



# Skin autofluorescence, a marker of advanced glycation end products and oxidative stress, is increased in recently preeclamptic women

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Received for publication March 10, 2006; revised June 13, 2006; accepted June 29, 2006

## KEY WORDS

Preeclampsia  
Advanced glycation  
end product  
Atherosclerosis  
Intima-media  
thickness

**Objective:** Advanced glycation end-products are considered to be markers of oxidative stress and to be involved in the atherosclerotic process. We investigated skin autofluorescence, which reflected advanced glycation end-product accumulation, in recently preeclamptic women and its relationship with intima-media thickness, which is a marker of atherosclerosis.

**Study design:** Skin autofluorescence of the arm and leg was measured in 26 preeclamptic women and 17 control subjects at 3 to 13 months after delivery. Lipid profiles, smoking habits, and intima-media thickness of 5 carotid and femoral artery segments were recorded.

**Results:** The preeclampsia group was younger and had higher values for blood pressure, insulin resistance, common femoral artery intima-media thickness, and skin autofluorescence of the leg. With the use of linear regression analysis, the difference in leg autofluorescence was explained only by preeclampsia. In the preeclampsia group, skin autofluorescence of the leg correlated with smoking and common femoral artery intima-media thickness.

**Conclusion:** These results support the hypothesis of accelerated atherosclerosis in recently preeclamptic women and the possible involvement of advanced glycation end-product accumulation.  
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Women with a history of preeclampsia appear to have an increased risk of cardiovascular manifestations in later life.<sup>1</sup> Recently, we reported increased arterial

intima-media thickness (IMT), particularly of the femoral arteries, in women with a recent history of early-onset preeclampsia that might indicate early atherosclerosis.<sup>2</sup>

Oxidative stress plays a central role in the cause of atherosclerosis.<sup>3</sup> Likewise, it is suggested that oxidative stress is a component of preeclampsia<sup>4</sup> that could provide the linkage between the decreased placental perfusion and the maternal syndrome through direct vascular damage and endothelial dysfunction.<sup>5,6</sup>

Presented at the 26th Annual Meeting of the Society for Maternal Fetal Medicine, January 30–February 4, 2006, Miami, FL.

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Advanced glycation end-products (AGEs) are the irreversible products of nonenzymatic glycation and the oxidation of proteins and lipids. In normal conditions, AGEs accumulate gradually in tissues and plasma with aging.<sup>7</sup> However, during oxidative and/or glyceemic stress, AGEs can be formed rapidly and promote atherosclerosis through the stimulation of the receptor for AGE (RAGE) and subsequent cytokine production.<sup>8</sup>

RAGE activation can induce pathophysiologic changes that are comparable with changes that are found in preeclampsia.<sup>9</sup> A recent study suggests elevated RAGE protein in preeclampsia<sup>9</sup> that may contribute to vascular endothelial dysfunction, a hallmark of preeclampsia.<sup>10,11</sup>

Tissue AGE levels can be assessed noninvasively by the autofluorescence reader (AFR; patent number PCT/NL99/00607, prototype of current AGE-reader; DiagnOptics BV Groningen, The Netherlands), a recently introduced and validated device that measures skin autofluorescence based on the fluorescent properties of a part of the various AGE molecules.<sup>12</sup> With the AFR, a close relation of skin autofluorescence was established not only with renal failure, diabetes mellitus, and its complications<sup>13,14</sup> but also with inflammation and oxidative stress in acute coronary syndromes.<sup>15</sup>

Our aim was to assess skin autofluorescence of arm and leg using the AFR in women who recently had early-onset preeclampsia and to investigate its relationship with IMT of carotid and femoral arteries, which are markers of early atherosclerosis.

## Methods

### Subjects

From January 2003 until April 2004, 27 consecutive women with a history of early onset (<34 weeks of gestational) preeclampsia and 17 women with uncomplicated pregnancies participated. Thirty-four of the 44 women who participated in this study also took part in our previous investigation that showed increased IMT in women with early-onset preeclampsia.<sup>2</sup> No study data were used in the clinical care of these study subjects and thus could not have affected the outcome of either investigation. Data of both studies were analyzed separately after the completion of all the measurements to prevent possible bias.

All women were white, had singleton pregnancies, and were tested between 3 and 11 months after delivery, at least 6 weeks after ending lactation. *Preeclampsia* was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy.<sup>16</sup> Women with pre-existing hypertension (blood pressure at <20 weeks of gestation of  $\geq 140/90$  mm Hg or using anti-hypertensives), diabetes mellitus, renal disease, or preeclampsia in previous pregnancies were excluded. None of the participants used medication during the studies.

The study was approved by the medical ethics committee of the University Medical Center Groningen, and all women gave informed consent.

### Measurements

A history of hypertension, diabetes mellitus, and renal diseases, smoking habits, drug therapy, weight, length, and family history (first degree) that related to premature cardiovascular diseases (men, <55 years old; women, <65 years old) was obtained by questionnaires. Blood pressure was measured (Korotkov V) at the end of the examinations.

Blood samples were taken after an overnight fast to measure blood glucose, creatinine level, and lipids (total and high-density lipoprotein-cholesterol, and triglycerides) in serum by standard laboratory methods. Low-density lipoprotein-cholesterol was calculated by the Friedewald formula. Conversion of blood glucose to plasma levels was calculated by multiplying the blood glucose levels by 1.11.<sup>17</sup> Insulin resistance was assessed by the homeostasis model assessment (HOMA) and calculated with the following formula: fasting insulin  $\times$  fasting plasma glucose/22.5.<sup>18</sup> Lipoprotein (a) was determined by nephelometry (BN ProSpec; Dade Behring Holding GmbH, Liederbach, Germany). Urinary albumin-creatinine ratio was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). *Microalbuminuria* was defined as an albumin-creatinine ratio of >3.4 g/mol in at least 2 of 3 specimens of urine that were voided immediately after awakening.<sup>19</sup>

### Assessment of IMT

IMT of the common carotid artery, internal carotid artery and carotid bulb, and common femoral and superficial femoral artery was obtained bilaterally with high-resolution B-mode ultrasound (Acuson 128XP10; Acuson Corporation, Mountain view, CA), as reported previously.<sup>2</sup> Offline video analysis was performed by a reader who was unaware of the clinical characteristics. *IMT* was defined as the mean distance between the intima and the media double-line pattern, as an average of left- and right-sided values and expressed in millimeters.

### Autofluorescence measurements

Skin autofluorescence was assessed by the AFR, as described previously.<sup>12,15</sup> The AFR illuminates approximately 4 cm<sup>2</sup> of skin with a light source that emits between 350 and 420 nm (peak excitation, approximately 370 nm). Light from the skin is measured between 300 and 600 nm with a spectrometer with a 50- $\mu$ m glass fiber (USB2000 fiber optic spectrometer; Avantes, Eerbeek, The Netherlands).

All measurements were performed in a semidark environment. After control measurements were made with the light off, autofluorescence was measured 3 times

at the volar side of the right lower arm and at the dorsal side of the right calf. The mean value of the 3 autofluorescence measurements at each location was used for analysis. Care was taken to perform the measurements at normal skin sites. Autofluorescence was calculated by dividing the average light intensity emitted per nanometer over the emission range (420–600 nm) by the average light intensity that is emitted per nanometer over the excitation range (300–420 nm) and was multiplied by a factor 100 for easy evaluation. Autofluorescence is expressed in arbitrary units (AU). Calculations were performed offline by automated analysis and were observer-independent. Day-to-day variability was found previously to be 5%.<sup>12</sup>

## Statistics

From the results of a previous study of diabetic patients and control subjects,<sup>12</sup> we calculated that, to detect a difference of 50% in autofluorescence in the preeclampsia group, a minimum of 10 women in each group was required ( $\alpha = .05$ ;  $\beta = .2$ ). We used the Shapiro-Wilke test to assess the normality of the data. For statistical analysis of autofluorescence and levels of triglycerides, insulin, creatinine, lipoprotein (a), and HOMA, we used logarithmic transformations to normalize distributions. Group differences were tested with the unpaired Student *t* test. Frequencies or categorical variables were compared with the  $\chi^2$ -test. Correlation analyses were performed by Pearson's test. Contribution of possible confounding variables to the association of having a preeclamptic pregnancy with autofluorescence was examined by linear regression analysis. A 2-sided probability value of  $<.05$  was considered to indicate statistical significance. Statistical analyses were performed with SPSS software (version 12.0.1; SPSS Inc, Chicago, IL). Box plots were used to show the medians, interquartile ranges, and outliers.

## Results

### Clinical and biochemical characteristics

Autofluorescence of 1 woman of the preeclampsia group was not available because of a temporary defect of the spectrometer; she was excluded from further analyses. Analyses are based on 26 recently preeclamptic women and 17 control subjects. Clinical characteristics of the subjects are summarized in Table I.

Ten women (38%) in the preeclampsia group and 7 control subjects (41%) used oral contraceptives ( $P = .3$ ). Biochemical and vascular data are shown in Table II.

### Autofluorescence

Figure 1 shows that the preeclampsia group had higher autofluorescence ( $P = .003$ ) of the leg (mean  $\pm$  SD,

**Table I** Clinical characteristics of the recently preeclampsia and control groups

Variable	Preeclampsia group	Control group	<i>P</i> value
N	26	17	
Age (y)*	30 $\pm$ 4	32 $\pm$ 3	.02
Body mass index (kg/m <sup>2</sup> )*	25 $\pm$ 5	23 $\pm$ 3	.1
Systolic blood pressure (mm Hg)*	128 $\pm$ 10	115 $\pm$ 9	<.001
Diastolic blood pressure (mm Hg)*	81 $\pm$ 9	68 $\pm$ 8	<.001
Current smoking (n)	11 (42%)	5 (29%)	.4
Family history of cardiovascular diseases (n)	16 (62%)	8 (47%)	.4
Primiparous women (n)	23 (88%)	12 (71%)	.1
Gestational age at delivery (wk)*	31 $\pm$ 3	40 $\pm$ 1	<.001
Small for gestational age infant: Birth weight <10th percentile (n)	18 (69%)	0	<.001
Interval delivery to day of study (mo)*	7 $\pm$ 3	6 $\pm$ 2	.3

\* Values are expressed as mean  $\pm$  SD.

1.8  $\pm$  0.5 AU) compared with the control group (1.4  $\pm$  0.5 AU). No difference was found for the autofluorescence of the arm between both groups (preeclampsia, 1.8  $\pm$  0.4 AU, vs control, 1.7  $\pm$  0.3 AU;  $P = .8$ ).

In the preeclampsia group, autofluorescence of the leg correlated significantly with autofluorescence of the arm ( $r = .5$ ;  $P = .01$ ), smoking ( $r = .4$ ;  $P = .04$ ), and common femoral artery IMT ( $r = .4$ ;  $P = .03$ ; Figure 2). In the control group, autofluorescence of the arm correlated with triglycerides ( $r = .6$ ;  $P = .01$ ), smoking ( $r = .5$ ;  $P = .04$ ), and superficial femoral artery IMT ( $r = .5$ ;  $P = .03$ ). All other clinical and biochemical variables did not show significant correlations with autofluorescence of either arm or leg, in either groups.

To estimate whether the differences in maternal age and HOMA scores between the 2 groups could have influenced the difference in autofluorescence of the leg, we conducted a linear regression analysis, with autofluorescence of the leg as the dependent variable and maternal age, HOMA score, and history of preeclampsia (yes or no) as independent variables. This analysis showed that a history of preeclampsia was the only significant ( $P = .008$ ) contributor for the difference in autofluorescence of the leg.

### Comment

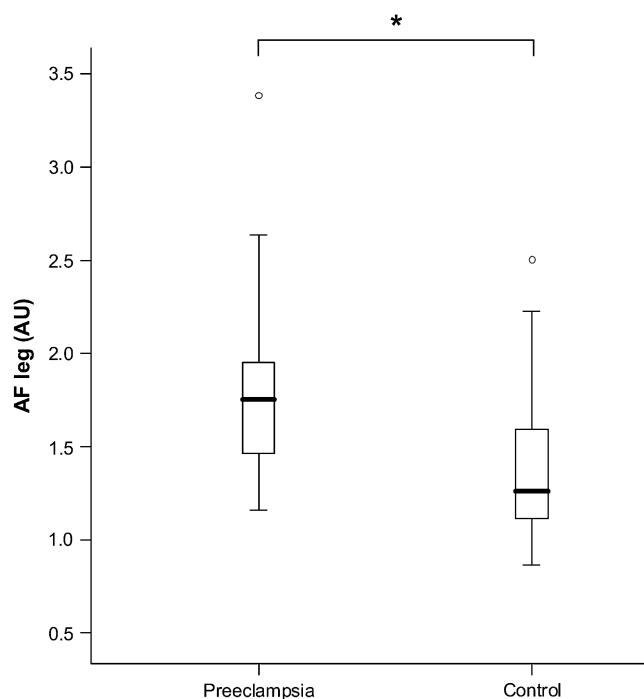
Our study shows higher autofluorescence of the leg in recently preeclamptic women compared with control subjects. The difference remained significant after

**Table II** Biochemical and vascular results of the recently preeclampsia and control groups

Variable	Preeclampsia group	Control group	P value
Total cholesterol (mmol/L)*	5.1 ± 0.8	4.9 ± 0.7	.5
High-density lipoprotein cholesterol (mmol/L)*	1.5 ± 0.3	1.5 ± 0.3	.8
Low-density lipoprotein cholesterol (mmol/L)*	3.0 ± 0.7	2.9 ± 0.6	.8
Triglycerides (mmol/L)*	1.3 ± 0.7	1.1 ± 0.3	.3
Lipoprotein (a) (mg/L) <sup>†</sup>	102 (7-593)	102 (24-1040)	.6
Creatinine (μmol/L)*	80 ± 8	84 ± 11	.3
Plasma glucose (mmol/L)*	5.0 ± 0.6	4.8 ± 0.4	.2
Insulin (mU/L)*	13 ± 6	7 ± 4	.002
Insulin resistance (HOMA)*	2.8 ± 1.5	1.6 ± 0.8	.001
Microalbuminuria (n)	2 (7%)	0	.2
Common carotid artery (mm)*	0.64 ± 0.07	0.63 ± 0.09	.6
Internal carotid artery (mm)*	0.55 ± 0.09	0.58 ± 0.10	.4
Carotid bulb (mm)*	0.68 ± 0.08	0.71 ± 0.07	.2
Common femoral artery (mm)*	0.60 ± 0.09	0.54 ± 0.06	.02
Superficial femoral artery (mm)*	0.55 ± 0.09	0.53 ± 0.07	.5

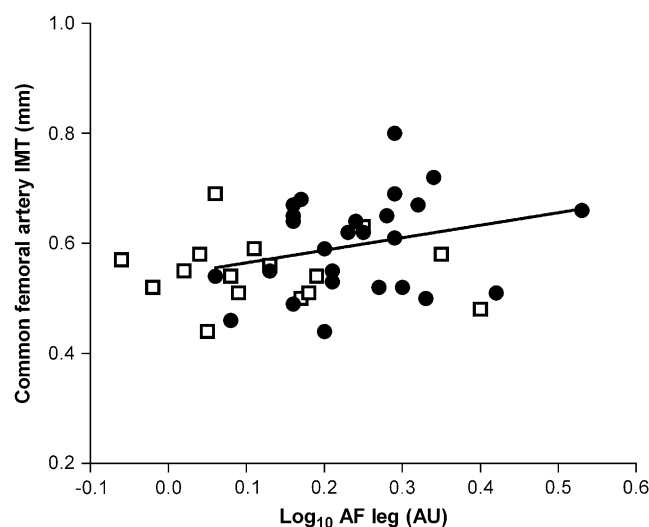
\* Values are expressed as means ± SD.

<sup>†</sup> Values are expressed as median (range).



**Figure 1** Box plots of autofluorescence of the leg in the preeclampsia and control groups. The *asterisk* denotes a probability value of .003.

correction for differences in HOMA scores and maternal age. In addition, a positive correlation with IMT of the common femoral artery and smoking was observed in the recently preeclamptic group. Remarkably, we did not find differences in autofluorescence of the arm between both groups. Autofluorescence was assessed with the AFR, a simple and rapid alternative to invasive measurement of AGE accumulation, which previously



**Figure 2** IMT of the common femoral artery is plotted against autofluorescence of the leg in recently preeclamptic women (*closed circles*) and control subjects (*open squares*). The *line* represents the relationship of recently preeclamptic women ( $r = .4$ ;  $P = .03$ ).

was validated by comparison of autofluorescence with the content of specific AGEs in extracts from skin biopsy specimens in groups of diabetic, hemodialysis, and control subjects.<sup>12,13</sup> Although the contribution of other skin fluorophores on autofluorescence cannot be excluded, the strong relations with both fluorescent and nonfluorescent skin AGE levels in biopsy specimens support the use of autofluorescence as a marker of the AGE pool.<sup>12,13</sup>

AGEs have been implicated as contributing factors in the progression of chronic, age-related diseases (such as atherosclerosis, end-stage renal disease, and diabetes



mellitus). AGEs have long been considered to be predominantly markers of glycemic stress.<sup>20</sup> However, in the last decade, the central role of reactive carbonyl compounds, which result from oxidative stress, in the formation of AGEs has become accepted.

To our knowledge, only 1 study has been performed on the relation between AGEs and preeclampsia. Cooke et al<sup>9</sup> compared RAGE protein expression in myometrial and omental vascular beds among normal pregnant women, women with preeclampsia, and nonpregnant women. Blood vessels from women with preeclampsia consistently had intense staining for RAGE. Because RAGE activation may be mediating the progression of diseases that are characterized by oxidative stress and inflammation by altering vascular cell function, our study supports the possible role of an abnormal AGE-RAGE interaction during and/or after preeclampsia.

Possibly more AGEs are formed or deposited in the skin during preeclampsia. An alternative possibility is that the AGEs are increased already before pregnancy and thereby contribute to vascular damage in general.

Because AGE accumulation is demonstrated in vascular walls and is known to be able to accelerate atherosclerosis, our findings of increased IMT of the femoral artery and increased AGEs might indicate accelerated atherosclerosis in the femoral vasculature of previously preeclamptic women.

As for the increased autofluorescence of the leg and not of the arm, one may speculate that the course of changes of AGE content in the skin between leg and arm are different: AGE levels in the leg have not yet returned to prepregnancy levels, in contrast to those in the arm. Another possibility may be that differences in hydrostatic and vascular pressures and/or permeability between arm and leg and between recently preeclamptic women and control subjects could have induced a different degree of AGE deposition.

Preeclampsia, in particular, in combination with a preterm delivery appears to be associated with an increased risk of future cardiovascular diseases.<sup>1</sup> Therefore, it would be worthwhile to have early screening tools to determine the individual cardiovascular risk and to identify subjects who might benefit from preventive measures, such as an improved lifestyle and risk-factor modification. In our small study, we found increased systolic and diastolic blood pressures, increased levels of fasting insulin, and insulin resistance that was assessed by HOMA but no differences in lipid levels, as noticed by other investigators.<sup>21,22</sup>

In summary, we observed elevated autofluorescence of the leg, which reflected increased AGE accumulation, in women with a recent history of early-onset preeclampsia and found a positive relation with the IMT of the femoral artery. AGEs may be a mediator in the accelerated atherosclerosis that is found in this high-risk group.

In combination with the existing markers, this technique may offer a simple and rapid noninvasive assessment of vascular tissue damage. Furthermore, the possible role of abnormal AGE-RAGE interaction in preeclampsia may offer a new linking pathophysiologic insight in the diverse manifestations of preeclampsia and deserves further investigation.

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