The relationship between endogenous secretory RAGE and cardiac autonomic function in prediabetes

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Abstract

Aims: The putative protective role of esRAGE for cardiac autonomic function (CAF) remain unclear. To address this question, the present study has assessed the relationship of serum AGEs, sRAGE and esRAGE, and tissue AGEs with CAF in a high-risk population without diabetes. Material and methods: Forty eight subjects of mean age 52.7±11.2years and mean BMI 28.4±6.3kg/m2, divided into 2 groups according to glucose tolerance: 16 with normal glucose tolerance (NGT) and 24 with prediabetes, were enrolled. A standard OGTT was performed. The glucose tolerance was defined according to 2006 WHO criteria. Fasting, 120-min glucose, lipids, creatinine and HbA1c were measured. eGFR was calculated (CKD-EPI). Fasting, 120-min insulin (ECLIA method), esRAGE, sRAGE and AGEs (ELISA method) were assessed. HOMA-IR was calculated. Tissue AGEs were assessed by skin autofluorescence (AGE-Reader, DiagnOpticsTM). CAF was evaluated with ANSAR, applying deep breathing, Valsalva and standing. Results: There was a significant decline in CAF in prediabetes in comparison to NGT. Serum and tissue AGEs, sRAGE and esRAGE levels were similar between groups. On the matrix analysis, both sympathetic and parasympathetic activity at baseline and after standing and sympathetic tone during Valsalva were positively related to esRAGE in prediabetes. Multivariate regression analysis showed that esRAGE is an independent contributor to sympathetic, parasympathetic and total autonomic tone in prediabetes accounting for about 28%, 34% and 35% of their variances, respectively. Conclusion: Our results have demonstrated that CAF is decreased in prediabetes. esRAGE, but not sRAGE, is reciprocally related to CAF, probably opposing the negative effects of glycation.

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Running title: esRAGE and autonomic function in prediabetes

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Results: There was a significant decline in CAF in prediabetes in comparison to NGT. Serum and tissue AGEs, sRAGE and esRAGE levels were similar between groups. On the matrix analysis, both sympathetic and parasympathetic activity at baseline and after standing and sympathetic tone during Valsalva were positively related to esRAGE in prediabetes. Multivariate regression analysis showed that esRAGE is an independent contributor to sympathetic, parasympathetic and total autonomic tone in prediabetes accounting for about 28%, 34% and 35% of their variances, respectively.

Conclusion: Our results have demonstrated that CAF is decreased in prediabetes. esRAGE, but not sRAGE, is reciprocally related to CAF, probably opposing the negative effects of glycation.

Key words: endogenous secretory RAGE, cardiac autonomic activity, prediabetes

What is already known about this topic?

1. Glycation is enhanced in the settings of hyperglycemia, even in early stages of dysglycemia, and presents a key pathological mechanism for diabetic neuropathy.

2. There is plasma soluble receptor for AGE comprising two forms - esRAGE and sRAGE, which has been reported to be involved in the prevention and progression of vascular complications and somatic neuropathy.

What does this article add?

1. The esRAGE concentration, but not sRAGE levels, was found to be inversely related to cardiac autonomic tone.

2. The esRAGE and sRAGE probably exerts different functions in the AGE-RAGE axis signaling.

3. The esRAGE probably serves as a negative feedback loop to oppose the damaging effects of glycation.

Introduction

Type 2 diabetes is the most prevalent and serious metabolic disease. Hyperglycemia initiates a vicious cycle of intra- and extracellular disturbances resulting in a broad spectrum of chronic micro- and macrovascular complications. Glycation is enhanced in the settings of increased glucose concentrations and presents a key pathological mechanism, significantly contributing to initiate and/or accelerate chronic diabetic complications. The accumulation of advanced glycation end products (AGEs) affects the target tissue structure and leads to a gradual decline in its function by a receptor-mediated process [1]. The receptor for AGEs (RAGE) is a cell surface multi-ligand receptor of the immunoglobulin superfamily expressed in macrophages, endothelial cells and several other cell types [2], including neural tissue [3]. RAGE signaling pathway is involved in the processes of protein turnover, tissue remodeling and inflammation [4-6] through inducing activation of nuclear factor-kB, increasing expression of cytokines and upregulation of adhesion molecules, evoking oxidative stress and neo-intimal proliferation [7-9]. Its significant role in the development and progression of macro- [10] and micro- [11] vascular complications has been demonstrated. The AGE-RAGE axis has been

shown to be one of the leading mechanisms linking microvascular disturbances and neuropathy [12]. In experimental models, it has been demonstrated [13] that AGEs induce endothelial dysfunction [14] and decrease blood flow to peripheral nerves [15-17]. An abnormal AGEs accumulation in peripheral nerves in diabetes [18, 19] exerts a toxic effect on Schwann, neuronal, vascular and mesangial cells [12, 20-23]. In humans, it has been shown that glycation is an important contributor to small-fiber sensory [24-26] and painful [27] neuropathy and diabetic foot [14, 28]. However, data on cardiac autonomic neuropathy are still conflicting [29, 30].

Since AGEs accumulation is not just a consequence of hyperglycemia, but represents cumulative metabolic burden, its impact is likely to exceed the states of hyperglycemia [31]. RAGE ligands include proinflammatory proteins (S100/calgranulins [8, 32] and high-mobility group 1 protein [33]) which might be a key factor linking AGE-RAGE axis with insulin resistance [34]. The responses of AGE-RAGE signaling pathway have been observed in nondiabetic high-risk population at early stages of dysglycemia and in the metabolic syndrome [35, 34]. Of note, AGEs-RAGE axis activity has been proven to be involved in human nondiabetic atherosclerosis [6, 36-38].

There is plasma soluble receptor for AGE (sRAGE) comprising two forms - an endogenous secreted isoform, a spliced variant lacking a transmembrane domain (esRAGE) [39], and the extracellular domain of wild-type RAGE cleaved from the cell membrane [40, 41]. It has been suggested that plasma soluble RAGE serves as a decoy for ligands binding to AGE [34] and plays an antagonistic role by competing with the cell surface receptor, thus opposing the AGE-RAGE signal cascade in vivo and in vitro [32, 33, 42]. However, there is some data that the component of sRAGE derived from proteolytic cleavage might be part of the regulatory process [40, 43].

The pivotal role of sRAGE and esRAGE for the prevention and progression of vascular complications [44] and somatic neuropathy has already been shown. However, their putative role in cardiac autonomic neuropathy, remains unclear in diabetes and indefinite in the state of prediabetes.

To address this question the present study has evaluated the circulating levels of serum AGEs, sRAGE and esRAGE, and tissue AGEs accumulation in the high-risk population with normal glucose tolerance (NGT) and prediabetes and their relationship with both sympathetic and parasympathetic activity.

We hypothesized that probably serum and tissue AGEs will be negatively, and sRAGE and esRAGE positively correlated with cardiac autonomic activity even at these early stages of dysglycemia.

Material and methods

1. Participants

Forty eight subjects of mean age 52.7±11.2 years and mean BMI 28.4±6.3 kg/m^2 were enrolled. They were divided into 2 groups according to glucose tolerance: 16 with normal glucose tolerance (NGT) and 24 with prediabetes (16 with impaired fasting glucose and 8 with impaired glucose tolerance).

Participants were recruited at the Department of Endocrinology, Division of Diabetology, Medical University of Sofia within an ongoing diabetes screening program. All subjects were informed about the aims of the study and the risks of participating and declared their written informed consent in accordance with the Helsinki Declaration and rules of Good Clinical Practice, as the study was approved by the Ethics Committee of the Medical University, Sofia.

2. Exclusion criteria

Subjects with previously diagnosed type 1 or type 2 diabetes or taking any medication for the indication of diabetes; previously diagnosed with arrhythmias or taking any medications for the indication of arrhythmia; taking any medications for the indication of dyslipidemia; with eGFR-EPI $<$ 60 ml/min/1.73m^2; at the age of $<$ 30 years or $>$ 70 years; with serious comorbidities, including kidney, liver, cardiovascular disease, thyroid or recent acute illness, were not eligible for the study.

3. Anthropometric parameters

Height (cm) and weight (kg) were measured and BMI was calculated, using the formula: weight (kg)/height $(m)^2$. Waist circumference was measured twice in the midline between the inferior margin of the 12^{th} rib and the iliac crest in the standing position after exhalation and averaged.

4. Functional test

Glucose tolerance was evaluated during a standard oral glucose tolerance test with 75 g anhydrous glucose after an overnight fast, at least 12 hours after the last meal, refraining from smoking, coffee and taking any medication prior to the test. Participants were on a diet regimen with 150 g of carbohydrate daily during the last 72 hours prior to the test. The test was initiated between 8.00-9.00 a.m. and the participants remained at a resting seated position throughout the test. Blood samples were taken at 0 and 120 minutes relative to glucose ingestion. The glucose tolerance was defined according to 2006 WHO criteria.

5. Laboratory tests

Fasting and 120-min postload plasma glucose were measured by a hexokinase enzyme method (Roche Diagnostics).

- Serum lipid parameters (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) were measured at fasting by an enzymatic colorimetric method (Roche Diagnostics).
- HbA1c (NGSP certified) was measured in whole blood samples using immunoturbidimetric method (Roche Diagnostics).
- Serum creatinine was measured at fasting by an enzymatic colorimetric method (Roche Diagnostics).
- Estimated Glomerular Filtration Rate (eGFR) was assessed using the CKD-EPI Creatinine Equation.
- Fasting and 120-min postload serum insulin were assessed by ECLIA method (Roche Diagnostics).
- Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated, using the formula: (fasting plasma glucose x fasting serum insulin) / 22.5.
- Endogenous secretory receptor for advanced glycation end products (esRAGE) was assessed by ELISA method (HumaReader HS).
- Soluble receptor for advanced glycation end products (sRAGE) was assessed by ELISA method (HumaReader HS).
- Serum advanced glycation end products (AGEs) were assessed by ELISA method (HumaReader HS).

6. Instrumental examinations

tissue advanced glycation products (AGEs)

AGEs accumulation was assessed non-invasively measuring the skin autofluorescence of ultraviolet light on the ventral side of the lower arm (AGE-Reader, DiagnOpticsTM).

cardiac autonomic function

Cardiac autonomic nervous system function was evaluated with ANX-3.0 autonomic monitoring system (ANSAR Medical Technologies, Inc., Philadelphia, PA). This is a software that computes both sympathetic and parasympathetic nervous system activity non-invasively, simultanuously and independently applying spectral analysis of respiratory activity with concomitant spectral analysis of heart rate variability. The system uses both "time-domain" and ,,frequency-domain" analysis at rest and during standard autonomic tests: Deep breathing challenge, Valsalva challenge, and Stand challenge. The analysis applied in the ANX-3.0 method is focused on the low-frequency range of the spectrum fixed between 0.04-0.15 Hz.

The examination of the cardiac autonomic function was performed under standard conditions including - at least 24 hours after the last dose of medications affecting autonomic function – antihypertensives, tricyclic antidepressants and SSRIs, refraining from coffee and smoking at least 12 hours prior to the test, at least 30 minutes after the last meal, in the time interval between 8.00 – 11.00 am.

7. Statistical analyses

Statistical analyses of the data was performed by SPSS 21.0 (SPSS, Chicago, USA). Descriptive statistics was used to describe the data in the two groups. The data are expressed as mean ± standard deviation (SD) and median (percentile 25% to 75%). Logarithmic transformation was used for the data with skewed distribution. One-way analysis of variance (One-way ANOVA) was used for comparison of the groups. Principal component analysis was performed to define a principal component variable for sympathetic, parasympathetic and total autonomic activity at rest and during autonomic tests. Partial correlation test, controlling for age and BMI, was applied to assess the relationship between sympathetic, parasympathetic and total autonomic activity and AGE/RAGE levels. Pearson correlation test was performed for the assessment of the relationship between cardiac autonomic function principal component variables and AGE/RAGE levels and estimated metabolic parameters. Multiple linear regression with stepwise forward method was used for the evaluation of the predictive value of esRAGE levels for sympathetic, parasympathetic and total autonomic activity. A p-value (two tailed) of less than 0.05 was considered statistically significant.

Results

Main characteristics of the groups are present in Table 1. Between-group differences were observed in age, BMI, waist circumference, plasma glucose levels during OGTT, serum insulin at fasting, HOMA-IR, and most of sympathetic and parasympathetic tone indices. All other parameters, including postload serum insulin, lipid levels, HbA1c, serum creatinine levels, eGFR, serum and tissue AGEs, sRAGE and esRAGE were not significantly different between the groups.

Table 2 provides data on the matrix analysis, including the whole cohort, and a separate analysis of the two subject groups. Both sympathetic and parasympathetic activity parameters at baseline and after standing and sympathetic tone index during Valsalva were positively related to esRAGE levels in the studied cohort and this relationship was consistent with the results in the prediabetes group but failed to achieve significance in the NGT group. sRAGE, serum AGEs and tissue AGEs accumulation showed no association with cardiac autonomic function.

Multivariate regression analyses were performed to estimate whether there was an independent relationship between esRAGE levels and cardiac autonomic function. On the multiple linear regression analyses with stepwise method, after controlling for age and BMI, esRAGE emerged as an independent contributor to sympathetic, parasympathetic and total autonomic tone in prediabetes accounting for about 28%, 34% and 35% of their variances, respectively (Table 3).

No correlation was found between tissue AGEs accumulation, serum AGEs, sRAGE and esRAGE serum levels and estimated metabolic parameters (Suppl. Table 1). Sympathetic, parasympathetic and total autonomic activity component variables showed an inverse correlation with age, HbA1c, LDL cholesterol, total cholesterol, triglycerides and eGFR (Suppl. Table 2).

Discussion

This study supports the importance of prediabetes as a category of increased risk for the development of cardiac autonomic dysfunction since both sympathetic and parasympathetic tone has been found to be declined in prediabetes in comparison to NGT.

Our results do not show any relationship between serum AGEs and the parameters of cardiac autonomic activity, which is in line with some reported data in type 2 diabetes with short [29] and long [45] duration. Tissue accumulation of the heterogeneous AGEs have also been measured indirectly by skin autofluorescence [46] and the results have been in consistence with the findings for the serum AGEs.

However, most studies in type 1 [24, 47] and type 2 [48] diabetes have uniformly confirmed the key role of glycation in the development and acceleration of diabetic neuropathy. The important role of glycation in the pathophysiology of both diabetic peripheral and autonomic neuropathy has been demonstrated, even before the clinical manifestation of neuropathy [30]. It has been suggested that the peripheral nerve fiber loss in diabetes is partly due to the AGEs accumulation [49]. RAGE has been demonstrated to be expressed in endothelial and Schwann cells of perineural and endoneural vessels [3, 49]. In experimental models AGEs

even at physiological concentration have been reported to decline the viability of Schwann cells [50] through facilitating of endothelial dysfunction [14], which may affect every component in the peripheral nervous system. The alterations in protein structure mediated directly by AGEs toxic effect or indirectly by AGE/RAGE cascade results firstly in functional (interruption of axonal transport) with consequent structural (the development of atrophy and degeneration) abnormalities in the peripheral nerves [15, 51]. In summary, the fulcrums of the negative effect of glycation in the nerve tissue are reduced Na+ K+-ATPase [15] and nitric oxide [16, 17] activity, and glycation of collagen and laminin [16] resulting in increased permeability of blood vessels [16], reduced nerve blood flow and hypoxia [17]. The impairment of endothelial cell function caused by AGE/RAGE axis even in prediabetes involves increased activity of NF-ϰB and activator protein-1, which enhance the expression of vascular cell adhesion molecule-1, tumor necrosis factor and interleukin-6 [52, 53].

These discrepancies are highly likely to be due to the variable role of hyperglycemia for AGEs and RAGE formation at these early stages of impaired glucose homeostasis and the predominant role of some confounding factors - low-grade inflammation, oxidative stress, insulin resistance and metabolic syndrome. RAGE activation is the consequence of both AGEs and different pro-inflammatory molecules, which exert a synergistic effect in the initiation and/or progression of diabetes chronic complications [54]. Therefore, it might be speculated that at these early stages of dysglycemia probably not hyperglycemia, but low-grade inflammation, as a consequence of insulin resistance, is the predominant stimulus for the development of late complications of diabetes.

With regard to sRAGE and esRAGE levels, it has been assumed that they are involved in the negative feedback regulation of RAGE-mediated signaling by blocking the RAGE. Their anti-inflammatory effect and role in the balance between oxidative stress and antioxidant defense have been suggested even in prediabetes [55]. Since data have shown the putative independent beneficial effect of statins and pioglitazone on sRAGE and esRAGE levels [56, 57], all screened subjects on lipid lowering treatment were excluded from the analysis in the current study.

Our findings have demonstrated no statistically significant difference in sRAGE and esRAGE levels between the groups with NGT and prediabetes, which is in consistence with some available data [58-60]. However, the predominant data have shown lower levels of sRAGE [55, 61] and esRAGE [55, 58, 61-63] in prediabetes, which is assumed to be linked to a loss of protection against low-grade inflammation in this high-risk population.

On a matrix analysis, both sympathetic and parasympathetic tone have been observed to be positively related to esRAGE, but not to sRAGE, in the studied cohort and in the prediabetes group. These findings support the conception for the protective role of esRAGE in the continuum of glycation process, preventing autonomic nerves from the deleterious effects of AGE/RAGE cascade. esRAGE has been considered a competitive inhibitor of AGE/RAGE signal pathways, since it acts as a decoy receptor for AGEs. Thus, esRAGE exhibits a feedback mechanism, by which inhibits AGE/RAGE cascade [32].

Our cohort consists of subjects with prediabetes and NGT, suggesting that the potential significance of esRAGE for the autonomic power is not confined to diabetes. Moreover, plasma esRAGE levels have been suggested to be more closely associated with early stage of dysglycemia, rather than overt diabetes [64-66]. Although an independent association between esRAGE levels and coronary artery disease has been confirmed in high-risk population without diabetes [67, 68] and decreased levels of esRAGE have been reported to predict cardiovascular mortality not only in diabetes, but also in prediabetes [69], it is unclear whether sRAGE levels are related to atherosclerosis. Both lower [70] and elevated [71] levels of sRAGE have been reported in subjects with coronary artery disease without diabetes.

Circulating sRAGE isoform seems to be under the control of different mechanism. An alternative splicing has been suggested to generate esRAGE and proteolytic shedding of cell-surface RAGE - to generate sRAGE, which might be the reason for distinct roles of these soluble forms in certain disease conditions. Thereafter, it is of paramount significance to distinguish the exact physiological relevance of both markers. Probably sRAGE and esRAGE are under distinct regulation and independently influence AGE/RAGE axis in different

manner.

Although the simultaneous evaluation of both sRAGE and esRAGE together with serum and tissue AGEs strengthen the analysis, our results share the limitations of a cross-sectional design with relatively small sample size.

In conclusion, our data have demonstrated that both sympathetic and parasympathetic activities are already declined in prediabetes. The esRAGE concentration, but not sRAGE levels, seems to be inversely related to autonomic tone, probably serving as a negative feedback loop to oppose the damaging effects of glycation. Larger prospective studies are needed to evaluate the causal relationship between autonomic function and circulating esRAGE and sRAGE, and to distinct their individual pathophysiological significances in different clinical settings.

Conflict of interest

There are no potential conflicts of interest relevant to this article.

Authorship

R.D., N.C., G.G., and T.T. have made substantial contributions to conception and design of the current study. R.D. and N.C. analyzed the data. R.D. have drafted the manuscript. T.T. have revised it critically. R.D., N.C., G.G., and T.T. have given final approval for the publication.

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Table 1. Main characteristics of the groups according to glucose tolerance - normal glucose tolerance (NGT) and prediabetes.

Parameter
Age (years)
BMI (kg/m^2)
Waist circumference (cm)
HbA1c (%)

Parameter
Plasma glucose at fasting (mmol/l)
Plasma glucose postload (mmol/l)
Insulin at fasting (uIU/l)
Insulin postload (uIU/l)
HOMA-IR index
Total cholesterol (mmol/l)
LDL cholesterol (mmol/l)
HDL cholesterol males (mmol/l)
HDL cholesterol females (mmol/l)
Triglycerides (mmol/l)
Serum creatinine (umol/l)
eGFR (CKD-EPI) (ml/min/1.73m^2)
tissue AGEs
serum AGEs (pg/ml)
esRAGE (pg/ml)
sRAGE (ng/ml)
LFa activity baseline (bpm^2)
RFa activity baseline (bpm^2)
Total autonomic activity baseline (bpm^2)
LFa activity deep breathing (bpm^2)
RFa activity deep breathing (bpm^2)
Total autonomic activity deep breathing (bpm^2)
LFa activity Valsalva maneuver (bpm^2)
RFa activity Valsalva maneuver (bpm^2)
Total autonomic activity Valsalva maneuver (bpm^2)
LFa activity standing (bpm^2)
RFa activity standing (bpm^2)
Total autonomic activity standing (bpm^2)
Data are mean ± SD or median and IQR. sRAGE – soluble receptor for advanced glycation end products; esRAGE – endog

Table 2. Correlation between sympathetic and parasympathetic activity and tissue and serum AGEs, sRAGE and esRAGE.

Parameter Partial Correlation, controlling for age and BMI	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Whole cohort	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**
Ln(LFa component variable)	**0.43**	**0.008**	-0.18	0.293	0.12	0.229	-0.06	0.739

Parameter Partial Correlation, controlling for age and BMI	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
Ln(RFa component variable)	**0.41**	**0.011**	-0.20	0.232	0.11	0.505	-0.11	0.526
Ln(LFa+RFa component variable)	**0.43**	**0.008**	-0.19	0.265	0.15	0.372	-0.08	0.634
Ln(LFa baseline)	**0.42**	**0.009**	-0.07	0.700	0.14	0.421	-0.19	0.251
Ln(RFa baseline)	0.29	0.080	-0.09	0.610	0.31	0.068	-0.19	0.253
Ln(LFa+RFa baseline)	**0.38**	**0.019**	-0.08	0.625	0.20	0.226	-0.18	0.279
Ln(LFa deep breathing)	0.05	0.780	-0.21	0.219	0.24	0.158	-0.32	0.058
Ln(RFa deep breathing)	0.19	0.269	-0.04	0.819	-0.12	0.465	-0.01	0.991
Ln(LFa+RFa deep breathing)	0.14	0.418	-0.09	0.618	-0.12	0.492	-0.01	0.948
Ln(LFa Valsalva)	0.15	0.377	-0.11	0.519	0.11	0.514	-0.17	0.322
Ln(RFa Valsalva)	0.09	0.598	-0.26	0.124	-0.04	0.804	-0.12	0.467
Ln(LFa+RFa Valsalva)	0.15	0.389	-0.13	0.454	0.09	0.600	-0.17	0.330
Ln(LFa standing)	**0.48**	**0.003**	-0.07	0.672	0.09	0.618	-0.22	0.199
Ln(RFa standing)	**0.36**	**0.028**	-0.13	0.429	0.21	0.204	**-0.38**	**0.021**
Ln(LFa+RFa standing)	**0.47**	**0.003**	-0.09	0.597	0.13	0.430	-0.28	0.0.95
NGT sub-group	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**
Ln(LFa component variable)	0.07	0.821	-0.21	0.504	-0.18	0.568	-0.09	0.791

Parameter Partial Correlation, controlling for age and BMI	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
Ln(RFa component variable)	0.28	0.386	-0.02	0.960	-0.11	0.743	-0.48	0.113
Ln(LFa+RFa component variable)	0.38	0.226	-0.01	0.987	0.01	0.989	-0.34	0.274
Ln(LFa baseline)	0.38	0.230	-0.34	0.280	-0.26	0.410	-0.19	0.550
Ln(RFa baseline)	0.37	0.241	-0.06	0.864	0.16	0.628	-0.29	0.370
Ln(LFa+RFa baseline)	0.46	0.135	-0.19	0.553	-0.17	0.597	-0.05	0.871
Ln(LFa deep breathing)	0.16	0.625	-0.03	0.933	0.50	0.097	**-0.76**	**0.004**
Ln(RFa deep breathing)	0.36	0.252	-0.22	0.490	-0.40	0.196	-0.37	0.240
Ln(LFa+RFa deep breathing)	0.36	0.248	-0.20	0.535	-0.36	0.256	0.41	0.189
Ln(LFa Valsalva)	-0.07	0.828	-0.10	0.761	0.14	0.657	-0.31	0.320
Ln(RFa Valsalva)	-0.07	0.818	-0.02	0.949	0.07	0.821	-0.43	0.159
Ln(LFa+RFa Valsalva)	-0.07	0.820	-0.09	0.775	0.14	0.665	-0.33	0.292
Ln(LFa standing)	0.55	0.062	**-0.61**	**0.036**	0.01	0.993	-0.51	0.094
Ln(RFa standing)	0.39	0.208	-0.41	0.189	0.12	0.708	-0.50	0.097
Ln(LFa+RFa standing)	0.54	0.066	**-0.58**	**0.047**	0.02	0.940	0.53	0.073
Prediabetes sub-group	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Predia sub-group**
Ln(LFa component variable)	**0.53**	**0.009**	-0.19	0.394	0.19	0.399	-0.14	0.536

Parameter Partial Correlation, controlling for age and BMI	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
Ln(RFa component variable)	**0.46**	**0.027**	-0.24	0.262	0.16	0.459	-0.04	0.840
Ln(LFa+RFa component variable)	**0.49**	**0.018**	-0.24	0.280	0.19	0.391	-0.05	0.834
Ln(LFa baseline)	**0.46**	**0.028**	-0.04	0.841	0.21	0.348	-0.29	0.180
Ln(RFa baseline)	0.24	0.280	-0.18	0.410	0.34	0.115	-0.15	0.498
Ln(LFa+RFa baseline)	0.38	0.072	-0.10	0.651	0.27	0.220	-0.24	0.274
Ln(LFa deep breathing)	-0.05	0.835	-0.27	0.206	0.16	0.463	-0.14	0.520
Ln(RFa deep breathing)	0.19	0.389	-0.12	0.592	-0.05	0.810	-0.16	0.480
Ln(LFa+RFa deep breathing)	0.14	0.529	-0.16	0.482	-0.04	0.843	-0.14	0.513
Ln(LFa Valsalva)	0.21	0.348	-0.24	0.274	0.09	0.670	-0.06	0.789
Ln(RFa Valsalva)	0.09	0.691	-0.34	0.116	-0.08	0.712	-0.02	0.922
Ln(LFa+RFa Valsalva)	0.19	0.375	-0.25	0.246	0.07	0.767	-0.05	0.830
Ln(LFa standing)	**0.48**	**0.022**	0.11	0.608	0.11	0.614	-0.10	0.648
Ln(RFa standing)	0.34	0.111	-0.09	0.668	0.24	0.277	-0.35	0.097
Ln(LFa+RFa standing)	**0.47**	**0.023**	0.08	0.732	0.17	0.446	-0.19	0.381

Parameter Partial Correlation, controlling for age and BMI	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasympathetic nervous system	LFa – sympathetic nervous system; RFa – parasym pathetic nervous system

Table 3. Multiple regression analysis for the predictive value of esRAGE for SNS and PSNS activity, controlling for age and BMI.

Cardiac autonomic function component variables	Stepwise forward regression Explanatory variables	Regression Coefficient (β)	SEM	SEM	P value	Coefficient of determination (R^2)	Coefficient of determination (R^2)
Whole cohort							
LFa+RFa component variable	esRAGE	0.001	0.001	0.0001	0.002	0.002	0.354
	Total cholesterol	-0.300	-0.300	0.121	0.018	0.018	0.438
RFa component variable	esRAGE	0.001	0.001	0.0001	0.007	0.007	0.336
LFa component variable	esRAGE	0.001	0.001	0.0001	0.004	0.004	0.282
Prediabetes	Prediabetes	Prediabetes	Prediabetes	Prediabetes	Prediabetes	Prediabetes	Prediabetes
LFa+RFa component variable	esRAGE	0.001	0.001	0.0001	0.014	0.014	0.280
RFa component variable	esRAGE	0.001	0.001	0.0001	0.024	0.024	0.286

Cardiac autonomic function component variables	Stepwise forward regression Explanatory variables	Regression Coefficient (β)	SEM	SEM	P value	Coefficient of determination (R^2)	Coefficient of determination (R^2)
LFa component variable	esRAGE	0.001	0.001	0.0001	0.007	0.007	0.282
	Total cholesterol	-0.447	-0.447	0.200	0.036	0.036	0.410
Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	Variables entered into the regression analysis: esRAGE, eGFR, triglycerides, total cholesterol, LDL cholesterol, HbA1c. Confounding variables: age and BMI. LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity

Supplemental Table 1. Correlations between estimated metabolic parameters and tissue AGEs accumulation, serum AGEs and sRAGE and esRAGE.

Parameter Pearson Correlation	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**

Parameter Pearson Correlation	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
Whole cohort	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**
Age	-0.20	0.188	0.07	0.631	-0.11	0.357	**0.34**	**0.024**
Waist circumference	0.17	0.255	0.19	0.203	0.01	0.999	-0.13	0.390
BMI	0.06	0.688	0.20	0.184	0.03	0.862	-0.13	0.389
Ln(HbA1c)	-0.22	0.145	0.20	0.189	0.11	0.465	0.11	0.464
Plasma glucose at fasting	0.20	0.180	0.21	0.164	-0.07	0.625	0.06	0.690
Plasma glucose postload	0.16	0.291	0.24	0.121	0.06	0.709	-0.02	0.912
Ln(Insulin at fasting)	0.04	0.793	0.18	0.245	-0.11	0.466	-0.25	0.108
Ln(Insulin postload)	0.05	0.726	0.22	0.146	-0.04	0.775	-0.01	0.949
HOMA-IR index	0.06	0.698	0.19	0.204	-0.11	0.455	-0.20	0.191
Total cholesterol	0.06	0.684	-0.16	0.287	0.08	0.584	0.32	0.037
LDL cholesterol	0.17	0.242	-0.11	0.466	0.12	0.415	**0.34**	**0.024**
Ln(HDL cholesterol)	-0.15	0.315	-0.02	0.895	0.01	0.987	**0.22**	**0.148**
Ln(Triglycerides)	0.01	0.998	-0.04	0.783	-0.02	0.891	-0.19	0.227
Ln(eGFR)	-0.16	0.303	-0.15	0.335	0.09	0.568	-0.16	0.295
NGT sub-group	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**	**NGT sub-group**		
Age	-0.23	0.387	0.02	0.930	-0.22	0.418	0.44	0.117
Waist circumference	0.27	0.310	0.44	0.089	-0.05	0.853	-0.09	0.758
BMI	0.18	0.509	0.41	0.117	-0.06	0.821	-0.07	0.804
Ln(HbA1c)	-0.34	0.196	0.23	0.398	0.11	0.680	0.03	0.920
Plasma glucose at fasting	0.03	0.902	0.35	0.180	-0.44	0.092	-0.10	0.747
Plasma glucose postload	0.03	0.907	0.24	0.370	-0.17	0.519	-0.23	0.425

Parameter Pearson Correlation	**esRAGE**	**esRAGE**	**Ln(serum AGEs)**	**Ln(serum AGEs)**	**Ln(sRAGE)**	**Ln(sRAGE)**	**Ln(tissue AGEs)**	**Ln(tiss AGEs)**
Insulin at fasting	0.07	0.804	0.20	0.467	-0.10	0.723	0.04	0.886
Insulin postload	-0.22	0.404	0.15	0.586	-0.09	0.747	0.11	0.702
HOMA-IR index	0.07	0.798	0.23	0.390	-0.15	0.592	0.06	0.840
Total cholesterol	0.03	0.911	0.11	0.692	0.35	0.183	0.42	0.133
LDL cholesterol	0.11	0.688	0.15	0.588	0.26	0.325	0.47	0.091
Ln(HDL cholesterol)	0.01	0.990	-0.16	0.553	0.16	0.553	-0.01	0.989
Ln(Triglycerides)	-0.39	0.132	0.10	0.715	-0.24	0.364	-0.24	0.418
Ln(eGFR)	0.37	0.155	0.17	0.541	0.13	0.644	-0.18	0.530
Prediabetes sub-group	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**	**Prediabetes sub-group**		
Age	-0.25	0.167	0.02	0.908	-0.04	0.842	0.32	0.088
Waist circumference	0.15	0.444	0.06	0.778	0.12	0.516	-0.28	0.135
BMI	0.01	0.961	0.07	0.706	0.16	0.386	-0.25	0.180
Ln(HbA1c)	-0.21	0.262	0.16	0.403	0.19	0.320	0.13	0.479
Plasma glucose at fasting	0.30	0.100	0.15	0.418	0.18	0.328	0.06	0.774
Plasma glucose postload	0.18	0.358	0.20	0.314	0.18	0.341	0.05	0.800
Insulin at fasting	0.01	0.971	0.13	0.479	-0.06	0.759	**-0.54**	**0.002**
Insulin postload	0.16	0.402	0.24	0.225	0.01	0.955	-0.13	0.521
HOMA-IR index	0.03	0.879	0.15	0.441	-0.04	0.821	**-0.52**	**0.004**
Total cholesterol	0.07	0.700	-0.32	0.084	0.02	0.918	0.26	0.173
LDL cholesterol	0.23	0.222	-0.29	0.121	0.10	0.579	0.12	0.526
Ln(HDL cholesterol)	-0.20	0.294	0.07	0.714	-0.10	0.586	**0.38**	**0.038**
Ln(Triglycerides)	-0.17	0.351	-0.18	0.350	0.14	0.439	-0.21	0.263

Parameter Pearson Correlation	esRAGE	esRAGE	Ln(serum AGEs)	Ln(serum AGEs)	Ln(sRAGE)	Ln(sRAGE)	Ln(tissue AGEs)	Ln(tiss AGEs)
Ln(eGFR)	-0.33	0.077	-0.25	0.191	0.03	0.878	-0.13	0.496

Supplemental table 2. Correlations between estimated metabolic parameters and sympathetic and parasympathetic activity.

Parameter Pearson Correlation	LFa component variable	LFa component variable	RFa component variable	RFa component variable	LFa+RFa component variable	LFa+RFa component variable
	r	p	r	p	r	p
Whole cohort	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**	**Whole cohort**
Age	**-0.30**	**0.050**	**-0.43**	**0.004**	**-0.6**	**0.012**
Waist circumference	-0.15	0.336	-0.15	0.325	-0.15	0.326
BMI	-0.19	0.214	-0.20	0.198	-0.20	0.166
Ln(HbA1c)	-0.22	0.152	**-0.33**	**0.028**	-0.23	0.125
Plasma glucose at fasting	-0.07	0.664	-0.15	0.321	-0.18	0.123
Plasma glucose postload	-0.13	0.404	-0.08	0.595	-0.07	0.654
Ln(Insulin at fasting)	-0.29	0.062	-0.18	0.261	-0.24	0.110
Ln(Insulin postload)	-0.24	0.132	-0.13	0.420	-0.19	0.206
HOMA-IR index	-0.28	0.066	-0.19	0.234	-0.25	0.091
Total cholesterol	-0.21	0.164	-0.19	0.212	-0.25	0.088
LDL cholesterol	-0.17	0.279	-0.19	0.220	-0.26	0.077
Ln(HDL cholesterol)	0.01	0.953	0.13	0.405	0.09	0.532
Ln(Triglycerides)	0.05	0.752	0.06	0.723	0.02	0.919
Ln(eGFR)	-0.03	0.860	0.10	0.540	0.13	0.402
NGT subgroup	**NGT subgroup**	**NGT subgroup**	**NGT subgroup**	**NGT subgroup**	**NGT subgroup**	**NGT subgroup**
Age	**-0.61**	**0.016**	**-0.62**	**0.013**	-0.40	0.126
Waist circumference	-0.34	0.218	-0.24	0.389	-0.04	0.886
BMI	-0.36	0.186	-0.33	0.231	-0.24	0.377
Ln(HbA1c)	-0.12	0.677	-0.26	0.352	-0.07	0.802
Plasma glucose at fasting	-0.32	0.246	-0.40	0.142	-0.17	0.543

Parameter Pearson Correlation	**LFa component variable**	**LFa component variable**	**RFa component variable**	**RFa component variable**	**LFa+RFa component variable**	**LFa+RFa component variable**
Plasma glucose postload	-0.02	0.953	-0.16	0.559	-0.18	0.509
Insulin at fasting	-0.37	0.176	-0.07	0.802	-0.17	0.538
Insulin postload	-0.29	0.299	-0.01	0.992	-0.18	0.517
HOMA-IR index	-0.41	0.130	-0.11	0.704	-0.20	0.461
Total cholesterol	-0.08	0.771	-0.04	0.897	-0.21	0.438
LDL cholesterol	-0.09	0.743	-0.07	0.800	-0.30	0.255
Ln(HDL cholesterol)	0.27	0.338	0.02	0.935	0.05	0.866
Ln(Triglycerides)	-0.29	0.289	-0.33	0.231	-0.33	0.214
Ln(eGFR)	**0.50**	**0.059**	**0.66**	**0.008**	**0.54**	**0.030**
Prediabetes subgroup	**Prediabetes subgroup**	**Prediabetes subgroup**	**Prediabetes subgroup**	**Prediabetes subgroup**	**Prediabetes subgroup**	**Prediabetes subgroup**
Age	-0.13	0.496	-0.33	0.078	-0.22	0.220
Waist circumference	-0.01	0.973	-0.02	0.921	-0.01	0.998
BMI	-0.04	0.854	-0.05	0.782	-0.05	0.800
Ln(HbA1c)	-0.22	0.258	-0.33	0.085	-0.20	0.266
Plasma glucose at fasting	-0.20	0.299	-0.07	0.718	-0.10	0.571
Plasma glucose postload	-0.10	0.624	-0.08	0.691	-0.02	0.912
Insulin at fasting	-0.21	0.289	-0.15	0.457	-0.14	0.442
Insulin postload	-0.19	0.354	-0.15	0.477	-0.13	0.493
HOMA-IR index	-0.18	0.366	-0.13	0.499	-0.13	0.498
Total cholesterol	**-0.41**	**0.028**	**-0.36**	**0.056**	**-0.37**	**0.035**
LDL cholesterol	**-0.37**	**0.050**	-0.32	0.092	**-0.34**	**0.057**
Ln(HDL cholesterol)	0.14	0.464	0.25	0.188	0.24	0.182
Ln(Triglycerides)	0.34	0.071	**-0.38**	**0.041**	**-0.36**	**0.045**
Ln(eGFR)	0.25	0.190	0.14	0.463	0.12	0.530

Parameter Pearson Correlation	**LFa component variable**	**LFa component variable**	**RFa component variable**	**RFa component variable**	**LFa+RFa component variable**	**LFa+RFa component variable**
LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity	LFa – sympathetic nervous system activity; RFa – parasympathetic nervous system activity

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