digital BP monitor (HEM-705CP, Omron Corporation, Tokyo, Japan) with each reading being followed by a further rest period of 3 min. The mean of the second and third measurements was calculated and used for the present analyses. Hypertension was defined as a systolic BP of ≥ 140 mmHg, a diastolic BP of ≥ 90 mmHg, or use of antihypertensive medication.

Nonfasting blood samples were drawn from the cubital vein in the supine position. The samples were taken between 0700 and 1600 h and analyzed immediately for all parameters except IGF1 and IGFBP3, for which the serum was stored at -80 °C. Internal quality controls were performed at least daily. Serum creatinine levels were measured with a modified Jaffé method, and total serum cholesterol was measured photometrically (both Dimension RxL HM Max, Siemens Healthcare Diagnostics, Bad Nauheim, Germany). Low- and high-density lipoprotein cholesterol (LDL- and HDL-cholesterol) levels were quantified by lipid electrophoresis (HELENA SAS-3 system, HELENA 7 BioSciences Europe, Tyne & Wear, UK). Triglycerides and glucose were determined enzymatically using reagents from Roche Diagnostics (Hitachi 717, Roche Diagnostics, Mannheim, Germany). Serum IGF1 and IGFBP3 levels were measured using a chemiluminescent immunometric assay on an Immulite 2500 analyzer (Siemens Immulite 2500; Siemens Healthcare Medical Diagnostics). The assays were performed according to the manufacturer's recommendations by skilled technical personnel. Measurements were carried out from April to May 2008. An aliquot of two levels of the manufacturer's control material (IGF-Control-Module, ref. LGCOC, lot 022, Siemens Healthcare Medical Diagnostics) was included within each series in single determination as reported previously (22). During the course of the study, the inter-assay coefficient of variation was 7.9% with a systematic deviation of $+2.5\%$ at the 64 ng/ml level for the IGF1 assay, and 4.6% with a systematic deviation of -2.7% at the 880 ng/ml level for the IGFBP3 assay.

Flow-mediated dilation

FMD was assessed as described previously (23). In brief, FMD of the brachial artery was assessed by measuring the increase in the brachial artery diameter during reactive hyperemia after transient forearm ischemia. The brachial artery was visualized using a 10 MHz linear array transducer (Cypress, Siemens, Erlangen, Germany). Ultrasonography was performed in a dark and quiet room. The participants lay quietly for 1 min before measurements. A BP cuff was placed around the right forearm 5 cm distally from the right antecubital crease. B-mode longitudinal images of the brachial

artery were obtained at the level of the antecubital fossa. After marking the optimal position of the transducer, baseline images of the brachial artery were digitally stored. Arterial flow to the forearm was interrupted by insufflation of the forearm cuff for 5 min by 200 or 50 mmHg above systolic BP, whichever was highest. Exactly 1 min after cuff deflation, B-mode longitudinal images of the brachial artery were obtained for FMD measurements. Examinations were performed and read by two observers. All ultrasound measurements in SHIP are performed offline and underlie strict quality management (19). Intrareader, intra-observer, interreader, and interobserver variability are evaluated in certification procedures. Before data collection, 25 images were measured twice by each participating reader, and 12 volunteers were examined twice by each participating observer. During data collection, observer certification procedures were repeated semi-annually for at least six volunteers. An interval of at least 24 h was required between examination and reading procedures. The number of images and volunteers was arbitrarily defined before the beginning of the study, and has been proven satisfactory from experience to demonstrate relevant reader and observer differences. All measurements of intrareader, intra-observer, interreader, and interobserver agreements with respect to FMD revealed a mean bias of $<5\%$ and $+2$ S.D. of bias of $<25\%$. The applied quality measures have been described elsewhere in detail (23). End-diastolic vessel diameters were measured from the anterior to the posterior M-line (i.e. the interface between the media and adventitia) of the vessel wall. Diameters were calculated from the average of three measurements of four consecutive cardiac cycles. Absolute FMD was calculated by subtracting baseline vessel diameters from postischemia vessel diameters. Relative changes were expressed as percentage of absolute FMD to baseline diameters. Values below the median of sex-specific distribution were considered decreased.

Statistical analysis

Data on quantitative characteristics are expressed as mean and S.D. Data on qualitative characteristics are expressed as absolute numbers and percent values. All analyses were done in male and female subjects separately. Comparisons between groups were made using Mann–Whitney's U test (continuous data) and χ^2-test (nominal data). Bivariate correlations between variables were determined by Pearson's correlation coefficient (r). Multivariable statistical analyses were performed using logistic regression analysis. Adjusted odds ratios (OR) and 95% confidence intervals (CI) are provided. Models were developed by adjusting for age, WC, diabetes mellitus, hypertension, liver and renal diseases, smoking status, and serum HDL- and LDL-cholesterol levels. To check for possible selection bias introduced by exclusion of subjects (i.e. nonparticipants of FMD examination), we applied inverse probability weighting according to major variables of difference between the study population and nonparticipants in further sensitivity analyses (24). A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using Stata 10 (Stata Corporation, College Station, TX, USA).

Results

Females had smaller baseline diameters of the brachial artery and higher FMD values than males (Table 1). Male subjects were more often current and ex-smokers, had larger WC and BMI, lower HDL-cholesterol values, higher triglyceride and glucose levels, and higher systolic and diastolic BP, and were more often hypertensive than women. No differences regarding the prevalence of diabetes mellitus, liver, and renal diseases became apparent between men and women. Mean serum IGF1 levels did not differ between men and women, whereas men had lower serum IGFBP3 levels and higher IGF1/IGFBP3 ratios compared to women.

Volunteers with sufficient data for this analysis (study participants) were younger, had less often diabetes mellitus, renal diseases, and hypertension, used less often antihypertensive medication, and had lower glucose levels than nonparticipants. Study participants revealed higher IGF1 and IGFBP3 levels and higher IGF1/IGFBP3 ratios than nonparticipants (Table 1).

We observed an inverse bivariate correlation between serum IGF1 levels and age in both women ($r=-0.447$; $P<0.001$) and men ($r=-0.404$; $P<0.001$). The inverse association between IGFBP3 levels and age was weaker in women ($r=-0.247$; $P<0.001$) than men ($r=-0.415$; $P<0.001$). There was a stronger negative correlation between IGF1/IGFBP3 ratio and age in women ($r=-0.379$; $P<0.001$) than in men ($r=-0.153$; $P<0.001$). Bivariate comparisons between serum IGF1 levels and FMD showed a positive association in female ($r=0.075$; $P=0.041$) and in male subjects ($r=0.203$; $P<0.001$) respectively. Furthermore, we revealed no association between serum IGFBP3 levels and FMD women ($r=0.027$; $P=0.466$), but a positive association in men ($r=0.130$; $P<0.001$). Finally, there was a weaker positive correlation between IGF1/IGFBP3 ratio and FMD in women ($r=0.086$; $P=0.020$) than in men ($r=0.151$; $P<0.001$).

In multivariable logistic regression analyses, there was an age-adjusted OR of 1.28 (CI 1.08–1.53; $P=0.005$) for decreased FMD in males with each decrement of IGF1 S.D. (S.D. = 51.9 ng/ml; Table 2). Adjustment for further potential confounders including WC, hypertension, diabetes, liver and renal diseases, smoking status, and serum HDL- and LDL-cholesterol did not influence the major results substantially.

Table 1 Selected characteristics of the study population and nonparticipants.

	Study population					
	Women $n=736$	Men $n=746$	P^a	All participants $n=1482$	Nonparticipants $n=1818$	P^a
Age (years)	51.1 (12.8)	53.1 (14.0)	0.009	52.1 (13.5)	56.4 (16.4)	<0.001
Sex (male)	–	–		746 (50.3)	843 (46.4)	0.023
Smoking status			<0.001			0.308
Never-smoker	418 (56.8)	216 (29.0)		634 (42.8)	744 (41.1)	
Ex-smoker	150 (20.4)	337 (45.2)		487 (32.9)	584 (32.3)	
Current smoker	168 (22.8)	193 (25.9)		361 (24.4)	483 (26.7)	
Body mass index (kg/m^2)	27.5 (5.2)	28.2 (4.0)	<0.001	27.8 (4.7)	28.1 (5.1)	0.493
Waist circumference (cm)	86.6 (12.9)	98.1 (11.5)	<0.001	92.4 (13.5)	92.9 (14.4)	0.443
Diabetes mellitus	51 (6.9)	67 (9.0)	0.145	118 (8.0)	252 (13.9)	<0.001
Liver diseases	16 (2.2)	11 (1.5)	0.314	27 (1.8)	34 (1.9)	0.918
Renal diseases	38 (5.2)	29 (3.9)	0.237	67 (4.5)	205 (11.3)	<0.001
Systolic blood pressure (mmHg)	125.8 (17.9)	135.5 (16.9)	<0.001	130.7 (18.1)	134.1 (21.3)	<0.001
Diastolic blood pressure (mmHg)	79.7 (9.5)	83.7 (10.4)	<0.001	81.7 (10.1)	81.1 (11.0)	0.007
Hypertension	299 (40.6)	416 (55.8)	<0.001	715 (48.3)	998 (54.9)	<0.001
Use of antihypertensive medication	244 (33.2)	269 (36.1)	0.240	513 (34.6)	853 (46.9)	<0.001
HDL-cholesterol (mmol/l)	1.35 (0.43)	1.02 (0.33)	<0.001	1.18 (0.42)	1.17 (0.43)	0.578
LDL-cholesterol (mmol/l)	3.50 (1.06)	3.55 (0.98)	0.172	3.52 (1.02)	3.53 (1.00)	0.832
Triglycerides (mmol/l)	1.52 (1.09)	2.17 (2.47)	<0.001	1.85 (1.94)	1.83 (1.51)	0.622
Glucose (mmol/l)	5.22 (1.11)	5.48 (1.40)	<0.001	5.35 (1.27)	5.66 (1.84)	<0.001
Use of sex hormones	127 (17.3)	–		127 (8.6)	156 (8.6)	0.991
Number of pregnancies						0.051
0	74 (10.1)	–		820 (55.3)	929 (51.1)	
1	155 (21.1)	–		155 (10.5)	186 (10.2)	
2	243 (33.0)	–		243 (16.4)	322 (17.7)	
≥3	264 (35.9)	–		264 (17.8)	381 (21.0)	
IGF1 levels (ng/ml)	148.3 (52.6)	145.5 (51.9)	0.298	146.9 (52.3)	137.2 (54.3)	<0.001
IGFBP3 levels (ng/ml)	4225.0 (867.2)	3974.6 (953.4)	<0.001	4099.0 (919.9)	3916.6 (1048.3)	<0.001
IGF1/IGFBP3 ratio	0.035 (0.010)	0.037 (0.010)	<0.001	0.036 (0.010)	0.035 (0.010)	0.006
Baseline diameter of *A. brachialis* (mm)	3.40 (0.45)	4.41 (0.52)	<0.001	3.91 (0.70)	–	–
FMD (%)	5.75 (4.28)	4.31 (3.23)	<0.001	5.03 (3.86)	–	–

Data are given as number (percentage) or mean (s.d.). HDL, high-density lipoprotein; LDL, low-density lipoprotein; IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor-binding protein-3; FMD, flow-mediated dilation.
[a]χ^2-test (nominal data) or Mann–Whitney *U* test (interval data).

In females, no significant association between serum IGF1 levels and FMD was identified after adjustment for age and further confounders (OR 0.88, CI 0.74–1.05; $P=0.147$). In both men and women, no association between serum IGFBP3 levels and FMD was present. Only in males, there was a higher adjusted OR of 1.24 (CI 1.06–1.46; $P=0.009$) in the full model for decreased FMD with each decrement of IGF1/IGFBP3 ratio s.d. (s.d.=0.0098; Table 2). No findings changed substantially after usage of inverse probability weighting according to age, diabetes mellitus, and hypertension.

Additional analyses were performed after excluding subjects with antihypertensive medication. In the fully adjusted model in males, there was an OR of 1.40 (CI 1.12–1.75; $P=0.003$) and an OR of 1.38 (CI 1.11–1.71; $P=0.003$) for decreased FMD with each decrement of serum IGF1 s.d. (s.d.=48.1 ng/ml) and IGF1/IGFBP3 ratio s.d. (s.d.=0.0093) respectively. The associations of IGF1 levels and IGF1/IGFBP3 ratio with FMD in women and serum IGFBP3 levels and FMD in women and men remained nonsignificant after these changes (Table 3). In further sensitivity analyses, height (of men and women) and current oral contraceptive use and number of pregnancies (in women) were used as additional confounding factors. These analyses did not change the main result of no association between IGF1 and IGFBP3 levels and IGF1/IGFBP3 ratio with FMD in women. In further analyses, multivariable models with an additional adjustment for serum TSH levels revealed apparently unchanged ORs for men of 1.26 (CI 1.06–1.50; $P=0.009$) and 1.40 (CI 1.12–1.75; $P=0.003$) and for women of 0.88 (CI 0.74–1.05; $P=0.147$) and 0.95 (CI 0.77–1.16; $P=0.601$) for subjects including and without antihypertensive medication respectively.

Discussion

In the present population-based study, we detected an association between low serum IGF1 levels and decreased FMD in men after adjustment for age. Adjustment for further confounding cardiovascular risk factors including abdominal obesity, diabetes mellitus, hypertension, liver and renal diseases, smoking status, as well as HDL- and LDL-cholesterol levels did not have significant impact on this major finding. Various antihypertensive medications such as

Table 2 The relationship between serum IGF1/IGFBP3 levels and decreased FMD in women and men.

	Decreased FMD[a]			
	Women		Men	
Models	OR (95% CI)	P^b	OR (95% CI)	P^b
IGF1 (S.D. decrement)				
Model 1	0.89 (0.75–1.06)	0.184	1.28 (1.08–1.53)	0.005
Model 2	0.88 (0.74–1.05)	0.147	1.27 (1.07–1.51)	0.008
IGFBP3 (S.D. decrement)				
Model 1	1.03 (0.88–1.20)	0.751	1.05 (0.89–1.24)	0.527
Model 2	1.01 (0.86–1.19)	0.912	1.05 (0.89–1.25)	0.550
IGF1/IGFBP3 ratio (S.D. decrement)				
Model 1	0.87 (0.74–1.03)	0.108	1.24 (1.06–1.45)	0.007
Model 2	0.87 (0.74–1.03)	0.103	1.24 (1.06–1.46)	0.009

Models: 1 (adjusted for age); 2 (+ adjusted for waist circumference, hypertension, diabetes mellitus, liver and renal diseases, smoking status, and HDL- and LDL-cholesterol). IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor-binding protein-3; FMD, flow-mediated dilation; OR, odds ratio; CI, confidence interval.
[a]<Median (<5.17% for women; <3.96% for men).
[b]Logistic regression.

β-blockers, ACE inhibitors, or angiotensin II receptor blockers modulate endothelial function (25). Exclusion of volunteers with current use of antihypertensive medication from the analysis revealed no significant impact on the association of IGF1 and FMD in our study. Since circulating IGF1 is predominantly bound by IGFBP3 (8), we performed separate analyses to detect a potential differing association between IGF1/IGFBP3 ratio and FMD. All of these findings were similar when the IGF1/IGFBP3 ratio was analyzed instead of IGF1 levels. This fact indicates the biological relevance of the association between IGF1 levels and FMD in men.

An association between low serum IGF1 levels and impaired endothelium-mediated vasodilation has been described previously in a population of 100 untreated, hypertensive subjects (18). Since the authors did not present data analysis separately for men ($n=65$) and women ($n=35$), it cannot be excluded that the observed association of IGF1 levels and acetylcholine-induced forearm blood flow in their whole population was due to the preponderance of male subjects. Other studies focussing on the relationship of serum IGF1 levels and endothelial function were even smaller and primarily confined to patients with disturbed GH secretion, such as acromegaly or GH deficiency (15–17). In patients with low (15, 16) and high (17) preinterventional serum IGF1 levels, normalization of IGF1 levels led to an improvement of previously reduced FMD. In these studies, gender-specific analysis was either not discussed or considered impossible due to the small number of patients (15). Our study comprised 1482 subjects with a broad age range (25–85 years) and IGF1 levels largely within the reference range, which points toward a relevant impact of IGF1 on the cardiovascular system in male subjects under physiological circumstances.

Table 3 The relationship between serum IGF1/IGFBP3 levels and decreased FMD in women and men without antihypertensive medication.

	Decreased FMD[a]			
	Women		Men	
Models	OR (95% CI)	P^b	OR (95% CI)	P^b
IGF1 (S.D. decrement)				
Model 1	0.96 (0.78–1.18)	0.695	1.35 (1.09–1.68)	0.005
Model 2	0.95 (0.77–1.16)	0.595	1.40 (1.12–1.75)	0.003
IGFBP3 (S.D. decrement)				
Model 1	1.04 (0.86–1.26)	0.677	1.09 (0.89–1.32)	0.410
Model 2	0.99 (0.81–1.21)	0.926	1.09 (0.88–1.34)	0.450
IGF1/IGFBP3 ratio (S.D. decrement)				
Model 1	0.93 (0.76–1.13)	0.440	1.29 (1.06–1.58)	0.011
Model 2	0.94 (0.76–1.15)	0.542	1.38 (1.11–1.71)	0.003

Models: 1 (adjusted for age); 2 (+ adjusted for waist circumference, diabetes mellitus, systolic and diastolic blood pressure, liver and renal diseases, smoking status, and HDL- and LDL-cholesterol). IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor-binding protein-3; FMD, flow-mediated dilation; OR, odds ratio; CI, confidence interval.
[a]<Median (<5.50% for women; <4.42% for men).
[b]Logistic regression.

The association between IGF1 and decreased FMD as a marker of preclinical atherosclerosis in men might represent a possible link to cardiovascular morbidity and mortality. Indeed, we have recently demonstrated that men with low IGF1 levels had an almost twofold higher risk of cardiovascular disease mortality compared to men with normal IGF1 levels (26). However, Andreassen *et al.* (27) recently described an increased mortality and risk of heart failure in elderly subjects with high IGF1 levels.

In contrast to men, serum IGF1 levels did not reveal any relevant influence on FMD in women in our analysis. In concordance with this finding, no association between serum IGF1 levels and mortality (26) and risk of myocardial infarction (28) was found in women. Few studies have addressed possible sex differences in cardiovascular risk factors in relation to the GH/IGF system. Sexual dimorphism exists in GH secretion: women in their fertile age have higher serum GH levels and lower serum IGF1 levels than men (29). Moreover in patients with GH deficiency receiving GH replacement therapy, sex-specific effects have been described (30). Men require lower GH doses than women (31), and have apparently greater benefits from the substitution with regard to improvement in body composition, lipid profile, and bone mass (32). While these findings are in good agreement with the sex-specific associations found in our study, the mechanisms underlying this sex specificity remain to be investigated.

Our study has several potential limitations and strengths that should be considered. Limitations arise from the cross-sectional study design, ruling out any interpretation on causal inferences in the association of IGF1 and FMD. Unfortunately, GH and insulin levels are not available for the SHIP-1 population. Thus, estimates of insulin resistance status are difficult to obtain. Generalization of our findings might be limited due to selection bias because our study population (with both complete examinations of FMD of sufficient quality and available IGF1 and IGFBP3 datasets) differed from the whole SHIP-1 population in some potential confounders. However, inverse probability weighting according to age, diabetes mellitus, and hypertension confirmed that our findings were not biased by the differences among these variables between study population and nonparticipants.

Major strengths are the population-based design, the accurate FMD measurement under strict quality management by standardized protocol and certified staff (23), the comprehensive and detailed assessment of metabolic and cardiovascular confounding factors (19, 20), and the size of the study population. The method of forearm ischemia induction of FMD precludes the potential contribution of ischemia of the brachial artery itself – in contrast to upper arm ischemia-induced FMD, which is known to induce more vasodilation (33, 34).

In conclusion, low serum IGF1 levels are associated with impaired endothelial function in males, which might represent a possible link to increased cardiovascular morbidity and mortality. In women, serum IGF1 is not associated with endothelial function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät und keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, den 07.11.2011

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Roberto Lorbeer

Wissenschaftliche Leistungen

Originalartikel

Lorbeer R, Dörr M, Ittermann T, Koch B, Ewert R, Rettig R, Nauck M, Felix SB, Wallaschofski H, Völzke H Serum Thyrotropin Levels and Blood Pressure Response to Exercise in a Population-based Study. Thyroid 2011; 21(8):829-35

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Poster

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Auszeichnung

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