HbA_{1c} Identifies Subjects With Prediabetes and Subclinical Left Ventricular Diastolic Dysfunction

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Context: Prediabetes is associated with subclinical cardiac changes associated with heart failure development.

Objective: We investigated diastolic function and its association with markers of glycation and inflammation related to cardiovascular disease in patients with prediabetes. We focused on individuals with prediabetes identified only by glycated hemoglobin A_{1c} [HbA_{1c}; 5.7% to 6.4% and normal fasting glucose (NFG) and normal glucose tolerance (NGT) after an oral glucose tolerance test (OGTT)].

Design: Cross-sectional study.

Setting: Departments of Clinical and Experimental Medicine and Cardiology, University of Catania, Catania, Italy.

Main Outcome Measures: HbA_{1c}, OGTT, Doppler echocardiography, soluble receptor for advanced glycation end products (sRAGEs), and endogenous secretory RAGE (esRAGE) were evaluated.

Patients: We recruited 167 subjects with NFG/NGT who were stratified according to HbA_{1c} level: controls (HbA_{1c} <5.7%) and HbA_{1c} prediabetes (HbA_{1c} 5.7% to 6.4%).

Results: Patients with HbA_{1c} prediabetes (n = 106) showed a lower peak mitral inflow in early diastole (E wave) to late diastolic atrial filling velocity (A wave) ratio (E/A ratio) than controls (n = 61) (1.10 ± 0.24 vs 1.18 ± 0.23; $P < 0.05$). They showed a higher left atrium volume (LAV) (28.4 ± 5 vs 22.1 ± 3; $P < 0.05$) and sphericity index (SI) (0.6 ± 0.06 vs 0.5 ± 0.05; $P < 0.05$). After multiple regression analyses, HbA_{1c}, sRAGE, and esRAGE were the major determinants of E/A ratio, LAV, and SI.

Conclusions: Subjects with HbA_{1c} prediabetes exhibited subclinical cardiac alterations associated with sRAGE, esRAGE, and HbA_{1c}. These subjects would not have been classified as having prediabetes on the basis of fasting glycemia or post-OGTT values. (*J Clin Endocrinol Metab* 102: 3756–3764, 2017)

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Abbreviations: AGE, advanced glycation end product; ApoB/ApoA1, Apolipoprotein B/Apolipoprotein A1; BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; E/A ratio, peak mitral inflow in early diastole (E wave) to late diastolic atrial filling velocity (A wave) ratio; E/e′ ratio, mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e′); ELISA, enzyme-linked immunosorbent assay; esRAGE, endogenous secretory receptor for advanced glycation end product; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; LAV, left atrium volume; LDL, low-density lipoprotein; LV, left ventricular; LVM, left ventricular mass; NFG, normal fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; RAGE, receptor for advanced glycation end product; RWT, relative wall thickness; SI, sphericity index; sRAGE, soluble receptor for advanced glycation end product; TDI, tissue Doppler imaging.

Type 2 diabetes mellitus is an established risk factor for heart failure development, independent of other conditions such as hypertension and coronary artery disease, and diastolic dysfunction represents one of the first manifestations of diabetic cardiomyopathy (1, 2). Previous data indicated that early detection of diabetic heart disease should be a clinical priority because timely prevention programs or medical intervention may avoid or delay the development of heart failure, which is a major cause of morbidity and mortality in these patients (3).

Events portending cardiovascular disease are under way before the formal diagnosis of diabetes, and a consistent body of studies has demonstrated the association between changes in cardiac function and early alterations of glucose homeostasis, such as prediabetes (4). According to these data, patients with prediabetes may represent a specific population in which prevention programs should be applied (5).

Previous evidence showed that subjects with prediabetes or with normal fasting glucose (NFG) and normal glucose tolerance (NGT) values are not a homogenous group, and they may present with different degrees of cardiometabolic risk (6). In recent studies, we analyzed the metabolic profiles of subjects with prediabetes identified according to glycated hemoglobin A_{1c} (HbA_{1c}) value (5.7% to 6.4%) and NFG/NGT values after an oral glucose tolerance test (OGTT). We found alterations of early markers of cardiovascular risk, an impaired inflammatory profile, and low vitamin D levels (7–10), suggesting an impaired cardiometabolic profile in these subjects, who would not have been classified as having prediabetes on the basis of fasting or post-OGTT value.

The pathogenic mechanism underlying myocardium impairment in patients with alterations of glucose homeostasis is most likely linked to multiple and complex metabolic reactions, including insulin signaling, lipotoxicity, and increased inflammatory state (11). Several studies showed the relevant role of advanced glycation end products (AGEs) and their receptors (RAGEs) in the pathogenesis of type 2 diabetes complications in various organs, including the heart (12). RAGE is also found in a soluble form (sRAGE) primarily formed by the proteolytic cleavage of membrane-bound RAGE and secondarily by a secreted, non−membrane-bound form of the receptor resulting from alternative splicing of the *RAGE* gene, which is known as endogenously secreted RAGE (esRAGE). esRAGE, which may contribute to the removal/neutralization of circulating ligands, thus functions as a decoy by competing with cell-surface RAGE for ligand binding (13). A critical role of the AGE/RAGE axis has been explored in diabetic cardiomyopathy, cardiac ischemia damage, and accelerated atherosclerosis (14); furthermore, in animal models, RAGE blockade protected the animals from the development of diastolic dysfunction, attenuating myocardial collagen expression (15).

Given the expected increased use of HbA_{1c} as a screening tool to identify individuals with alterations of glycemic homeostasis, it is clinically important to evaluate the ability of HbA_{1c} to identify patients who have early alterations of myocardial function. In this study, we evaluated diastolic function and examined its association with markers of glycation and inflammation strictly related with cardiovascular disease in patients with prediabetes identified only with HbA_{1c}.

Research Design and Methods

Study subjects

We recruited 167 subjects (age range, 18 to 65 years) who had no previous diagnosis of diabetes and who attended our university hospital for diabetes and cardiovascular risk evaluation. Inclusion criteria were an age range between 18 and 65 years; body mass index (BMI) between 18.5 and 40 kg/m^2; baseline systolic blood pressure (BP) level <150 mm Hg; baseline diastolic BP level <100 mm Hg, and Caucasian race. Exclusion criteria were a history of diabetes; impaired fasting glucose level; impaired glucose tolerance (IGT); new-onset type 2 diabetes according to fasting glucose level, OGTT, or HbA_{1c} value; history of overt cardiovascular events (stroke, ischemic heart disease, chronic obstructive peripheral arteriopathy, or heart failure), anemia or hemoglobinopathies, use of medications affecting glucose metabolism, malignant disease, and drug or alcohol abuse.

All patients underwent a physical examination and review of their clinical history, smoking status, and alcohol consumption. After a 12-hour fast, the participants had standard hematological and clinical biochemistry parameters measured [fasting glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, high-sensitivity C-reactive protein (hs-CRP)] and a 75-g OGTT administered, as previously described (16). Patients with NFG/NGT were recruited. At the second visit, blood was drawn for HbA_{1c} measurement and evaluation of the AGE/RAGE axis. Patients with an HbA_{1c} value <5.7% were considered control subjects; patients with an HbA_{1c} value between 5.7% and 6.4% were considered prediabetic subjects according to the American Diabetes Association (17). An echocardiographic study was performed within 1 week from the first visit by a single operator who was blinded to the clinical data.

Body weight and height were measured, and BMI was calculated as weight (kg)/[height (m^2)]. BP level was measured with a calibrated sphygmomanometer after 10 minutes of resting. Low-density lipoprotein (LDL) cholesterol concentrations were estimated using the Friedewald formula.

Biochemical analyses

Plasma glucose, serum total cholesterol, triglyceride, HDL cholesterol, and hs-CRP were measured using available enzymatic methods as previously described (18).

To quantify plasma concentrations of sRAGE (Human sRAGE enzyme-linked immunosorbent assay [ELISA]; Biovendor, Brno, Czech Republic), esRAGE (B-Bridge esRAGE ELISA Kit, Cupertino, CA), and S100A12 (ELISA Kit for S1000A12; Cloud-Clone Corp., Houston, TX), fasting blood samples were collected and specimens were centrifuged and stored at −80°C. Commercially available ELISA kits were used according to the manufacturers' instructions. The interassay and intra-assay coefficients of variation ranged from 5.5% to 8.8% and from 2.6% to 5.3%, respectively, for sRAGE and from 5.9% to 7.5% and from 0.7% to 1.5%, respectively, for esRAGE and were <10% and 12%, respectively, for S100A12.

HbA_{1c} value was measured via high-performance liquid chromatography using the National Glycohemoglobin Standardization Program and was standardized to the Diabetes Control and Complications Trial assay reference (19). Chromatography was performed using a certified automated analyzer (HLC-723G7 hemoglobin HPLC analyzer; Tosoh Corp., Tokyo, Japan), normal range 4.25% to 5.9% (23 to 41 mmol/mol).

Cardiac ultrasound examination

Echocardiographic examination was performed with all subjects in left lateral decubitus using a GE Vivid 9 Ultrasound system (GE Healthcare, Horten, Norway) equipped with a multifrequency MS5 probe. Two-dimensional guided M-mode parameters were measured by a single operator who was blinded to clinical data, according to the recommendations of the American Society of Echocardiography (20). Left atrium volume (LAV) was measured from the apical four- and two-chamber views using the Simpson rule. Careful attention was paid to avoid left atrial foreshortening. LAV was then indexed for body surface area to obtain the LAV index.

Left ventricular mass (LVM) was calculated using the Devereux formula and normalized by body surface area (LVM index). Partition values for left ventricular hypertrophy were taken with the cutoff of 115 g/m^2 for men and 95 g/m^2 for women. Finally, calculation of relative wall thickness (RWT) with the equation [(2 left ventricular [LV] posterior wall thickness)/(LV internal diameter at end-diastole)] permitted categorization of an increase in LVM as either concentric (RWT >0.42) or eccentric (RWT ≤0.42) hypertrophy and allowed the identification of concentric remodeling (normal LVM with increased RWT).

From the parasternal long-axis view, the following parameters were obtained: LV end-diastolic thickness of interventricular septum and posterior wall and LV end-diastolic and end-systolic diameters. RWT was calculated using the formula RWT = 2 LV end-diastolic thickness of posterior wall/LV end-diastolic diameter.

For the assessment of mitral inflow velocities, the sampling volume of pulsed-wave Doppler was placed at the tip level of the mitral leaflets from the apical four-chamber view. The following parameters were measured: the peak mitral inflow in early diastole (E wave), the late diastolic atrial filling velocity (A wave), and their ratio (E/A ratio).

Tissue Doppler imaging (TDI) in the pulsed-wave Doppler modality was used for the assessment of myocardial velocities and was applied at the septal and lateral sides of the mitral annulus obtaining systolic myocardial velocities (S′ wave) and early and late diastolic myocardial velocities (E′ and A′ waves, respectively). All the TDI measurements were obtained by averaging the values at the septal and lateral mitral annulus. Timings of LV isovolumic contraction, ejection, and isovolumic relaxation were measured from the pulse wave-TDI traces, obtaining the myocardial performance index as in this formula: LV myocardial performance index = (LV isovolumic contraction timing + LV isovolumic relaxation timing)/LV ejection timing (21). Finally, the ratio between mitral peak velocity of early filling (E) and early diastolic mitral annular velocity (E′) (E/e′ ratio) was calculated and considered as an index of LV filling pressures (22).

Mitral regurgitation (when present) was evaluated and graded according to American Society of Echocardiography criteria (23).

The sphericity index (SI) was calculated as the ratio between the greater cross-sectional diameter and the greater longitudinal diameter of the LV in the end-diastolic apical four-chamber view. This index was used as an indicator of geometry change.

Statistical analyses

The sample size was calculated according to the E/A ratio using a level of significance (α) set to 5% and power (1-β) set to 80%. We based the power calculation on previous studies examining the E/A ratio among patients with early alteration of glucose homeostasis and controls (24). The estimated sample size was 50 patients per group. Statistical comparisons of clinical and biomedical parameters were performed using StatView 6.0 for Windows. Data are given as means ± standard deviation or median (interquartile range). Each variable's distributional characteristics, including normality, were assessed with the Kolmogorov-Smirnov test. Statistical analyses included the unpaired t test for continuous variables and the χ^2 test for noncontinuous variables. A P value <0.05 was considered statistically significant. When necessary, numerical variables were logarithmically transformed to reduce skewness, and values were expressed as median and interquartile range.

Simple regression analysis was performed to relate E/A ratio, E/e′ ratio, LAV, and SI to the clinical and metabolic characteristics of the patients (age, BMI, sex, smoking status, systolic and diastolic BP, total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, Apolipoprotein B/Apolipoprotein A1 (ApoB/ApoA1) ratio, homeostatic model assessment for insulin resistance (HOMA-IR), HbA_{1c}, fasting glycemia, uric acid, hs-CRP, S100A12, sRAGE, and esRAGE). To identify variables independently associated with variations of E/A ratio, E/e′ ratio, LAV, and SI, we performed multiple regression analyses including the variables reaching statistical significance in simple regression analysis. The variance inflation factor was used to check for the problem of multicollinearity in multiple regression analysis.

The local ethics committee approved the study. Informed consent was obtained from each participant.

Results

In total, 167 subjects participated in the study. The study population was divided into two groups on the basis of fasting glucose, OGTT, and HbA_{1c} levels according to American Diabetes Association recommendations: 61 control subjects (HbA_{1c} value <5.7% and

with NFG/NGT) and 106 patients with HbA_{1c} prediabetes (HbA_{1c} value 5.7% to 6.4% and with NFG/NGT).

The clinical and biochemical characteristics of the study subjects are presented in Table 1. Although in the range of NFG and NGT, subjects with HbA_{1c} prediabetes showed higher fasting and 2-hour postload glycemia than controls (89.2 ± 6.2 vs 85.3 ± 8 mg/dL; $P < 0.05$ and 121.1 ± 8.9 vs 112.3 ± 7.7 mg/dL; $P < 0.05$, respectively). Furthermore, they had higher uric acid plasma level (5.1 ± 1.5 vs 4.4 ± 1.1 mg/dL; $P < 0.05$) and ApoB/ApoA1 ratio (0.73 ± 0.22 vs 0.63 ± 0.24; $P < 0.05$).

There were no differences among the groups in the number and class of antihypertensive drugs used in hypertensive patients ($P = 0.68$). Only two patients in the control group and five patients in the prediabetic group were in double therapy. No triple-therapy patients were represented in this study. Four patients in the control group and 10 patients in the prediabetic group ($P = 0.74$) were considered to have new-onset hypertension (systolic BP level >135 and <150 mm Hg) without any therapy.

Circulating levels of hs-CRP and S100A12 were higher in subjects with HbA_{1c} prediabetes than in controls [0.18 (0.08 to 0.39) vs 0.14 (0.07 to 0.15) mg/dL, $P < 0.05$; 6.9 ± 5.2 vs 4.7 ± 2.9 ng/mL, $P < 0.05$, respectively] (Table 1). esRAGE plasma level was significantly lower in subjects with HbA_{1c} prediabetes than in controls (0.41 ± 0.18 vs 0.56 ± 0.23 ng/mL; $P < 0.05$). As shown in Table 1, sRAGE plasma levels were slightly lower in the group with prediabetes without statistical significance (1.30 ± 0.53 vs 1.45 ± 0.62 ng/mL; $P < 0.07$).

Echocardiographic parameters according to HbA_{1c} levels are reported in Table 2 for the study population. Patients with HbA_{1c} prediabetes exhibited a lower E/A ratio than controls (1.10 ± 0.24 vs 1.18 ± 0.23; $P < 0.05$). Furthermore, they showed a higher left atrium area and volume (28 ± 5 vs 22 ± 3; $P < 0.05$ and 15.5 ± 3.6 vs 13.2 ± 2.5; $P < 0.05$, respectively). The E/e′ ratio significantly increased from controls to patients with prediabetes (7.2 ± 2.5 vs 7.8 ± 2.2; $P < 0.05$). Interestingly, the SI was significantly higher in patients with prediabetes (0.6 ± 0.06 vs 0.5 ± 0.05; $P < 0.05$).

Simple regression analysis was performed to test the relationships between echocardiographic parameters and different clinical variables.

Table 1. Clinical Characteristics and Inflammatory Variables of the Study Population According to HbA_{1c} Level

	Controls (HbA_{1c} Value <5.7% and NFG/NGT) (n = 61)	HbA_{1c} Prediabetes (HbA_{1c} Value 5.7% to 6.4% and NFG/NGT) (n = 106)	*P* Value
Clinical variables			
Age, y	48 ± 8.4	50.4 ± 8.9	0.1
BMI, kg/m^2	28.4 ± 3.1	28.7 ± 3.9	0.63
Fasting glucose, mg/dL	85.3 ± 8	89.2 ± 6.2	0.00
30-min postload glucose, mg/dL	135.2 ± 12.3	140.4 ± 15.6	0.01
60-min postload glucose, mg/dL	139.8 ± 15.1	162 ± 21.3	0.01
90-min postload glucose, mg/dL	126.5 ± 11.2	142.1 ± 18.1	0.00
2-h postload glucose, mg/dL	112.3 ± 7.7	121.1 ± 8.9	0.01
Fasting insulin, μU/mL	5.8 (4.1–9.6)	6.7 (4.8–10)	0.22
Total cholesterol, mg/dL	201.1 ± 41.5	207.7 ± 43	0.25
HDL cholesterol, mg/dL	49.7 ± 11.5	47.6 ± 37.7	0.31
Triglycerides, mg/dL	101 (67–118)	102 (71–160)	0.28
LDL cholesterol, mg/dL	130.3 ± 38	135 ± 37.7	0.47
Systolic BP, mm Hg	119 ± 13.6	122.4 ± 13	0.78
Diastolic BP, mm Hg	70.1 ± 9.9	72.2 ± 9.8	0.29
ApoA1, mg/dL	143.5 ± 22.3	138.3 ± 24.7	0.31
ApoB, mg/dL	89.4 ± 28.5	96.1 ± 23.3	0.22
ApoB/ApoA1 ratio	0.63 ± 0.24	0.73 ± 0.22	0.04
Uric acid, mg/dL	4.4 ± 1.1	5.1 ± 1.5	0.01
HOMA-IR	1.5 (0.81–2.3)	1.9 (1–2.5)	0.16
Active smokers	33%	33%	0.45
Hypertension	32%	34%	0.53
Sex (M/F), %	35%	38%	0.35
Inflammatory markers			
hs-CRP, mg/dL	0.14 (0.07–0.15)	0.18 (0.08–0.39)	0.04
S100A12, ng/mL	4.7 ± 2.9	6.9 ± 5.2	0.05
sRAGE, ng/mL	1.45 ± 0.62	1.30 ± 0.53	0.07
esRAGE, ng/mL	0.56 ± 0.23	0.41 ± 0.18	0.00

Data are presented as mean ± standard deviation or median (interquartile range). Smoking was quantified (number of cigarettes and years smoked), and smoking status was classified as active and nonsmoker. Hypertension was defined as systolic BP level >135 mm Hg or diastolic BP level >85 mm Hg or as taking any hypertension medications.

Table 2. Echocardiographic Variables of the Study Population According to HbA_{1c} Level

	Controls (HbA_{1c} Value <5.7% and NFG/NGT) (n = 61)	HbA_{1c} Prediabetes (HbA_{1c} Value 5.7% to 6.4% and NFG/NGT) (n = 106)	*P* Value
Echo Doppler parameters			
E/A ratio	1.18 ± 0.23	1.10 ± 0.24	0.04
DT, m/s	181 ± 14	188 ± 15	0.21
IVRT, m/s	74 ± 7	70 ± 8	0.35
Tissue Doppler parameters			
Septal e′, cm/s	12.2 ± 2.0	11.3 ± 1.8	0.45
Lateral e′, cm/s	18.8 ± 1.6	16.8 ± 1.8	0.32
E/e′ ratio	7.2 ± 2.2	7.8 ± 2.5	0.04
Echo M-mode 2D parameters			
IVS diastolic dimension, cm	1.02 ± 0.15	1.07 ± 0.18	0.45
PW diastolic dimension, cm	1.08 ± 0.12	1.09 ± 0.19	0.71
LVM, g	143 ± 14	153 ± 16	0.32
RWT	0.40 ± 0.6	0.41 ± 1.1	0.50
LV internal dimension			
Diastolic dimension, mm	46 ± 3.1	47.1 ± 3.7	0.6
Systolic dimension, mm	27.1 ± 4.3	28.3 ± 4.7	0.7
LV volumes, biplane			
LV EDV, mL	82 ± 8.0	87 ± 7.5	0.36
EF, %	61 ± 6	63 ± 5	0.5
FS, %	38 ± 3	40 ± 2	0.48
LAV/BSA, mL/m^2	22 ± 3	28 ± 5	0.01
Left atrium area, mm	13.2 ± 2.5	15.5 ± 3.6	0.02
SI	0.5 ± 0.05	0.6 ± 0.06	0.01
MAPSE, mm	15 ± 1	15 ± 2	0.6
TAPSE, mm	22 ± 3	21 ± 3	0.2

Data are presented as mean ± standard deviations.

Abbreviations: 2D, two-dimensional; BSA, body surface area; DT, deceleration time; EF, ejection fraction; FS, fractional shortening; IVRT, isovolumetric relaxation time; IVS, interventricular septal thickness; LV EDV, left ventricular end-diastolic volume; MAPSE, mitral annular plane systolic excursion; PW, pulse wave; TAPSE, tricuspid annular plane systolic excursion.

The E/A ratio was associated with age ($r = -0.22$; $P < 0.005$), BMI ($r = 0.33$; $P < 0.0001$), total cholesterol ($r = 0.18$; $P < 0.02$), LDL cholesterol ($r = 0.15$; $P < 0.05$), HbA_{1c} ($r = 0.2$; $P < 0.02$), uric acid ($r = 0.24$; $P < 0.01$), HOMA-IR ($r = 0.18$; $P < 0.02$), hs-CRP ($r = 0.35$; $P < 0.001$), S100A12 ($r = 0.23$; $P < 0.01$), sRAGE ($r = -0.28$; $P < 0.01$), and esRAGE ($r = -0.22$, $P < 0.02$). To identify variables independently associated with variations of the E/A ratio, variables reaching statistical significance were inserted in a multiple regression model. To avoid the problem of multicollinearity between variables (total cholesterol, non−HDL cholesterol, and LDL cholesterol), only LDL cholesterol was used as an independent variable in the model. The multivariate analysis including each of the components reaching a *P* value < 0.05 in the simple regression analysis showed a correlation only with age, HbA_{1c}, and esRAGE (Table 3).

We then performed further analyses focusing only on patients with HbA_{1c} prediabetes (n = 106). In simple regression analysis, the E/A ratio was associated with age ($r = -0.24$; $P < 0.01$), BMI ($r = -0.20$; $P < 0.008$), HbA_{1c} ($r = -0.18$; $P < 0.05$), S100A12 ($r = -0.23$; $P < 0.01$), hs-CRP ($r = -0.17$; $P < 0.03$), and sRAGE ($r = 0.33$; $P < 0.02$). The multivariate analysis showed a correlation only with age, HbA_{1c}, S100A12, and sRAGE (Table 4).

The E/e′ ratio showed a significant relationship with age ($r = 0.21$; $P < 0.05$), systolic BP ($r = -0.19$; $P < 0.05$), LDL cholesterol ($r = 0.18$; $P < 0.05$), HbA_{1c} ($r = 0.20$; $P < 0.05$), HOMA-IR ($r = 0.18$; $P < 0.05$), and hs-CRP ($r = 0.16$; $P < 0.05$) in a simple regression analysis. Multiple regression analysis including significant variables showed an independent association between E/e′ ratio and age, systolic BP, HbA_{1c} and hs-CRP (Table 3).

Simple regression analysis showed a significant relationship between LAV and BMI ($r = 0.22$; $P < 0.005$), HDL cholesterol ($r = -0.15$; $P < 0.05$), ApoB/ApoA1 ratio ($r = 0.25$; $P < 0.02$), HbA_{1c} ($r = 0.18$; $P < 0.02$), and sRAGE ($r = -0.21$; $P < 0.05$). Multiple regression analysis including significant variables showed an independent association between LAV, HbA_{1c}, and sRAGE (Table 3). When we analyzed only patients with HbA_{1c} prediabetes, we found significant relationships between LAV and BMI ($r = 0.23$; $P < 0.001$), HDL cholesterol ($r = -0.22$; $P < 0.02$), HbA_{1c} ($r = 0.26$; $P < 0.005$), HOMA-IR ($r = 0.18$; $P < 0.02$), and sRAGE ($r = -0.31$; $P < 0.01$). Multiple regression analysis showed an independent association between LAV, HbA_{1c}, and sRAGE (Table 4).

In the simple regression analysis, SI was significantly related with total cholesterol ($r = 0.28$; $P < 0.005$), LDL

Table 3. Multiple Regression Analysis Evaluating E/A Ratio, E/e′ Ratio, LAV, and SI as Dependent Variables

	Coefficient β	*P* Value
E/A ratio		
Age, y	−0.03	0.02
BMI, kg/m^2	−0.036	0.10
LDL cholesterol, mg/dL	−0.04	0.19
HbA_{1c}, %	−0.39	0.04
Uric acid, mg/dL	−0.43	0.22
HOMA-IR	0.12	0.49
hs-CRP, mg/dL	0.09	0.48
S100A12, ng/mL	−2.7	0.41
sRAGE, ng/mL	0.39	0.07
esRAGE, ng/mL	−0.43	0.02
E/e′ ratio		
Age, y	0.001	0.01
Systolic BP, mm Hg	0.24	0.02
LDL cholesterol, mg/dL	0.16	0.21
HbA_{1c}, %	0.14	0.02
HOMA-IR	−0.002	0.17
hs-CRP, mg/dL	0.02	0.01
LAV/BSA		
BMI, kg/m^2	0.4	0.43
HDL cholesterol mg/dL	0.24	0.1
ApoB/ApoA1 ratio	0.03	0.31
HbA_{1c}, %	0.67	0.01
sRAGE, ng/mL	−0.2	0.03
SI		
LDL cholesterol, mg/dL	1.2	0.04
Fasting glycemia, mg/dL	0.01	0.16
HbA_{1c}, %	0.21	0.02

Abbreviation: BSA, body surface area.

cholesterol ($r = 0.29$; $P < 0.004$), non-HDL cholesterol ($r = 0.29$; $P < 0.004$), HbA_{1c} ($r = 0.20$; $P < 0.01$), and fasting glycemia level ($r = 0.16$; $P < 0.04$). To identify variables independently associated with variations of SI, variables reaching statistical significance were inserted in a multiple regression model. To avoid the problem of multicollinearity between variables (total cholesterol, LDL cholesterol, and non−HDL cholesterol), only LDL cholesterol was used as an independent variable in the model. Multiple regression analysis showed that SI was independently associated only with HbA_{1c} and LDL cholesterol (Table 3).

Finally, we performed subgroup analysis on the HbA_{1c} prediabetes group; SI was significantly related with total cholesterol ($r = 0.31$; $P < 0.002$), LDL cholesterol ($r = 0.30$; $P < 0.002$), fasting glucose level ($r = 0.18$; $P < 0.05$), and sRAGE ($r = -0.29$; $P < 0.05$). Multiple regression analysis showed that SI was independently associated only with LDL cholesterol and sRAGE (Table 4).

Discussion

In this study, we examined selected echocardiographic parameters and their association with inflammatory factors strictly bounded with cardiovascular risk in

Table 4. Multiple Regression Analysis Evaluating E/A Ratio, LAV, and SI as Dependent Variables for the Prediabetic Group

	Coefficient β	*P* Value
E/A ratio		
Age, y	−0.14	0.001
BMI, kg/m^2	−0.09	0.43
HbA_{1c}, %	−0.23	0.001
S100A12, ng/mL	−0.22	0.01
Hs-CRP, mg/dL	−0.03	0.14
sRAGE, ng/mL	0.31	0.03
LAV/BSA		
BMI, kg/m^2	−0.15	0.5
HDL cholesterol, mg/dL	0.13	0.21
HbA_{1c}, %	0.26	0.007
HOMA-IR	0.06	0.90
sRAGE, ng/dL	−0.21	0.005
SI		
LDL cholesterol, mg/dL	0.01	0.03
HbA_{1c}, %	0.01	0.24
sRAGE, ng/dL	0.6	0.002

Abbreviation: BSA, body surface area.

subjects with prediabetes according only to HbA_{1c} level (HbA_{1c} level of 5.7% to 6.4% and NFG/NGT).

The main finding of this study is that subjects with HbA_{1c} prediabetes exhibited a lower E/A ratio than controls; moreover, we found that the relationship between HbA_{1c} and E/A ratio was independent of other confounding factors. These data confirm a consistent body of previous studies demonstrating the relationship between early alteration of glucose homeostasis and subclinical alterations of cardiac function in different populations with early metabolic alterations, such as insulin resistance, high 1-hour postload glycemia, and new-onset and overt type 2 diabetes (4, 5, 25, 26).

Accordingly, we also showed an independent association between E/e′ ratio and HbA_{1c} value. These results are in line with those of previous studies involving subjects with alteration of glucose homeostasis. Stahrenberg *et al.* (5) showed that glucose metabolism was associated with diastolic dysfunction and that HbA_{1c} value was associated with E/e′ ratio in multivariate analyses. Furthermore, a study conducted on the ARIC (Atherosclerosis Risk In the Communities) study population reported that although the E/e′ ratio was mostly in the normal range, it was also positively associated with HbA_{1c} value (27). This result is clinically relevant, underlining the role of HbA_{1c} as a marker of asymptomatic diastolic dysfunction that is the most prominent characteristic of diabetic cardiomyopathy (28).

In recent studies, we found alterations of subclinical markers of cardiovascular risk in the same population (persons with HbA_{1c} value 5.7% to 6.4% and NFG/NGT) with no significant differences compared with persons with impaired fasting glucose/IGT and new-onset

type 2 diabetes (8). The role of HbA_{1c} in predicting cardiovascular disease in subjects without diabetes has been supported by other authors. Selvin *et al.* (29) found a significant increase in the incidence of cardiovascular events in subjects with an HbA_{1c} value substantially lower than that used for the diagnosis of diabetes. Moreover, a recent prospective study showed a significant increase in coronary heart disease (CHD) and cardiovascular disease in subjects with prediabetes identified by HbA_{1c} value with respect to subjects with normoglycemia (30).

Interestingly, in this study, subjects with HbA_{1c} prediabetes exhibited a higher LAV (another parameter strictly bounded with diastolic function) than controls. In agreement with our data, Dinh *et al.* (4) showed an increase in LAV from NGT to IGT and type 2 diabetes; furthermore, the study indicated that HbA_{1c} was significantly correlated with LAV and E/e′ ratio, parameters indicative of LV diastolic dysfunction with elevated filling pressure, even in subjects without a history of diabetes. The authors suggested that LAV reflects the cumulative effect of different long-duration contributors to LV diastolic function and is less vulnerable to acute changes in preload and afterload, which may have an acute effect on diastolic function (4).

We recently reported that patients with HbA_{1c} prediabetes showed lower esRAGE plasma levels than controls, and these levels were independently related with early markers of cardiovascular disease (9). However, the relationship between diastolic function and the AGE/RAGE axis in patients with prediabetes was not explored. In this study, we found an independent relationship between low esRAGE plasma levels and E/A ratio, suggesting that glycosylation and inflammation may play a role in heart damage.

Multiple studies have reported the measurement of plasma or serum levels of soluble forms of RAGE in human patients with different diseases, including heart failure, with controversial findings. Raposeiras-Roubín *et al.* (31) examined the correlation between total sRAGE level and severity of heart failure; they found that sRAGE levels were higher with increasing degrees of heart failure. Other authors reported higher total levels of sRAGE in patients with heart failure; however, in agreement with our data, they found lower esRAGE levels in patients with impaired cardiac function vs controls (32). Conversely, Falcone *et al.* (33) reported an independent association between low levels of sRAGE and CHD in men without diabetes, and low levels of sRAGE have been associated with risk of diabetes, CHD, and mortality (33, 34).

The mechanisms underlying these controversial results regarding different trends between total and esRAGE levels have yet to be determined. However, it is essential to note that all of these findings may have been affected by the recruitment modalities of the different studies; indeed, plasma levels of all soluble forms of RAGE may be modulated by renal function and common drug treatments such as statins, angiotensin receptor blockers, and angiotensin-converting enzyme inhibitors.

In addition to showing a higher SI in patients with HbA_{1c} prediabetes, we found that SI was independently related with HbA_{1c} levels. These data may be relevant from a clinical point of view; indeed, adverse remodeling of the left ventricle has been associated with worse prognostic outcome (35). In particular, LV sphericity has been associated with decreased survival after acute myocardial infarction (36). Another recent study conducted in a multiethnic population free of cardiovascular disease at baseline showed that higher sphericity was a strong predictor of incident heart failure and atrial fibrillation (37).

In this study, patients with HbA_{1c} prediabetes had slightly higher glycemic values at every OGTT time point compared to controls (Table 1). These data offer a possible explanation for the higher HbA_{1c} values of these patients and underline the importance of HbA_{1c} in identifying this high-risk population. The association between low esRAGE plasma levels and HbA_{1c} was observed in previous studies in subjects with and without diabetes (38, 39). However, the cross-sectional design of these studies does not allow determination of a causal relationship between low esRAGE levels and higher HbA_{1c} levels. In a recent study, we observed decreased messenger RNA expression of full-length *RAGE* and *esRAGE* in patients with prediabetes and type 2 diabetes mellitus compared with controls; however, it is still unclear whether the mechanism for alternative splicing to generate esRAGE is regulated by environmental or genetic factors (38).

Finally, we did not perform particular matching during the recruitment of patients. All participants were referred to our outpatient clinic for type 2 diabetes screening and cardiovascular risk evaluation because of overweight, obesity, first-degree relatives with diabetes, or other cardiovascular risk factors. The exclusion of patients with previous cardiovascular disease, uncontrolled cardiovascular risk factors, high/low BMI, and older age (according to our exclusion criteria) constrained the mean anthropometric characteristics of these patients in a quite narrow range. Furthermore, all patients had NFG/NGT. Differences (statistically but not clinically) would most likely be evident in a larger population; however, this was outside the scope of our study.

Several potential limitations of this study should be highlighted. First, although our patients were clinically free from CHD, we did not screen for the presence of

CHD using coronary angiography, and the lack of information on coronary status is a potential source of bias. Second, the cross-sectional design of this study did not permit any conclusions on causality. Third, although the E/A ratio has important diagnostic and prognostic implications, it should be interpreted in conjunction with clinical characteristics and other echocardiographic parameters to guide patient management.

In conclusion, subjects with HbA_{1c} prediabetes exhibited early subclinical cardiac alterations, such as lower E/A ratio, higher LAV value, and impaired SI. Given the expected increase in use of HbA_{1c} as a screening tool to identify alterations in glycemic homeostasis, these data may have important clinical implications for patients at increased risk for cardiovascular disease.

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