ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 2, 2012

DOI: 10.1089/ars.2012.4525

Could Receptors for Advanced Glycation End Products Be Considered Cardiovascular Risk Markers in Obese Children?

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Abstract

Early development of increased cardiovascular risk in obese children and the possible related cardiovascular diseases into adulthood have been shown; however, the underling pathogenetic mechanisms implicated are not yet completely defined. Receptors for advanced glycation end products (RAGE) pathway play a pivotal role in the genesis of abnormality of arterial wall. However, whether obese prepubertal children present impaired levels of endogenous and soluble secretory receptor for advanced glycation end products (esRAGE/sRAGE) and whether an association exists between RAGE levels and carotid intima media thickness (cIMT) are not yet evaluated in this age group. We note that esRAGE and sRAGE were significantly lower in obese children than controls and were independently related to cIMT. Our findings lead to the hypothesis that RAGE system seems to be related to the development of atherosclerosis even in obese prepubertal children. *Antioxid. Redox Signal*. 17, 187–191.

Obesity and Atherosclerosis in Prepubertal Children

OBESITY IS ONE of the most important public health issues, representing the sixth most important risk factor contributing to the overall burden of diseases worldwide (2). In particular, fat mass accumulation in infancy induces chronic metabolic and inflammatory alterations. Of note, these findings finally induce an increase of carotid intima media thickness (cIMT) that is universally accepted as the primary step in the natural history of atherosclerosis (1, 7).

Previous studies have clearly demonstrated that obesity and related insulin resistance (IR) induce impaired oxidant–antioxidant status and chronic inflammation. These alterations seem to promote endothelial dysfunction, such as increased cellular activation and platelet aggregation, muscular cell proliferation, and increased expression of adhesion molecules, which in turn results in proatherosclerotic abnormalities of vascular wall (7). According to these findings, we confirmed that obese children presented significantly higher cIMT (Table 1) than healthy controls. In addition, IR status and oxidant status were explored in our pediatric population. Fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) values were significantly higher, while whole-body insulin sensitivity index (WBISI) levels were

Innovation

During the last years, a growing interest has been placed on the role of receptors for advanced glycation end products (RAGE) system in the development of several disorders, including complications related to metabolic and cardiovascular diseases. In addition, growing evidences have supposed a relevant role of AGE/RAGE system in the development of atherosclerosis *in vitro* and *in vivo* studies; however, no data have been shown in children. In the present study, we observed that obese prepubertal children presented an impairment of the RAGE system compared with healthy controls. Further, to the best of our knowledge, this is the first report demonstrating an independent association between RAGE system and increased carotid intima media thickness in a very young age group. The novelty of the present study is that circulating forms of AGE receptors could be reliable markers of cardiovascular diseases even in pediatric population. The characterization of new possible mechanisms involved in the development of atherosclerosis in children could help in the identification of new therapeutic strategies of cardiovascular diseases in children.

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Table 1. Baseline Clinical Characteristics and Levels of Biochemical Parameters of Obese Prepubertal Children and Controls

	Obese prepubertal children	*Prepubertal controls*	p
Anthropometric measurements			
Age	7.8 ± 1.4	7.1 ± 1.8	NS
Gender	27M/23F	20M/17F	NS
Height-SDS	1.11 ± 0.97	1.12 ± 1.04	NS
Weight (Kg)	41.9 ± 1.2	27.8 ± 7.3	0.0001
BMI (Kg/m^2)	24.1 ± 4.2	16.6 ± 1.6	0.0002
BMI-SDS	5.59 ± 2.88	0.38 ± 0.85	0.0009
WC (cm)	74.2 ± 11.9	55.6 ± 6.1	0.0001
SBP (mmHg)	104 ± 8	102 ± 8	NS
DBP (mmHg)	66 ± 9	66 ± 7	NS
Lipid profile			
Total cholesterol (mg/dl)	165 ± 13	163 ± 14	NS
HDL cholesterol (mg/dl)	52 ± 11	53 ± 12	NS
LDL cholesterol (mg/dl)	101 ± 16	95 ± 12	NS
Triglyceride (mg/dl)	82 ± 37	70 ± 30	NS
IR			
Fasting insulin (μU/ml)	10.16 ± 6.31	3.78 ± 1.44	0.0003
Fasting glycemia (mg/dl)	83 ± 7	85 ± 7	NS
HOMA-IR	2.12 ± 1.30	0.80 ± 0.32	0.0001
WBISI	8.05 ± 4.60	15.67 ± 6.67	0.0006
Oxidant status			
PGF-2α (ng/ml)	7.84 ± 3.02	1.72 ± 0.82	0.0001
cIMT			
Right cIMT (mm)	0.42 ± 0.064	0.29 ± 0.11	0.0005
Left cIMT (mm)	0.42 ± 0.065	0.32 ± 0.07	0.0004
Mean cIMT (mm)	0.42 ± 0.063	0.31 ± 0.07	0.0005

Data are mean ± standard deviation.

M, male; F, female; SDS, standard deviation score; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; cIMT, carotid intima media thickness; HOMA-IR, homeostasis model assessment of insulin resistance; WBISI, whole-body insulin sensitivity index; PGF-2α, prostaglandin F-2alpha; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant.

significantly lower in obese children than in controls (Table 1). In addition, when oxidant status was assessed, obese children presented significantly higher levels of prostaglandin F-2alpha (PGF-2α) than controls (Table 1).

Interestingly, recent *in vivo* and *in vitro* studies have evaluated new possible pathogenetic mechanisms involved in the development of atherosclerosis, remarking the existence of unexplored views implicated in this process.

Advanced Glycation End Product/Receptor for Advanced Glycation End Product Pathways and Atherosclerosis

Recent advances in the realm of vascular biology have identified several novel pathways linked to onset and progression of atherosclerosis. Among them the advanced glycation end products/receptor for advanced glycation end products (AGE/RAGE) system has been shown to play a pivotal role in the development of cardiovascular diseases (8). In fact, RAGE is strategically expressed on the surface of a large number of cells implicated in plaque formation and progression, such as endothelial cells, vascular smooth muscle cells, monocyte/macrophages, and lymphocytes (9). Experimental evidences demonstrate that RAGE engagement with its ligands promotes cellular activation, leading to the induction of oxidative stress and a broad spectrum of proatherosclerotic intracellular signaling. The activation of these different pathways promotes the migration of circulating monocyte/macrophages and lymphocytes into arterial wall and induces the release of other cytokines and proteases (9). These findings raise the intriguing hypothesis that RAGE–ligand interaction could play a key role in genesis and progression of cardiovascular diseases (8, 9).

Is There an Impairment of RAGE System in Obese Prepubertal Children?

Of note, recent studies demonstrated that obesity is associated with low levels of soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) (3) and that an impairment of the RAGE system represents an important risk factor for the development of atherosclerosis (4, 8). However, to the best of our knowledge, no reports have assessed the levels of esRAGE and sRAGE in obese prepubertal children compared with normal-weight peers. Thus, in the present study, we evaluated differences in terms of levels of these markers in obese prepubertal children compared with normal-weight controls. Significant differences were documented in terms of sRAGE and esRAGE between the two groups. In details, obese children had significantly lower levels of sRAGE (Fig. 1) and esRAGE (Fig. 2) compared with healthy controls. These data underlined the important role that obesity could play on an impairment of the RAGE system even in this young age group.

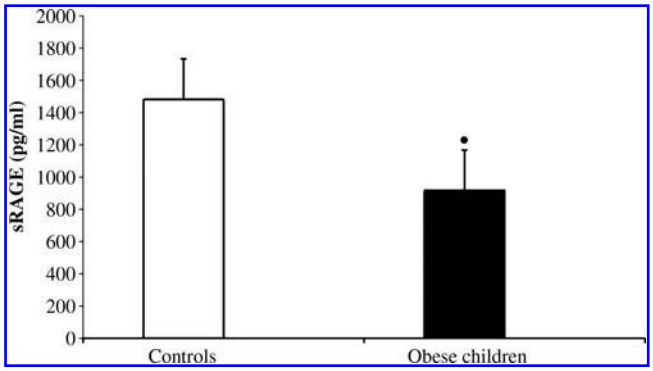

FIG. 1. Soluble receptor for advanced glycation end products (sRAGE) levels in obese children and normal-weight controls.

Is RAGE System Associated with the Development of Atherosclerosis in Children?

Interestingly, during the last years, AGE/RAGE system has been proven to be involved in the pathogenesis and progression of microvascular and macrovascular lesions in diabetic patients (4, 6). In fact, in this population, low levels of these receptors and a direct relationship between levels of RAGE and markers of microvascular diabetic complications have been identified (6).

Based on the link between hyperglycemia, AGE, and their receptors, several experimental studies confirmed the relationship between vascular impairment and AGE/RAGE pathway (6, 8, 9). In this respect, Falcone *et al.* (5) demonstrated that in adult obese patients with angiographically documented cardiovascular disease, low levels of sRAGE were independently associated with the presence of cardiovascular disease. However, no studies have investigated the role of RAGE system in the development of atherosclerosis in children. In agreement with the previous study (7), we demonstrated for the first time, an independent association between the RAGE pathway and cIMT. In details, a linear regression analysis was performed in order to investigate the effect of both sRAGE (models A and B) and esRAGE (models C and D) on increased cIMT independently of other confounding factors (Table 2). HOMA-IR, PGF-2α, and sRAGE were significantly and independently related to mean cIMT (model A). In addition, WBISI, PGF-2α, and sRAGE were significantly and independently related to mean cIMT (model B). When esRAGE was introduced in the model, only PGF-2α and both HOMA-IR (model C) and WBISI (model D) were significantly and independently related to mean cIMT, while no association was detected between esRAGE and mean cIMT. These findings highlight the possible role of RAGE system in the development of endothelial dysfunction even in obese prepubertal children, suggesting that circulating forms of AGE receptors could be reliable markers of cardiovascular disease in pediatric population.

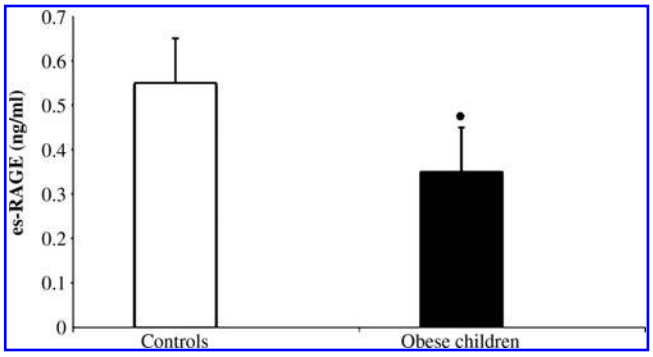

FIG. 2. Endogenous secretory receptor for advanced glycation end products (esRAGE) levels in obese children and normal-weight controls.

Table 2. Multiple Stepwise Linear Regression Analysis to Evaluate Correlation Between Carotid Intima Media Thickness (mm) and Other Main Parameters Within Obese Children

Dependent variable cIMT	*Beta*	p
Independent variables		
Model A (BMI-SDS, age, and gender)		
PGF-2α	0.465	0.001
sRAGE	0.240	0.013
HOMA-IR	0.216	0.023
Model B (BMI-SDS, age, and gender)		
PGF-2α	0.452	0.001
WBISI	−0.312	0.001
sRAGE	−0.195	0.038
Model C (BMI-SDS, esRAGE, age, and gender)		
PGF-2α	0.513	0.001
HOMA-IR	0.28	0.003
Model D (BMI-SDS, esRAGE, age, and gender)		
PGF-2α	0.488	0.001
WBISI	−0.377	0.002

sRAGE, soluble receptor for advanced glycation end products; esRAGE, endogenous secretory receptor for advanced glycation end products.

Although RAGE system activation seems a new component of pathways implicated in the increasing of cIMT, it is still not completely clear which one is the real trigger in the activation of AGE receptor in obese patients. It seems reasonable to hypothesize that the effect of RAGE in euglicemic patients with atherosclerosis may be dependent, at least in part, on molecular interactions between AGE receptors and different proinflammatory ligands, as different calcium binding proteins or amphoterin (5, 9).

Limitations of the Study

It might be argued that our study has some limitations. First, given the cross-sectional nature of our study, we were unable to prove causality between RAGE system impairment and atherosclerosis. Therefore, prospective studies are required to clarify the role of sRAGE and esRAGE levels in the genesis and progression of cIMT. The second limitation is defined by the reliability and applicability of cIMT as a sign of cardiovascular disease. In fact, other methods such as arterial flow-mediated dilatation and arterial distensibility are proposed as new and more specific markers of inherent atherosclerotic risk. However, both of these methods require

appropriate collaboration by the patients, which is difficult to be obtained from prepubertal children.

Conclusions and Future Directions

In conclusion, in this study, decreased sRAGE and esRAGE levels have been shown in obese prepubertal children. Notably, AGE-RAGE pathway seems to be independently associated to early proatherosclerotic alterations of the vascular wall even in obese prepubertal children.

In view of the increasing prevalence of cardiovascular diseases in pediatric population, further longitudinal studies are needed in order to completely clarify the natural history of arterial wall dysfunction and the pathogenetic mechanisms of its development.

Notes

Study population

We recruited 50 obese prepubertal children (27M/23F) who had been referred to the Obesity Clinic of the Department of Pediatrics, University of Chieti, Italy.

All subjects were affected by severe essential obesity (body mass index [BMI] > 2 standard deviation [SD] for the mean age and gender). None had other chronic diseases (diabetes, endocrine disorders, hereditary diseases, or systemic inflammation) or was taking any medication. A detailed medical and family history was obtained from all subjects and a complete physical examination was performed, including anthropometric parameters and staging of puberty. As a control group we recruited 37 normal-weight (BMI between −2 and +2 SD) healthy children comparable for age, gender, and pubertal stage (20M/17F), who had been admitted to the Department of Pediatrics, of University of Chieti, for minor diseases. All subjects, who agreed to participate in the study, were evaluated at least 8 weeks after discharge for any minor disease, when blood and urinary samples, and anthropometric and instrumental measurements were taken.

Ethical Committee of University of Chieti approved this study. Parental informed consent and child assent were obtained from all subjects.

Anthropometric measurements

Body weight was determined to the nearest 0.1 kg and height was measured with Harpenden stadiometer to the nearest 0.1 cm. As fatness indexes, we used the BMI-standard deviation score (SDS) for age and gender and waist circumference (WC). BMI-SDS was calculated using the following formula: SDS = [individual's measurement − population mean]/population SDS (3), while WC was measured at its smallest point between iliac crest and rib cage (3).

In all subjects, pubertal stage was defined on the basis of breast development in girls and genital development in boys, according to Tanner's criteria (all patients had prepubertal characteristics corresponding to stage 1).

Blood pressure was evaluated as previously reported (3).

Laboratory procedures

Baseline plasma glucose, insulin levels, and lipid profile (total cholesterol, triglycerides [TG], high-density lipoprotein [HDL] cholesterol, and low-density lipoprotein [LDL] cholesterol) were measured. Multiple aliquots of blood samples were collected and stored at −80°C for later assessment of sRAGE and esRAGE. All subjects performed an overnight urine collection before blood sampling. Urinary samples were added with the antioxidant 4-hydroxy-tempo (Sigma Chemical Co.) and multiple aliquot samples were stored at −80°C until analysis.

Glucose and insulin analysis

Plasma glucose level was determined by using the glucose oxidase method and plasma insulin was measured with two-site immunoenzymometric assay (AIA-PACK IRI; Tosoh). The limit of detection was 0.5 μU/ml with intra- and inter-assay coefficients of variation < 7% for quality control.

Oral glucose tolerance test and IR indices

Oral glucose tolerance test was performed according to standard procedures, as previously described (3). Subsequently, IR indices (HOMA-IR and WBISI) were calculated as previously reported (3).

Lipid analysis

Serum total cholesterol, HDL cholesterol, and TG concentrations were determined by calorimetric enzymatic method. LDL cholesterol was calculated according to the Friedewald formula (LDL cholesterol = total cholesterol − HDL cholesterol − TG/5).

esRAGE and sRAGE

The serum concentration of sRAGE was measured in duplicate by using the B-Bridge sRAGE enzyme-linked immunosorbent assay (ELISA) kit (which determines the total pool of all soluble forms of RAGE, including esRAGE) (manufactured by Daiichi Fine Chemicals and distributed by B-Bridge Int.). Human circulating esRAGE was measured in duplicate using the B-Bridge esRAGE kit (Daiichi Fine Chemicals/B-Bridge Int.). Measurements were performed following the manufacturer's instructions. The intra-assay coefficient of variation (CV) for repeated esRAGE and sRAGE measurements ranged from 3.5% to 6.7% and from 3.2% to 7.1%, respectively.

Urinary isoprostanes

Urinary samples were added with the antioxidant 4-hydroxy-tempo (Sigma Chemical Co.) and multiple aliquot samples were stored at −80°C until the analysis was performed. PGF-2α levels were evaluated in triplicate by an immunoenzymatic method (ELISA; Oxford Biomedical Research, enzyme immunoassay for urinary isoprostane) (7).

Carotid ultrasonography

High-resolution B-mode ultrasonography of the right and left carotid arteries was performed with a linear 14 mHz transducer for Philips Sonos. Children were examined in the supine position with the head turned slightly to the left and right. The common, internal, and external carotid arteries were identified by combined B-mode and color-Doppler ultrasound examinations. A careful search was performed to obtain an optimal visualization of the vessel wall

demonstrating the typical double lines representing the intima-media layer. cIMT was defined as the distance between the leading edge interface of the far wall and the leading edge of the median adventitia interface of the far wall, as previously described (1, 7).

The ultrasonic protocol requires the visualization of the near and far walls of the right and left common carotid, internal carotid arteries, and bifurcation in three different projections—anterior, lateral, and posterior—for a total of ~15 carotid segments per patient. All procedures were performed according to recent recommendations proposed by the American Heart Association (1).

Statistical analysis

Given the non-normal distribution of the variables, differences in clinical and metabolic variables were assessed by the Mann–Whitney *U*-test. Differences in gender prevalence between groups were assessed by χ^2 test. Correlations were assessed by the Spearman rank correlation.

Linear regression analysis was performed to assess the possible independent association between cIMT, RAGE system, and the other main parameters by using four models: A, B, C, and D. In the models A and B, cIMT was used as the dependent variable and sRAGE, IR indexes (using HOMA-IR in the model A and WBISI in the model B), PGF-2α, age, gender, and SDS-BMI were used as the independent variables. In the models C and D, cIMT was used as the dependent variable and esRAGE, IR indexes (using HOMA-IR in the model C and WBSI in the model D), PGF-2α, age, gender, and SDS-BMI were used as the independent variables.

All data were expressed as mean ± SD. The statistical significance level was $p < 0.05$.

All calculations were made with the computer program Statistical Package for the Social Science (SPSS), version 16.0 software for Windows.

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Date of first submission to ARS Central, January 20, 2011; date of acceptance, January 21, 2011.

Abbreviations Used

AGE/RAGE = advanced glycation end products/receptor for advanced glycation end products
BMI = body mass index
cIMT = carotid intima media thickness
DBP = diastolic blood pressure
esRAGE = endogenous secretory RAGE
HDL = high-density lipoprotein
HOMA-IR = homeostasis model assessment of insulin resistance
IR = insulin resistance
LDL = low-density lipoprotein
PGF-2α = prostaglandin F-2alpha
SBP = systolic blood pressure
SD = standard deviation
SDS = standard deviation score
sRAGE = soluble RAGE
TG = triglyceride
WBISI = whole-body insulin sensitivity index
WC = waist circumference