The interplay between fasting glucose, echocardiography, and biomarkers

Pathophysiological considerations and prognostic implications

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The Interplay between Fasting Glucose, Echocardiography, and Biomarkers: Pathophysiological Considerations and Prognostic Implications

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1. LIST OF PAPERS
The thesis is based on the following papers:


2. ABBREVIATIONS
ARIC: Atherosclerosis Risk In the Community Study
BNP: brain natriuretic peptide
CI: confidence interval
C-index: concordance index
DHS: Dallas Heart Study
DT: E-wave deceleration time
FHS: Framingham Heart Study
FPG: fasting plasma glucose
HbA1c: hemoglobin A1c (glycosylated hemoglobin)
HR: hazard ratio
Hs-TnT: high-sensitivity (cardiac) troponin T
ICD: International Classification of Diseases
IFG: impaired fasting glucose
IQR: interquartile range
LV: left ventricle / left ventricular
LVEF: left ventricular ejection fraction
LVH: left ventricular hypertrophy
LVM: left ventricular mass
LVMI: left ventricular mass index
MESA: Multi-Ethnic Study of Atherosclerosis
MPP: Malmö Preventive Project
MPP-RES: Malmö Preventive Project Re-Examination Study
NFG: normal fasting glucose
NT-proBNP: N-terminal prohormone of brain natriuretic peptide
RWT: relative wall thickness
SCORE: Systematic Coronary Risk Evaluation
SHS: Strong Heart Study
TnI: (cardiac) troponin I
TnT: (cardiac) troponin T

3. RESEARCH IN CONTEXT
Despite declining age-adjusted mortality rates, cardiovascular disease is still the leading cause of death worldwide (1). Traditional cardiovascular risk stratification tools that employ clinical risk factors, including Systematic Coronary Risk Evaluation (SCORE) and Framingham Risk Score, although easily applied, are limited by their modest discriminative abilities (2-5). As such, robust cardio-
vascular risk assessment, including our understanding of the complex interplay between risk factors, in the primary preventive setting, remains incomplete. Phenotypical heterogeneity may be even greater among subjects with hyperglycemic conditions, i.e., prediabetes and diabetes, which is troublesome, given the dramatic global rise in mean fasting glucose levels, and the strong association with adverse cardiovascular outcomes (6–12). The unmet need for refinement or re stratification of risk based on these conventional prediction models is further emphasized by our entrance into the era of precision medicine after the realization that one size simply does not fit all (13–15). Potential tools for closing these gaps and increasing our understanding of the pathways from risk factors through subclinical changes to manifest disease include imaging modalities and circulating biomarkers (16–20).

4. BACKGROUND

4.1 Glycemic Status and Cardiovascular Disease

Type 2 diabetes is a multifaceted, chronic disease, pathophysiologically characterized by a progressive decrease in pancreatic insulin secretion and a concomitant increase in peripheral insulin resistance, leading to hyperglycemia. The condition may be diagnosed based on fasting or random plasma glucose, the 2-hour plasma glucose during a 75 g oral glucose tolerance test, or glycated hemoglobin (HbA1c) (21–23). In most cases, a confirmatory plasma glucose during a 75 g oral glucose tolerance test, or glycated hemoglobin (HbA1c) (21–23) may be considered as prediabetes and are associated with a high risk of progression to manifest diabetes (21,23,24). Intermediate levels of HbA1c may be considered as pre-diabetes as well (25). Type 2 diabetes has reached epidemic proportions globally, and the burden continues to increase (26). This is worrisome, given the adverse contributions to health-related costs and disability-adjusted life years through its influence on multiple organ systems (12,27,28). The leading cause of death in diabetes is cardiovascular disease (29). There is a graded increase in cardiovascular risk with worsening glycemic status, and complications include coronary artery disease, heart failure, cerebrovascular disease, and peripheral artery disease (6–8,30–34). Therefore, diabetes is considered a major risk factor for, or perhaps even a risk equivalent to, cardiovascular disease (35–37).

4.2. Left Ventricular Structure and Function

Since the course from risk factors to manifest cardiovascular disease is highly variable, subclinical, i.e., asymptomatic anatomical and functional, alterations may resemble intermediate mechanistic links connecting the two, and as such, potentially improve risk discrimination (38). The presence of subclinical cardiovascular damage significantly raises the likelihood of developing symptomatic cardiovascular disease (39–42). Two such examples include left ventricular hypertrophy (LVH) and diastolic dysfunction, both of which can be evaluated using transthoracic echocardiography. LVH is defined as an inappropriately increased left ventricular mass (LVM), whereas diastolic dysfunction is the inability of cardiac myofibrils to rapidly or completely return to their resting length and is characterized by delayed active relaxation and increased LV stiffness. The conditions are interrelated as diastolic abnormalities are commonly observed among persons with LVH (43–46). Furthermore, both conditions are frequently encountered in the general population (47–53) and are predictive of cardiovascular morbidity and mortality in subjects with and without diabetes (54–57). The risk may be further modified by the magnitude of LVM and the LV geometric pattern, categorized according to the absence or presence of LVH and the relative wall thickness (RWT), a measure of the relationship between LV wall thickness and cavity diameter (58–61).

4.3. Potential Implications of Glycemic Status on Left Ventricular Structure and Function

One explanation for the poor prognosis among patients with diabetes is their susceptibility to develop a distinct primary myocardial condition, diabetic cardiomyopathy, predisposing them to an increased risk of heart failure (35). Diabetic cardiomyopathy is defined as ventricular dysfunction in patients with diabetes, occurring independently of hypertension, coronary artery disease, and other recognized causes and is characterized by increased LVM, concentric changes, and predominantly diastolic dysfunction (62–66). These cardiac abnormalities are common findings among patients with diabetes and may result from continuous metabolic stress, in line with the observation that their prevalence and severity seem directly related to the duration of diabetes (55,67–75). Indeed, the presence of subclinical cardiovascular damage in diabetes may act as a significant risk modifier and translates to a substantially increased event risk (76–78). However, it remains controversial whether there is a graded association between glycemic status, particularly when assessed in the fasting state, and LV structural and functional abnormalities, i.e., whether the risk increases along the diabetic continuum, from normal glycemic status through prediabetes to manifest diabetes (79–86). Importantly, although abnormal glycemic status accentuates risk in patients with known heart failure, its prognostic impact on subclinical cardiac abnormalities in apparently healthy subjects is unknown (87–90).

4.4. Cardiovascular Biomarkers

Despite the high prevalence of subclinical cardiac abnormalities, routine echocardiographic screening among asymptomatic patients with diabetes is not recommended (91,92). The same applies to asymptomatic individuals without diabetes. The availability of circulating cardiovascular biomarkers, e.g., natriuretic peptides and cardiac troponins, and their link to key pathophysiological processes, structural and functional myocardial alterations among others, makes their use as risk modifiers across the spectrum of cardiovascular risk theoretically attractive, even in subjects who have been stratified according to their echocardiographic findings (93–105). Brain natriuretic peptide (BNP) is released predominantly from ventricular myocytes in response to increased wall stress, i.e., volume expansion with resultant pressure overload, and has vaso dilatory and diuretic properties (107). Circulating levels of the physiologically inert N-terminal fragment of its prohormone (NT-proBNP) may be measured instead (108). Cardiac troponins I (TnI) and T (TnT) form part of the contractile apparatus in myocytes and are released upon disruption of normal cell membrane integrity (109). Their myocardial specificity makes them the preferred markers of ischemia and necrosis; however, the introduction of highsensitivity troponin (hs-Tn) assays has facilitated detection of circulating levels in a considerable proportion of individuals without established cardiovascular disease as well (110–112). Finally, natriuretic peptides and troponins may be independent predictors of adverse outcomes in subjects both with and without diabetes, but their overall contribution to risk prediction models, particularly in low risk, apparently healthy subjects, is less clearly established (16,17,113–131).

4.5. Potential Implications of Glycemic Status on Cardiovascular Biomarkers
Patients with diabetes are prone not only to heart failure, but also coronary artery disease, which is often silent (6-8,35). In fact, adverse cardiac alterations may already be present at the time of diagnosis of diabetes (132,133), making NT-proBNP and hs-TnT rational candidates for biomarkers in this setting. Nevertheless, glycemic status may affect the strength of the association between biomarkers and echocardiographic findings, perhaps implying that this should be taken into account when attempting biomarker-based risk prediction (134,135). The proportion of individuals with elevated concentrations of cardiovascular biomarkers may further be higher in the presence of diabetes, but seems to be influenced by diabetes duration and risk factor burden (98,116,121,123,136-139), and, similar to that noted for echocardiographic abnormalities, it is unclear whether hyperglycemia and biomarker concentrations exhibit a graded relationship (139-143). Finally, it is uncertain whether the predictive capabilities of NT-proBNP and hs-TnT differ across the glycemic spectrum, in apparently healthy subjects.

5. OBJECTIVES
1. To examine whether greater fasting plasma glucose (FPG) levels were independently associated with LVM and/or geometric pattern, in apparently healthy, elderly subjects with preserved LVEF.

2. To examine whether greater FPG levels were associated with LV diastolic dysfunction, independently of increased left ventricular mass index (LVM), in apparently healthy, elderly subjects with preserved LVEF.

3. To examine whether FPG levels modified the prognostic role of abnormal LVM, geometric pattern, and diastolic dysfunction, in predicting cardiovascular morbidity and mortality, in apparently healthy, elderly subjects with preserved LVEF.

4. To examine whether greater FPG levels were independently associated with concentrations of NT-proBNP and hs-TnT, in apparently healthy, elderly subjects with preserved LVEF.

5. To define the incremental prognostic value of NT-proBNP and hs-TnT for predicting incident cardiovascular outcomes, beyond traditional risk factors and subclinical echocardiographic abnormalities, in apparently healthy, elderly subjects with preserved LVEF. Additionally, to explore the associations of NT-proBNP and hs-TnT with key echocardiographic measures of LV structure and function, including effects of FPG, in these subjects.

6. To examine whether greater FPG levels modified the prognostic abilities of NT-proBNP and hs-TnT in predicting cardiovascular morbidity and mortality, in apparently healthy, elderly subjects with preserved LVEF.

6. METHODS
6.1. Study Population
The study population was derived from the echocardiography subsample (n=1,792) of the Malmö Preventive Project Re-Examination Study (MPP-RES) (2002-2006, n=18,238) (82). MPP-RES was the second phase of the Malmö Preventive Project (MPP) (1974-1992, n=33,346), a population-based screening program that included inhabitants from Malmö, Sweden, who belonged to prespecified birth cohorts between 1921-1949 (144). All participants answered a computer-based, self-administered questionnaire on lifestyle and medical history, including medication. Blood pressure and heart rate were recorded twice after 5 minutes of supine rest, with values averaged for the analyses. Height, weight, waist and hip circumferences were measured, and body mass index and body surface area (DuBois formula) were calculated. Blood samples were drawn after an overnight fast for analysis of plasma glucose, plasma lipids, and biobank storage for genetic analysis. Additional blood samples for cardiovascular biomarker assessments were drawn at the time of echocardiography, approximately 6 months after the initial screening visit. Samples were stored at -20°C.

Subjects, who underwent echocardiography, were randomly chosen from the three categories defined by baseline FPG, i.e., normal fasting glucose (NFG), IFG, and diabetes, including use of anti-diabetic medication, with oversampling in the two latter groups. The original echocardiography subsample was stratified into patients defined as individuals with known cardiovascular disease (n=300), those who were on cardiovascular (n=864), anti-diabetic (n=329), or lipid-lowering medication (n=464), or had a reduced LVEF < 50% (n=99) and apparently healthy subjects, the latter being the focus of this thesis. Slight between-paper sample size variations were due to certain additional exclusion criteria, including missing echocardiographic variables (n=158), missing biomarkers (n=31), or incomplete follow-up (n=6), but comprised of 670-693 subjects.

MPP-RES was approved by the Regional Ethical Review Board and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

6.2. Fasting Plasma Glucose
The majority of analyses took into account the aforementioned FPG-based selection of study subjects. Excluding those already on anti-diabetic medication, the definitions of NFG, IFG, and diabetes resembled the World Health Organization criteria (21): NFG: a single FPG ≤ 6.0 mmol/L; IFG: a single FPG between 6.1-6.9 mmol/L, or one measurement 7.0-11.0 mmol/L and a separate measurement ≤ 6.9 mmol/L; newly diagnosed diabetes: a single FPG > 11.1 mmol/L or two separate measurements ≥ 7.0 mmol/L. Given the age-range of the study population, it was presumed that all cases comprised of type 2 diabetes, and no clear distinction was made throughout this thesis or its related publications.

6.3. Echocardiography
Six experienced technicians (biomedical scientists) performed all echocardiograms using either a 3V2c transducer (Acuson Sequoia, Mountain View, CA, USA) or an S3 transducer (Sonos 5500 Philips, Andover, MA, USA) and further did offline analyses of the images, using a mean of 3-5 cycles. Intra- and interobserver variabilities have been reported previously (82).

LVEF estimates were based on visual quantification. For LVM assessment, end-diastolic, 2-dimensional linear measurements in the parasternal long-axis view, at the tips of the mitral valve leaflets, perpendicular to the LV long axis, were used (46). Calipers were positioned on the interface between myocardial wall and cavity and the interface between myocardial wall and pericardium, respectively, obtaining the thickness of the interventricular septum, LV internal diameter, and thickness of the posterior wall. The Devreux formula was used for LVM calculation, with LVM obtained by indexing for body surface area. Cut-offs employed for LVH were LVM > 95 g/m² (females) and > 115 g/m² (males). LV geometric characterization was achieved by combining LVM and relative wall thickness (RWT, (2*posterior wall thickness)/LV internal diameter): normal: absence of LVH and RWT ≤ 0.42; concentric remodeling:
absence of LVH and RWT > 0.42; eccentric LVH: presence of LVH and RWT ≤ 0.42; concentric LVH: presence of LVH and RWT > 0.42.

For evaluation of LV diastolic function, transmitral pulsed Doppler flow with a 1-3 mm sample volume placed between the tips of the mitral valve leaflets and tissue Doppler imaging with the sample volume positioned within 1 cm of the septal and lateral borders of the mitral annulus, both in the apical four-chamber view, were used (44). E, A, and E-wave deceleration time (DT) were obtained by pulsed Doppler imaging, and septal and lateral é by tissue Doppler imaging. Age-appropriate partitions of these parameters were then used for classification: normal diastolic function: septal é ≥ 8 cm/s and/or lateral é ≥ 10 cm/s; diastolic dysfunction: septal é < 8 cm/s and lateral é < 10 cm/s; grade 1 dysfunction: diastolic dysfunction + DT ≥ 240 ms, E/A ratio < 0.8, averaged E/é ratio ≤ 12; grade 2 dysfunction: diastolic dysfunction + DT 140-240 ms, E/A ratio 0.8-1.5, averaged E/é ratio ≥ 9; grade 3 dysfunction: diastolic dysfunction + DT < 140 ms, E/A ratio > 1.5, averaged E/é ratio ≥ 13. All cases of E/é ratio < 9 were classified as normal (DT < 240ms and E/A ratio ≥ 0.8), or grade 1 dysfunction (the remainder). In case of E/é ratio ≥ 9 and ≤ 12, subjects were only categorized as grade 1 or grade 2 dysfunction if both DT and E/A ratio fitted the same category, with the remainder labeled undetermined diastolic dysfunction. If E/é ratio was > 12, subjects were categorized as grade 2 or 3 diastolic dysfunction, which were grouped together for the analyses, due to the low number of subjects with grade 3 dysfunction. Finally, planimetry in the apical four-chamber view during end-systole allowed for estimation of left atrial area.

6.4. Biomarkers
NT-proBNP was measured with an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Chemistry, Akerhus University Hospital, Lorenskog, Norway. An elevated concentration was defined as ≥ 15 pmol/L. Hs-TnT was analyzed at the Department of Clinical Biochemistry, Regional Hospital of Northern Jutland, Hjørring, Denmark, between May-June 2013, using an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Basel, Switzerland). A concentration ≥ 14 ng/L (99th percentile of a healthy reference population) was used as partition.

6.5. Outcomes
In papers III-IV, a composite of coronary events (myocardial infarction, stable ischemic heart disease, percutaneous coronary intervention, coronary artery bypass grafting), heart failure, stroke, and all-cause mortality constituted the primary clinical outcome. Non-fatal events were defined according to International Classification of Diseases (ICD-9 and ICD-10) codes, with validities in the Swedish National Inpatient Register previously reported (145). Mortality data were obtained through the National Registry on Causes of Mortality at the Swedish Central Bureau of Statistics. Follow-up was terminated on December 31, 2014.

6.6. Statistical Methods
The statistical software packages IBM SPSS Statistics versions 22-23 (IBM, Armonk, NY, USA) and Stata/IC versions 13-14 (StataCorp LP, College Station, TX, USA) were used for all analyses.

6.6.1. Summary Statistics
Continuous variables were plotted as histograms, and distributions were visually inspected. Means, medians, skewness, and kurtosis were examined as well. Summary statistics were presented as means and standard deviations (variables with a clear or approximate Gaussian distribution) or medians and interquartile ranges (IQR) (variables with a clear non-Gaussian distribution). Categorical variables were presented as counts with corresponding percentages. Group-wise comparisons were performed using independent samples t-test, one-way analysis of variance, Mann-Whitney U test, Kruskal-Wallis test, Pearson’s χ²-test, and Fisher’s exact test, as appropriate.

6.6.2. Cross-Sectional Analyses
In papers I-II, associations between echocardiographic variables and risk factors were examined using multivariable linear regression, binary logistic regression, and ordinal logistic regression models. In all cases, potential explanatory variables underwent univariable analysis. Variables that were statistically significant or deemed clinically appropriate, including FPG category, were entered into the final multivariable regression models, with application of stepwise backward elimination for optimization (P-retention < 0.20 except for FPG category, which was kept in the models regardless of its P-value). Correlations between LV structure (mass and geometry) and diastolic function were further tested with Pearson’s product moment correlation (r) or Kendall’s tau-b (τ). In paper IV, associations between biomarkers and echocardiographic variables were assessed using sex- and age-adjusted multivariable linear regression models or binary logistic regression models. Moderately to severely positively skewed variables (DT, E/A ratio, averaged E/é ratio, NT-proBNP, and Hs-TnT) were natural logarithmically transformed before inclusion in the regression models.

6.6.3. Survival Analyses
In papers III-IV, the risk associated with abnormal LV structure and function, and abnormal biomarker levels, respectively, were tested using Cox proportional-hazards regression models, with time to first composite endpoint as the underlying timescale. The proportional-hazards assumption was examined using Schoenfeld residuals. Possible covariates included SCORE variables (age, sex, smoking status, systolic blood pressure, total cholesterol), kidney function (cystatin C), FPG category, and presence of LVH and/or grade 2 or 3 diastolic dysfunction. For biomarkers, hazard ratios (HR) were reported for both the assay-specific cut-offs and the upper quartile versus the three lower quartiles.

6.6.4. Discrimination and Reclassification
In paper IV, discrimination abilities for prediction models, with and without addition of the biomarkers as continuous variables, were described and compared using Harrell’s concordance index (C-index), with the assumption that in case of two simultaneously terminating lifetimes, neither could be said to have survived the other (uncensored policy) (146). The ability of each biomarker to improve prognostication was also tested with the continuous (category-free) net reclassification improvement, which is not affected by category cut-offs and event rates (147).

6.6.5. Interaction Analyses
Interactions (effect modifications) were tested using the likelihood-ratio test for regression models with and without the interaction term and most often displayed using Forest plots.

6.6.6. Statistical Significance
A two-sided P-value < 0.05 was considered statistically significant. No adjustments for multiple comparisons were made, and all findings are to be considered exploratory (hypothesis-generating).
7. RESULTS

7.1. Paper I

7.1.1. Left Ventricular Mass Index
LVMI was significantly greater among subjects with diabetes than in subjects with NFG or IFG (mean 90 +/- 26 g/m² vs. 85 +/- 20 g/m²; P = 0.01), but there was no significant difference between subjects with IFG versus NFG (mean 87 +/- 21 g/m² vs. 84 +/- 19 g/m²; P = 0.08). Although FPG category was not a significant predictor of LVMI on multivariable analysis (Table 1), greater LVMI was only associated with higher NT-proBNP levels among subjects with IFG or diabetes (P < 0.001 for interaction) (Figure 1). There was a weak correlation between LVMI and diastolic function (r = 0.171; P < 0.001).

7.1.2. Left Ventricular Hypertrophy and Geometry
LVH in its entirety was not more commonly observed among subjects with diabetes than those without (14% vs. 12%, P = 0.60). The adjusted multivariable regression model is shown in Table 2. As such, FPG category was not associated with LVH on multivariable analysis, but significantly interacted with the association between LVH and NT-proBNP (P = 0.01 for interaction) (Figure 2). LVH and diastolic function were weakly, albeit significantly, correlated (τ = 0.185; P < 0.001). Of note, concentric LVH was significantly more prevalent among subjects with diabetes (11% vs. 6%, P = 0.03) and also displayed the strongest correlation with grade 2 or 3 diastolic dysfunction (τ = 0.280; P < 0.001), with a significant interaction analogous to that described for LVH (P = 0.02 for interaction).

7.2. Paper II

7.2.1. E/é
E/é was significantly greater among subjects with diabetes than those without (median 8 (IQR: 6-11) vs. 7 (IQR: 5-10); P = 0.03), but lower among subjects with IFG than those with NFG (median 7 (IQR: 6-9) vs. 8 (IQR: 6-10); P = 0.04). Moreover, FPG category was not a significant determinant of E/é on multivariable analysis (Table 3), but did interact with the association between E/é and systolic blood pressure (P = 0.001 for interaction), E/é and NT-proBNP (P < 0.001 for interaction), and E/é and LVMI (P = 0.02 for interaction) (Figure 3). The direction of the interaction for LVMI was the opposite of what was seen for systolic blood pressure and NT-proBNP.

7.2.2. Diastolic Dysfunction
The prevalence of grade 2 or 3 diastolic dysfunction was significantly greater among subjects with diabetes than those without (25% vs. 16%; P = 0.02), and there was no significant difference between subjects with IFG versus those with NFG (13% vs. 18%; P = 0.08). As seen for E/é, FPG category was not a significant determinant of overall diastolic function on multivariable analysis (Table 4), but significantly interacted with the association between E/é and systolic blood pressure (P = 0.03 for interaction), and E/é and NT-proBNP (P = 0.005 for interaction) (Figure 4).

Table 1. Multivariable linear regression model for prediction of left ventricular mass index (adjusted r² = 0.204).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>β-coefficient (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.25 (-0.04 to 0.55)</td>
<td>0.10</td>
</tr>
<tr>
<td>Female sex</td>
<td>-11.01 (-14.49 to -7.53)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index, per kg/m²</td>
<td>0.99 (0.44 to 1.53)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmHg</td>
<td>0.20 (0.03 to 0.37)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pulse rate, per min⁻¹</td>
<td>-0.26 (-0.38 to -0.13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Log(NT-proBNP), per log(pro-A)</td>
<td>5.59 (3.71 to 7.48)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviation: NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

Figure 1. The association between NT-proBNP and left ventricular mass index, according to fasting plasma glucose category.

Table 2. Binary logistic regression model for prediction of left ventricular hypertrophy (pseudo-r² = 0.126).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>1.65 (0.999 to 2.11)</td>
<td>0.05</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.46 (0.87 to 2.47)</td>
<td>0.15</td>
</tr>
<tr>
<td>Body mass index, per kg/m²</td>
<td>1.08 (1.01 to 1.15)</td>
<td>0.04</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmHg</td>
<td>1.02 (1.004 to 1.03)</td>
<td>0.009</td>
</tr>
<tr>
<td>Log(NT-proBNP), per log(pro-A)</td>
<td>1.88 (1.39 to 2.50)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ES = estimate; CI = confidence interval; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

Figure 2. The association between NT-proBNP and left ventricular hypertrophy, according to fasting plasma glucose category.

Table 3. Multivariable linear regression model for prediction of diastolic function (adjusted r² = 0.204).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>β-coefficient (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.12 (-0.04 to 0.29)</td>
<td>0.10</td>
</tr>
<tr>
<td>Female sex</td>
<td>-1.12 (-1.44 to -0.80)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index, per kg/m²</td>
<td>0.24 (0.08 to 0.40)</td>
<td>0.01</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmHg</td>
<td>0.20 (0.03 to 0.37)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pulse rate, per min⁻¹</td>
<td>-0.26 (-0.38 to -0.13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Log(NT-proBNP), per log(pro-A)</td>
<td>5.59 (3.71 to 7.48)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviation: NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

Figure 3. The association between NT-proBNP and diastolic function, according to fasting plasma glucose category.

P = 0.01 for interaction.

Abbreviations: ES = estimate; CI = confidence interval; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

7.2. Paper II

7.2.1. E/é
E/é was significantly greater among subjects with diabetes than those without (median 8 (IQR: 6-11) vs. 7 (IQR: 6-10); P = 0.03), but lower among subjects with IFG than those with NFG (median 7 (IQR: 6-9) vs. 8 (IQR: 6-10); P = 0.04). Moreover, FPG category was not a significant determinant of E/é on multivariable analysis (Table 3), but did interact with the association between E/é and systolic blood pressure (P = 0.001 for interaction), E/é and NT-proBNP (P < 0.001 for interaction), and E/é and LVMI (P = 0.02 for interaction) (Figure 3). The direction of the interaction for LVMI was the opposite of what was seen for systolic blood pressure and NT-proBNP.

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The prevalence of grade 2 or 3 diastolic dysfunction was significantly greater among subjects with diabetes than those without (25% vs. 16%; P = 0.02), and there was no significant difference between subjects with IFG versus those with NFG (13% vs. 18%; P = 0.08). As seen for E/é, FPG category was not a significant determinant of overall diastolic function on multivariable analysis (Table 4), but significantly interacted with the association between E/é and systolic blood pressure (P = 0.03 for interaction), and E/é and NT-proBNP (P = 0.005 for interaction) (Figure 4).
Table 3. Multivariable linear regression model for prediction of log(E/e) (adjusted $r^2 = 0.317$).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>$\beta$-coefficient (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.03 (0.02 to 0.03)</td>
<td>$&lt;$ 0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.12 (0.07 to 0.18)</td>
<td>$&lt;$ 0.001</td>
</tr>
<tr>
<td>Body mass index, per kg/m$^2$</td>
<td>0.006 (-0.005 to 0.01)</td>
<td>0.34</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmHg</td>
<td>0.001 (0.0003 to 0.003)</td>
<td>0.03</td>
</tr>
<tr>
<td>Left ventricular mass index, per g/m$^2$</td>
<td>0.002 (0.001 to 0.003)</td>
<td>$&lt;$ 0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose category</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal fasting glucose (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>-0.006 (-0.06 to 0.04)</td>
<td>0.85</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.01 (-0.02 to 0.12)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Figure 3. The associations between risk factors and E/e, according to fasting plasma glucose category. For visual purposes, the $\beta$-coefficients are shown for every 10-unit increase.

Systolic blood pressure: P = 0.001 for interaction; NT-proBNP: P < 0.001 for interaction; LVMI: P = 0.02 for interaction.
Abbreviations: ES = estimate; CI = confidence interval; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

Table 4. Ordered logistic regression model for prediction of worse diastolic function (pseudo-$r^2 = 0.158$).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>1.20 (1.18 to 1.23)</td>
<td>$&lt;$ 0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.45 (0.87 to 2.17)</td>
<td>0.07</td>
</tr>
<tr>
<td>Left ventricular mass index, per g/m$^2$</td>
<td>1.01 (1.01 to 1.02)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fasting plasma glucose category</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal fasting glucose (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>0.08 (0.54 to 1.23)</td>
<td>0.36</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.12 (0.67 to 1.87)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Figure 4. The associations between risk factors and worse diastolic function, according to fasting plasma glucose category. For visual purposes, the odds ratios are shown for every 10-unit increase.

Systolic blood pressure: P = 0.03 for interaction; NT-proBNP: P = 0.005 for interaction.
Abbreviations: ES = estimate; CI = confidence interval; NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

7.3. Paper III
7.3.1. Fasting Plasma Glucose
Although a graded, positive relationship between FPG category and outcome was indicated, this was not statistically significant: unadjusted analyses; NFG: baseline; IFG: HR, 1.14 (95% CI: 0.79-1.66), P = 0.47; diabetes: HR, 1.32 (95% CI: 0.83-2.10), P = 0.25 (P = 0.23 for trend) (Table 5 and Figure 5).

7.3.2. Left Ventricular Mass and Geometry
LVMI was not significantly associated with cardiovascular event risk (HR, 1.05 (95% CI: 0.97-1.14) per 10 g/m$^2$ increase, P = 0.20). Similar results were observed for LVH (HR, 1.36 (95% CI: 0.85-2.09), P = 0.21) and for the individual geometric patterns (P $\geq$ 0.09 for all) (Table 5 and Figure 6). However, a significant interaction was found between FPG category and concentric LVH (P $<$ 0.001 for interaction), i.e., concentric LVH only predicted risk among subjects with IFG or diabetes (Figure 7).

7.3.3. Diastolic Dysfunction
Greater E/e (HR, 1.06 (95% CI: 1.01-1.11), P = 0.02), any diastolic dysfunction (HR, 1.66 (95% CI: 1.18-2.32), P = 0.003), grade 1 dysfunction, and grade 2 or 3 dysfunction (P $\leq$ 0.01 for both) were all significantly associated with an increased event risk on univariable analysis, whereas undetermined dysfunction was not (P = 0.97) (P = 0.002 for trend) (Table 5 and Figure 8). FPG category significantly interacted with the association between adverse risk and any diastolic dysfunction, grade 2 or 3 dysfunction, and undetermined dysfunction (P $\leq$ 0.04 for all interactions), with diastolic dysfunction being a better predictor of adverse risk among subjects with hyperglycemia (Figure 7).

Table 5. Unadjusted and adjusted hazard ratios for fasting plasma glucose and echocardiographic measures of left ventricular structure and function (composite of coronary events, heart failure, stroke, and all-cause mortality).
All hazard ratios are adjusted for SCORE variables (age, sex, smoking status, systolic blood pressure, total plasma cholesterol). No events occurred in the group of subjects with NFG and concentric LVH (n=16); therefore, that subgroup is not depicted in the figure. Abbreviations: HR = hazard ratio; CI = confidence interval.
7.4. Paper IV

7.4.1. N-terminal Prohormone of Brain Natriuretic Peptide

NT-proBNP concentrations did not significantly differ between subjects with and without diabetes (median 8 (IQR: 5-15) pmol/L vs. 8 (IQR: 5-15) pmol/L; P = 0.46), but were significantly lower among subjects with IFG (median 7 (IQR: 4-13) pmol/L) than among those with NFG (median 10 (IQR: 5-18) pmol/L; P = 0.001 for difference) or diabetes (median 8 (IQR: 5-16) pmol/L; P = 0.009 for difference). However, IFG did not remain significantly associated with NT-proBNP on multivariable analysis (Table 6), and there were no significant interactions.

7.4.2. High-Sensitivity Troponin T

Hs-TnT concentrations were significantly greater among subjects with diabetes than those with NFG or IFG (median 9 (IQR: 6-11) ng/L vs. 7 (IQR: 5-9) ng/L; P < 0.001), but there was no difference between subjects with IFG and NFG, respectively (median 7 (IQR: 5-9) ng/L vs. 7 (IQR: 5-9) ng/L; P = 0.87). Furthermore, hs-TnT was significantly greater among subjects with diabetes on multivariable analysis, although without any significant interactions (Table 7).

7.4.3. Echocardiographic Correlates

Both NT-proBNP and hs-TnT displayed significant associations with several echocardiographic measures, particularly those of LV structure, although effect sizes were generally unimpressive. Associations with concentric LVH were stronger than those for eccentric LVH. Among measures of diastolic function, the most consistent results were observed for grade 2 or 3 diastolic dysfunction. Table 8 shows an extract of these associations. The effects of FPG on associations between NT-proBNP and echocardiographic measures have been addressed in previous sections. In addition, FPG significantly interacted with the association between hs-TnT and LVMI (P = 0.02 for interaction) and LVH (P = 0.04 for interaction), but not E/e (P > 0.99 for interaction) and diastolic function (P = 0.73 for interaction) (Figures 9 and 10).

7.4.4. Prediction of Cardiovascular Events

Subjects with high concentrations (upper quartile) of NT-proBNP or hs-TnT were at greater risk compared to those with low concentrations (lower three quartiles) (Figures 11 and 12). The addition of NT-proBNP to traditional risk factors, fasting glucose, cystatin C, and echocardiography provided a significant discrimination improvement for prediction of cardiovascular events (C-index: 0.721 vs. 0.714, P = 0.045) and a positive net reclassification improvement. However, no such isolated benefit was found for hs-TnT (C-index: 0.715 vs. 0.714; P = 0.82) (Table 9).

7.4.5. Prognostic Impact of Fasting Plasma Glucose on N-terminal Prohormone of Brain Natriuretic Peptide

Although the prognostic ability of NT-proBNP seemed stronger among subjects with diabetes, no significant interactions were detected (P ≥ 0.24) (Figure 13).

7.4.6. Prognostic Impact of Fasting Plasma Glucose on High-Sensitivity Troponin T

Similar to NT-proBNP, a stronger prognostic ability of hs-TnT among subjects with diabetes was indicated, but without any significant interactions (P ≥ 0.23) (Figure 13).

Table 6. Multivariable linear regression model for prediction of log(NT-proBNP) (adjusted r² = 0.290).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>β-coefficient [95% confidence interval]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.06 (0.05 to 0.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.37 (0.23 to 0.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, per kg/m²</td>
<td>-0.02 (-0.04 to -0.00)</td>
<td>0.002</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmhg</td>
<td>0.01 (0.002 to 0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse rate, per min⁻¹</td>
<td>-0.01 (-0.01 to -0.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose category</td>
<td>Normal fasting glucose (reference)</td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>-0.11 (-0.24 to 0.02)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.08 (-0.10 to 0.26)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Abbreviation: NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

Table 7. Multivariable linear regression model for prediction of log(hs-TnT) (adjusted r² = 0.260).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>β-coefficient [95% confidence interval]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.03 (0.01 to 0.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>-0.28 (-0.36 to -0.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, per kg/m²</td>
<td>0.02 (0.01 to 0.03)</td>
<td>0.03</td>
</tr>
<tr>
<td>Systolic blood pressure, per mmhg</td>
<td>0.003 (-0.005 to 0.009)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C, per mg/L</td>
<td>0.05 (0.01 to 0.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose category</td>
<td>Normal fasting glucose (reference)</td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>0.11 (-0.07 to 0.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.11 (-0.01 to 0.24)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Abbreviation: hs-TnT = high-sensitivity troponin T.

Table 8. Age- and sex-adjusted associations between biomarkers and echocardiographic variables.

<table>
<thead>
<tr>
<th>Echocardiographic variable</th>
<th>NT-proBNP</th>
<th>High-sensitivity troponin T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular mass index</td>
<td>0.21 (0.01)</td>
<td>0.23 (0.001)</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>0.37 (0.04)</td>
<td>0.42 (0.001)</td>
</tr>
<tr>
<td>Concentric remodeling</td>
<td>-0.06 (0.06)</td>
<td>-0.04 (0.04)</td>
</tr>
<tr>
<td>Eccentric hypertrophy</td>
<td>0.015 (0.02)</td>
<td>0.28 (0.05)</td>
</tr>
<tr>
<td>Concentric hypertrophy</td>
<td>0.38 (0.002)</td>
<td>0.58 (0.002)</td>
</tr>
<tr>
<td>Averaged E/e</td>
<td>0.047 (0.02)</td>
<td>0.06 (0.10)</td>
</tr>
<tr>
<td>Diastolic dysfunction</td>
<td>0.11 (0.03)</td>
<td>0.28 (0.02)</td>
</tr>
<tr>
<td>Grade 1 dysfunction</td>
<td>-0.06 (0.04)</td>
<td>0.17 (0.21)</td>
</tr>
<tr>
<td>Grade 2 or 3 dysfunction</td>
<td>0.013 (0.01)</td>
<td>0.39 (0.02)</td>
</tr>
<tr>
<td>Undetermined dysfunction</td>
<td>0.038 (0.08)</td>
<td>0.64 (0.007)</td>
</tr>
</tbody>
</table>

For associations between two continuous variables, standardized β-coefficients (P-values in brackets) are reported.

For associations between one categorical (echocardiography) and one continuous (biomarker) variable, β-coefficients for standardized values of biomarkers (P-values in brackets) are reported.

Figure 9. The association between hs-TnT and left ventricular mass index, according to fasting plasma glucose category.
P = 0.02 for interaction.
Abbreviations: ES = estimate; CI = confidence interval; hs-TnT = high-sensitivity troponin T.

Figure 10. The association between hs-TnT and left ventricular hypertrophy, according to fasting plasma glucose category.

Abbreviations: ES = estimate; CI = confidence interval; hs-TnT = high-sensitivity troponin T.

Figure 11. Kaplan-Meier survival curves for the composite of coronary events, heart failure, stroke, and all-cause mortality, according to NT-proBNP concentrations.

Abbreviation: NT-proBNP = N-terminal prohormone of brain natriuretic peptide.

P = 0.04 for interaction.
Abbreviations: ES = estimate; CI = confidence interval; hs-TnT = high-sensitivity troponin T.

Figure 12. Kaplan-Meier survival curves for the composite of coronary events, heart failure, stroke, and all-cause mortality, according to hs-TnT concentrations.

Abbreviation: hs-TnT = high-sensitivity troponin T.

Table 9. Hazard ratios, C-indices, and net reclassification improvement for NT-proBNP and hs-TnT (composite of incident coronary events, heart failure, stroke, and all-cause mortality).

<table>
<thead>
<tr>
<th>Model</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
<th>C-index</th>
<th>P-value for difference</th>
<th>Net reclassification improvement (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.38 (1.10-1.73)</td>
<td>0.01</td>
<td>1.31 (1.04-1.66)</td>
<td>0.03</td>
<td>0.690</td>
<td>0.12</td>
<td>0.270 (0.194-0.457)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.58 (1.29-1.94)</td>
<td>0.02</td>
<td>1.47 (1.20-1.76)</td>
<td>0.04</td>
<td>0.720</td>
<td>0.63</td>
<td>0.238 (0.163-0.313)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.57 (1.37-1.82)</td>
<td>0.02</td>
<td>1.49 (1.29-1.73)</td>
<td>0.05</td>
<td>0.720</td>
<td>0.67</td>
<td>0.177 (0.108-0.255)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.37 (1.16-1.62)</td>
<td>0.02</td>
<td>1.47 (1.26-1.71)</td>
<td>0.04</td>
<td>0.720</td>
<td>0.67</td>
<td>0.120 (0.054-0.225)</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.35 (1.20-1.49)</td>
<td>0.02</td>
<td>1.43 (1.25-1.63)</td>
<td>0.06</td>
<td>0.721</td>
<td>0.64</td>
<td>0.173 (0.098-0.258)</td>
</tr>
</tbody>
</table>

For prespecified cut-off
b) For quartile 4 versus quartiles 1-3
c) With versus without biomarker (continuous)
d) For biomarker (continuous), bootstrapped confidence interval (1,000 replications)

Model 1: Adjusted for age and sex
Model 2: Adjusted for SCORE variables (age, sex, smoking status, systolic blood pressure, total plasma cholesterol)
Model 3: Adjusted for SCORE variables, left ventricular hypertrophy and/or diastolic dysfunction
Model 4: Adjusted for SCORE variables, fasting plasma glucose category, and cystatin C
Model 5: Adjusted for models 3+4

TABLE 9. Hazard ratios, C-indices, and net reclassification improvement for NT-proBNP and hs-TnT (composite of incident coronary events, heart failure, stroke, and all-cause mortality).
Abbreviations: NT-proBNP = N-terminal prohormone of brain natriuretic peptide; hs-TnT = high-sensitivity troponin T; CI = confidence interval.

Figure 13. The associations between biomarker concentrations and the composite of coronary events, heart failure, stroke, and all-cause mortality, according to fasting plasma glucose category.

Additional interaction analyses for absence versus presence of diabetes were non-significant (P = 0.06 for both biomarkers (quartile 4 versus quartiles 1-3)).

8. DISCUSSION

8.1. Impact of Glycemic Status on Left Ventricular Structure

Manifest diabetes is generally regarded as an independent risk factor for increased LVMI (67-69,72); however, in accordance with the magnetic resonance imaging based results from the Multi-Ethnic Study of Atherosclerosis (MESA) (81), we did not see a graded association between FPG category and LV structure. Conversely, traditional markers of hyperglycemia were associated with LVMI and RWT in both the Strong Heart Study (SHS) (79,84,85) and the Framingham Heart Study (FHS) (80). For example, De Marco et al. found progressively greater LVMI and RWT among obese adolescents and young adults with IFG or diabetes (84). Capaldo et al. observed greater LVMI and RWT in persons with isolated IFG or combined IFG and impaired glucose tolerance (85), and independent associations for impaired glucose tolerance were demonstrated in FHS (80). There are several possible methodological explanations for these inconsistencies. Despite IFG and impaired glucose tolerance being equally predictive of incident type 2 diabetes, the concordance between these two categories is limited, and postload glucose may better predict cardiovascular morbidity and mortality than fasting glucose (24). The development of adverse myocardial changes is temporally related to hyperglycemia (73,75), and the time spent with glycemic disturbances in our study was presumably short as we excluded subjects on anti-diabetic medication at the time of screening. In addition, data from SHS referred to a specific ethnic cohort, i.e., American Indians. Lastly, the importance of hyperglycemia may decrease with age, and the subjects in the present study were on average older than those examined in SHS. Further adding to the premise that the mode of assessment for glucose metabolism and selected partitions are important factors to consider when attempting to demonstrate cardiovascular differences across the hyperglycemic spectrum, is a recent report from the Atherosclerosis Risk in the Community (ARIC) Study, which showed graded associations between LV abnormalities and glycemic category, based on HbA1c, for some, but not all markers of LV structure, i.e., septal wall thickness and RWT were greater and LV internal diameter smaller, but LVMI was not significantly different in subjects with prediabetes versus those with normal glycemic status (86).

In our study, diabetes was primarily associated with concentric LVH, and concentric LVH further displayed the strongest correlation with diastolic dysfunction. Concentric changes are more prevalent in the setting of hyperglycemia (67-69,79,80), and concentric LVH may further be associated with a higher risk of morbidity and mortality than the other LV geometric patterns (59-61). Additionally, the effects of antihypertensive treatment on LVH regression seem attenuated among patients with diabetes (148,149), and the cardiovascular role of isolated, strict glucose-lowering is also unclear (150-155). Formation of advanced glycation end products and their cross-linking with collagen may in part account for these treatment failures (156). All things considered, the presence of LVH in diabetes may represent a more harmful type of LVH than that seen in other conditions.

8.2. Impact of Glycemic Status on Left Ventricular Function

Evidence regarding the association between glycemic status and LV diastolic function is less abundant than that for LV structure. Similar to the observations for LV structure, and in line with SHS (84), we did not find a graded effect of FPG category on diastolic function. In contrast, in the Diagnostic Trial on Prevalence and Clinical Course of Diastolic Dysfunction and Diastolic Heart Failure, the prevalence and severity of diastolic dysfunction increased along the diabetic continuum (83). However, in the latter study, all individuals without known diabetes were subjected to an oral glucose tolerance test, the implications for which have been described in the previous section, and the study population was further enriched by selecting subjects with risk factors for or even manifest heart failure. Finally, in ARIC, the majority of measures of diastolic function did not significantly differ between individuals with normal glycemic status and prediabetes, whereas significant changes were reported for most measures when comparing patients with diabetes versus those without (86). Since diabetes per se is considered a risk factor for diastolic dysfunction, we speculate that the significant differences observed between subjects with diabetes and those without might be due to a threshold effect of hyperglycemia on myocardial stiffening.

Higher systolic blood pressure was predominantly associated with worse diastolic function among subjects without diabetes, strengthening the idea of an independent effect of diabetes, whereas greater LVMI showed a significantly stronger association with worse diastolic function among patients with abnormal glycemic status. It is likely that hyperglycemia augments the myocardial stiffening caused by LVH, possibly through a reduction in coronary flow reserve, which has proven associations with LVH, diastolic dysfunction, and diabetes (157). Nevertheless, whether the lower coronary flow reserve among patients with diabetes is a direct consequence of hyperglycemia or due to insulin resistance, endothelial dysfunction, or increased sympathetic activity, is not known. Although the absence of an association between systolic blood pressure and diastolic function among subjects with diabetes disagrees with previously demonstrated synergistic effects of diabetes and hypertension (158), an explanation may be provided by the
Cardiovascular Continuum paradigm, i.e., ageing, whether physiological or pathological, brought on by cardiovascular risk factors, including diabetes, ultimately results in the same adverse cardiovascular alterations, and accelerated effects are seen in the presence of multiple risk factors (159-161). This theory and our findings are supported by the strong relationship between age and diastolic function in this elderly cohort, a presumed late onset of hyperglycemia, and a probable dominating effect of diabetes over other cardiovascular risk factors. Lastly, a theoretical framework, complementing the Cardiovascular Continuum, has been proposed, in which diastolic dysfunction can be considered the final common pathway for various conditions, including overweight, hypertension, and diabetes, which induce a state of inflammation, coronary microvascular endothelial dysfunction, and cardiomyocyte hypertrophy and stiffening (162).

8.3. Prognostic Impact of Glycemic Status on Left Ventricular Structure and Function

Several studies have examined the cross-sectional relationships between prediabetes, diabetes, and LV structural and functional alterations; however, we have demonstrated for the first time that glucoseemic status, in a graded fashion, modifies the risk of incident events associated with subclinical echocardiographic abnormalities. Specifically, concentric LVH and diastolic dysfunction were more strongly associated with an adverse prognosis in subjects with IFG or diabetes compared to subjects with NFG. These observations fit well the aforementioned Cardiovascular Continuum concept (159-161), including the tendency towards risk factor clustering in diabetes (12), not all of which can be adjusted for. Moreover, we used a composite endpoint, including all-cause mortality, and even non-cardiovascular mortality may be elevated among patients with diabetes (163). Still, the underlying mechanisms, specifically on the cardiac level, remain speculative. As alluded to previously, the presence of LVH in diabetes may represent a more deleterious form of LVH, due to its often-concentric nature and strong association with hypertension and diastolic dysfunction, but notably, the results were independent of systolic blood pressure. These findings are also consistent with previously proposed theories regarding the etiology and pathogenesis of structural and functional echocardiographic alterations in patients with diabetes, including inflammation and endothelial dysfunction (162), decreased coronary flow reserve (157), and formation of advanced glycation end products (156).

8.4. Impact of Glycemic Status on Cardiovascular Biomarker Concentrations

Prior studies have shown higher hs-TnT concentrations among patients with diabetes compared to subjects without (98,116,121,137,138,142,143). Examples include the Dallas Heart Study (DHS), in which the proportion of patients with diabetes increased across hs-TnT categories (98), and the Women’s Health Study, in which hs-TnT concentrations and the fraction with detectable hs-TnT concentrations, but not elevated above the upper reference limit, i.e., the 99th percentile, were significantly higher among women with a history of diabetes versus those without (116). Our results confirmed these observations in older, low risk individuals, but hs-TnT did not vary between subjects with NFG and IFG. Although such a difference was seen in ARIC (142), only glycemic status defined by means of Hba1c, not FPG, was significantly associated with hs-TnT. Further supporting our results, and the prior notion that the method for measuring and defining glycemic status should be taken into consideration when exploring associations with subclinical cardiovascular disease, significant differences between subjects with diabetes versus those without, but not between normal glycemic status and prediabetes, defined as IFG or impaired glucose tolerance, were shown by Zheng et al. (143). Finally, the relationship between Hba1c, and hs-TnT may be steepest in the narrow range that defines prediabetes, providing a rationale for the more robust findings for diabetes (142). Potential underlying mechanisms for the increased hs-TnT levels in non-acute settings largely mimic those proposed for hyperglycemia-related LV structural and functional alterations, including microvascular dysfunction or damage (164), advanced glycation end products (156), low grade inflammation (165), myocardial fibrosis (166), or perhaps even silent epicardial coronary artery disease (167).

The case may be more complicated for NT-proBNP since concentrations were lowest in the IFG group, and without any measurable difference between subjects with diabetes and NFG. Indeed, previous results concerning glycemic status have been somewhat inconsistent (136,139-141,168-172), while inverse relationships of natriuretic peptides with body mass index and other metabolic risk factors have been consistently shown in population-based studies, including FHS (168,171) and DHS (170). Wang et al. showed additive, negative effects of obesity and diabetes on BNP levels (168), whereas Das et al. found inverse associations between both BNP and NT-proBNP with body mass index and a closer correlation with lean mass than with fat mass (170). This suggests that the presence of natriuretic peptide clearance receptors in adipose tissue, which are believed not to bind NT-proBNP, is not the only explanation (173). Altered cardiac synthesis and/or secretion seems more probable (174), and sex may play an additional role (141,168). Finally, and in agreement with its hemodynamic properties, NT-proBNP is elevated in the presence of subclinical cardiac damage (95-97,99-102,104-106,134,135), which is more common in the presence of diabetes. Although it is difficult to draw definite conclusions regarding the influence of isolated hyperglycemia on NT-proBNP, lower concentrations should generally be expected, and interpretation in relation to the overall metabolic profile seems reasonable.

8.5. Impact of Glycemic Status on the Associations between Echocardiography and Cardiovascular Biomarkers

Significant, albeit modest, correlations between biomarkers and echocardiography were expected in this apparently healthy cohort (98,100,134,135). This highlights the beliefs that individual biomarkers cannot be recommended as screening tools solely for LV dysfunction due to limited sensitivity (94,97,98,101,175), that risk estimates based on imaging studies and circulating biomarkers should be considered complimentary (100,105), and further fits the said clinical and pathophysiological complexities associated with circulating levels of NT-proBNP and hs-TnT. We found stronger correlations with LV structure than with diastolic function, perhaps due to the inherent challenges associated with non-invasive assessment of diastolic function (44,176).

NT-proBNP concentrations were primarily related to LVMi and LVH among subjects with hyperglycemia, extending previous findings from the Hooray Study, in which stronger cross-sectional associations between BNP and diastolic function, and BNP with temporarily worsening diastolic function and increasing LVMi, were observed among patients with diabetes compared to those without (134,135). Possibly, the association between NT-proBNP and LV size only appeared stronger in subjects with IFG due to their lower NT-proBNP concentrations, whereas the robust association in patients with diabetes stemmed from relatively lower NT-proBNP.
concentrations in relation to their higher prevalence of LV abnormalities (168,171,177-180). This lends support to the concept that similar NT-proBNP levels indicate a worse LV state and prognosis in diabetes. This could also apply to hs-TnT, despite its higher baseline levels among patients with diabetes as similar patterns for LVM and LVH were observed. It has further been speculated that an increased production of natriuretic peptides might precede overt echocardiographic abnormalities, resembling damage by the mechanisms mentioned previously (157,181,182). The fact that the interaction between NT-proBNP and E/e′ followed a reverse pattern would support this concept as well as the hypothesis that LVH precedes diastolic dysfunction, i.e., the impact of hemodynamic measures on myocardial relaxation may fall beneath that of metabolic factors, once LVM has been sufficiently increased. Although this contradicts the findings from the Hoorn Study, their lack of Doppler measurements for assessment of diastolic function obscures direct comparisons (134,135).

8.6. Prognostic Impact of Glycemic Status on Cardiovascular Biomarkers

Troponins and natriuretic peptides deliver incremental prognostic information among high risk individuals, particularly those with known cardiovascular disease (128,183,184), but the evidence for apparently healthy subjects is ambiguous, i.e., very large sample sizes have usually been needed to show minor improvements, if any, in risk stratification for individual biomarkers (115,127,130,185), and there is further evidence of reporting bias (186). The observed modest model improvement provided by NT-proBNP beyond clinical risk factors and structural and functional echocardiographic changes is intriguing and extends prior findings from MESA (99), DHS (100), and the Cardiovascular Health Study (105), in which LVH with concomitantly elevated concentrations of NT-proBNP and/or hs-TnT was associated with a more severe disease course. As an example, in DHS, which oversampled African Americans, NT-proBNP and hs-TnT provided incremental prognostic value beyond, and interacted with, LVH determined by magnetic resonance imaging (100). The findings were independent of LVM, leading the authors to propose that elevations of these biomarkers may represent adverse remodeling from LVH to manifest heart failure, not merely greater LVM in persons with higher biomarker concentrations. Furthermore, NT-proBNP and hs-TnT displayed distinct correlations with markers of LV structure. Likewise, in our study, hs-TnT seemed a better predictor of LV diastolic function than NT-proBNP and vice versa. However, hs-TnT showed no benefit as an individual marker in our sample in its entirety, possibly related to ethnicity, use of both LVH and diastolic dysfunction factors, but lower utility of biomarkers, when comparing persons free from known cardiovascular disease and on no cardiovascular medications may have introduced another selection bias, i.e., healthy cohort effect. Additionally, the low event rate with associated low power to detect prognostic associations was a limitation and prompted us to use a composite endpoint. It should be noted, however, that these limitations would tend to decrease rather than increase the sensitivity of our analyses, thus highlighting the importance of the positive findings.

9. LIMITATIONS
9.1. Study Population

Despite the relatively high participation rate of 72% in MPP-RES, it may be argued that the study subjects did not represent a truly random population sample, since individuals who agree to participate in such screening studies may be healthier than the general population. The applicability of the results in females may be limited by the fact that 70% of subjects in the present study were male, and the generalizability to groups other than elderly Caucasians is also unknown. Our exclusion of more than half of the original study population to obtain a cohort of apparently healthy subjects free from known cardiovascular disease and on no cardiovascular medications may have introduced another selection bias, i.e., healthy cohort effect. Additionally, the low event rate with associated low power to detect prognostic associations was a limitation and prompted us to use a composite endpoint. It should be noted, however, that these limitations would tend to decrease rather than increase the sensitivity of our analyses, thus highlighting the importance of the positive findings.

9.2. Fasting Plasma Glucose

The subgroup division was based on FPG, in most cases a single measurement. The addition of an oral glucose tolerance test or HbA1c measurements would have been desirable for several reasons. For instance, impaired glucose tolerance and diabetes defined by oral glucose tolerance testing are more common among women than men, whereas IFG is more often seen in men (24). FPG exhibits greater fluctuation than HbA1c, which provides an estimate of the average glucose levels, and possibly sustained myocardial affection, over a 2-3-month period (22); however, the latter was not routinely measured during the screening process due to financial limitations. Furthermore, prognostic associations might have been affected by subjects having been captured early during hyperglycemia, and use of anti-diabetic medication, perhaps initiated soon after screening, could not be accounted for. Therefore, the use of FPG would tend to decrease the sensitivity, not specificity, of the analyses.
9.3. Echocardiography

2D-guided linear LV measurements have well-documented prognostic properties and are particularly useful for studying large populations. However, the method utilizes basal dimensions only, and LV changes that occur along the long axis of the chamber cannot be accounted for. The Devereux formula for calculating LVM assumes normal LV geometry and cubes the linear measurements (46). Consequently, even small errors may significantly influence the estimated mass. The exclusion of individuals with reduced LVEF < 50% is likely to have accounted for distorted LV geometry except discrete upper septal thickening, which is particularly common in elderly, hypertensive subjects (188). It is possible that in our study population, this phenomenon may have attenuated the prognostic role of LVMI.

Preload increases e velocity in normal subjects, and as such, its utility in these individuals may be limited (189). In addition, lateral E/é may be better than septal E/é for estimation of LV filling pressures in subjects with preserved LVEF > 50%. However, we used the averaged E/é due to lack of information on regional dysfunction. Finally, a more robust grading of diastolic dysfunction could have been attained if we had been able to include the left atrial volume index (44). Nevertheless, we used strict criteria for E/é, and the uncertain prognostic differences between grade 1 dysfunction and grade 2 or 3 dysfunction in our cohort serve as a general reminder of the difficulties associated with echocardiographic diagnosis and grading of diastolic dysfunction (176).

Therefore, it may be argued, that these limitations pertaining to echocardiography, would, similar to what was noted for the study population and FPG, tend to increase the likelihood of false negative findings.

9.4. Biomarkers

Biomarkers were examined in blood samples which had been frozen for up to 10 years, potentially influencing concentrations and presumably leading to underestimation of their prognostic abilities (190).

10. CONCLUSIONS

1. Subjects with newly diagnosed diabetes had greater LVMI and a higher prevalence of concentric LVH, but only on univariable analysis.

2. Subjects with newly diagnosed diabetes had greater E/é and a higher prevalence of grade 2 or 3 diastolic dysfunction, but the associations were not independent of LVMI. FPG category significantly interacted with the association between E/é and LVMI, and LVMI was primarily associated with higher E/é in subjects with IFG or diabetes.

3. Subclinical echocardiographic abnormalities, particularly those of LV diastolic function, were associated with an increased risk of incident cardiovascular events. FPG category was not by itself associated with the outcome, but modified the prognostic role of concentric LVH and diastolic dysfunction, i.e., these alterations were more strongly associated with adverse prognosis in subjects with IFG or diabetes.

4. Subjects with IFG had significantly lower NT-proBNP concentrations than subjects with NFG or newly diagnosed diabetes on univariable, but not multivariable, analysis. Diabetes was independently associated with higher hs-TnT concentrations on multivariable analysis.

5. Subjects with high concentrations of NT-proBNP and hs-TnT were at greater risk of incident cardiovascular events compared to those with low concentrations. NT-proBNP provided a significant discrimination improvement beyond traditional risk factors and subclinical echocardiographic abnormalities, whereas hs-TnT did not. NT-proBNP and hs-TnT were significantly associated with several echocardiographic parameters, particularly those of LV structure, and less so for diastolic function, but effect sizes were generally modest. Associations were modified by FPG category.

6. FPG category did not significantly modify the prognostic role of NT-proBNP and hs-TnT, but the totality of the data suggests that glycemic status should be considered when interpreting biomarker concentrations.

11. PERSPECTIVES

Cardiovascular risk assessment in the primary preventive setting remains challenging and may be both affected and complicated by glycemic status. Numerous potential strategies have not been properly studied or validated, and most attempts to enhance risk stratification have yielded insignificant to modest effects. Given the results of this thesis, the following may be considered for future observational and interventional research:

1. The adverse myocardial alterations in diabetes might stem from extended exposure to hyperglycemia and obesity, and the presence of IFG may provide a window of opportunity to prevent or reduce LVH and diastolic dysfunction by halting progression into manifest diabetes.

2. Targeting both systemic and cardiovascular comorbidities at an early stage, including blood pressure lowering, regression of LVH, glucose-lowering, weight reduction, lipid-lowering, and anti-inflammatory therapy, may delay the progression of subclinical diastolic dysfunction to overt heart failure.

3. Subclinical echocardiographic abnormalities may be particularly deleterious in subjects with hyperglycemia, represent a key mechanistic link between diabetes and overall cardiovascular risk, and may thus be used to enrich study populations in future clinical trials of such individuals.

4. Different prognostic roles of traditional risk factors and biomarkers according to a priori risk should be considered. Multi-marker strategies may have a prognostic role among low risk subjects, but there is a need for validation of specific biomarker combinations in different populations and across different risk categories.

5. Biomarkers may be used to enrich study populations in future clinical trials of primary preventive strategies, regardless of baseline glycemic status, but more data regarding the potential benefit of biomarker-based intervention, including initiation or intensification of diagnostic, therapeutic, and monitoring strategies, is needed, particularly among low risk subjects.

6. Biomarkers may be more representative of subclinical echocardiographic abnormalities among subjects with hyperglycemia, but should be considered complimentary, rather than as replacements to, echocardiography, regardless of glycemic status.
12. SUMMARY

12.1. Background

Traditional cardiovascular risk stratification tools that employ clinical risk factors are limited by their modest discriminative abilities. As such, robust cardiovascular risk assessment, including our understanding of the complex interplay between risk factors, in the primary preventive setting, remains incomplete. Phenotypical heterogeneity may be even greater among subjects with hyperglycemic conditions, i.e., prediabetes and diabetes, which is worrisome, given the dramatic global rise in mean fasting glucose levels, and the strong association with adverse cardiovascular outcomes. The unmet need for refinement or restratification of risk based on these conventional prediction models is only emphasized by our entrance into the era of precision medicine. Potential tools for closing these gaps and increasing our understanding of the pathways from risk factors through subclinical changes to manifest disease include echocardiography and circulating biomarkers.

12.2. Objectives

1) To examine whether greater fasting plasma glucose (FPG) levels were associated with left ventricular mass (LVM), geometric pattern, diastolic function, and concentrations of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T (hs-TnT) in apparently healthy, elderly subjects with a preserved LV ejection fraction \( \geq 50\%; 
2) To examine whether FPG levels modified the prognostic role of abnormal LVM, geometric pattern, diastolic dysfunction, NT-proBNP, and hs-TnT, in predicting cardiovascular morbidity and mortality; 3) To define the incremental prognostic value of NT-proBNP and hs-TnT for predicting incident cardiovascular outcomes, beyond traditional risk factors, glycemic status, and subclinical echocardiographic abnormalities; 4) To explore the associations of NT-proBNP and hs-TnT with key echocardiographic measures of LV structure and function, including the effects of FPG levels.

12.3. Methods

The thesis was based on a series of cross-sectional and prospective observational studies. The study population was derived from the echocardiography subsample (n=1,792) of the Malmö Preventive Project Re-Examination Study (MPP-RES) (2002-2006, n=18,238), a population-based screening program that included inhabitants from Malmö, Sweden, who belonged to prespecified birth cohorts between 1921-1949. Subjects, who underwent echocardiography, were randomly chosen from the three categories defined by baseline FPG, i.e., normal fasting glucose, impaired fasting glucose, and diabetes, including use of anti-diabetic medication. Blood samples for cardiovascular biomarker assessments were drawn at the time of echocardiography and kept frozen until analysis. Outcome data were obtained through national and local registries. The original echocardiography subsample was stratified into patients and apparently healthy subjects, the latter being the focus of this thesis.

12.4. Results

1) Subjects with diabetes had a greater prevalence of concentric LV hypertrophy (LVH), grade 2 or 3 diastolic dysfunction, and higher hs-TnT concentrations. Subjects with impaired fasting glucose had the lowest NT-proBNP concentrations. LVMi was primarily associated with diastolic function in subjects with hyperglycemia; 2) LV diastolic dysfunction was associated with an increased risk of incident cardiovascular events, but did not provide discriminative improvement. Concentric LVH and diastolic dysfunction were more strongly associated with adverse prognosis in subjects with hyperglycemia. High concentrations of NT-proBNP and hs-TnT predicted incident cardiovascular events, with no effect modification by FPG; 3) NT-proBNP, but not hs-TnT, provided discriminative improvement beyond traditional risk factors, FPG, and LVH and/or diastolic dysfunction; 4) NT-proBNP and hs-TnT were associated with several echocardiographic parameters, but effect sizes were generally modest. Associations between biomarkers and echocardiographic measures were affected by hyperglycemia.

12.5. Conclusions

FPG influenced the interplay between subclinical echocardiographic abnormalities, circulating biomarkers, and cardiovascular outcomes at multiple stages, in this cohort of apparently healthy, elderly subjects. Newly diagnosed diabetes, but not impaired fasting glucose, was associated with adverse subclinical changes. The associations between structural echocardiographic abnormalities and biomarker concentrations were stronger in subjects with hyperglycemia. NT-proBNP, but not echocardiographic measures or hs-TnT, provided discriminative improvement on top of traditional cardiovascular risk factors. FPG further modified the prognosis related to echocardiographic alterations, but not that predicted by biomarkers. Therefore, FPG should be considered when assessing markers of subclinical cardiovascular alterations.

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