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Title page

Title:

Worsening diastolic function is associated with elevated fasting plasma glucose and increased left ventricular mass in a supra-additive fashion in an elderly, healthy, Swedish population.

Short title:

Diastolic dysfunction in relation to fasting plasma glucose and left ventricular mass.

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All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Conflicts of interest

The authors declare no conflict of interest.

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Abbreviations:

DD: diastolic dysfunction

LV: left ventricular / left ventricle

DM: diabetes mellitus

HF: heart failure

HbA_{1c}: hemoglobin A_{1c} (glycosylated hemoglobin)

LVMI: left ventricular mass index

MPP: Malmö Preventive Project

MPP-RES: Malmö Preventive Project Re-Examination Study

FPG: fasting plasma glucose

ICD: International Classification of Diseases

NFG: normal fasting glucose

IFG: impaired fasting glucose

WHO: World Health Organization

LVEF: left ventricular ejection fraction

ASE: American Society of Echocardiography

LAA: left atrial area

DT: E-wave deceleration time

EAE: European Association of Echocardiography

NT-proBNP: N-terminal prohormone of brain natriuretic peptide

IQR: interquartile range

ANOVA: analysis of variance

BMI: body mass index

LDL: low-density lipoprotein

HDL: high-density lipoprotein

OGTT: oral glucose tolerance test

CFR: coronary flow reserve

Abstract:

Aims To examine whether increasing fasting plasma glucose (FPG) levels were associated with worsening left ventricular (LV) diastolic function, independently of LV mass index (LVMI) in elderly, otherwise healthy subjects.

Methods and results We tested cross-sectional associations between echocardiographically determined averaged E/é ratio / diastolic function, LVMI, cardiovascular risk factors, and FPG categorized as normal (NFG), impaired (IFG), and new-onset diabetes mellitus (DM), in 483 men and 208 women aged 56-79 years without overt cardiovascular disease, who received no cardiovascular, anti-diabetic, or lipid-lowering drugs and had a preserved LV ejection fraction > 50%. Median E/é was significantly higher among subjects with diabetes than those without (8 vs. 7; $p = 0.03$), as was the prevalence of grade 2 or 3 diastolic dysfunction (25% vs. 16%; $p = 0.02$). E/é and diastolic function were significantly associated with LVMI ($p \leq 0.002$), but not FPG category, on multivariable analysis. However, interaction analyses revealed that increasing LVMI was primarily associated with worsening diastolic function (higher E/é) in subjects with FPG > 6mmol/l ($\beta = 0.005$ for IFG and DM vs. 0.001 for NFG; $p = 0.02$), whereas increasing systolic blood pressure was primarily associated with worsening diastolic function (higher E/é) in subjects with FPG ≤ 6.9 mmol/l ($\beta = 0.005$ for NFG and 0.003 for IFG vs. -0.001 for DM; $p = 0.001$).

Conclusion Diastolic dysfunction was significantly more prevalent among patients with DM than those without. The importance of LVMI increased, but the importance of systolic blood pressure decreased with higher FPG category.

1. Introduction

Diastolic dysfunction (DD) is the inability of the cardiac myofibrils to rapidly or completely return to their resting length and is characterized by delayed active relaxation and increased left ventricular (LV) stiffness [1]. The condition is most often caused by ischemic heart disease and/or hypertension with subsequent concentric remodeling or hypertrophy of LV [2]. LV hypertrophy and DD are common findings among patients with diabetes mellitus (DM), with the presence and severity of LV DD being directly correlated to the duration of DM [3-5]. The associations are independent of concomitant hypertension and ischemic heart disease. Therefore, it seems that patients with DM are predisposed to a primary myocardial disease, diabetic cardiomyopathy, defined as ventricular dysfunction occurring in a diabetic patient, independently of a recognized cause [3].

DD in patients with DM is associated with both subsequent development of heart failure (HF) and increased mortality [4,5]. The mechanisms through which DD develops and progresses to overt HF in patients with DM are not clearly understood, but may be partially associated with increased LV mass [3]. Keeping in mind the negative results of recent clinical trials regarding the management of HF with preserved ejection fraction [6,7], a better understanding of the pathogenesis of DD is essential for development of novel therapeutic strategies that can prevent or delay this process.

LV diastolic function can be non-invasively evaluated by Doppler echocardiography, and a wide range of specific echocardiographic indicators of diastolic function exist [8]. The combination of assessing mitral annulus motion with tissue Doppler during early diastole (ϵ) and early passive mitral inflow velocity (E) provides an acceptable estimate of LV filling pressure, with $E/\epsilon < 8$ being associated with normal filling pressure, whereas values $> 12-15$ are associated with elevated filling pressures. Moreover, studies have demonstrated a reduction of ϵ in type 2 DM and an independent correlation between increased E/ϵ and both glycosylated hemoglobin (HbA_{1c}), mortality, and the risk of development of overt HF [4,5,9-12]. Therefore, the E/ϵ ratio may be used to detect and follow the progression of LV DD in patients with DM.

The purpose of this study was: 1) To examine whether worsening glucometabolic status was associated with worsening LV diastolic function, independently of increased LV mass index (LVMI); and 2) To identify other risk factors independently associated with worsening LV diastolic function.

2. Methods

The study was a cross-sectional study.

2.1 Study population

The study subjects were derived from the Malmö Preventive Project (MPP, 1974-1992, n = 33,346), a population-based cohort study with the aim of screening for cardiovascular risk factors, alcohol abuse, and breast cancer among inhabitants in Malmö, Sweden, born 1921-1949 [13]. A re-examination study (MPP-RES, n = 18,238) was conducted between 2002-2006, during which the participants answered a questionnaire on lifestyle, medical history, and medication. Blood pressure and pulse rate were recorded twice in the supine position after 5 minutes of rest (with the values averaged for the analyses), and height, weight, waist and hip circumferences were measured. Moreover, blood samples were drawn after overnight fasting for analysis of plasma glucose, serum lipids, and storage in a biobank. In a subsample of 1,792 individuals from MPP-RES, an echocardiography was carried out. These subjects were randomly selected from groups defined by fasting plasma glucose (FPG), with oversampling in groups of subjects with impaired fasting glucose (IFG) and DM. Both MPP and MPP-RES were approved by the Ethics Committee of Lund University, Sweden and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2 Prevalent cardiovascular disease or diabetes mellitus

Subjects with prevalent cardiovascular disease (n = 300) and/or those on cardiovascular (n = 864), anti-diabetic (n = 329) or lipid-lowering therapy (n = 464) were excluded in the present study (total excluded n = 1029). Prevalent cardiovascular disease was defined by the *International Classification of Diseases* (ICD-9 and ICD-10) codes gathered from the Swedish Hospital Discharge Registry as well as local hospital and study registries and encompassed previous myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, HF, stroke, or atrial fibrillation and/or flutter.

2.3 Glucometabolic status

The definitions of normal fasting glucose (NFG), IFG, and DM were based on the *World Health Organization* (WHO) criteria [14]: NFG was defined as a single FPG ≤ 6.0 mmol/L; IFG was defined as 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; and new-onset DM was defined as a single FPG ≥ 11.1 mmol/L or two separate measurements ≥ 7.0 mmol/L.

2.4 Echocardiography

Echocardiography was conducted with a 3V2c transducer (Acuson Sequoia, Mountain View, CA) or an S3 transducer (Sonos 5500 Philips, Andover, MA). LV ejection fraction (LVEF) was quantified visually. LV mass calculations were based on 2-dimensional images in the parasternal long-axis view at the level of the mitral valve tips during end-diastole, using the formula recommended by the *American Society of Echocardiography (ASE)*, and indexed for body surface area, obtaining LVMI [15]. Left atrial area (LAA) during end-systole was obtained by planimetry in the apical four-chamber view. LV diastolic function was assessed in the apical four-chamber view using transmitral pulsed Doppler flow with a 1-3 mm sample volume placed between the tips of the mitral valve leaflets (obtaining E, A, and E-wave deceleration time (DT)) and tissue Doppler imaging with the sample volume positioned within 1 cm of the septal and lateral borders of the mitral annulus (obtaining both septal and lateral ϵ and averaging the values for the analyses). A mean of 3-5 cycles was used. The intra- and interobserver variabilities are reported elsewhere [16]. Diastolic function was graded according to the recommendations of the *European Association of Echocardiography (EAE) and ASE*, using age-appropriate cut-off values of septal ϵ , lateral ϵ , E-wave DT, E/A, and averaged E/ ϵ [8]. If septal ϵ was ≥ 8 and/or lateral ϵ was ≥ 10 , subjects were classified as having normal diastolic function. If septal ϵ was < 8 and lateral ϵ was < 10 , subjects were classified as having DD, and the values of E-wave DT, E/A, and E/ ϵ were used for grading subjects into grade 1, 2 or 3 DD (table 1). If E/ ϵ was ≥ 9 and ≤ 12 , subjects were only classified as having either grade 1 or 2 DD if the values of both E-wave DT and E/A fitted the same category. Equivocal cases, i.e. subjects who were in a transitional state between grade 1 and 2 DD with E/ ϵ ≥ 9 and ≤ 12 , but E/A and E-wave DT pointing in opposite directions, were classified as undetermined DD. If E/ ϵ was > 12 , subjects were classified as having either grade 2 or 3 DD. Finally, all subjects with E/ ϵ < 9 were classified as either normal (E-wave DT < 240 ms and E/A ≥ 0.8) or grade 1 DD (all other subjects), even if they did not strictly fulfill the primary ϵ criteria for normal diastolic function. Grade 2 and 3 DD were grouped together, since only one individual fulfilled the criteria for grade 3 DD. Subjects with LVEF $\leq 50\%$ were excluded in the present study (n = 29). Moreover, 43 subjects were excluded due to missing echocardiographic variables.

2.5 Biomarkers

In the echocardiography subcohort, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was analyzed using an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Chemistry, Akershus University Hospital, Lorenskog, Norway.

2.6 Statistical analysis

Continuous variables were summarized by means and standard deviations (approximately normally distributed variables) and medians and interquartile ranges (IQR) (non-normally distributed variables), whereas categorical variables were presented by frequencies and corresponding percentages. Group-wise comparisons were performed using one-way analysis of variance (ANOVA), Kruskal-Wallis test, and Pearson's χ^2 -test or Fisher's exact test (depending on cell frequencies), respectively. Since E/é was moderately positively skewed, the association between E/é and various risk factors was assessed by multivariable linear regression after natural log-transformation of E/é. The association between diastolic function and risk factors was assessed by ordered logistic regression. In order to define potential explanatory variables for E/é and worsening diastolic function, univariate regressions were applied on the following demographic and clinical variables: age, sex, smoking status, body mass index (BMI), waist circumference, systolic blood pressure (SBP), pulse rate, FPG category, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, NT-proBNP, and LVMI. Statistically and clinically significant variables were included in the final multivariable linear and ordered logistic regression models, and stepwise subset selection was applied for adjustment of these models with an entry level of 0.2. The significance level for the univariate analyses was 5%. Analyses were carried out using IBM SPSS Statistics 22 (IBM, Armonk, New York, USA) and Stata/IC 13 (StataCorp LP, College Station, Texas, USA).

3. Results

3.1 Characteristics of the study population

The final study cohort (figure 1) comprised 691 subjects (483 men and 208 women), including 344 individuals with NFG, 240 with IFG, and 107 with new-onset DM. Subjects were middle-aged or elderly with a median age of 66 (IQR 60-70) years and mildly hypertensive with a mean SBP of 147 +/- 20 mmHg. Mean BMI was 24.0 +/- 3.2 kg/m², and values of total and LDL cholesterol were 5.9 +/- 1.0 mmol/L and 3.9 +/- 0.9 mmol/L, respectively. Median averaged E/é was 7 (IQR 6-10), and 18% (when excluding subjects with undetermined DD) had grade 2 or 3 DD. Worsening

glucometabolic status (i.e. higher FPG category) was associated with increasing BMI, waist circumference, SBP, pulse rate, triglycerides, LVMI, and LAA, and decreasing HDL cholesterol, whereas the presence of DM was associated with increased E/é, male sex, and a greater prevalence of both grade 2 or 3 DD and $E/\acute{e} \geq 13$. Table 2a and 2b show the baseline characteristics of the subjects categorized according to FPG category and diastolic function, respectively, whereas table 2c shows the characteristics of the subjects with undermined DD.

3.2 Determinants of the averaged E/é ratio

E/é was significantly higher in subjects with DM than those without (8 (IQR: 6-11) vs. 7 (IQR: 6-10), $p = 0.03$), Mann-Whitney U test). In univariate analyses, an increasing value of E/é was associated with higher age, BMI, SBP, total cholesterol, NT-proBNP, LVMI, and female sex. The adjusted linear regression model is presented in table 3a and included age, BMI, SBP, LVMI, and sex, whereas FPG category was forced into the model. In a separate model, E/é was significantly associated with LVMI ($\beta = 0.004$ (95% CI: 0.002 to 0.005), $p < 0.0001$), but not FPG category (NFG: reference; IFG: $\beta = -0.06$ (95% CI: -0.12 to -0.007), $p = 0.03$; DM: $\beta = 0.05$ (95% CI: -0.03 to 0.13), $p = 0.2$). The same was true when subjects were subdivided into two glucometabolic categories only, i.e. DM vs. NFG or IFG. However, FPG category significantly interacted with the association between E/é and SBP ($p = 0.001$), NT-proBNP ($p < 0.001$), and LVMI ($p = 0.02$) (figure 2a).

3.3 Determinants of worsening diastolic function

Grade 2 or 3 DD was more prevalent among subjects with diabetes than those without (25% vs. 16%, $p = 0.02$, Pearson's χ^2 -test), as was $E/\acute{e} \geq 13$ (15% vs. 6%, $p = 0.001$, Pearson's χ^2 -test). In univariate analyses, worsening diastolic function was associated with higher age, BMI, NT-proBNP, LVMI, and female sex. The adjusted ordered logistic regression model is presented in table 3b and included age, LVMI, and sex, whereas FPG category was forced into the model. In a separate model, diastolic function was significantly associated with LVMI (OR = 1.02 (95% CI: 1.01 to 1.03), $p < 0.0001$), but not FPG category (NFG: reference; IFG: OR = 0.59 (95% CI: 0.40 to 0.86), $p = 0.006$; DM: OR = 1.02 (95% CI: 0.64 to 1.63), $p = 0.9$). Considering only DM vs. NFG or IFG yielded similar results. FPG category significantly interacted with the association between diastolic function and SBP ($p = 0.03$), and NT-proBNP ($p = 0.005$) (figure 2b).

4. Discussion

The main findings of our study were: 1) A significant greater prevalence of grade 2 or 3 DD among subjects with new-onset DM than those without; 2) A significant positive association between worsening diastolic function and increasing LVMI; 3) Significant interactions, i.e. effect modifications, between glucometabolic status and other cardiovascular risk factors, including surrogate markers of hemodynamic load, i.e. SBP, NT-proBNP, and LVMI, in the prediction of diastolic function, with increasing SBP and NT-proBNP being predominantly associated with worsening diastolic function among subjects without DM, whereas increasing LVMI showed a stronger association with worsening diastolic function (as determined by higher E/\dot{e}) among patients with IFG or DM.

Prevalence measurements of asymptomatic or pre-clinical DD, i.e. DD with normal systolic function and no symptoms of HF, are not easily obtained due to differences in defining DD and HF [2]. The prevalence of pre-clinical DD in the general adult Caucasian population is estimated to be 20-30%, but only 5-10% for moderate or severe DD [17-20]. In patients with DM, the reported prevalence varies between 20-60% [5]. In accordance with the algorithm provided in the *EAE-ASE* recommendations, the primary steps in grading diastolic function in the present study were septal and lateral \dot{e} [8]. Although this by itself could lead to an overestimation of subjects with grade 2 DD, an averaged $E/\dot{e} \geq 9$ was also required. Most other investigators used $E/\dot{e} \geq 10$ as cut-off, and the greater number of subjects with moderate or severe DD in our study may be partially explained by the fact that $E/\dot{e} \geq 9$ and < 10 was common (8%). Using averaged $E/\dot{e} \geq 13$ (the recommended cut-off for predicting increased LV filling pressure in subjects with preserved LVEF) as the sole criterion for DD yielded estimates much closer to those shown in previous similar studies.

Regarding risk factors for pre-clinical DD in subjects without overt cardiovascular comorbidities, our results generally agreed with previous findings, since increasing age, SBP, BMI, LVMI, NT-proBNP levels, DM, and female sex were all significantly associated with worsening diastolic function, at least on univariate analysis [5,17-19]. We did not find a graded effect of FPG category on DD prevalence or severity, even though IFG represented an intermediate state between NFG and DM concerning other risk factors. This complied with the *Strong Heart Study*, which, however, did not include E/\dot{e} and only examined subjects below 40 years of age [21], but was in contrast to the *Diagnostic Trial on prevalence and clinical course of diastolic dysfunction and diastolic heart failure*, in which both DD prevalence and severity increased along the diabetic continuum [11]. In the latter study, however, all subjects without a history of DM underwent an oral glucose tolerance test (OGTT), and although the risk of future development of type 2 DM is the same

for individuals with either IFG or impaired glucose tolerance (IGT), the latter seems to be a better predictor of cardiovascular mortality, and perhaps pre-clinical cardiac damage as well [22]. Furthermore, subjects were required to either have risk factors for diastolic HF or manifest chronic HF, strengthening the chance of finding such associations. Our detection of a significant difference in both E/é and the prevalence of grade 2 or 3 DD between subjects with DM and those without, raises the question of whether a certain threshold level of glucose exists, at which the adverse effect on myocardial function is accelerated.

Interestingly, FPG category significantly interacted with the association between surrogate markers of hemodynamic load and diastolic function, i.e. increasing SBP and NT-proBNP were predominantly associated with worsening diastolic function among subjects without DM, whereas increasing LVMI showed a significantly stronger association with worsening diastolic function among patients with IFG or DM. Although speculative, the latter may suggest that glucose levels in the diabetic range somehow affect the myocardium by amplifying the deteriorating effects of increasing LV mass on myocardial relaxation, i.e. the presence of DM intensifies the myocardial stiffening caused by LV hypertrophy. Our findings could be explained by the following observations [23]: 1) Pathological LV hypertrophy results in decreased coronary flow reserve (CFR), and there is an association between the magnitude of CFR reduction and DD; 2) Coronary flow reserve is also decreased in patients with DM as either a direct consequence of hyperglycemia or due to insulin resistance, endothelial dysfunction, or increased sympathetic activity; 3) DM by itself is associated with increased LV mass, which may be due to microvascular damage causing myocardial damage and reactive fibrosis and/or hypertrophy.

Our results also indicate that among patients with DM, increased LVMI may have a much greater influence on myocardial stiffening than increased SBP and other traditional risk factors, which seemed to matter mainly in subjects without DM. Although this finding disagrees with previous studies showing a synergistic effect of DM and hypertension [24], an explanation may be provided by the *Cardiovascular Continuum*, i.e. the concept that both physiological and pathological ageing brought on by cardiovascular risk factors, e.g. DM, result in similar disturbances in LV structure and function [25-27]. In other words, impaired glucose metabolism can be considered an accelerator for physiological ageing. The subjects in the present cohort were relatively old, and the strong positive association between age and diastolic function in addition to the presumed late onset of glucometabolic disturbances could explain the lack of an association between FPG category and diastolic function on multivariable analysis. In patients with DM; however,

one would still expect the presence of DM to dominate over other traditional risk factors, which may explain the non-significant association between SBP and diastolic function in these particular individuals. The observation that increasing SBP was only associated with increasing E/ϵ and worsening diastolic function in the younger half of our study population supports this hypothesis (results not shown). In addition, the chances of finding an additive or synergistic effect was attenuated by our exclusion of patients receiving anti-diabetic or cardiovascular medication as well as the abovementioned old age of the subjects, whereby traditional risk factors, e.g. hypertension, may have already exerted their effects, perhaps by having increased LV mass.

Despite the established association between DM and LV DD, the pathophysiological mechanisms by which type 2 DM impairs myocardial function have not been fully elucidated, and other hypotheses do exist. A theoretical framework has been proposed in which DD can be thought of as a process of different conditions, e.g. overweight, hypertension, and DM, inducing a state of both systemic and microvascular inflammation causing cardiomyocyte hypertrophy and stiffening through coronary microvascular endothelial dysfunction [28]. This theory, which suggests that several independent risk factors may play a role, also fits well the described results. Looking at tables 3a and 3b and taking into account this hypothesis, a multidisciplinary therapeutic approach aimed at both systemic and cardiovascular comorbidities, initiated at an early stage, including lowering of blood pressure, regression of LV hypertrophy and/or concentric LV geometry, lowering of elevated plasma glucose, weight reduction, lipid-lowering, and anti-inflammatory therapy, e.g. with statins, may delay the progression of asymptomatic DD to overt HF.

4.1 Limitations

Although the participation rate of 72% in MPP-RES is considered high, one may still argue that the study subjects did not represent a truly random population sample since people who agree to take part may be healthier than the general population. 70% of the subjects in the present study were male, which may limit the applicability of the results in females. Moreover, our exclusion of more than half of the original study population in order to get a cohort of apparently healthy subjects in whom possible associations are not affected by medication may as mentioned earlier introduce a selection bias. The subgroup division was based on FPG, in most cases only a single measurement. The addition of an OGTT and/or HbA_{1c} measurements would have been preferable, as IGT and DM defined by OGTT is more common among women than men [22], and HbA_{1c} provides an estimate of the average glucose levels, and

perhaps sustained myocardial affection, over a 8-12 week period [29]. The oversampling of subjects with either IFG or DM also prevented us from using FPG as a continuous variable.

The usefulness of E/e' velocity in normal subjects may be limited, as preload increases e' in these subjects [30]. Moreover, lateral E/e' may be superior to septal E/e' for predicting LV filling pressure in subjects with LVEF > 50%, but our choice of using averaged E/e' was due to the lack of information on regional dysfunction. Also, although increased E/e' is indicative of elevated LV filling pressure, it should not be used as a stand-alone parameter when drawing conclusions about LV DD. Our grading of DD could; however, have been more robust, had we also been able to incorporate the left atrial volume index [8]. Finally, the cross-sectional nature of our study prevents us from making finite inferences about causality.

5. Conclusion

In conclusion, although apparently healthy, elderly subjects with new-onset DM had higher values of averaged E/e' and a greater prevalence of grade 2 or 3 DD, the associations were not independent of LVMI. However, FPG category significantly interacted positively with the association between E/e' and LVMI, with LVMI predominantly being associated with higher E/e' in subjects with IFG or DM.

References

1. Zile MR, Baicu CF, Bonema DD. Diastolic heart failure: definitions and terminology. *Prog Cardiovasc Dis* 2005;47:307-313.
2. Wan SH, Vogel MW, Chen HH. Pre-clinical diastolic dysfunction. *J Am Coll Cardiol* 2014;63:407-416.
3. Huynh K, Bernardo BC, McMullen JR, Ritchie RH. Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther* 2014;142:375-415.
4. From AM, Scott CG, Chen HH. Changes in diastolic dysfunction in diabetes mellitus over time. *Am J Cardiol* 2009;103:1463-1466.
5. From AM, Scott CG, Chen HH. The development of heart failure in patients with diabetes mellitus and pre-clinical diastolic dysfunction a population-based study. *J Am Coll Cardiol* 2010;55:300-305.
6. Massie BM, Carson PE, McMurray JJ et al. Irbesartan in patients with heart failure and preserved ejection fraction. *N Engl J Med* 2008;359:2456-2467.
7. Pitt B, Pfeffer MA, Assmann SF et al. Spironolactone for heart failure with preserved ejection fraction. *N Engl J Med* 2014;370:1383-1392.
8. Nagueh SF, Appleton CP, Gillebert TC et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur J Echocardiogr* 2009;10:165-193.
9. Boyer JK, Thanigaraj S, Schechtman KB, Perez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am J Cardiol* 2004;93:870-875.
10. Shishehbor MH, Hoogwerf BJ, Schoenhagen P et al. Relation of hemoglobin A1c to left ventricular relaxation in patients with type 1 diabetes mellitus and without overt heart disease. *Am J Cardiol* 2003;91:1514-7, A9.
11. Stahrenberg R, Edelmann F, Mende M et al. Association of glucose metabolism with diastolic function along the diabetic continuum. *Diabetologia* 2010;53:1331-1340.
12. Di Bonito P, Moio N, Cavuto L et al. Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging. *Diabet Med* 2005;22:1720-1725.
13. Berglund G, Nilsson P, Eriksson KF et al. Long-term outcome of the Malmo preventive project: mortality and cardiovascular morbidity. *J Intern Med* 2000;247:19-29.
14. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. Report of a WHO/IDF consultation. 2006.
http://whqlibdoc.who.int/publications/2006/9241594934_eng.pdf?ua=1

15. Lang RM, Bierig M, Devereux RB et al. Recommendations for chamber quantification. *Eur J Echocardiogr* 2006;7:79-108.
16. Leosdottir M, Willenheimer R, Plehn J et al. Myocardial structure and function by echocardiography in relation to glucometabolic status in elderly subjects from 2 population-based cohorts: a cross-sectional study. *Am Heart J* 2010;159:414-420.e4.
17. Redfield MM, Jacobsen SJ, Burnett JC, Jr, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA* 2003;289:194-202.
18. Abhayaratna WP, Marwick TH, Smith WT, Becker NG. Characteristics of left ventricular diastolic dysfunction in the community: an echocardiographic survey. *Heart* 2006;92:1259-1264.
19. Mureddu GF, Agabiti N, Rizzello V et al. Prevalence of preclinical and clinical heart failure in the elderly. A population-based study in Central Italy. *Eur J Heart Fail* 2012;14:718-729.
20. Lam CS, Lyass A, Kraigher-Krainer E et al. Cardiac dysfunction and noncardiac dysfunction as precursors of heart failure with reduced and preserved ejection fraction in the community. *Circulation* 2011;124:24-30.
21. De Marco M, de Simone G, Roman MJ et al. Cardiac geometry and function in diabetic or prediabetic adolescents and young adults: the Strong Heart Study. *Diabetes Care* 2011;34:2300-2305.
22. Unwin N, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med* 2002;19:708-723.
23. Galderisi M. Diastolic dysfunction and diabetic cardiomyopathy: evaluation by Doppler echocardiography. *J Am Coll Cardiol* 2006;48:1548-1551.
24. Russo C, Jin Z, Homma S et al. Effect of diabetes and hypertension on left ventricular diastolic function in a high-risk population without evidence of heart disease. *Eur J Heart Fail* 2010;12:454-61.
25. Dzau VJ, Antman EM, Black HR et al. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part I: Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). *Circulation* 2006;114:2850-2870.
26. Dzau VJ, Antman EM, Black HR et al. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part II: Clinical trial evidence (acute coronary syndromes through renal disease) and future directions. *Circulation* 2006;114:2871-2891.

27. O'Rourke MF, Safar ME, Dzaou V. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. *Vasc Med* 2010;15:461-468.
28. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol* 2013;62:263-271.
29. World Health Organization. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. Abbreviated Report of a WHO Consultation. 2011.
http://www.who.int/diabetes/publications/report-hba1c_2011.pdf?ua=1
30. Firstenberg MS, Levine BD, Garcia MJ et al. Relationship of echocardiographic indices to pulmonary capillary wedge pressures in healthy volunteers. *J Am Coll Cardiol* 2000;36:1664-1669.

Figures and legends

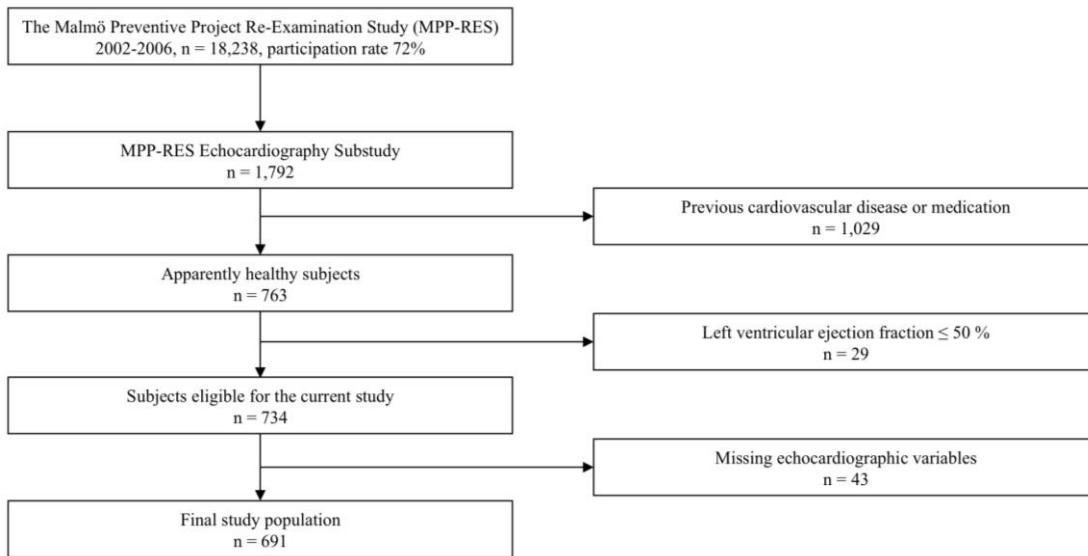


Figure 1: Flowchart showing the study population selection.

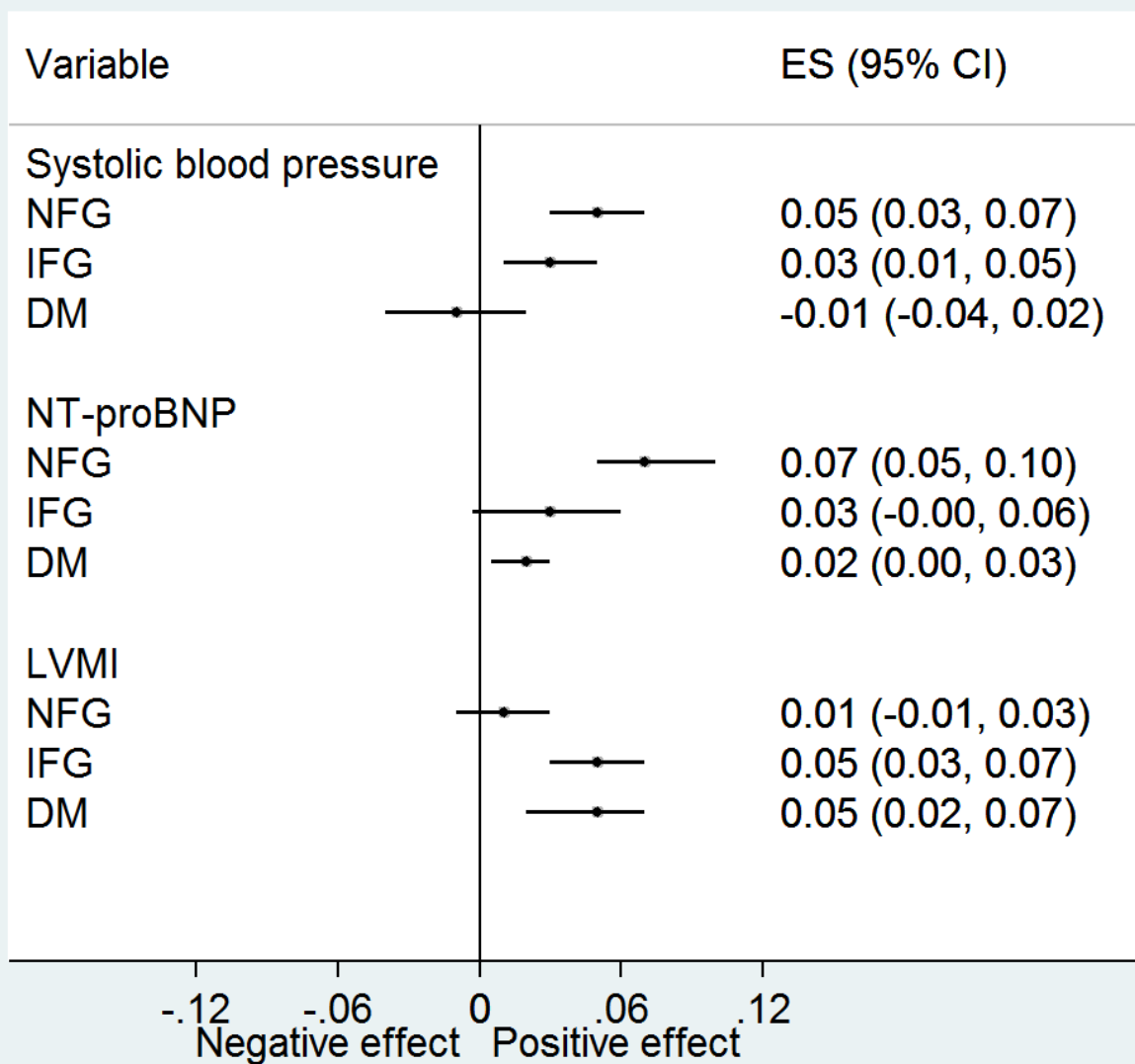


Figure 2a: Forest plot showing the interactions for the linear regression analysis.

For visual purposes, the regression coefficients are shown for every 10 unit increase. NFG: normal fasting glucose; IFG: impaired fasting glucose; DM: diabetes mellitus; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; LVMI: left ventricular mass index; ES: estimate; CI: confidence interval.

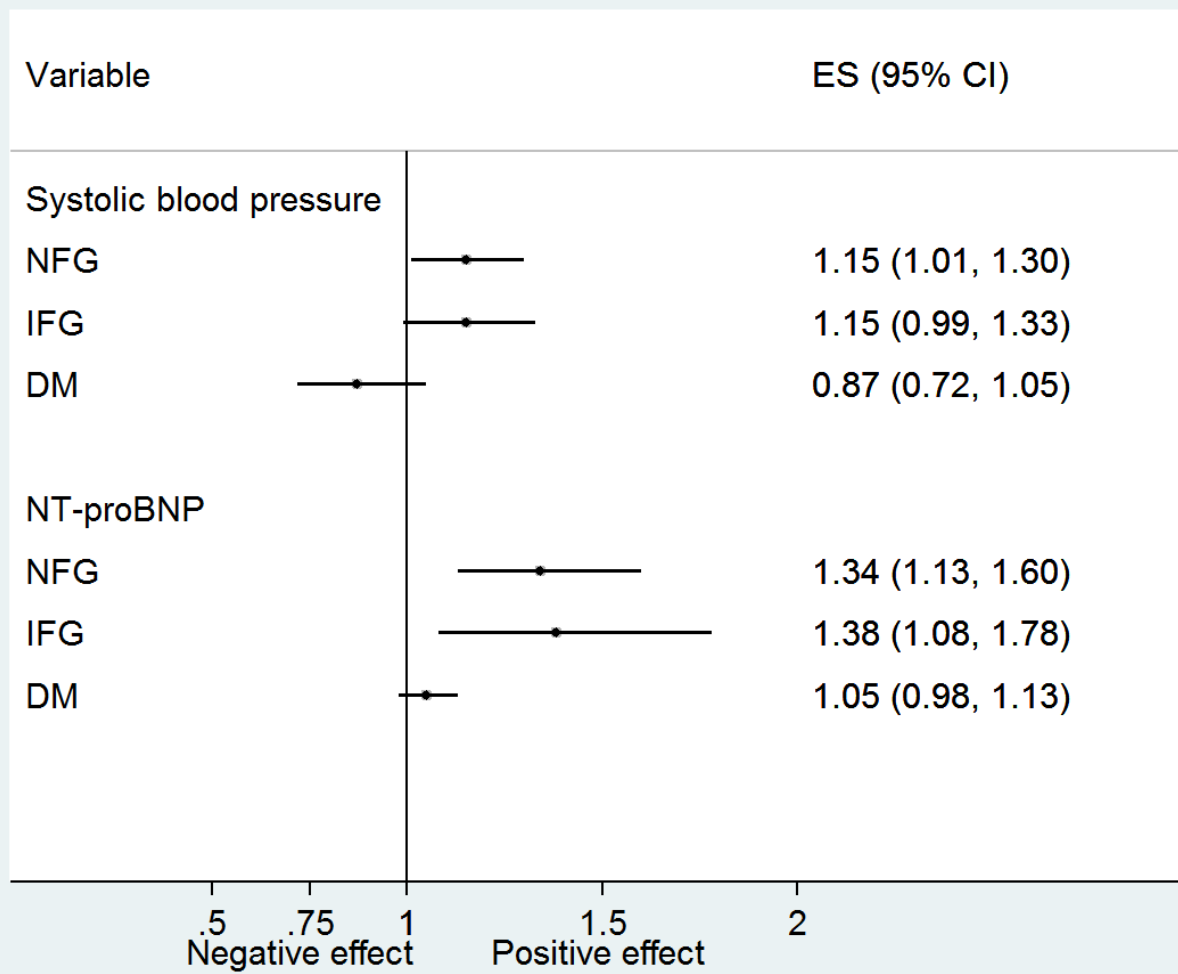


Figure 2b: Forest plot showing the interactions for the ordered logistic regression analysis.

For visual purposes, the odds ratios are shown for every 10 unit increase. NFG: normal fasting glucose; IFG: impaired fasting glucose; DM: diabetes mellitus; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; ES: estimate; CI: confidence interval.

	Grade 0 (normal)	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Septal $\dot{\epsilon}$ (cm/s)	≥ 8	< 8	< 8	< 8
Lateral $\dot{\epsilon}$ (cm/s)	≥ 10	< 10	< 10	< 10
E-wave DT (ms)	140-240	≥ 240	140-240	< 140
E/A	0.8-1.5	< 0.8	0.8-1.5	> 1.5
E/ $\dot{\epsilon}$	< 9	≤ 12	≥ 9	≥ 13

Table 1: Scheme for grading diastolic dysfunction.

Variable	All subjects (n = 691)	Normal fasting plasma glucose (n = 344)	Impaired fasting plasma glucose (n = 240)	Diabetes mellitus (n = 107)	P-value for difference between FPG categories
Male sex	483 (70 %)	206 (60 %)	193 (80 %)	84 (79 %)	< 0.0001*
Age (years)	66 (60-70)	67 (61-70)	64 (59-67)	65 (62-69)	0.002 [§]
Active smoking	120 (17 %)	54 (16 %)	46 (19 %)	20 (19 %)	0.5*
BMI (kg/m ²)	24.0 +/- 3.2	23.5 +/- 3.1	23.9 +/- 2.8	25.6 +/- 3.8	< 0.0001 [†]
Waist circumference (cm)	96 +/- 11	92 +/- 11	98 +/- 10	102 +/- 11	< 0.0001 [†]
Systolic blood pressure (mmHg)	147 +/- 20	143 +/- 18	150 +/- 21	155 +/- 23	< 0.0001 [†]
Pulse rate (beats per minute)	73 +/- 12	71 +/- 12	74 +/- 12	77 +/- 13	< 0.0001 [†]
FPG at first visit (mmol/L)	6.1 (5.4-6.5)	5.4 (5.1-5.7)	6.3 (6.2-6.5)	8.1 (7.4-9.2)	< 0.0001 [§]
Total cholesterol (mmol/L)	5.9 +/- 1.0	5.9 +/- 0.9	5.9 +/- 1.0	6.0 +/- 1.1	0.4 [†]
LDL cholesterol (mmol/L)	3.9 +/- 0.9	3.9 +/- 0.9	3.9 +/- 0.9	4.0 +/- 1.0	0.5 [†]
HDL cholesterol (mmol/L)	1.3 (1.1-1.6)	1.4 (1.2-1.7)	1.3 (1.0-1.5)	1.1 (0.9-1.4)	< 0.0001 [§]
Triglycerides (mmol/L)	1.2 (0.8-1.6)	1.0 (0.8-1.4)	1.2 (0.9-1.6)	1.7 (1.2-2.3)	< 0.0001 [§]
NT-proBNP (pmol/L)	8 (5-15)	10 (5-17)	7 (4-13)	9 (5-16)	< 0.0001 [§]
LVMI (g/m ²)	86 +/- 21	83 +/- 18	87 +/- 21	90 +/- 26	0.01 [†]
EF (%)	62 +/- 6	62 +/- 6	61 +/- 5	61 +/- 5	0.02 [†]
Septal é (cm/s)	8 +/- 3	8 +/- 3	9 +/- 3	8 +/- 3	0.1 [†]
Lateral é (cm/s)	11 +/- 4	11 +/- 3	11 +/- 4	11 +/- 4	0.04 [†]
E-wave DT (ms)	220 +/- 50	221 +/- 51	218 +/- 48	224 +/- 51	0.6 [†]
E/A	0.9 +/- 0.2	0.9 +/- 0.2	0.9 +/- 0.2	0.9 +/- 0.2	0.5 [†]
E/é	7 (6-10)	8 (6-10)	7 (6-9)	8 (6-11)	0.02 [§]
E/é ≥ 13	51 (7 %)	21 (6 %)	14 (6 %)	16 (15 %)	0.005*
LAA (cm ²)	19 +/- 3	19 +/- 3	19 +/- 3	20 +/- 4	0.001 [†]
Diastolic dysfunction	116 (18 %)	60 (18 %)	29 (13 %)	30 (25 %)	0.02*

Table 2a: Baseline characteristics according to fasting plasma glucose category.

Categorical variables (male sex, active smoking, E/é ≥ 13, grade 2 or 3 diastolic dysfunction) are given as n (%), whereas continuous variables are given as mean +/- SD (approximately normally distributed variables, i.e. BMI, waist circumference, systolic blood pressure, pulse rate, total cholesterol, LDL cholesterol, LVMI, EF, septal é, lateral é, DT, E/A, and LA area) or median (IQR) (non-normally distributed variables, i.e. age, FPG, HDL cholesterol, triglycerides, NT-proBNP, and E/é).

*Pearson's χ^2 -test; [†]One-way ANOVA; [§]Kruskal-Wallis test.

Variable	Normal diastolic function (n = 456)	Grade 1 (mild) diastolic dysfunction (n = 90)	Grade 2 or 3 (moderate or severe) diastolic dysfunction (n = 116)	P-value for difference between categories
Male sex	344 (75 %)	61 (68 %)	59 (51 %)	< 0.0001*
Age (years)	63 (58-67)	69 (65-74)	70 (67-74)	< 0.0001 [§]
BMI (kg/m ²)	23.7 +/- 3.1	24.0 +/- 3.1	24.9 +/- 3.4	0.001 [†]
Systolic blood pressure (mmHg)	146 +/- 20	146 +/- 18	149 +/- 20	0.2 [†]
FPG at first visit (mmol/L)	6.1 (5.5-6.5)	5.7 (5.3-6.3)	6.0 (5.2-7.1)	0.07 [§]
Total cholesterol (mmol/L)	5.9 +/- 1.0	6.0 +/- 0.9	6.0 +/- 1.0	0.4 [†]
NT-proBNP (pmol/L)	7 (4-13)	10 (6-18)	15 (7-23)	< 0.0001 [§]
LVMI (g/m ²)	83 +/- 20	90 +/- 22	92 +/- 22	< 0.0001 [†]
EF (%)	61 +/- 5	62 +/- 6	65 +/- 6	< 0.0001 [†]
Septal é (cm/s)	10 +/- 2	6 +/- 1	6 +/- 1	< 0.0001 [†]
Lateral é (cm/s)	13 +/- 3	8 +/- 2	7 +/- 2	< 0.0001 [†]
E-wave DT (ms)	214 +/- 44	268 +/- 52	207 +/- 50	< 0.0001 [†]
E/A	1.0 +/- 0.2	0.7 +/- 0.1	0.9 +/- 0.2	< 0.0001 [†]
E/é	6 (5-8)	8 (7-9)	13 (11-15)	< 0.0001 [§]
E/é ≥ 13	3 (1 %)	0	48 (41 %)	< 0.0001*
LAA (cm ²)	19 +/- 3	18 +/- 4	20 +/- 4	< 0.001 [†]

Table 2b: Baseline characteristics according to diastolic function.

Only variables that were significant in at least one univariate analysis or were relevant for grading diastolic function are shown. Categorical variables (male sex, E/é ≥ 13) are given as n (%), whereas continuous variables are given as mean +/- SD (approximately normally distributed variables, i.e. BMI, systolic blood pressure, total cholesterol, LVMI, EF, septal é, lateral é, E/A, DT, and LA area) or median (IQR) (non-normally distributed variables, i.e. age, FPG, NT-proBNP, and E/é).

*Pearson's χ^2 -test; [†]One-way ANOVA; [§]Kruskal-Wallis test.

Variable	Undetermined diastolic dysfunction (n = 29)
Male sex	19 (66 %)
Age (years)	69 (67-73)
BMI (kg/m ²)	24.4 +/- 3.5
Systolic blood pressure (mmHg)	157 +/- 23
FPG at first visit (mmol/L)	5.3 (5.8-6.2)
Total cholesterol (mmol/L)	6.2 +/- 1.1
NT-proBNP (pmol/L)	11 (7-21)
LVMI (g/m ²)	85 +/- 19
EF (%)	64 +/- 7
Septal é (cm/s)	6 +/- 1
Lateral é (cm/s)	8 +/- 2
E-wave DT (ms)	220 +/- 47
E/A	0.9 +/- 0.3
E/é	11 (10-11)
LAA (cm ²)	20 +/- 4

Table 2c: Baseline characteristics in subjects with undetermined dysfunction.

Only variables that were significant in at least one univariate analysis or were relevant for grading diastolic function are shown. Categorical variables (male sex, E/é ≥ 13) are given as n (%), whereas continuous variables are given as mean +/- SD (approximately normally distributed variables, i.e. BMI, systolic blood pressure, total cholesterol, LVMI, EF, septal é, lateral é, E/A, DT, and LA area) or median (IQR) (non-normally distributed variables, i.e. age, FPG, NT-proBNP, and E/é).

Risk factor	Beta-coefficient (β) (95% CI)	P-value
Age (per year)	0.03 (0.02 to 0.03)	< 0.0001
Female sex	0.13 (0.07 to 0.18)	< 0.0001
BMI (per kg/m ²)	0.006 (-0.002 to 0.01)	0.1
Systolic blood pressure (per mmHg)	0.001 (0.0003 to 0.003)	0.01
Left ventricular mass index (per g/m ²)	0.002 (0.001 to 0.003)	< 0.0001
Fasting plasma glucose (FPG) category		
Normal fasting glucose (NFG) (reference)		
Impaired fasting glucose (IFG)	-0.006 (-0.06 to 0.04)	0.8
Diabetes mellitus (DM)	0.05 (-0.02 to 0.12)	0.2

Table 3a: Multivariable linear regression model for the prediction of $\log(E/\acute{e})$ (adjusted $r^2 = 0.318$).

The following risk factors were significantly associated with $\log(E/\acute{e})$ in univariate analysis only: total cholesterol and NT-proBNP.

Risk factor	Odds ratio (OR) (95% CI)	P-value
Age (per year)	1.20 (1.16 to 1.25)	< 0.0001
Female sex	1.45 (0.97 to 2.17)	0.07
Left ventricular mass index (per g/m ²)	1.01 (1.01 to 1.02)	0.002
Fasting plasma glucose (FPG) category		
Normal fasting glucose (NFG) (reference)		
Impaired fasting glucose (IFG)	0.82 (0.54 to 1.25)	0.4
Diabetes mellitus (DM)	1.12 (0.67 to 1.87)	0.7

Table 3b: Ordered logistic regression model for the prediction of worsening diastolic function (pseudo-r² = 0.158).

The following risk factors were significantly associated with diastolic function in univariate analysis only: BMI and NT-proBNP.