Plasma Advanced Glycation End Products (AGEs), Receptors for AGEs and Their Correlation with Inflammatory Markers in Middle School-Age Children

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Key Words

N^ε-(carboxymethyl)lysine · Soluble RAGE · Endogenous secretory RAGE · Pediatric obesity · Insulin resistance

Abstract

Aim: Advanced glycation end products (AGEs) and/or their receptors (RAGE) are significantly positively correlated with adiposity, inflammation, dyslipidemia, and insulin resistance in adults. However, the relationships between AGEs, RAGE, and adiposity-related comorbidities in children have not been well studied. **Methods:** In a cross-sectional study of 88 children (age 11–15 years) from the New York area enrolled in the Reduce Obesity and Diabetes (ROAD) study, we examined the correlation of the AGE N^ε-(carboxymethyl)lysine (CML), soluble RAGE (sRAGE), and endogenous secretory RAGE (esRAGE) with adiposity, inflammatory markers [interleukin-6 (IL-6), C-reactive protein, tumor necrosis factor- α], adiponectin, lipids, insulin sensitivity, and insulin secretory capacity. **Results:** Pediatric CML levels were ~20% below average adult levels. CML was significantly ($p < 0.05$) positively correlated with age and insulin sensitivity and negatively with adiposity, dyslipidemia and IL-6. sRAGE correlated pos-

itively with esRAGE and negatively with adiposity and IL-6. Both sRAGE and esRAGE correlated negatively with insulin secretory capacity. **Conclusion:** Our findings suggest that unlike adults, CML is negatively associated with adiposity and adiposity-related comorbidity risk in children. As in adults, sRAGE and esRAGE were, to varying degrees, negatively correlated with body fatness and risk factors for adiposity-related comorbidities.

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Introduction

The increasing prevalence of pediatric obesity (and its attendant comorbidities) has become one of the most important public health issues in the United States [1]. Many obesity-related complications, such as fatty liver and type 2 diabetes that were previously thought to be exclusively adult diseases are now frequently seen in children [2]. Obesity in childhood has been reported to be a predictor of mortality and adiposity-related comorbidity risk in adults even if pediatric obesity does not persist into adulthood [1].

The pathogenesis of adiposity-related comorbidities is most likely the result of multiple convergent and coordinate pathways that affect endothelial function, vascular tone, insulin sensitivity, insulin secretory capacity and any of the other multiple systems that are potentially adversely affected by increasing adiposity [3]. One of the well-characterized pathways in adults involves the generation of oxidative stress and subsequent induction of inflammation and thrombosis via accumulation of advanced glycation end products (AGEs) which are a product of nonenzymatic glycation [4]. In humans, AGEs have been chemically characterized by the detection of pentosidine, methylglyoxal and N^ε-(carboxymethyl)lysine (CML) [4].

AGEs interact with the receptor RAGE, which is a 35-kDa transmembrane specific cell surface protein in the immunoglobulin superfamily that is expressed in a number of cell lines including endothelial, mesangial, smooth muscle, and mononuclear cells [5]. The AGE-RAGE interactions cause activation of the transcription factor nuclear factor- κ B, inducing oxidative stress and inflammatory reactions and increased RAGE expression in multiple tissues [5]. Isoforms of the full-length RAGE, which lack the transmembrane and signaling domain, are found in the extracellular domains in two different forms, soluble RAGE (sRAGE) and a C-truncated variant known as endogenous secretory RAGE (esRAGE) [6, 7], which are detectable in plasma. Both esRAGE and sRAGE are able to bind circulating RAGE ligands and act as ligand decoys, thus potentially diminishing the proinflammatory effects were these ligands to interact with the full-length cell surface RAGE. In adults, lower circulating concentrations of sRAGE and esRAGE are associated with conditions such as type 1 diabetes, obesity, and rheumatoid arthritis that are characterized by high concentrations of circulating proinflammatory ligands [7].

The relationship of AGEs, sRAGE and esRAGE to markers of other adiposity-related disease states has been studied extensively in the adult population. AGEs are positively correlated with circulating concentrations of C-reactive protein (CRP), albuminuria, and components of the metabolic syndrome [waist circumference, insulin resistance, blood pressure, HDL cholesterol (HDL) and triglycerides (TG)] in adults [8]. Circulating concentrations of RAGE are positively correlated with the level of AGEs and increased expression and circulating concentrations of inflammatory markers such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) and IL-6 [9].

In children, the links between AGEs, their receptors (sRAGE and esRAGE) and obesity and adiposity-related comorbidities are not well defined. Few pediatric studies

report varying results as to the correlations of AGEs, their receptors, adiposity and adiposity-related comorbidity risk [7, 10, 11]. Our initial hypothesis was that in children the relationship of AGEs and RAGE to adiposity, inflammation, lipids, and glucose homeostasis would be similar to that seen in adults [12].

Materials and Methods

Study Overview

This is part of a multi-site 5-year study of a multi-ethnic population of middle school students in 5 New York City public schools implemented by a collaborative research consortium called the Reduce Obesity and Diabetes (ROAD) project which was assembled through the Academy for Medical Development and Collaboration (AMDeC, New York, N.Y., USA) [13]. The collaborators were researchers at Winthrop University Hospital, Columbia University Medical Center, Mt. Sinai Medical Center, Cohen Children's Medical Center, and Maimonides Medical Center. This study was approved by the Institutional Review Board of all relevant institutions as well as the New York City Board of Education and is consistent with guiding principles for research involving humans [14]. Written informed consent was obtained from the parents or legal guardians, and written informed assent was obtained from each subject. Detailed methods for this study have been previously published [13].

Subjects

An initial population of 619 subjects was divided into 4 groups: African-American, Caucasian, Hispanic, and Asian-American. For this sub-study, subjects were selected from initial screening tests performed in the fall of the first year they were enrolled. Within each group, subjects were ranked from the lowest to the highest values for measures of insulin secretion and resistance [glucose disposal index (GDI), acute insulin response (AIR), homeostasis model of assessment for insulin resistance (HOMA-IR)]. Subjects in the first 3 groups with the highest HOMA-IR in each 10th percentile rank were chosen, followed by the highest GDI in each 10th percentile rank to generate a total of 22–25 subjects per group. In the case of Asian-Americans, a similar procedure was used for the highest values of HOMA-IR and GDI in each 5th percentile rank to generate a total of 120 subjects to include a mixed population of East Asians (from China, Japan, Taiwan, and Korea) and South Asians (from India, Pakistan, Sri Lanka, Bangladesh).

After identification of this cohort, prospective data exclusion criteria were outliers for fasting insulin values or inflammatory markers (CRP, TNF- α , or IL-6) >3 standard deviations (SD) above the mean, assuming that such participants were probably not fasting or were ill. Exclusion criteria were fasting insulin >30 mIU/ml, CRP >32.6 pg/ml, TNF- α >9.6 pg/ml, or IL-6 >4.2 pg/ml. Twelve subjects were excluded based on these specific indices, and an additional 2 subjects were excluded because of insufficient sample collection. As described in 'Results', circulating concentrations of CML are significantly lower in children than in adults and an additional 18 data sets were not included in the analysis due to CML values outside of the lowest range of the assay. All of these exclusions were made prospectively, and the final population for which

data are analyzed consisted of 88 students (51 males, 37 females). Height, weight, body mass index (BMI), age, gender, and ethnicity/race of enrolled subject populations for each school were compared with the known demographics for that school to ascertain that there was no bias in subjects who consented to enroll [15].

Procedures

Comorbidity risk was assessed by family history, anthropometry, and biochemical evaluation of lipids, glucose homeostasis, and inflammation. A medical and family history questionnaire including age, ethnicity, gender, family history of obesity, hypertension, and diabetes, as well as the subject's current medication and health problems, was completed by each participant and their caregivers. This information was then reviewed with the parent or legal guardian either in person or over the phone. All students underwent anthropometric measurements including weight (light clothes, no sweaters, jackets or shoes), height, and waist circumference (obtained at the level just above the iliac crest) on the morning of the study. Body composition was analyzed by bioelectrical impedance (Body Fat Analyzer, Model HBF-306, Omron, Gays Mills, Wisc., USA).

Students were studied in the fasting state between 09.00 and 10.00 for fasting glucose, insulin, CRP, IL-6, total cholesterol, HDL, LDL cholesterol (LDL), and TG levels, as well as adiponectin (ACRP30), sRAGE, esRAGE and CML. Students received a standard intravenous glucose load (0.5 g/kg, maximum 25 g) over 3–4 min, and blood was drawn for insulin at 1, 3 and 5 min after glucose administration.

Laboratory Analyses

CML is one of the most prevalent AGEs, and is considered a representative marker of glycosylation in numerous animal and human studies [5]. Serum CML was measured by enzyme-linked immunosorbent assay (ELISA; MicroCoat GmbH, Bernried, Germany), with intraassay precision of 3.0% and interassay precision of 4.0%. sRAGE was measured by ELISA (R&D Systems, Minneapolis, Minn., USA) with intraassay precision of 4.7–6.0% and interassay precision of 6.1–8.7%, and esRAGE was measured by ELISA (B Bridge International) with intraassay precision of 3.0% and interassay precision of 5.0%. Total cholesterol was determined by autoanalyzer (Integra 400 Plus, Roche Diagnostics, Indianapolis, Ind., USA). Sensitivity is 0.12 mg/dl. Intra- and interassay precision is 0.5 and 1.9%, respectively. HDL was determined by autoanalyzer (Integra 400 Plus). Sensitivity is 3 mg/dl. Intra- and interassay precision is 1.0 and 1.3%, respectively. TG were determined by autoanalyzer (Integra 400 Plus). Sensitivity is 8.85 mg/dl. Intra- and interassay precision is 1.6 and 1.9%, respectively. LDL-direct was calculated from total cholesterol, HDL and TG using the Friedewald formula. Glucose was determined by autoanalyzer (Integra 400 Plus). Sensitivity is 2.16 mg/dl. Intra- and interassay precision is 0.8 and 1.4%, respectively. Insulin was measured by CLIA (Immulinite 1,000, Siemens Healthcare Diagnostics, Deerfield, Ill., USA). Sensitivity is 2 mIU/ml. Intra- and interassay precision is 4.3 and 5.3% respectively. Adiponectin was measured by radioimmunoassay (Millipore, Billerica, Minn., USA). Sensitivity is 0.8 ng/ml. Intra- and interassay precision is 6.2 and 6.9%, respectively. IL-6 was measured by ELISA (R&D Systems). Sensitivity is 0.04 pg/ml. Intra- and interassay precision is 7.2 and 7.8%, respectively. CRP was measured by turbidimetrics (Roche Diagnostics). Sensitivity is 0.04 pg/ml. Intra- and interassay precision is 1.3% and 3.1

pg/ml, respectively. TNF- α was measured by ELISA (R&D Systems). Sensitivity is 0.5 pg/ml. Intra- and interassay precision is 5.9 and 12.6%, respectively. Estradiol and testosterone were measured by CLIA (Immulinite 1,000, Siemens Healthcare Diagnostics). Sensitivities for estradiol and testosterone are 15 pg/ml and 15 ng/dl, and intraassay precisions are 5.1 and 8.9%, respectively.

Calculations

Fat mass was calculated from the weight and percent body fat using the following equation: [fat mass = (weight in kg) $\times$ (% body fat)/100]. Insulin sensitivity was measured as the Qualitative Insulin Sensitivity Check Index [QUICKI, (1/log fasting insulin $\times$ log fasting glucose)] [16]. As in our previous work [15], QUICKI was prospectively chosen as the primary index of insulin sensitivity based on the work of Katz et al. [16].

Phase 1 insulin release was measured as AIR (mean rise in insulin 3 and 5 min after i.v. dextrose) [17]. Because insulin sensitivity and insulin release are significantly positively correlated [15, 18], insulin secretory capacity adjusted for insulin sensitivity was calculated as the GDI [$\log_{10}(\text{AIR} \times \text{fasting glucose}/\text{fasting insulin})$] as described previously [15].

Statistical Analyses

Data are presented as mean $\pm$ SD unless otherwise indicated. Whenever possible, data were converted into z-scores to control for the effects of age and gender. This was done for BMI and waist circumferences as indices of adiposity and truncal adiposity, and for total cholesterol, TG, HDL, and LDL for lipids. More specifically, BMI z-scores were calculated using the EpiInfo 2,000 program from the Centers for Disease Control which is based on growth charts smoothed using the LMS method [19]. Z-scores for laboratory values and waist circumference were calculated as z-score (or SD score) = (observed value – median value of the reference population)/SD value of reference population [20, 21].

Multiple linear regression analyses were performed, and partial correlation coefficients were calculated using the entire data set for $\log_{10}$ CML, sRAGE and esRAGE as independent variables, and measures of glucose homeostasis, lipids, and inflammation as individual dependent variables. Similar analyses were conducted using BMI Z-score as an independent variable and $\log_{10}$ CML, sRAGE and esRAGE as a dependent variable. This technique significantly reduced the number of comparisons being made. CML data were log-transformed to ensure normality of distribution. Our initial hypotheses were that CML, sRAGE and esRAGE are significantly correlated with fat (BMI z-score), truncal adiposity (waist circumference z-score), insulin resistance (QUICKI), insulin secretory capacity (GDI), lipids and inflammatory markers. Because these dependent variables are all interrelated, post-hoc adjustments, which assume that the tests are independent, were not made [22–24]. Correlation coefficients were calculated among: (1) $\log_{10}$ CML, sRAGE and esRAGE as independent variables, (2) measures of adiposity as dependent variables, and (3) measures of glucose homeostasis and inflammation as dependent variables. In the event that both circulating concentrations of $\log_{10}$ CML, sRAGE and esRAGE and other variables were found to be significantly correlated with adiposity, we performed an analysis of covariance (ANCOVA) adding age, gender, and percent body fat as covariates. Statistical significance was prospectively defined as $P < 0.05$. All p values < 0.10 are reported. Concentrations of CML, sRAGE and esRAGE in this study were compared with data from

adult studies because of the possibility of nonlinearity of the relationships of these variables to fat mass and risk factor of adiposity-related comorbidities [25].

Results

Clinical and metabolic characteristics of subjects who were included or excluded (based on prospectively set criteria) are presented in table 1. The only significant difference between the included and excluded groups was that AIR and HOMA-IR were significantly higher in the excluded group, most likely due to exclusion based on elevated fasting insulin levels.

Anthropometric and Gender Data

Fifty one males and 37 females were included in the sub-study. Forty-four percent of students (23 males, 16 females) were overweight (BMI >85th percentile for age and gender) or obese (BMI >95th percentile for age and gender) and 31% of students reported a first- or second-degree relative with type 2 diabetes mellitus. Compared to females, males were significantly taller [160 (10) vs. 155 (7) cm, $p = 0.013$], had a higher fasting glucose level [94 (7) vs. 91 (7) mg/dl, $p = 0.045$], and significantly lower circulating concentrations of adiponectin [11.1 (4.0) vs. 14.4 (4.7) $\mu\text{g/ml}$, $p = 0.001$]. No significant gender differences were detected in CML, sRAGE, or esRAGE levels, lipids, or variables related to anthropometry, glucose homeostasis, lipids, or inflammatory status.

Correlation of Log_{10} CML with Anthropometric Data and Metabolic Markers

CML data were not normally distributed and therefore correlation analyses are presented only in reference to log_{10} (CML; see tables 1 and 2). Log_{10} CML was significantly positively correlated with age ($r = 0.42$, $p < 0.0001$; fig. 1a), and negatively correlated with waist circumference z-score ($r = -0.24$, $p = 0.02$; fig. 1b). Log_{10} CML was negatively and weakly correlated with percent fat ($r = -0.2$, $p = 0.07$). CML and log_{10} CML were significantly positively correlated with age ($r = 0.42$, $p < 0.0001$) and QUICKI ($r = 0.23$, $p = 0.03$). Possible effects of puberty were examined by looking for correlations of testosterone in males and estradiol in females with CML, sRAGE, and esRAGE. No significant correlations were observed with testosterone levels in males or estradiol levels in females.

When analyzed relative to biochemical data, log_{10} CML was significantly negatively correlated with LDL ($r = -0.23$, $p = 0.03$) and IL-6 ($r = -0.32$, $p = 0.003$). Be-

Table 1. Anthropometric data for all study subjects

Variable	Subjects included	Subjects excluded
Demographics		
Total	88	32
Males/females	51/37	21/11
Age, years	12.9 (0.9)	13.0 (0.9)
Weight, kg	57.8 (15.3)	56.9 (16.2)
Height, cm	158.4 (8.8)	159.6 (9.9)
BMI, kg/m^2	22.7 (4.7)	22.1 (5.4)
BMI z-score	0.87 (0.95)	0.78 (1.10)
Waist, cm	77.4 (12.8)	76.2 (12.9)
Waist z-score	0.63 (1.0)	0.56 (1.1)
Body fat, %	28.3 (7.9)	26.7 (7.3)
Fat mass, kg	17.1 (8.3)	16.7 (7.9)
Lipids		
Total cholesterol, mg/dl	164 (30)	168 (37)
Cholesterol z-score	0.12 (0.84)	0.13 (0.92)
TG, mg/dl	77 (37)	74 (29)
TG z-score	-0.01 (0.65)	-0.03 (0.77)
HDL, mg/dl	54 (12)	50 (12)
HDL z-score	0.27 (0.87)	0.22 (0.95)
LDL, mg/dl	95 (26)	104 (32)
LDL z-score	0.06 (0.39)	0.19 (0.42)
Glucose homeostasis		
Glucose, mg/dl	93 (7)	94 (6)
Insulin, mIU/ml	9.4 (5.2)	12.0 (11.4)
QUICKI	0.35 (0.03)	0.34 (0.06)
GDI	2.88 (0.28)	2.95 (0.33)
AIR, mIU/ml	88.3 (70.1)	148.7 (154.9)*
HOMA-IR	2.45 (4.51)	2.95 (2.62)*
Inflammatory markers		
Adiponectin, $\mu\text{g/ml}$	12.5 (4.5)	10.5 (5.6)
IL-6, pg/ml	1.05 (0.68)	0.92 (0.55)
CRP, $\mu\text{g/ml}$	3.97 (5.16)	2.77 (2.67)
TNF- α , pg/ml	1.93 (1.57)	2.15 (1.62)
Sex steroids		
Testosterone (males), ng/dl	121.4 (163.5)	157.7 (197.5)
Estradiol (females), pg/ml	33.1 (41.9)	46.1 (59.2)
CML, $\mu\text{g/ml}^1$	423.9 (153.8)	
Log_{10} CML ¹	2.60 (0.15)	
sRAGE, pg/ml ¹	1,095.7 (427.7)	
esRAGE, pg/ml ¹	310.4 (129.8)	

Values are expressed as mean (SD) unless otherwise indicated.

* $p < 0.05$ vs. included group.

¹ CML, sRAGE, and esRAGE data not reported for excluded group since the majority were excluded based on values for these variable that were outside the range of assay sensitivity.

cause log_{10} CML was negatively correlated with body fatness and age, data were also analyzed by multiple regression analysis to 'correct' for BMI z-score. After this correction, the correlation between log_{10} CML and LDL (semipartial $r = -0.22$, $p = 0.047$) and IL-6 (semipartial $r = -0.31$, $p = 0.005$; fig. 1c) remained significant (table 2).

Table 2. Correlation of log₁₀ CML, sRAGE and esRAGE with significant and near-significant independent variables

	Log ₁₀ CML		sRAGE, pg/ml		esRAGE, ng/ml	
	r	p	r	p	r	p
Age (years)	0.42	<0.0001				
BMI, kg/m ²			-0.30	0.004		
BMI z-score						
Percent body fat	-0.2	0.07	-0.30	0.003		
Fat mass (kg)			-0.36	0.0007		
Waist (cm)			-0.22	0.04		
Waist z-score	-0.24	0.02	-0.23	0.03		
HDL (mg/dl)						
HDL z-score						
LDL (mg/dl)	-0.23	0.03	-0.19	0.07		
LDL z-score			-0.19	0.08		
TG (mg/dl)						
Glucose (mg/dl)						
Insulin (mIU/ml)						
QUICKI	0.23	0.03				
GDI			-0.22	0.06		
HOMA-IR	-0.20	0.061				
AIR (mIU/ml)			-0.42	0.0003	-0.33	0.006
Adiponectin (μg/ml)						
IL-6 (pg/ml)	-0.32	0.003	-0.18	0.09		
CRP (μg/ml)						
TNF-α (pg/ml)						
sRAGE (pg/ml)	0.20	0.06			0.64	<0.0001
Testosterone (males; ng/dl)						
Estradiol (females; pg/ml)					-0.25	0.081

Correlation of sRAGE and esRAGE with Anthropometric Data and Metabolic Markers

sRAGE was significantly negatively correlated with multiple indices of body fatness [% fat ($r = -0.3$, $p = 0.003$), and fat mass ($r = -0.36$, $p = 0.0007$), fig. 2a, b] but not with BMI z-score, dyslipidemia [LDL ($r = -0.2$, $p = 0.07$), LDL z-score ($r = -0.19$, $p = 0.08$)], or indices of insulin release [AIR ($r = -0.42$, $p = 0.0003$), fig. 2c, and GDI ($r = -0.22$, $p = 0.06$)]. Because sRAGE was negatively correlated with body fatness, data were also analyzed by multiple regression to adjust for BMI z-score. After this correction, sRAGE remained significantly negatively correlated with AIR (semipartial $r = -0.40$, $p < 0.001$), and a trend was noticed toward a negative correlation with GDI (semipartial $r = -0.21$, $p = 0.057$, NS) but was no longer significantly correlated with any lipid values.

esRAGE was significantly negatively correlated with phase 1 insulin release (AIR; $r = -0.33$, $p = 0.006$; table 2; fig. 3a) and was significantly positively correlated with sRAGE ($r = 0.64$, $p < 0.0001$; table 2; fig. 3b).

Discussion

The major findings of this study are: (1) As in adults, sRAGE and esRAGE are, to varying degrees, negatively correlated with body fatness (both BMI and waist z-scores), insulin resistance, insulin secretory capacity, LDL, and IL-6. (2) Unlike adults, CML is negatively associated with adiposity, LDL, and IL-6. To the best of our knowledge, this is the largest pediatric study to simultaneously examine the association between serum CML, sRAGE and esRAGE and adiposity, glucose homeostasis,

Fig. 1. Correlation of $\log_{10}$ CML with chronological age (a), waist circumference Z-score (b), and IL-6 (c).

and inflammation in a diverse population of healthy middle school-age children in early to mid-adolescence. Sebekova et al. [10] studied a small cohort of 36 children and adolescents (4–18 years of age) and found a significantly lower level of plasma CML in obese children in comparison to their lean counterparts with no intergroup difference in sRAGE concentration (plasma esRAGE was not evaluated). Similar to the study by Sebekova et al. [10], we

Fig. 2. Correlation of sRAGE with percent body fat (a), fat mass (b), and AIR (c).

found an inverse relationship between $\log_{10}$ CML and the multiple markers of adiposity and adiposity-related comorbidity risk factors (IL-6, and LDL). However, Sebekova did not find the inverse association between sRAGE and indices of body fatness and insulin release seen in our study and in many adult studies [26]. In contrast to Sebekova et al. [10], a recent study of 130 prepubertal children (younger than those examined in the present study)

Fig. 3. Correlation of esRAGE with AIR (a), and sRAGE (b).

by Chiavaroli et al. [11] reported significant negative correlations of both sRAGE and esRAGE with adiposity and insulin resistance and also noted independently lower concentrations of sRAGE and esRAGE in children who were at increased risk of adiposity-related comorbidities by virtue of being small or large for gestational age. The present study demonstrates these same negative correlations of CML, sRAGE, and esRAGE with absolute adiposity (fat mass) were not reciprocated by significant correlations relative to adiposity (BMI Z-score), nor were they evident when subjects were overweight (BMI >85th percentile), obese (BMI >95th percentile) or not overweight (BMI <85th percentile). These analyses suggest that in peripubertal children, circulating concentrations of advanced glycation products and their receptors are more closely related to fat mass than to age- and gender-adjusted fatness.

The majority of adult and animal studies found a direct correlation between CML, body fatness and compo-

nents of metabolic syndrome, unlike the findings in the present study [8, 9, 11, 27–29]. Gugliucci et al. [8] noted that serum AGE levels were directly correlated with BMI, waist circumference and TG in 37 healthy obese and overweight adults following weight loss after being on a low calorie diet. Despite the significant positive correlation that we observed between CML and age, there were no significant correlations observed between CML and sex steroids, suggesting that the increasing CML with age may be primarily a temporal effect (perhaps reflecting dietary CML) rather than an effect of sexual maturation. Tahara et al. [29] demonstrated that serum AGE levels were independently positively correlated with the HOMA-IR index in adult nondiabetic subjects. However, consistent with our findings, a large study of community-dwelling men and women recently showed that body fat mass, fasting plasma glucose, oral glucose tolerance, and insulin resistance were not significant determinants of serum CML [30]. Whether serum CML concentrations are affected by fat mass is not known. In adipocytes, CD36, a scavenger receptor, has the ability to bind AGE [31]. As a result, fat mass could possibly affect circulating AGE concentrations through a role in the catabolism and breakdown of AGE. This speculation is based on previous in vitro studies of both murine and human adipocytes that showed binding of AGE with CD-36 is followed by endocytosis and degradation of adipocytes. Therefore, this could be a physiologic mechanism to clear AGE from the circulation [31].

Although our extended statistical analysis showed a negative correlation between $\log_{10}$ CML and LDL, it is still possible that glycation of lipids ultimately contributes to the unfavorable lipid profile in overweight and obese children and adolescents. Stitt et al. [32] showed that tissue AGE correlates with concentrations of plasma AGE-modified LDL in euglycemic, normolipidemic adults with atherosclerosis compared to young, normal controls. Therefore, it is worthwhile to consider that over a time period of many years, advanced glycation also may contribute to the age-related development of atherosclerosis in the general population since we also found a direct correlation between $\log_{10}$ CML and chronological age in our study population. Moreover, Galler et al. [33] noted serum levels of fluorescent AGEs, but not of CML-AGEs, to be significantly higher in children and adolescents with diabetes compared to control subjects, and that subjects with diabetes and high serum cholesterol and TG also had significantly higher levels of serum fluorescent AGEs. Whether other forms of AGEs, rather than CML, play a role in dyslipidemia associated with diabetes in pe-

diatric population remains to be determined. A recent observational study showed that LDL did not differ by tertiles of serum CML in adults [32].

A key question is whether AGEs are a cause or a symptom of adiposity and its related comorbidities. In a murine model of type 2 diabetes (*Lepr^{db/db}*) [27] and in diet-induced obese [28] mice, administration of a low-versus high-AGE diet resulted in improved glucose homeostasis, inflammation, and lipid panels. The animals fed the high-AGE diet were ultimately fatter and had more visceral fat than the low-AGE diet mice. The improvements in glucose homeostasis, inflammation, and lipids were evident before the weight differences between dietary groups were apparent in *Lepr^{db/db}* mice [27] and disproportionately improved relative to a small difference in body weight in the diet-induced obese mice [28]. The disproportionately large improvement in adiposity-related comorbidity risk factors compared to differences in body composition between the mice fed high- and low-AGE diets suggests that the AGE itself not only favors weight gain but also worsens complications of that gain.

Studies, mainly in adults, indicate that CML promotes inflammation and is also a marker of ongoing inflammation. Incubation of human macrophages with CML has been shown to induce production of proinflammatory cytokines [34], and CML production is induced at sites of inflammation in the adult population [35]. However, studies of healthy children and adults [10, 36] have not reported significant correlations of CML with circulating concentrations of proinflammatory cytokines even though both CML and pro-inflammatory cytokines are elevated in pediatric and adult inflammatory conditions and ingestion of high CML diets in adults has been shown to be correlated with higher circulating concentrations of proinflammatory cytokines [37, 38]. No studies, to our knowledge, have suggested a negative correlation of CML with inflammatory markers. One hypothesis is that our findings could be related to the type of population included in this study. The children included in our study were not selected previously for being at high risk of having elevated levels of cytokines.

Why then might a healthy population of children show a different pattern of association of CML with adiposity-related comorbidity risk factors than adults, or compared with adults and children with inflammatory conditions? The simplest explanation would be that there are age-related differences in the absolute or relative circulating concentrations of CML, sRAGE, and or esRAGE which place them outside the range on the dose-response curve

at which 'adult' associations with adiposity-related comorbidity risk are evident. CML levels in our population of healthy middle school students were ~20% below average adult levels ($p < 0.001$), while circulating concentrations of sRAGE and esRAGE are approximately 70% higher than those reported in healthy adults (both $p < 0.001$) [25]. Another possibility, particularly relevant to the negative correlation of CML and IL-6, would be possible age- and health-related differences in the type of AGE-receptor expressed. Activation of the AGE receptor 1 (AGER1) suppresses cell inflammation and oxidant stress, and its expression is downregulated in inflammatory conditions, such as diabetes, and negatively associated with circulating concentrations of AGEs [39]. Restriction of intake of AGEs is associated with increased AGER1 and sirtuin (SIRT1) and increased insulin sensitivity [40]. In addition, AGER1 expression diminishes with age [41]. It is possible that within the range of low CML and increased AGER1 in this population of healthy children, there is actually a negative correlation of circulating CML, and thereby increased AGER1 activation, with inflammation and insulin sensitivity. At higher CML and lower AGER1 levels, the greater relative activation of other AGE receptors might increase oxidant stress rather than decrease it. In addition, as suggested by Hudson et al. [42], different genetic backgrounds are probably the explanation for the different CML levels as well as different amount of receptors found, considering that the majority of the population studied is non-Caucasian.

In our study population, we did not observe any significant correlation between $\log_{10}$ CML and sRAGE or esRAGE. Although CML is used in most studies, and serum level of sRAGE may reflect tissue RAGE expression in nondiabetic healthy subjects, it is not exactly known which of the different AGE proteins is responsible for or associated with increased oxidative stress and inflammatory markers [43]. As discussed earlier, the relationship between AGEs and their receptors may be quite different in the range of lower concentration of AGEs. AGEs are a complex and heterogeneous group of proteins, and only the structures of some AGEs have been identified. Best characterized are pentosidine and CML. However non-AGEs, such as S100A12 proteins, have also been associated with activation of AGE receptors and could mediate the initiation of the inflammatory cascade. So far, no universally accepted method to detect AGEs or an internationally recognized standard unit of measurement exists [44]. One of the most common methods of measurement used is competitive ELISAs. Recently, a competitive ELISA using a monoclonal antibody recognizing CML

became commercially available, and this may facilitate AGE determination in clinical practice [5]. AGEs have also been detected by other methods such as fluorescence spectroscopy or more advanced fast-protein liquid chromatography, high-performance liquid chromatography, and immunohistochemistry [5, 45].

The strength of this study is the inclusion of a diverse population of healthy children. Most studies examining AGEs and their receptors have involved adults or individuals with chronic inflammatory diseases such as diabetes, kidney disease and atherosclerosis where there is a broader and higher range of AGEs than are evident in this study [8, 9, 11, 20]. The weaknesses of this study are largely related to its cross-sectional design. In addition, in this study the AGE content of the children's diet was unknown. As a cross-sectional study, it does not elucidate the causal relationships between serum CML, sRAGE and anthropometric, metabolic, and inflammatory variables, i.e. issues better examined in a longitudinal study. Because CML concentrations could potentially be influenced by increased glomerular filtration rate with increased BMI, another limitation of this study is that renal function test was not measured in this observational study. Although this type of study could potentially be carried out in all age groups, our study is limited to middle school students for several reasons. Students in this age group are likely to reflect all stages of puberty, permitting the assessment of the effects of progression of puberty in relationship to predictor and outcome variables. A similar study of older students would represent a developmentally less diverse population and is also more likely to be confounded by other variables such as pregnancy,

substance abuse, and T2DM. Younger students would also be less diverse and would have questionable ability to reliably assent to biochemical testing. We also recognize that while this early to mid-pubertal age group may represent the most logical choice for this study, it could account as a limitation for this study.

In summary, our study included 88 middle school-age children, representing a wide range of insulin resistance with multi-ethnic/-racial background. To our knowledge, this is the largest multi-ethnic/-racial study investigating the correlation between AGE, sRAGE, esRAGE and markers of obesity-related comorbidity risk factors in healthy adolescents. While AGEs have been implicated as a possible cause of oxidative stress in adult studies, the role of AGE level in healthy nonobese adolescents is less clear. It may be that the effects of AGEs are cumulative and may not have immediate deleterious effects on healthy children, at least at the time they are in middle school. At what time in life the correlations found in adults begin to occur will require future longitudinal studies of children as they grow into adulthood.

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