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The endothelial functional is impaired by advanced glycation end products (AGE) in eutrophic and obese healthy adults

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Abstract

Background: An endogenous increase in advanced glycation products (AGE - *advanced glycation end product*) has been correlated with coronary artery disease (CAD) for some time, but the role of exogenous AGEs consumed through foods with any degree of processing that involves temperature increase, has not yet been fully elucidated. We aimed to verify if the dietary intake of AGE (dAGE) and the formation of fluorescent AGE in the skin correlate with the presence of endothelial dysfunction and subclinical atherosclerosis during the aging process.

Design: Observational, quantitative, descriptive cross-sectional study

Methods: 30 adults (age 54 ± 9.7 years; non-smokers, without diabetes mellitus (DM), and without coronary artery disease were stratified by Body Mass Index (BMI) range (normal weight <25 Kg/m^2, between 25 and 29.9 Kg/m^2 overweight and obesity >30 Kg/m^2), and evaluated about dietary intake and AGE formation and cardiovascular parameters related to endothelial function, and subclinical atherosclerosis. We used Carotid Doppler Ultrasound to calculate intimal medial thickness (IMT), flow-mediated dilation (FMD) to verify endothelial function, Skin Autofluorescence (SAF) AGE reading to measure AGE formation. We collected 03 24-hour dietary recalls on different days to calculate dietary AGE intake.

Results: dAGE and SAF was homogeneous in the 3 evaluated groups (eutrophic = 17743 ± 6088 kU/day ; 3,0 ± 0,7 AU , overweight = 20555 ± 4901 kU/day ; 2,7 ± 0,5 AU, obese = 18424 ± 8778 kU/day; 2,7 ± 0,7 AU; *p*=0.63; p=0,58). dAGE was inversely correlated with the FMD in the eutrophic group (r=-0.679, p<0.05). SAF was inversely orreated with FD in the obesegroup (r=-0,662, p= <0,05).

Conclusion: In healthy elderly, higher dietary AGE and the fluorescent AGE in the skin are independently associated with endothelial dysfunction, suggesting a contribution to the atherosclerosis process.

Keywords: Advanced glycation end products; Vascular endothelium; Aging; Atherosclerosis

1. Introduction

Cardiovascular diseases (CVD) stand out for being the major cause of worldwide mortality, and its incidence is even higher in the elderly population. Coronary artery disease (CAD) is one of its primary forms and remains the most responsible for both high morbidity and mortality of this type of pathology. Studies show a prevalence of angina in 12

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to 14% of men, and in 10 to 12% of women 65 to 84 years old. Therefore, the identification of asymptomatic individuals at risk of acute events such as acute myocardial infarction (AMI) and death is essential for both treatment and prevention (1). The aging process contributes to structural changes in the vascular wall, extracellular matrix (ECM) remodeling, endothelial dysfunction, deposits of substances such as lipids, calcium, advanced glycation ending products (AGE), among others. According to Malachias et al. (2016) (2), vascular aging promotes changes in the vessel wall microarchitecture, and some important ones, such as the aorta, lose their distensibility and probably due to structural changes in the middle vessel layer, such as elastin fatigue failure, as well as collagen deposits and calcification, resulting in an increased vessel diameter and intimal medial thickening (IMT) (2). Saremi et al. (2017) (3), consider that the formation of AGEs may be present in this pathophysiology, impairing the integrity and function of blood vessels, since they may cause tightening of the vasculature, extinction of nitric oxide (NO) activity, increased endothelin-1 production through activation of nuclear factor kappa B (NF-κB) and increased modification by low density lipoprotein (LDL) glycoxidation (3). Additionally, the interaction of AGEs with vessel wall components increases vascular permeability, the expression of procoagulant activity, and the generation of reactive oxygen species (ROS). AGEs modulate the early stages of atherogenesis, triggering an inflammatory-proliferative process and contributing to the spread of inflammation and vascular disturbance in an established disease (3).

The Maillard reaction is responsible for the formation of AGEs in foods through preparation involving high temperatures but also occurs endogenously in normal aging processes. In addition to the Maillard reaction, endogenous AGEs are formed from glucose autoxidation, Shiff base fragmentation, and intra- and intermolecular rearrangements of Amadori products promoting the formation of carbonyl species such as 1-deoxyglycosone and 3-deoxyglycosone, highly reactive oxoaldehydes with an vital ability to react with proteins and lipids in reactions that mostly involve oxidizing events (4).

The amount of AGE present in the body is due to an increase in production through endogenous reactions (including cellular metabolism, glucose autoxidation, and lipid peroxidation) and failures in detoxification of these compounds through their excretion of urine or feces (5).

It is well known that the aging process inexorably progresses with increased arterial stiffness (6,7); and endothelial dysfunction (8). However, the mechanisms involving these processes with advanced glycation end products are not yet fully elucidated. Therefore, we aimed to verify if the dietary intake of AGE and the formation of fluorescent AGE in the skin correlate with the presence of endothelial dysfunction and/or subclinical atherosclerosis during the aging process.

2. Material and methods

2.1. Case Study

This is an observational, quantitative, descriptive cross-sectional study that included 30 individuals of both sexes, 40 years old or older, with no pre-established coronary disease, no diagnosis of DM, and non-smokers. We collected general identification data, age, weight, height, blood pressure, waist circumference (WC), and biochemical data such as lipid profile, fasting glucose, and glycated hemoglobin (A1C).

2.2. Anthropometry

Weight and height were measured according to a previously validated protocol (9). The Data was used to calculate body mass index (BMI) defined as weight in kilograms divided by height in square meters (Kg/m^2). The individuals were classified as Eutrophic, overweight, and obese according to the criteria used by the Brazilian Association for the Study of Obesity (10). The WC was measured using an inelastic, flexible tape with a precision of 1cm, adopting the midpoint between the last rib and the iliac crest as an anatomical reference. The patient was evaluated with a relaxed abdomen, with arms crossed at shoulder height, and with the feet placed together (10).

2.3. AGE Consumption Assessment

The 24-hour dietary recall was obtained in three different days, allowing the estimation of food intake according to the literature standards (11). Individual records were analyzed, detailing portion sizes and food preparation, as this interferes with the formation of the AGEs. Consumption was estimated from a database containing 549 foods with respective AGE values based on the content of the AGE and very-long-chain fatty acid (VLCFA) and were expressed as AGE equivalents (Eq) per day (AGE Eq = 1000 kilounits (KU) The AGE content of the diets was calculated using the tables created by Uribarri et al. (12)

2.4. Skin Autofluorescence Measurement

The participants' AGE quantification was performed with the skin autofluorescence reader. AGE Reader ONE (*AGE Reader, Diagnoptics, Groningen, Netherlands*). The noninvasive method is clinically validated, and the device-measured AGE values correlate with the skin biopsy AGE content (13).

2.5. Biochemical Tests

Biochemical data from the participants' medical records were collected upon inclusion after signing the informed consent form through the database of the original medical service. They are fasting glucose, HbA1c, total cholesterol, and fractions (HDL, LDL, and VLDL), and triglycerides. The considered exams were performed less than three months before the date of inclusion.

2.6. Subclinical Atherosclerosis Evaluation

Carotid ultrasonography was performed using a high-resolution *Eco-color Doppler* (GE, Model VIVID-S5) with a 9 MHz multi-frequency linear transducer. All examinations were performed by the same observer, who had no access to the clinical data of the individuals submitted for the exam. The IMT measurement was performed automatically on the posterior wall of the right and left common carotid arteries: at least 1 cm proximal to the flow divider – referred to as the bifurcation. (14), with patient in supine position and head slightly raised. A longitudinal exploration in the anteroposterior and coronal planes of the right and left common carotid arteries was performed. The distance between the two acoustic interfaces is considered to be the IMT measurement, as described in Freire et al. (2009) (14). Three measurements were taken, including the thickest area, and the mean was used to determine the IMT. The presence of plaque was defined according to Markussis et al. (1992)(15) and Silva et al. (2012)(16), as an area of arterial wall thickness greater than 1.2 mm, or a thickness 0.5 mm greater than the adjacent IMT area, or as a diffuse wall thickness greater than 1.5 mm (15,16).

2.7. Endothelial Function Assessment

The FMD examination was performed on the right arm of the individual after 15 minutes of rest. The diameter of the brachial artery, approached above the antecubital fossa, was measured using ultrasound B-mode images (Sequoia Echocardiography System, version 6.0, Acuson, Siemens, CA, USA) equipped with a multi-frequency linear transducer (7-12 MHz) at the end of the diastole. After a resting basal measurement, the pneumatic tourniquet is inflated until brachial artery blood flow is undetected by the *Doppler*, the attained pressure is maintained for 5 minutes. The absence of flow causes ischemia and consequent dilation by the reduction of vascular resistance via autoregulation mechanisms. Increased flow through the brachial artery (called reactive hyperemia) is then induced by a rapid release of pressure (deflated tourniquet), causing the dilation of the low resistance artery to accommodate the flow. The echo evaluation is maintained for 1 minute. Flow-mediated dilatation measurement is calculated by comparing the basal diameter of the artery and the diameter after hyperemia.

2.8. Statistical Analysis

The data obtained were compiled in an Excel file and subjected to statistical treatment. After passing the Kolmogorov normality test, data were analyzed using the Pearson correlation by total sampling and by BMI range; variance analysis using ANOVA - and when their assumptions were not confirmed we used Kruskal-Wallis -, with a Tukey or Dunn's Post Hoc Test, respectively. In all inferences, a significance level of 95% was maintained for a type I error ($p<0.05$). All analyses were performed with the help of Graph Prism 6.0 software.

3. Results

3.1. Sample Characterization

In total, 30 patients (men/women: 10/20, age 54 + 6.7 years). Table 01 shows the clinical characteristics of the evaluated population. Both the mean and median BMI were above the cut-off values for age-independent eutrophy (25Kg/m2 for adults and 27Kg/m2 for the elderly). The WC, FMD, and AGE consumption, which are outside of the reference standards (WC: ≤94 cm for men - ≤80 cm for women; FMD: >6.87%; AGE consumption <15000 KU/day).

Table 1 Descriptive characteristics of the sample

Variable	Mean	SD	Median	Minimum Value	Maximum Value
Age	54	9.7	52	41	73
Weight (Kg)	76.1	18.6	74.8	52.3	135
BMI (Kg/m^2)	28.8	5.9	27.8	20.6	50.2
WC (cm)	96.2	13	96	78	139
Blood Glucose (mg/dL)	90.8	8.8	89	76	108
HbA_{1c} (%)	5.5	0.4	5.5	4.7	6.2
TC (mg/dL)	201	47	206	117	323
LDL (mg/dL)	111	32	114	50	167
HDL (mg/dL)	57	15	54	28	88
TG (mg/dL)	136	84	108	49	372
SAF (AU)	2.8	0.7	2.7	1.4	4.6
FMD (%)	3.5	3.1	3.3	-3.8	17.9
IMTr (mm)	0.78	0.14	0.77	0.60	1.28
IMTl (mm)	0.76	0.1	0.77	0.56	1.05
AGE CONSUMPTION (KU/DAY)	18459	6766	18423	5456	33123

n=sample number. SD: standard deviation; BMI: body mass index; WC: waist circumference, HbA1c: glycated hemoglobin, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglycerides, SAF: skin autofluorescence, AU: arbitrary units, FMD: flow-mediated dilatation, IMTr: intima-medial thickness carotid artery right, IMTl: intima-medial thickness carotid artery left, AGE: advanced glycation end products, KU: kilo units, Kg/m2: kilograms per square meter

3.2. Post-BMI Stratification Characteristics

After stratification by BMI, the three subgroups (eutrophic, overweight, and obesity) consisted of patients with very homogeneous parameters regarding the consumption and formation of AGE. (p=0.63; p=0.58), but with significant age variance among all groups (p<0.001), a significantly higher IMTl eutrophic measurement than the obese mean (p=0.02). The average AGE consumption calculated in our study was above the values recommended by Urribari et al. (2007), around 15000 KU/day in all groups. These values are described in **Table 2**.

Table 2 Analysis of the differences between the variances of mean cardiovascular parameters, inflammation, and consumption, or formation of AGE, according to nutritional status

Variable	Eutrophic n=10		Overweight n=10		Obese n=10		Model Coefficient	*p*
	MEAN	SD	MEAN	SD	MEAN	SD		
Age (years)	**61**	9	**52**	8	**46**	6	9.705[F]	**<0.001***
FMD (%)	1.6	3.9	4.3	5.5	5.1	3.8	1.652[F]	0.21
IMTr (mm)	**0.84**	0.07	0.74	0.11	**0.77**	0.2	7.71[H]	**0.02*****
IMTl (mm)	0.78	0.07	0.76	0.14	0.75	0.1	0.7[H]	0.7
SAF (AU)	3.0	0.7	2.7	0.5	2.7	0.7	0.55[F]	0.58
AGE Consumption (KU/DAY)	17743	6088	20555	4901	18424	8778	0.47[F]	0.63

Eutrophic (18.5/22≤BMI≤24.9/27); Overweight (25.0/27.1≤BMI≤29.9/32) Obesity (BMI≥30.0 - or more) according to ABESO criteria. FMD: flow-mediated dilation, IMTr intima-medial thickness carotid artery right, IMTl intima-medial thickness carotid artery left, SAF: skin autofluorescence, AU: arbitrary units, AGE: advanced glycation end products, KU: kilo units. SD = standard deviation. F = ANOVA single factor with Tukey Post Hoc Test. H = Kruskal-Wallis, with Dunn's Post Test

3.3. Associations and Correlations

Tables 3, 4, and 5 show the Pearson correlation according to the BMI range and variables related to age, AGE consumption and formation, endothelial function, and subclinical atherosclerosis.

Table 3 Correlation between consumption, AGE formation, and cardiovascular parameters of eutrophic patients

n=10	Age	SAF	dAGE	IMTr	IMTl
FMD	**0.645***	-0.034	**-0.679***	0.256	-0.100
Age		0.120	-0.593	**0.684***	0.463
SAF			-0.068	0.269	**0.669***
dAGE				**-0.690***	-0.375
IMTr					**0.723***

SAF = skin autofluorescence, dAGE = dietary AGE, FMD = flow-mediated dilation, IMTr = intima-medial thickness carotid artery right, IMTl = intima-medial thickness carotid artery left. Data were analyzed using the Pearson correlation. * Indicates a significant difference, P<0.05

Table 4 Correlation between consumption, AGE formation, and cardiovascular parameters of overweight patients

n=10	Age	SAF	dAGE	IMTr	IMTl
FMD	0.617	-0.037	0.171	0.092	0.104
Age		0.564	-0.215	0.351	0.348
SAF			0.050	0.438	0.410
dAGE				-0.312	-0.320
IMTr					0.999

SAF = skin autofluorescence, dAGE = dietary AGE, FMD = flow-mediated dilation, IMTr = intima-medial thickness carotid artery right, IMTl = intima-medial thickness carotid artery left. Data were analyzed using the Pearson correlation.

Table 5 Correlation between consumption, AGE formation, and cardiovascular parameters of obese patients

n=10	Age	SAF	dAGE	IMTr	IMTl
FMD	-0.360	**-0.662***	0.102	-0.094	-0.525
Age		-0.174	-0.349	0.358	**0.636***
SAF			-0.191	0.194	0.196
dAGE				-0.453	-0.354
IMTr					0.297

SAF = skin autofluorescence, dAGE = dietary AGE, FMD = flow-mediated dilation, IMTr intima-medial thickness carotid artery right, IMTl = intima-medial thickness carotid artery left. Data were analyzed using the Pearson correlation. * Indicates a significant difference, P<0.05

4. Discussion

The results found to correlate the consumption of advanced glycation end products (AGEs) inversely with the measurement of endothelial function (FMD) (*r* = -0.679), in individuals with a eutrophic body mass index (BMI) (n=10). Corroborating our results is the work of Urribari et al., 2007(17), who observed significant increases in serum AGEs occurring in conjunction with lower FMD measurements in diabetic (n=44) and non-diabetic (n=10) subjects after a single AGE-enriched drink. His patients in the non-diabetic group, like ours, had an average BMI of 25 Kg/m^2, but there were patients younger (43 years) than ours (61 $\pm$ 9 years old). The researchers concluded that repeated or chronic

exposure to high-AGE diets could, over time, lead to and/or accelerate vascular disease. Another study by Negrean et al., 2007 (18), verified the effects of an AGE-rich meal on acute vascular dysfunction, quantified by decreased flow-mediated vasodilation (FMD) of the brachial artery. In this study a single meal, differing by 5.5 times in the AGE content, induced a more profound and longer-lasting impairment in FMD, but the patients had diabetes.

The inverse correlation (*r*= -0.690) with the IMT values observed in this group may be related to a larger number of sRAGE receptors, whose role in the inverse correlation with atherosclerosis was demonstrated in the work of Grauen Larsen et al. (2018)(19) where sRAGE was negatively correlated with mean intimal thickening progression and risk reduction for major cardiovascular events. In this study, sRAGE was not quantified, so we considered them only as a hypothesis.

The accumulation of AGEs, represented by skin autofluorescence (SAF), correlated significantly with higher IMT in this same eutrophic subgroup (*r*=0.669). Den Decker et al. (2013) (13) similarly observed a correlation of (SAF) with an increase in IMT across the BMI-independent sample, but unlike our sample, in their study, BMI was also correlated with a greater thickness in the intimal mean of the carotid arteries.

In our sample, the age was correlated with an increased IMT (r=0.451; p<0.001). This association of aging with a greater increase in IMT was similarly observed in the Brazilian Longitudinal Study of Adult Health (ELSA Brazil study), which found the same phenomenon in all groups evaluated regardless of gender and race (20). Lim et al. (2012) (21) also observed that although male gender, diabetes mellitus, hypertension, and increased insulin resistance were associated with subclinical atherosclerosis, advanced age was considered the most prominent predictor after multiple factor controls. In our analysis we also observed, through the Chi-Squared Test an increased risk of carotid thickening with an age of over 60 years (*RR*=1.9; p<0.05) **Table 06**.

Overweight a patient groups showed no significant correlations between dAGE consumption and cardiovascular parameters.

Excessive dAGE consumption may be important when combined with aging, even in the absence of body composition changes, diabetes, or smoking.

Table 6 Chi-Squared Test

n=30	**Carotid Thickness (IMTr or IMTl >0.9mm)**		**Endothelial Dysfunction (DMF <6,8 %)**	
	RR	CI (95%)	*RR*	CI
Age (60 years and over)	**1.9***	1.203 - 4.035	1.02	0.7459 - 1.395
AGE CONSUMPTION (>1500 KU/day)	0.37	0.1067 - 1.259	0.9	0.5631 - 1.339
SAF (>2.7 AU)	1.07	0.6826 - 1.585	0.67	0.1293 - 3.438
BMI (Kg/m²)	0.82	0.4578 - 1.210	0.4	0.2048 - 0.7814

*p<0.05; RR = relative risk; CI = confidence interval. The Data was analyzed using a Chi-Squared Test with the Yates correction for frequencies with n<5.; BMI: body mass index; KU: kilo units; SAF: skin autofluorescence, AU: arbitrary units, FMD: flow-mediated dilation; IMTr: intima-medial thickness carotid artery right, IMTl: intima-medial thickness carotid artery left; AGE: advanced glycation end products.

5. Conclusion

In healthy elderly people, the AGEs are independently associated with endothelial dysfunction, suggesting a contribution to the atherosclerosis process.

Final Considerations

Our study had some limitations, including the small sample size and participants with dyslipidemia and hypertension. Further studies will be needed to clarify the relationship between dAGE intake and worsening endothelial function.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors of the manuscript entitled "The endothelial functional is impaired by advanced glycation end products (AGE) in eutrophic and obese healthy adults" declare that we have no conflict of interest financial, commercial, political, academic and, personal.

Statement of ethical approval

The study was submitted to the Ethics Committee of the São Judas Tadeu University and HC-FMUSP (co-participant entity) and approved (No. 2,593,580) and the CAAE Number: 79991417.9.0000.0089, with the consent of InCor-HCFMUSP.

Statement of informed consent

All participants signed an Informed Consent Form (ICF) according to the Ethics Committee criteria, and the data was collected following the Medical Treatment Agreement and the Personal Data Protection Act, per the ethical medical standards.

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Authors' Contributions

RHMP and AMSL designed the study. RHMP conducted data collection with the assistance and expertise of RSP, LAB, and VH. JLA and LAB provided the medical knowledge to analyze the results of the cardiac exams, RHMP performed the statistical analysis of the data. RHMP, AMSL, MP wrote the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript.

References

[1] Cesar L a, Ferreira JF, Armaganijan D, Gowdak LH, Mansur a P, Bodanese LC, et al. DIRETRIZ DE DOENÇA CORONÁRIA ESTÁVELGuideline for stable coronary artery disease. Arq Bras Cardiol [Internet]. 2014;103(2 Suppl 2):1–56.

[2] Malachias MVB, Souza WKSB, Plavnik FL, Rodrigues CIS, Brandão AA, Neves MFT, et al.. 7a Diretriz Brasileira De Hipertensão Arterial. Arq Bras Cardiol. 2016;107(3).

[3] Saremi A, Howell S, Schwenke DC, Bahn G, Beisswenger PJ, Reaven PD. Advanced glycation end products, oxidation products, and the extent of atherosclerosis during the VA diabetes trial and follow-up study. Diabetes Care. 2017;40(4):591–8.

[4] Torres NMPDO, Xavier JDA, Goulart MOF, Alves R. B.; Freitas R. A Química dos Produtos Finais de Glicação Avançada. Rev. Virtual Quim., 2018, 10 (2), 375-392

[5] Lin J-A, Wu C-H, Yen G-C. Perspective of Advanced Glycation End Products on Human Health. J Agric Food Chem [Internet]. 2018;66(9):2065–70.

[6] Kaplon RE, Gano LB, Seals DR, Accinni R, Rosina M, Bamonti F, et al. Vascular endothelial function and oxidative stress are related to dietary niacin intake among healthy middle-aged and older adults. J Appl Physiol [Internet]. 2014;116(2):156–63.

[7] Lakatta EG, Levy D. Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: Part I: Aging arteries: A "set up" for vascular disease. Circulation. 2003;107(1):139–46.

[8] Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. Clin Sci [Internet]. 2011;120(9):357–75.

[9] Lohman TG, Roche AF. Anthropometric standardization reference manual. Books HK, editor. Illinois; 1988.

[10] Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica. Diretrizes brasileiras de obesidade. 4. ed. São Paulo: ABESO, 2016.

[11] Fisberg, RM; Marchioni, DML; Colucci, ACAl. Assessment of food consumption and nutrient intake in clinical practice: [review].Arq. bras. endocrinol. metab ; 2009;53(5): 617-624.

[12] Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, et al. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. J Am Diet Assoc. 2010;110(6).

[13] Den Dekker MAM, Zwiers M, Van Den Heuvel ER, De Vos LC, Smit AJ, Zeebregts CJ, et al. Skin autofluorescence, a non-invasive marker for AGE accumulation, is associated with the degree of atherosclerosis. PLoS One. 2013;8(12).

[14] Freire, CMV, de Alcantara, ML, dos Santos, SN, do Amaral, SI, Veloso, O, Porto, CLL, Rabischoffsky, A. Recomendação para a Quantificação pelo Ultrassom da Doença Aterosclerótica das Artérias Carótidas e Vertebrais: Grupo de Trabalho do Departamento de Imagem Cardiovascular da Sociedade Brasileira de Cardiologia – DIC - SBC. ARQUIVOS BRASILEIROS DE CARDIOLOGIA - IMAGEM CARDIOVASCULAR, 2009;28. https://doi.org/10.5935/2318-8219.20150018.

[15] Markussis V, Beshyah SA, Fisher C, Sharp P, Nicolaides AN, Johnston DG. Detection of premature atherosclerosis by high-resolution ultrasonography in symptom-free hypopituitary adults. Lancet. 1992;340(8829):1188–92.

[16] Silva LR, Stefanello JMF, Pizzi J, Timossi LS, Leite N. Aterosclerose subclínica e marcadores inflamatórios em crianças e adolescentes obesos e não obesos. Rev Bras Epidemiol. 2012;15(4):804–16.

[17] Uribarri J, Stirban A, Sander D, Cai W, Negrean M, Buenting CE, et al. Single oral challenge by advanced glycation end products acutely impairs endothelial function in diabetic and nondiabetic subjects. Diabetes Care. 2007;30:2579–82.

[18] Negrean M, Stirban A, Stratmann B, Gawlowski T, Horstmann T, Gotting C, et al. Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Clin Nutr [Internet]. 2007;85(5):1236–43.

[19] Grauen Larsen H, Marinkovic G, Nilsson PM, Nilsson J, Engström G, Melander O, et al. High Plasma sRAGE (Soluble Receptor for Advanced Glycation End Products) Is Associated With Slower Carotid Intima-Media Thickness Progression and Lower Risk for First-Time Coronary Events and Mortality. Arterioscler Thromb Vasc Biol. 2019;39(5):925–33.

[20] Santos EHS, Santos Neto PJ dos, Santos I de S. Carotid intima-media thickness in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): a narrative review. Sao Paulo Med J [Internet]. 2018;136(1):64–72.

[21] Lim S, Jeong IK, Park KS, Lim S, Choi HJ, Khang AR, et al. Subclinical atherosclerosis in a community-based elderly cohort: The Korean Longitudinal Study on Health and Aging. Int J Cardiol. 2011;155(1):126–33.