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Reduced soluble receptor for advanced glycation end-products (sRAGE) scavenger capacity precedes pre-eclampsia in Type 1 diabetes

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Disclosure of interests

None to declare.

Contribution to authorship

TJL is the main corresponding author and conceived and designed the study. YY, KFH, VK, AC, AJJ, AJN, PAT, HS, TH, BL, SKG, MKM, SMH, JAS, JRS and MW contributed to the design, conducted the study and contributed to the Results and Discussion sections. AB and CEA analysed the data and contributed to the Results and Discussion sections.

Details of ethics approval

The study was approved by the Institutional Review Board (IRB) at the University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA, the affiliation of the Principal Investigator (IRB #11642).

Supporting information

The following supplementary materials are available for this article:

Table S1. Raw measures.

Table S2. Log-transformed measures.

Table S3. Measures adjusted for prandial status, duration of diabetes, HbA1c, parity and mean arterial pressure.

Additional Supporting Information may be found in the online version of this article.

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Abstract

Objective—Increased advanced glycation end-products (AGEs) and their soluble receptors (sRAGE) have been implicated in the pathogenesis of pre-eclampsia (PE). However, this association has not been elucidated in pregnancies complicated by diabetes. We aimed to investigate the serum levels of these factors in pregnant women with Type 1 diabetes mellitus (T1DM), a condition associated with a four-fold increase in PE.

Design—Prospective study in women with T1DM at 12.2 ± 1.9 , 21.6 ± 1.5 and 31.5 ± 1.7 weeks of gestation [mean $\pm$ standard deviation (SD); no overlap] before PE onset.

Setting—Antenatal clinics.

Population—Pregnant women with T1DM ($n = 118$; 26 developed PE) and healthy nondiabetic pregnant controls ($n = 21$).

Methods—Maternal serum levels of sRAGE (total circulating pool), *N*^ε-(carboxymethyl)lysine (CML), hydroimidazolone (methylglyoxal-modified proteins) and total AGEs were measured by immunoassays.

Main outcome measures—Serum sRAGE and AGEs in pregnant women with T1DM who subsequently developed PE (DM PE+) versus those who remained normotensive (DM PE-).

Results—In DM PE+ versus DM PE-, sRAGE was significantly lower in the first and second trimesters, prior to the clinical manifestation of PE ($P < 0.05$). Further, reflecting the net sRAGE scavenger capacity, sRAGE : hydroimidazolone was significantly lower in the second trimester ($P < 0.05$) and sRAGE : AGE and sRAGE : CML tended to be lower in the first trimester ($P < 0.1$) in women with T1DM who subsequently developed PE versus those who did not. These conclusions persisted after adjusting for prandial status, glycated haemoglobin (HbA1c), duration of diabetes, parity and mean arterial pressure as covariates.

Conclusions—In the early stages of pregnancy, lower circulating sRAGE levels, and the ratio of sRAGE to AGEs, may be associated with the subsequent development of PE in women with T1DM.

Keywords

Advanced glycation end-products; diabetes; hydroimidazolone; *N*^ε-(carboxymethyl)lysine; pre-eclampsia; pregnancy; soluble RAGE

Introduction

Pre-eclampsia (PE), a serious hypertensive complication of pregnancy, is known to occur in the setting of reduced placental perfusion because of inadequate cytotrophoblast invasion and vascular remodelling in the placental bed during early pregnancy, and culminating with diffuse maternal endothelial dysfunction later in pregnancy.¹ PE is known to have a much higher prevalence in women with diabetes than in those without (~20% versus ~5%).^{2,3} The mechanism leading to an increased risk of PE in women with diabetes is not fully understood, nor is an early detection measure available. In a prospective, multicentre study of pregnant women with pre-gestational Type 1 diabetes mellitus (T1DM), we have shown recently that circulating anti-angiogenic factors⁴ and antioxidant carotenoids⁵ are predictive of PE, and thus could contribute mechanistically to the increased prevalence in

diabetes. However, these serum markers were not typically present until early in the third trimester, soon before the clinical manifestation of PE.^{4,5} We have also shown higher plasma cholesterol-rich lipoprotein particles in early pregnancy (first and/or second trimesters) in women with T1DM who subsequently developed PE versus those who remained normotensive.⁶ As part of our continuing efforts to identify early markers of PE in women with diabetes, we have explored the serum levels of soluble receptors for advanced glycation end-products (sRAGE) and several measures of advanced glycation end-products (AGE) in the same study cohort.

As a heterogeneous class of compounds, AGEs are formed when amino groups on proteins and other macromolecules are glycated nonenzymatically by reducing sugars. Their formation involves a complex cascade of reactions under conditions mediated by oxidative stress and carbonyl stress.⁷ In these reactions, the initial glycation product fructose-lysine is subject to dehydration and rearrangement, forming products such as *N*^ε-(carboxymethyl)lysine (CML) and pentosidine. CML may be derived from lipids as well as from carbohydrates,⁸ and may also be formed nonoxidatively.⁹ Another product, methylglyoxal-derived hydroimidazolone (MG-H),¹⁰ is formed when methylglyoxal, a reactive carbonyl-containing intermediate product derived from the oxidation of carbohydrates or lipids, reacts with arginine residues. MG-H comprises three isomers: MG-H1, MG-H2 and MG-H3. Hydroimidazolones, particularly MG-H1, are the quantitatively dominant AGE *in vivo*.¹¹

AGEs are now established as major factors in the pathogenesis of diabetic vascular complications and, in the absence of diabetes, are also implicated in renal failure and aging.^{7–12} In diabetes, hyperglycaemia, enhanced oxidative stress and dyslipidaemia enhance directly the formation of AGEs. AGEs exert cell-mediated effects via RAGE, a multiligand transmembrane receptor of the immunoglobulin superfamily.^{13–15} AGE–RAGE interactions result in inflammation, oxidative stress, vascular hyperpermeability, enhanced thrombogenicity and reduced vasorelaxation, leading to homeostatic perturbation of the vasculature. Three RAGE splice variants have been identified: full-length, N-terminal-truncated and C-terminal-truncated RAGE.¹⁶ The C-terminal-truncated sRAGE protein is produced and secreted into the circulation by human endothelial cells and pericytes. The balance between the levels and actions of AGE/RAGE/sRAGE may be central in AGE-mediated pathology. Recent evidence, especially from prospective studies by Nin et al.,^{17–19} has shown significant positive associations between circulating sRAGE and AGEs, and cardiovascular disease, in nonpregnant populations with T1DM. These associations were stronger in the presence of endothelial and renal dysfunction, and inflammation. Similar findings of increased sRAGE in predicting cardiovascular mortality have also been reported in a larger study of Finnish adults with T1DM.²⁰ PE is a disease of the vasculature, with endothelial dysfunction serving as the convergence of both maternal and placental factors.^{21,22} Several cross-sectional studies in pregnant women without diabetes have shown elevated RAGE and AGEs to be associated with vascular dysfunction in PE versus controls.^{23–28} However, these studies have not addressed whether an altered sRAGE–AGE system precedes or is a consequence of PE. Furthermore, no data have been reported on the causal associations of sRAGE and AGEs with PE in T1DM, a condition associated with a four-fold increase in PE.^{2,3}

Thus, in the present study, we evaluated the total circulating levels of sRAGE, together with CML, hydroimidazolone and total AGEs, in a prospective cohort of pregnant women with established T1DM.⁴ Our goal was to determine whether sRAGE, and/or measures of sRAGE combined with measures of AGE, can serve as early markers of PE within the context of T1DM, and hence to define potential pathogenic mechanisms. We included a small group of normotensive pregnant women without diabetes to provide reference values,

and to enable a secondary comparison between 'healthy' women with and without diabetes. Both by design and necessity (resource issues for a prospective study), our study does not address PE in the absence of T1DM.

Methods

The participants, study design and enrolment criteria have been described previously.⁴ Briefly, the study was conducted according to Declaration of Helsinki principles and was approved by the Institutional Review Boards of all participating institutions (Australia, Norway and USA). All participants provided written informed consent. Over a 4-year period, 151 pregnant women with established Type 1 diabetes and 24 pregnant women without diabetes were recruited in their first trimester (~12 weeks) and followed throughout pregnancy. Clinical data and blood and urine specimens were collected at 12.2 ± 1.9 , 21.6 ± 1.5 and 31.5 ± 1.7 weeks of gestation [mean $\pm$ standard deviation (SD); no overlap]. These three visits corresponded to late first, mid-second and early third trimesters and, in the case of women with PE, all three preceded the onset of PE.

Of 151 women with T1DM, 133 provided samples at all study visits and completed pregnancy. Of these, 26 developed PE, 12 developed pregnancy-induced hypertension (PIH) and 95 remained normotensive. Three of the 95 were excluded because of the later detection of proteinuria in urine samples collected at visit 1. Of the 24 healthy controls without diabetes, 21 completed the entire pregnancy with no hypertensive complications; three were excluded on account of PE (one), miscarriage (one) or loss to follow-up (one). Thus, 92 women with T1DM who remained normotensive, 26 women with T1DM who developed PE and 21 controls without diabetes were included in the final analyses. Our group without diabetes was recruited to serve as a reference control, and we aimed for the number of participants in this group to be approximately equal to the expected number of PE cases in the cohort with diabetes.

Blood was collected after an overnight fast and, in women with T1DM, prior to insulin administration. Actual prandial status was recorded. Serum was centrifuged promptly and stored at -80°C . Urine was collected over 24 hours, the volume was recorded and an aliquot was stored at -80°C . Specimens were accumulated at the study centres, and then shipped in batches, frozen on dry ice, to the University of Oklahoma Health Sciences Center (OUHSC), Oklahoma City, OK, USA, where they were again stored at -80°C until analysis.

Diagnosis of PE and gestational hypertension

The definitions of PE and gestational hypertension were as described previously.¹ Briefly, PE was defined as new-onset hypertension ($>140/90$ mmHg) after 20 weeks of gestation in a previously normotensive woman, accompanied by proteinuria (>300 mg/24 hours).

Laboratory measures

Glycated haemoglobin (HbA1c) was measured at each centre by the DCA2000 method (Bayer Diagnostics, Elkhart, IN, USA). Urine analyses were carried out at the OUHSC Clinical Laboratory. Serum sRAGE levels were measured at OUHSC, and levels of AGEs were determined at the University of Oslo, Oslo, Norway.

Serum sRAGE (total circulating pool) was measured by a DuoSet enzyme-linked immunosorbent assay (ELISA) kit (DY1145) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). In brief, a monoclonal mouse anti-human RAGE (extracellular domain) antibody was coated onto microplate wells overnight. Serum samples (100 μl) and standards in duplicate were pipetted into the wells and incubated for 2 hours. After washing away unbound substances, a biotinylated goat anti-human RAGE

(extracellular domain) antibody was added to the wells for 2 hours. Following a wash to remove unbound antibody reagent, streptavidin conjugated to horseradish peroxidase was added to the wells for 20 minutes, and the wells were washed again. The colour was developed by the addition of the substrate solution (1 : 1 mixture of H₂O₂ and tetramethylbenzidine), with a reaction duration of 20 minutes. The colour development was stopped with 2 N H₂SO₄, followed by an optical density reading at 450 nm subtracting the readings at 570 nm. The low limit of quantification (LOQ) was 62.5 pg/ml. The intra-assay coefficient of variation (CV) was 3%, and the inter-assay CV was 5%, for internal controls for the sRAGE assay.

Serum levels of CML, MG-H and total AGE were determined by previously published methods developed by Hanssen and coworkers^{29–31} using competitive immunoassays with the Delayed Europium Lanthanide Fluorescence Immunoassay (DELFLIA) system. One CML, hydroimidazolone or AGE unit was defined as the competitive activity of 1 µg of CML MG-BSA or AGE standard, according to Makita et al.³² The final serum concentrations were corrected for total protein concentration. The data presented are the means of duplicates. The intra-assay CV was 8.7% for the AGE assay, 6% for the CML assay and 12% for the hydroimidazolone assay. The inter-assay CV was 15% for the AGE assay, 20% for the CML assay and 21% for the hydroimidazolone assay. All assays were conducted by laboratory personnel blind to the sample identification codes.

Statistical analysis

Results were expressed as the mean $\pm$ SD in text and tables, and as the mean $\pm$ standard error of the mean (SEM) in the figures. Our analyses included three groups of pregnancies: ‘diabetic PE’ (i.e. developed PE, DM PE+), ‘diabetic normotensive’ (DM PE–) and ‘nondiabetic normotensive’ (DM–). The latter group was included to provide normal ranges for the measures. We excluded PIH ($n = 12$) from the analyses to study clearly defined PE (DM PE+) versus DM PE–.

Our primary aim was to identify risk indicators for PE in women with T1DM. Therefore, our primary analysis considered differences at each visit (visits 1–3) between the DM PE+ and DM PE– groups. Differences in clinical characteristics were analysed by *t*-tests, followed by Mann–Whitney nonparametric tests; conclusions were the same either way, and so the *t*-test results are presented. Generalised linear models (GLMs) were used to analyse these differences. Serum AGE and sRAGE were normally distributed and analysed without transformation; CML and hydroimidazolone were skewed, and were analysed without (Table S1) and with (Table S2) logarithmic transformation. The conclusions were unchanged by transformation, and the untransformed statistics are presented in the figures. Analyses of the ratios between sRAGE and its ligands, reflecting net sRAGE scavenger capacity, were conducted after logarithmic transformation. The following covariates were considered in the GLMs, and were selected on the basis of differences at baseline and/or their known associations with PE: prandial status, duration of diabetes, HbA1c, body mass index (BMI), parity and mean arterial pressure. As reported previously,^{5,6} fasting is challenging for pregnant women with T1DM, and so data have been adjusted for the actual prandial status. Adjusted means after prandial status, duration of diabetes, HbA1c, BMI, parity and mean arterial pressure were considered are given in Table S3. No adjustments were made to the α level for multiple comparisons between groups or across time points and, instead, a hierarchy of questions of primary interest (i.e. comparisons between DM PE+ and DM PE– groups) was specified. A secondary comparison was exploratory, and addressed differences between DM PE– and DM– pregnancies to discern changes attributable to diabetes. All tests were two-tailed, with $P < 0.05$ considered as significant. Statistical analyses used SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Clinical characteristics at visit 1

The 133 women with T1DM and the 21 women without T1DM (~95% are white Europeans) have been described previously.⁴ Table 1 briefly summarises the clinical characteristics of the women included in the present analyses at visit 1. No differences were observed across groups with regard to age, blood pressure, urinary microalbumin or gestational age at visit 1. HbA1c was higher in women with than without diabetes, but did not differ significantly between the two groups of women with diabetes. DM PE+ women had a younger age of diabetes onset ($P < 0.01$) and longer diabetes duration ($P < 0.05$) than DM PE- women. In our T1DM cohort, the women were documented as not having PE up to and including visit 3 (~31.5 weeks of gestation).

As reported previously, during gestation, the mean arterial blood pressure started to increase from visit 2, most markedly in the DM PE+ group.⁴ The urine microalbumin : creatinine ratio remained constant amongst all groups during the first three visits, but was greatly increased at term in DM PE+ pregnancies,⁴ consistent with the clinical development of PE.

Serum sRAGE and AGE

In DM PE+ versus DM PE- groups, the serum levels of sRAGE were significantly lower at visits 1 and 2 ($P < 0.05$, Figure 1a). However, values were similar between these two groups at visit 3. Tables S1 (raw measures) and S2 (log-transformed measures) also show similar observations in the case of serum sRAGE. Analyses of serum levels of CML, hydroimidazolone and total AGE showed no statistically significant differences between DM PE+ and DM PE- groups at any visit (Figure 1b–d), although levels were generally higher in DM PE+ versus DM PE-. These conclusions persisted in multiple regression analyses, except in the case of BMI which exerted an independent effect in diminishing the significantly lower sRAGE in DM PE+ versus DM PE- (Table S3). Secondary analyses revealed no significant differences in these parameters between DM PE- and DM- groups.

Ratio of sRAGE to its ligands

As in the development of AGE-mediated vascular damage, the overall balance between sRAGE and its ligands may be more critical than the concentration of any one independently, we analysed the ratios of serum sRAGE to CML, hydroimidazolone and immunoreactive AGEs. Serum sRAGE : CML (Figure 2a) tended to be lower in DM PE+ versus DM PE- at visit 1 ($P < 0.1$). In the second trimester, sRAGE : hydroimidazolone (Figure 2b) was significantly lower in DM PE+ versus DM PE- ($P < 0.05$). Consistent with these data, the ratio between sRAGE and total nonspecific AGE (sRAGE : AGE, Figure 2c) also showed a lower trend in DM PE+ versus DM PE- at visit 1 ($P < 0.1$). These conclusions persisted in covariate analyses (Table S3). Secondary analyses revealed no significant differences in these ratios between DM PE- and DM- groups.

Discussion

In the present study, our goal was to determine whether, early in pregnancy, serum levels of sRAGE and the ratios of sRAGE to the AGEs, CML, hydroimidazolone and total AGE, might provide markers and mechanistic clues for the development of PE in women with T1DM. In our cohort of women with T1DM, all were documented as not having PE up to and including visit 3 (~31.5 weeks of gestation). The results showed that sRAGE levels were significantly lower at early stages of gestation in DM PE+ relative to DM PE- pregnancies. Furthermore, the serum sRAGE : hydroimidazolone ratio was significantly lower in the second trimester, and the serum sRAGE : CML and sRAGE : AGE ratios

tended to be lower in the first trimester. These findings suggest that, during the early stages of pregnancy, lower circulating sRAGE, and/or its ratio with putative ligands, may be associated with subsequent PE in pregnancies complicated by T1DM.

Our findings of lower sRAGE in pregnant women with T1DM who subsequently developed PE (compared with those who remained normotensive) are novel and clearly different from those found in previous cross-sectional studies of pregnant women without diabetes.^{23–26,28} The latter showed elevated sRAGE and/or AGE associated with the presence of PE. They do not provide data on the levels of sRAGE in early pregnancy, nor do they address levels in the presence of T1DM. It is believed that sRAGE binds to AGEs and other RAGE ligands, acting as a feedback and ‘sink mechanism’ to limit their activation of transmembrane RAGE receptors and downstream signalling in vascular cells.^{15,16} Thus, a lower level of sRAGE in the circulation could conceivably confer susceptibility to vascular complications, whereas a higher level of sRAGE may prevent the development of vascular complications. A growing body of evidence in nonpregnant women with diabetes supports this relationship. The circulating level of sRAGE was shown to be lower in patients with T1DM with macro- or microvascular complications than in those without,³³ whereas, in animal studies, the detrimental effects of AGEs on vascular complications were reversible on treatment with exogenous sRAGE.^{13,34} In contrast, in more recently reported large cohorts of subjects with T1DM, higher serum sRAGE has been associated with increased cardiovascular events.^{17–20} The patients in these studies were older, had existing complications of diabetes and had less favourable HbA1c levels than those in our cohort. In contrast, sRAGE has been shown to be inversely associated with BMI in patients with Type 2 diabetes.³⁵ These observations might support the association, in our study, of baseline BMI with serum sRAGE in women with diabetes. Those who developed PE tended to have higher baseline BMI than those who remained normotensive. It must also be remembered that serum measures are unlikely to reflect directly what is happening in specific tissues with regard to complex systems such as AGE–RAGE, and the affected tissues in PE are not the same as those in coronary heart disease. For reasons such as these, different associations may be found between sRAGE and the different complications of diabetes.

As sRAGE may play a role in the prevention of vascular tissue injury by scavenging circulating or tissue AGEs, thereby reducing the opportunity for AGEs to activate transmembrane RAGE, we reasoned that the ratio of sRAGE to its ligands may reflect ‘net scavenging capacity’ and provide an index of susceptibility to vascular complications, including PE, in patients with diabetes. Our results are consistent with this hypothesis. Thus, the serum ratios between sRAGE and CML, hydroimidazolone and the nonspecific total AGE were decreased in the DM PE+ versus DM PE– groups in the first and/or second trimester. The data suggest that serum sRAGE : ligand ratios may be associated with PE risk in women with pre-gestational T1DM.

A fraction of the sRAGE pool is produced via alternative splicing mechanisms, and is termed ‘endogenous sRAGE’ (esRAGE).³⁶ There is evidence from cross-sectional studies of women without diabetes that circulating sRAGE and esRAGE may represent distinct markers and play differential roles in the pathogenesis of PE. Fasshauer et al.²⁵ showed that the serum concentration of esRAGE was more than three-fold higher in women with PE than in control subjects at 29–31 weeks of gestation. Oliver et al.²⁶ also reported similar findings of significantly higher maternal serum and amniotic fluid sRAGE and esRAGE in women with severe PE versus healthy pregnant controls at 27–32 weeks of gestation. Kwon et al.²⁷ added to these observations by showing significantly elevated esRAGE : sRAGE in PE cases at approximately 33 weeks of gestation versus healthy pregnant controls. However, in these cross-sectional studies, as esRAGE levels were not measured before the onset of PE or at an earlier gestational age, and only in women without diabetes, it is not possible to

define whether there are differential roles of sRAGE and esRAGE in predicting PE, or differences in the levels of these species in the presence of PE. In contrast, our longitudinal study used samples obtained before the onset of PE, and showed that, in women with T1DM, maternal serum sRAGE levels in early pregnancy were lower in those with subsequent PE than in those who remained normotensive. Our findings are in agreement with those of Pertyska-Marczewska et al.,³⁷ who reported that plasma sRAGE levels were significantly lower in the first trimester in women with versus without T1DM. In our study, sRAGE levels were indeed lower in DM PE+ versus DM- pregnancies during the first two trimesters, although the differences were not statistically significant. This points to an interesting question: whether T1DM lowers systemic levels of sRAGE in early pregnancy, thus lowering the threshold for the development of PE. Further investigation in a cohort with a larger DM- control group would shed more light on this question.

Increased levels of AGEs have been reported in umbilical cord blood,³⁸ in placenta²⁴ and in skin (by autofluorescence)³⁹ in women without diabetes in association with PE. Harsem et al.,⁴⁰ however, did not find an elevation of circulating AGE, despite the presence of increased 8-isoprostane levels (reflecting increased oxidative stress) in pregnancies complicated by PE. In the present study, we evaluated longitudinal changes in serum levels of two specific AGE species (CML and hydroimidazolone) and total nonspecific AGE throughout gestation. We did not detect statistically significant changes in the two AGE species during pregnancy, and the levels of AGE were only increased mildly in the DM PE+ versus DM PE- pregnancies, without reaching statistical significance. The data therefore suggest that serum AGE levels alone cannot serve as reliable markers for PE risk.

Bierhaus & Nawroth⁴¹ further suggested that, as the circulating level of sRAGE is lower than K_d , it would not be sufficient to scavenge CML-modified AGE in a biological context. However, it is very likely that the levels of sRAGE within the immediate vicinity of affected tissues, where sRAGE is released from cells and where vascular injury takes place, may be higher than in the circulation. In this regard, measurable changes in serum sRAGE levels may be reflective of those in affected tissues, and therefore may still be of biological significance. The reduced sRAGE scavenger capacity in early pregnancy in the DM PE+ group is of particular interest: it may not only serve as an early disease marker, but may also shed light on the underlying pathogenic mechanisms. PE is likely to be multifactorial in nature. An unfavourable sRAGE : ligand ratio at the first and second trimesters might contribute to a milieu which leads to an unfavourable angiogenic/anti-angiogenic balance and reduced antioxidant status, which we observed at the third trimester, shortly before PE onset.^{4,5}

In summary, we have determined the serum level of sRAGE and its ratio to the advanced glycation products, CML, hydroimidazolone and total AGE, in an established prospective cohort of pregnant women with pre-gestational T1DM. No specific site effects were noted in any of our outcome variables in this multicentre trial. Our results do not address causality, but indicate that, during the early stages of pregnancy, lower circulating sRAGE levels and lower ratios of sRAGE to hydroimidazolone are associated with subsequent PE in pregnant women with T1DM. Our study is limited by its small size, the absence of pre-pregnancy samples and the absence of data to define sRAGE and AGEs in other tissues, such as placenta and/or maternal myometrium, which are involved in the pathogenesis of PE (although the latter is a theoretical weakness, as these tissues cannot be obtained in a prospective study). Our findings need to be confirmed in larger cohorts, and other future studies should address the role of both sRAGE and esRAGE in PE in Type 2 and gestational diabetes, and should pursue the mechanisms and preventative measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Serum levels of soluble receptor for advanced glycation end-products (sRAGE) (a), *N*^ε-(carboxymethyl)lysine (CML) (b), hydroimidazolone (c) and nonspecific advanced glycation end-products (AGE) (d) during gestation. Values (means ± SEM) were plotted against the average gestational age at each visit. For illustrative purposes, three groups of women are shown: □, normotensive women without diabetes (DM-) (*n* = 21); ○, normotensive women with diabetes (DM PE-) (*n* = 92); ●, women with diabetes and PE (DM PE+) (*n* = 26). Values significantly different between subject groups are also indicated: the primary analysis compared DM PE+ and DM PE- groups: **P* < 0.05; †*P* < 0.1; two-tailed *t*-test.

Figure 2. Ratios of soluble receptor for advanced glycation end-products (sRAGE) versus advanced glycation end-products (AGE) during gestation: (a) N^{ϵ} -(carboxymethyl)lysine (CML); (b) hydroimidazolone; (c) nonspecific AGE. For illustrative purposes, three groups of women are shown: $\square$, normotensive women without diabetes (DM-) ($n = 21$); $\circ$, normotensive women with diabetes (DM PE-) ($n = 92$); $\bullet$, women with diabetes and PE (DM PE+) ($n = 26$). Values significantly different between subject groups are also indicated: the primary analysis compared DM PE+ and DM PE- groups: $*P < 0.05$; $\dagger P < 0.1$; two-tailed t -test. Statistical analyses were conducted after logarithmic transformation.

Table 1
General clinical profile of women with and without Type 1 diabetes mellitus (DM)

	<i>n</i>	Diabetic			<i>P</i>	Nondiabetic (DM-)
		Pre-eclamptic (DM PE+)	DM PE+ versus DM PE-	Normotensive (DM PE-)	DM PE- versus DM-	
Age (years)	26	29 ± 6	0.39	30 ± 5	0.10	32 ± 5
Age at DM onset (years)		12 ± 7	0.0082	17 ± 8		-
BMI (kg/m ²)		28 ± 6	0.06	26 ± 5	0.04	24 ± 4
Duration of DM (years)		16 ± 7	0.036	13 ± 8		-
HbA1c (%)		7.3 ± 1.1	0.087	6.8 ± 1.1	<0.0001	5.3 ± 0.3
Blood pressure (mm Hg)	Systolic	115 ± 13	0.35	112 ± 12	0.88	112 ± 9
	Diastolic	67 ± 9	0.82	67 ± 8	0.89	66 ± 8
Urine microalbumin (mg/l)		7.2 ± 9.9	0.64	6.2 ± 7.1	0.067	4.5 ± 1.7
Urine creatinine (mg/dl)		73.1 ± 43.4	0.77	76.1 ± 36.8	0.44	69.5 ± 32.2
Urine creatinine (μmol/l) [SI]		6466 ± 3835	0.77	6726 ± 3251	0.44	6144 ± 2848
Microalbumin/creatinine ratio		13.2 ± 20.3	0.88	7.9 ± 7.1	0.40	7.1 ± 2.6
Gestational age (weeks)	Visit 1	12.2 ± 2.0	0.73	12.1 ± 1.8	0.19	12.7 ± 1.7
	Visit 2	21.9 ± 1.7	0.28	21.5 ± 1.4	0.81	21.5 ± 1.2
	Visit 3	31.7 ± 1.7	0.62	31.4 ± 1.7	0.38	31.2 ± 1.1

BMI, body mass index; PE, pre-eclampsia.

Measurements refer to visit 1 unless otherwise indicated. Values are means ± standard deviation (SD). Adapted from Yu et al.⁴