Soluble Receptor for Advanced Glycation End Products and Its Correlation with Vascular Damage in Adolescents with Obesity

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Keywords

Advanced glycation end products · Soluble receptor for advanced glycation end products · Obesity · Vascular damage

Abstract

Objective: The aim of this study was to evaluate soluble receptor for advanced glycation end products (sRAGE) and advanced glycation end products (AGEs) in adolescents with and without obesity (OB) and their correlation with vascular damage. **Methods:** This is a cross-sectional study with 15–19 years old adolescents: 33 with OB and 33 with normal weight (NW), each group included 17 male and 16 female. Lipid profile, insulin, carboxymethyllysine (CML), sRAGE, total AGEs, and dietary AGEs intake (dAGEs) were evaluated. Vascular damage was measured by flow-mediated vasodilation (FMD) and arterial stiffness index (I β). Homeostatic model assessment-insulin (HOMA-IR) and atherogenic index (AI) were calculated. **Results:** The group with OB had higher triglycerides (TG; $p < 0.0001$), AI ($p < 0.001$), HOMA-IR ($p < 0.0001$), dAGEs intake ($p < 0.0001$), lower CML ($p = 0.05$), total AGEs ($p < 0.01$), sRAGE ($p < 0.001$), and FMD ($p < 0.002$). In the total group, sRAGE correlated with AI ($r = -0.26$ $p = 0.037$); in the NW

group, CML correlated with I β ($r = -0.36$; $p = 0.037$); and in the group of adolescents with OB, sRAGE correlated with FMD ($r = -0.37$; $p = 0.037$) and I β ($r = 0.47$; $p = 0.006$), while CML and total AGEs correlated with AI, $p = 0.007$ and $p < 0.01$, respectively). **Conclusions:** The group of adolescents with OB showed higher cardiometabolic risk as shown by higher TG, AI, HOMA-IR, and lower sRAGE and FMD. sRAGE correlated negatively with FMD and positively with I β , so it could be suggested as a biochemical marker of impaired endothelial function.

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Introduction

Obesity (OB) is one of the major causes of cardiovascular disease morbidity and mortality, and its prevalence has rapidly increased worldwide [1]. The pathological processes and risk factors associated with atherosclerosis, including vascular endothelial dysfunction, begin during childhood and adolescence [2].

Arterial stiffness [3] and flow-mediated vasodilation (FMD) [4] are considered as an early clinical indicator of vascular damage. Greater arterial stiffness has been report-

ed in children with OB at the carotid artery [5, 6], and it has also been reported lower arterial stiffness measurement in children with OB with a possible cardiometabolic dysfunction [7]. In adolescents with no additional metabolic or clinical risk factors, FMD may be useful in identifying these children with early signs of atherosclerotic development [8]. Adolescents with OB have shown higher atherogenic index (AI), a measurement used to predict cardiovascular risk [9]. Among the factors that could play a role in atherogenesis, the advanced glycation end products (AGEs)/receptor for advanced glycation end products (RAGE) axis has been shown to play an important role in the development of cardiovascular diseases [10]. AGEs can be formed endogenously as a consequence of metabolism, and they can be ingested through food (dietary AGEs [dAGEs]) [11]. AGEs could increase vascular and myocardial stiffening and could stimulate inflammation and oxidative stress through the RAGE [12]. RAGE has a C-truncated secreted isoform, called soluble RAGE (sRAGE). AGEs also bind to sRAGE, and it has been proposed that sRAGE interacts competitively with RAGE as a decoy for AGEs, preventing hyperactivation of RAGE and its dire inflammatory consequences [13]. However, studies on sRAGE concentrations in cardiovascular disease are contradictory in adults [14, 15]. There has been a paucity of research on the subject conducted in younger populations. Studies on prepubertal children showed that OB is associated with low levels of sRAGE [16, 17] and suggest that these pathways in the process of atherosclerosis begin during adolescence or even earlier [18]. We carried out this study to evaluate sRAGE and AGEs in adolescents with and without OB and its correlation with vascular damage. To the best of our knowledge, the present study is the first to assess the levels of AGE/RAGE axis and markers of vascular damage such as FMD and arterial stiffness in adolescents.

Material and Methods

Subjects

This is a cross-sectional study of 66 adolescents: 33 with OB and 33 with normal weight (NW). They were recruited between October 2015 and July 2016 from schools in the city of Leon, Mexico. Anthropometric evaluations were performed following standardized methods, and the groups were classified based on their body mass index (BMI) according to Cole et al. [19] tables. Participants were male and female between 15 and 19 years old, with stage IV or V of Tanner scale, with no history of chronic diseases, tobacco smoking, and no evidence of any acute infection. This study was approved by the institutional Ethical Committee at the University of Guanajuato (CIBIUG-P-28-2015). Both adolescents and their parents or tutors signed an informed consent form.

Data Collection

The evaluation of blood pressure (BP) was according to the National High Blood Pressure Education Program, and noninvasive endothelial function assessment was performed following standardized methods [20, 21].

A venous blood sample was obtained after 12 h of fasting, and serum was processed the same day to measure glucose (GOD-PAP™, Lakeside, Mexico City) lipids by enzymatic methods (Spinreact, Spain). AI was calculated as (total cholesterol/high density lipoprotein-cholesterol [HDL-C]). Serum aliquots were stored at -80 °C until further analyses. Insulin was measured by ELISA (ALPCO™, Salem, NH, USA), and homeostatic model assessment-insulin (HOMA-IR) was defined as above 95% percentile, according to a previous report in Mexican adolescents [22].

AGE-sRAGE Determinations

sRAGE levels were determined using a commercial ELISA kit (Quantikine ELISA kit; R&D Systems, Minneapolis, MN, USA). Carboxymethyllysine (CML) was measured in serum by an ELISA, using non-cross-reactive monoclonal antibodies (4G9) raised against a synthetic standard, CML-BSA (donated by the group of Mount Sinai, New York, NY, USA) [23]. The total AGEs in serum were measured by fluorescence, using a Biotek Synergy H1, and top fluorescence was recorded at Excitation: λ 370 nm, Emission: λ 440 nm against blanks and expressed in AU [24].

Dietary Intake

Energy, macronutrients, and dAGEs intake were estimated from 3-day 24-h recalls (1 day of the weekend and 2 days of the week). The content of daily dAGEs was estimated with a food database with the AGE content [11]; dAGEs were expressed in kilounits (KU). Nutrients and energy were estimated with the software Food Processor (2015®).

Vascular Damage Assessment

Brachial artery FMD was measured with the aid of a pneumatic cuff which was placed on the forearm and was inflated to above systolic pressure for 5 min to induce ischemia. On deflation of the cuff, the increased flow results in stress. The stress activates endothelial nitric oxide synthase to release nitric oxide which diffuses into the smooth muscle cells causing relaxation and vasodilation. The ultrasound images of the brachial artery were obtained at baseline and after the release of the cuff. The percentage change in brachial artery diameter from baseline to the maximum increase in diameter represents FMD, and an increase <10% in the diameter of the artery suggests the presence of arterial disease [20].

Arterial stiffness index ($I\beta$). Stiffness index was calculated according to Mackenzie's formula: stiffness index = ratio of $\ln(\text{systolic/diastolic pressures})$ to (relative change in artery diameter); $I\beta = (\ln[Ps/Pd])/([Ds-Dd]/Dd)$ (P, pressure; D, artery diameter; s, systolic; d, diastolic) [21]. The ultrasound assessments were made using a ACUSON X150™ 2-dimensional ultrasound machine (Siemens, Mexico City, Mexico).

AI was calculated as (total cholesterol/HDL-C).

Statistical Analysis

Results are expressed as mean $\pm$ SD for continuous variables and as the median and interquartile range for variables with a skewed distribution. Differences between groups were evaluated by the student *t* test or Mann-Whitney U test. Pearson's correla-

Table 1. Clinic and metabolic characteristics of adolescents

Variables	OB (<i>n</i> = 33)	NW (<i>n</i> = 33)	<i>p</i> value
Age, years	16.94±1.32	16.63±1.51	0.44
Gender, female/male, <i>n</i>	17/16	17/16	–
Systolic BP, mm Hg	116.09±7.38	108.97±8.49	<0.001
Diastolic BP, mm Hg	72.79±6.77	68.60±6.83	0.02
Anthropometric measurements			
Weight, kg	89.46±14.23	57.14±8.18	<0.0001
Height, cm	164.87±8.70	162.62±7.44	0.26
BMI, kg/m ²	32.77±3.89	21.58±2.26	<0.0001
Waist circumference	98.50±9.50	72.50±5.33	<0.0001
Lipid and glucose metabolism			
TC, mmol/L	4.33±0.95	3.96±0.76	0.08
HDL-C, mmol/L	1.50±0.26	1.64±0.13	<0.007
TG*, mmol/L	1.48 (1.14–1.68)	0.85 (0.62–1.16)	<0.0001
AI	2.95±0.67	2.43±0.48	<0.001
Glucose, mmol/L	5.04±0.59	4.94±0.54	0.49
Insulin, µIU/mL ^a	15.64 (11.11–23.25)	7.78 (6.48–10.14)	<0.0001
HOMA-IR ^a	3.30 (2.54–5.33)	1.78 (1.31–2.20)	<0.0001
Diet			
Kcal/day	2,548.59±713.98	2,240.33±752.24	0.09
Protein, g/day	95.70±27.18	91.35±27.18	0.53
Lipids, g/day	98.54±31.14	86.67±27.18	0.13
Carbohydrates, g/day	324.83±105.17	279.43±108.03	0.09
dAGEs (KU)/day	14,577.03±5,492.12	8,525.67±3,309.93	<0.0001
AGE/RAGE axis			
CML, µg/mL	1.49±1.16	1.99±0.87	0.05
sRAGE, pg/mL	1,306.10±505.75	1,800.04±605.94	0.0006
Total AGEs F (AU) ^a	1,256.75 (1,092.50–1,469.75)	1,424 (1,304.00–1,620.50)	0.008
Vascular damage			
FMD	20.70±6.77	27.19±9.83	0.003
Arterial stiffness index (Iβ)	2.84±2.59	1.98±0.94	0.08

^a Mann-Whitney U test for nonparametric variables. Data are shown as mean ± SD for normally distributed variables or as median and interquartile range for non-Gaussian variables. BP, blood pressure; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin; CML, carboxymethyl-lysine; sRAGE, soluble receptor for advanced glycation end products; total AGEs F, total advanced glycation end products by fluorescence; FMD, flow-mediated vasodilation; AI, atherogenic index; TC/HDL-C, total cholesterol/high-density lipoprotein-cholesterol; dAGEs, dietary AGEs; OB, obesity; NW, normal weight.

tion analysis was used to determine the univariate correlation between the different variables in the study and the Spearman correlation for nonparametric variables. All analyses were performed using Statistica 7 software (StatSoft Inc., Tulsa, OK, USA). Significance was defined as a value of $p < 0.05$.

Results

The clinical and biochemical characteristics of both groups according to BMI are shown in Table 1. Both systolic and diastolic BPs were significantly higher in adolescents with OB and showed significantly higher triglycer-

ides (TG; $p < 0.0001$), AI ($p < 0.001$), HOMA-IR ($p < 0.0001$), and dAGEs (<0.0001). In contrast, HDL-C ($p < 0.007$), sRAGE ($p < 0.0006$), and total AGEs ($p < 0.008$) were lower than in the group with NW. FMD was significantly lower in the group with OB ($p < 0.003$), while arterial stiffness index was higher in this group with a marginal p value ($p < 0.08$).

The main outcome variables were analyzed by gender. Males showed significantly higher levels of sRAGE ($1,772.7 \pm 644.6$ vs. $1,346.4 \pm 494.9$ pg/mL $p < 0.0003$) and total AGEs (AU) 1,423 (1,310.5–1,548.0) versus 1,213 (1,080.00–1,500.50), when compared to females. No significant differences were found for other variables, and

Table 2. Correlations between AGE/RAGE axis and markers of vascular damage in the group with OB

AGE/RAGE axis	Vascular damage					
	FMD		arterial stiffness (I β)		AI	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
CML	-0.05	0.80	0.16	0.39	0.46	0.007
Total AGEs F	0.18	0.48	0.14	0.48	0.47	<0.01
sRAGE	-0.37	0.037	0.47	0.006	0.05	0.76

AGE/RAGE, advanced glycation end products/receptor for advanced glycation end products; FMD, flow-mediated vasodilation; AI, atherogenic index; CML, carboxymethyl-lysine; sRAGE, soluble receptor for advanced glycation end products; total AGEs F, total advanced glycation end products by fluorescence.

no significant differences were found according to the Tanner stage.

Regarding AGE/RAGE axis, only a negative correlation between sRAGE and CML ($r = -0.35$, $p = 0.044$) was found in the NW group. In addition, a negative correlation between sRAGE and AI ($r = -0.26$; $p = 0.037$) in the total group and between CML and arterial stiffness ($r = -0.36$; $p = 0.037$) in the NW group were found.

In the group with OB (Table 2), CML and total AGEs correlated significantly with AI (Fig. 1; $p = 0.007$ and $p < 0.01$, respectively). sRAGE correlated negatively with FMD ($p = 0.037$) and positively with arterial stiffness ($p = 0.006$; Fig. 2). These correlations were maintained after adjusting for BMI and insulin.

Discussion

In this study, we show that serum CML, total AGEs, and sRAGE are lower, while dAGEs consumption are higher in adolescents with OB compared to subjects with NW and that sRAGE is negatively associated with FMD and positively associated with arterial stiffness. Moreover, we also show that structural vascular damage (measured by arterial stiffness) and impaired endothelial function (FMD) are already present in adolescents with OB and associate in part with the AGE/RAGE axis.

Regarding the AGE/RAGE axis, our results are in agreement with those reported by Sebeková et al. [25] who found significantly lower levels of CML and AGE-associated fluorescence in children/adolescents with OB compared with their counterparts with NW and with another study that showed lower CML with adiposity [26]. A previous study in our groups with metabolically healthy adolescents did not show significant differences

in serum CML [27]. On the other hand, our results are in contrast with the assumption that OB is related to enhance oxidative stress and micro-inflammation, and this would be reflected by higher plasma AGE levels [26]. The most plausible explanation lies in the nature of the compounds measured. The most important MG-derived AGE is MG hydroimidazolone-1, and this has not been measured in previous studies in obese children nor in ours. Additionally, lower levels of CML and total AGEs have been explained due to enlargement of adipose tissue mass in OB [28]. A contributory feature of this is a likely decreased glycation of albumin due to the shift of albumin from plasma to interstitial fluid. An association with inflammation is also expected as trans-capillary albumin escape rate increases with increased capillary permeability in vascular inflammation [28, 29].

A study in adults showed that fat mass is inversely associated with serum CML suggesting that serum CML concentration is strongly affected by body fat. This association could be explained by the fact that CML is preferentially deposited in fat tissue or because adipocytes affect the metabolism of AGE [30]. In that sense, circulating CML does not reflect total body content of CML and notably the flux, which may well be increased. The increased flow of AGEs is partially quenched by sRAGE, which could then reduce their steady-state concentration. Sustained engagement of RAGE decreases glyoxalase 1 activity, the main detoxifier of MG, creating another vicious cycle [31].

Regarding dAGEs intake, there is only one study where dAGEs consumption was evaluated in adolescents; Saha et al. [32] reported that higher consumption of dAGEs is associated with an increase in the odds to have the metabolic syndrome components, specifically waist circum-

Fig. 1. Correlations in the OB group. The figure shows the correlations find between AI with CML (a) and with total AGEs (b). AI, atherogenic index; CML, carboxymethyllysine; AGEs, advanced glycation end.

Fig. 2. Correlations in the OB group. The figure shows the correlations find between sRAGE with FMD (a) and with arterial stiffness (b). FMD, flow-mediated vasodilation; sRAGE, soluble receptor for advanced glycation end products.

ference, elevated TG, and decreased HDL. These results are similar to those reported in adults where, in addition to MetS [33], they suggested dAGEs participation in the development of insulin resistance [34]. It should be noted that these MetS components, insulin and HOMA, are significantly increased in the OB group.

Our study confirms that sRAGE is lower in adolescents with OB. It has been postulated that a higher AGE flux can decrease sRAGE levels; on the other hand, it is

known that sRAGE has other ligands besides AGEs that could be decreasing it, so the results of sRAGE could be reflecting greater binding of AGEs and the other ligands, probably the higher consumption of AGEs in the diet added to the endogenous formation of AGEs favors the greater use of sRAGE [35].

Our results are in agreement with de Giorgis et al. [16] and D'Adamo et al. [17] who evaluated obese prepubertal children and found lower levels of sRAGE compared

with NW and with Chih-Tsueng He et al. [36] who demonstrate that BMI was an independent predictive factor for plasma sRAGE levels in adolescents; however, they did not find an association with HOMA-IR. Another study [37] also found that adolescents with OB had reduced sRAGE and greater HOMA-IR, which are associated with cardiovascular risk. The results suggested that sRAGE levels may reflect preclinical altered metabolism that could later lead to vascular complications and diabetes [37]. It has been posited that IR may play a role in downregulation of sRAGE [38], which is also supported by our data. Therefore, IR would come first, an excess of glucose and its metabolites, mainly MG would increase cellular AGE formation and spill-over of partially digested modified AGE peptides and free adducts to the bloodstream.

To ascertain that early modification in vascular damage may be linked to the AGE/RAGE axis, we also evaluated FMD and arterial stiffness, which define endothelial dysfunction and impaired structure of the vessels, respectively, in the first phase of the atherosclerotic process [8, 39]. Further, we also evaluated the AI that has been used as a cardiovascular risk predictor based on several epidemiological studies [40, 41].

Indeed, AGE/RAGE axis parameters in the group with OB are associated with vascular damage even in adolescents, which is another novelty of our results. We found a negative correlation between sRAGE and FMD and a positive correlation with arterial stiffness in the group with OB; these results are in agreement with Villegas et al. [42], who studied adults with newly diagnosed diabetes; other authors like Kajikawa et al. [43] found that in adults, endothelial dysfunction is positively associated with sRAGE and negatively associated with serum levels of AGEs. However, after adjustment for age, sex, and BMI, among other confounder variables, neither AGEs nor sRAGE correlated with FMD, while Fujisawa et al. [15], in a cohort study, found a higher risk for cardiovascular disease when sRAGE was higher. There is no consensus about these results, probably reflecting chronic [44] versus acute changes [42] and the clinical significance of sRAGE as a biomarker of cardiovascular risk that might differ considerably depending on the subjects' background, presence or absence of diabetes, and age. Although the reports in the literature are controversial, most of the literature supports that sRAGE is associated with greater cardiovascular risk. In agreement with our results, a study in adults demonstrated that serum sRAGE level was independently correlated with a marker of central aortic stiffness and suggested a potential role of RAGE

in the pathogenesis of aortic stiffness [45]. CML and total AGEs are positively correlated with the AI, which have been considered as cardiovascular risk factors in adults [24].

Our results and other studies demonstrate that OB is associated with low levels of sRAGE [17] and that an impairment of the RAGE system represents an important risk factor for the development of atherosclerosis [12]. There is evolving evidence that clinical indicators of atherosclerosis such as intima-media thickness, arterial stiffness, and endothelial dysfunction are altered in children with OB, although the strength of the associations and mechanisms by which these effects are mediated have not been fully elucidated [8]. However, more studies are warranted to determine the mechanisms involved in the associations between AGE/RAGE components and markers of vascular damage.

This study has some limitations. The sample size, although with sufficient power, is relatively small, yet it showed clear differences in sRAGEs and FMD. A more complete picture of the changes found in the AGE/RAGE axis can be obtained with future studies evaluating MG hydroimidazolone-1 and the different forms of sRAGEs: esRAGE and their ratios, and prospective studies are required to clarify the role of sRAGE in the genesis and progression of vascular damage.

Conclusion

The group of adolescents with OB showed higher cardiometabolic risk as shown by higher TG, AI, HOMA-IR, and lower sRAGE and FMD.

Moreover, we also show that impaired endothelial function is already present in adolescents with OB and that sRAGE is associated with FMD and arterial stiffness and CML and total AGEs are associated with AI.

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Statement of Ethics

This study was approved by the institutional Ethical Committee at the University of Guanajuato (CIBIUG-P-28-2015). Both adolescents and their parents or tutors signed an informed consent form.

Disclosure Statement

The authors declare that there is no conflict of interests regarding this publication.

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Author Contributions

R.R.-M., C.L.-C., S.S.-M., A.G., and M.E.G.-S.: participated in study design, data collection, data analysis, literature search, generation of figures, and writing of the manuscript. R.R.-M., A.G.-O., R.C., Y.B., and A.G.: carried out experiments. All authors were involved in data interpretation, writing the manuscript, and had final approval of the submitted and published versions.

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