European Journal of Nutrition

Relationship between soluble receptor for advanced glycation end products (sRAGE), body composition and fat distribution in healthy women

--Manuscript Draft--

Manuscript Number:	EJON-D-16-00369R2
Full Title:	Relationship between soluble receptor for advanced glycation end products (sRAGE), body composition and fat distribution in healthy women
Article Type:	Original Contribution
Keywords:	adipose tissue; adipose tissue distribution; epicardial adipose tissue; receptor for advanced glycation end products; subcutaneous adipose tissue; visceral adipose tissue
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Abstract:	Purpose Soluble receptor for advanced glycation end products (sRAGE) is a decoy receptor which sequesters RAGE ligands and acts as a cytoprotective agent. To date, it is unclear whether the lower sRAGE levels observed in obesity are a marker of increased overall adiposity or reflect increases in particular fat depots. Therefore, we evaluated in healthy women the relationship among sRAGE and indicators of adiposity, including abdominal visceral (VAT) and epicardial visceral (EAT) adipose tissues, to explore the potential role of sRAGE as an earlier biomarker of cadiometabolic risk. Methods Plasma sRAGE levels were quantified by an enzyme-linked immunosorbent assay in 47 healthy women. Total fat mass (FM) and fat-free mass were estimated with bioimpedance analysis. Anthropometric measures and biochemical data were recorded. Subcutaneous adipose tissue (SAT), VAT and EAT volumes were measured by magnetic resonance imaging. Results Obese women had lower sRAGE levels compared to normal weight women. sRAGE levels were also lower in women with a waist circumference (WC) larger than 80 cm. Correlation analyses indicated an inverse association of sRAGE with body

	mass index and FM. Concerning adipose tissue distribution, sRAGE inversely correlated with WC, EAT and VAT depots. In a multiple stepwise regression analysis, performed to emphasize the role of fat distribution, EAT volume was the only predictor of sRAGE. Conclusions Lower sRAGE levels reflect accumulation of visceral fat mainly at the epicardial level and is present in advance of metabolic complications in adult women. sRAGE quantification might be an early marker of cardiometabolic risk.
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Dear Dr Dozio,

Thank you for revising your manuscript. You will see that the reviewers are now satisfied barring some minor revisions. Please undertake this change, highlighting the revised text in your submitted manuscript. The revised paper will not be subject to further review but will be assessed editorially

Your revision is due by 24-09-2016.

To submit a revision, go to http://ejon.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Ian Rowland, PhD
Editor-in-Chief
European Journal of Nutrition

Dear Editor,
Please find below the specific answers to Reviewer's comments.
As required, all the changes have been highlighted in the revised version of our manuscript. I hope that with these further improvements, the manuscript would be now suitable for publication.

My personal Best

Elena Dozio

Reviewers' comments:

Reviewer #1: There have been several improvements/corrections to this manuscript. Thank you. The tables/figures are much improved. However, there are still several small areas that have not been addressed. See below.

Pg 2, line 33 "Aknowledgments" is still misspelled. Should be "Acknowledgments"
The word has been corrected.

Pg 2, abstract: recommend English editing of the results section. Some of the use of the singular sounds awkward to English-speaking ears. Example of revised passage: "Obese women had lower sRAGE levels compared to normal weight women". Also recommend changing "strong inverse association" to "inverse association". Avoid overstating the relationship. In the "conclusions" revise sentence as follows: "and is present in advance of metabolic complications in adult women".
According to Reviewer suggestion we have changed "sRAGE level" with "sRAGE levels" all over in the manuscript. In the abstract, "strong inverse association" has been changed into "inverse association" . In the conclusions, both in the abstract and at the end of the manuscript, we have revised the conclusion sentence has suggested.

Pg 4 line, 105-106 still says "among" instead of "between".
Among has been changed with between.

Pg 4, line 109: appropriate references were added to the statement in line 96 but not in 109, in which the relationship between low sRAGE and obesity is specifically identified. Include the citation for these here also.
Reference citation has been added as suggested.

Pg 7, lines 190-196: In my opinion, this section is still general. Rather than adding great detail to the figure, tables, I would recommend detailing here what was done. Somewhat better though.
Additional details have been added in this method section. The section now sounds as follows:

"Statistical analysis
Qualitative variables are summarized as numbers and percentages; quantitative variables are expressed as mean with standard deviation (SD) or median and interquartile range (IQR). The normality of data distribution was assessed by the Kolmogorov-Smirnoff test. T-test or Mann-Whitney test were used to compared sRAGE levels after women were classified according to the median value of EAT, VAT and SAT volumes or the WC cut off value of 80 cm. For group-wise comparison (three groups), ANOVA or Kruskal-Wallis tests followed by Bonferroni or Dunns tests were used as appropriate. To test the univariate association between sRAGE and the other variables, Pearson (for normal-distributes data) or Spearman (for non-normal distributed data) correlation tests were used, as appropriate. Stepwise regression analysis was performed to test the independent association between sRAGE and indices of fat distribution. All statistical analyses were performed using STATISTIX 7.0 (Analitical Software, Tallahassee, FL) and GraphPAd Prism 5.0 biochemical statistical package (GraphPad Software, San Diego, CA). A p value < 0.05 was considered significant."

Pg 8, line 205: still says "Obese woman". Should be "Obese women".
Corrected.

Pg 8, line 208: reword: "After women classification", should say "after participants were classified according to BMI . . ."
Corrected.

Pg 8, line 214-215: reword: "after women classification" should say "after women were classified . . . "
Corrected.

pg 9, line 225, should say "did not reach statistical significance"
Corrected.

Pg 9, lines 241, still refers just to 4 studies, instead of the several which establish this relationship.
Additional and previous cited references have been added as suggested.

Pg 11, line 292, I would be consistent about using/not using the word "healthy" to describe these women.
293: reword: "In obese women without metabolic disease, lower sRAGE levels reflect accumulation of visceral fat mainly at the epicardial level and are present in advance of the appearance of metabolic complications"
Corrected.

Relationship between soluble receptor for advanced glycation end products (sRAGE), body composition and fat distribution in healthy women

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Abbreviations

AGE, advanced glycation end products; BMI, body mass index; CAD, coronary artery disease; EAT, epicardial adipose tissue; HOMA-IR, homeostasis model assessment of insulin resistance; LAP, lipid accumulation product; RAGE, receptor for advanced glycation end product; sRAGE, soluble receptor for advanced glycation end products; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Keywords

Adipose tissue; adipose tissue distribution; epicardial adipose tissue; receptor for advanced glycation end products; subcutaneous adipose tissue; visceral adipose tissue

Acknowledgments

The authors thank Dr. Elena Costa, I.R.C.C.S. Policlinico San Donato, Milan, Italy, for clinical chemistry data and Prof. Simona Villani, Unit of Biostatistics and Clinical Epidemiology, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy, for biostatistical support.

The study was supported by funds from Italian Ministry for Health "Ricerca Corrente" IRCCS Policlinico San Donato.

Conflicts of Interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

All authors have read the journal's authorship agreement, and the manuscript has been reviewed and approved by all named authors.

Abstract

Purpose Soluble receptor for advanced glycation end products (sRAGE) is a decoy receptor which sequesters RAGE ligands and acts as a cytoprotective agent. To date, it is unclear whether the lower sRAGE levels observed in obesity ~~is~~ are a marker of increased overall adiposity or reflect~~s~~ increases in particular fat depots. Therefore, we evaluated in healthy women the relationship among sRAGE and indicators of adiposity, including abdominal visceral (VAT) and epicardial visceral (EAT) adipose tissues, to explore the potential role of sRAGE as an earlier biomarker of cadiometabolic risk.

Methods Plasma sRAGE levels ~~w~~were~~as~~ quantified by an enzyme-linked immunosorbent assay in 47 healthy ~~cumenorreich~~ women. Total fat mass (FM) and fat-free mass were estimated with bioimpedance analysis.

Anthropometric measures and biochemical data were recorded. Subcutaneous adipose tissue (SAT), VAT and EAT volumes were measured by magnetic resonance imaging.

Results Obese women had lower sRAGE levels compared to normal weight women. sRAGE levels ~~was~~ were also lower in women with a waist circumference (WC) larger than 80 cm. Correlation analyses indicated an ~~strong~~ inverse association of sRAGE with body mass index and FM. Concerning adipose tissue distribution, sRAGE inversely correlated with WC, EAT and VAT depots. In a multiple stepwise regression analysis, performed to emphasize the role of fat distribution, EAT volume was the only predictor of sRAGE.

Conclusions Lower sRAGE levels reflect~~s~~ accumulation of visceral fat mainly at the epicardial level and is present in advance ~~the appearing~~ of metabolic complications in adult women. sRAGE quantification might be an early marker of cardiometabolic risk.

Introduction

Receptor for advanced glycation end products (receptor for AGE, RAGE) is a cell-surface protein initially identified as a receptor for N-carboxymethyllysine-modified proteins, one of the major AGE *in vivo* [1], but also able to bind other nonglycated molecules, like S100/calgranulin family of peptides, High Mobility Group Box 1 protein (HMGB1), amyloid-β peptide and macrophage-1 antigen (Mac-1) [2-4]. Ligand engagement of RAGE leads to the activation of the nuclear transcription factor NF-κB which results in the onset of the inflammatory response [5]. Besides the cell surface form, RAGE also exists as a soluble circulating molecule (sRAGE), a decoy receptor able to prevent ligand binding at cellular level and therefore the induction of the inflammatory response [6]. Some recent studies demonstrated that increased expression of the cell-surface form promotes inflammation, adipocyte hypertrophy and insulin resistance [7,8]. Concerning the soluble form, sRAGE has been determined to have an inverse association with obesity, metabolic syndrome, atherosclerosis, coronary artery disease (CAD) and diabetes [9-17]. These observations suggest sRAGE as a possible marker for metabolic dysfunction and risk. Conversely, in some studies on long-term diabetes (both type 1 and 2) increased sRAGE levels ~~was~~ were proposed as a predictive biomarker of cardiovascular diseases (CVDs) and all-cause of mortality [15,18,19]. In this regard, it needs to be pointed out that there are different circulating RAGE forms contributing to the overall sRAGE levels: the endogenous secretory RAGE (esRAGE), an alternatively-spliced form of RAGE, and the cleaved form of the membrane receptor, cRAGE [6,11-13,19-22]. What is measured and whether the different forms have the same activities may account for some of the differences between the studies.

Accurate assessment of body fat distribution is considered critical for assessing the risk of CVDs, mainly CAD [23,24]. Although most attention has been traditionally given to the relationship among intra-abdominal fat accumulation and CAD, in the last years epicardial adipose tissue (EAT), the visceral fat located around the heart, received great consideration mainly due to its anatomical proximity to the myocardium and its endocrine activity [25-28].

To date, it is unclear whether the lower sRAGE levels observed in obesity [9,12,16,17] ~~is~~ are a marker of increased overall adiposity or instead reflect~~s~~ increases in particular visceral fat depots, important in CVDs risk estimation.

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Therefore, in a group of adult women without known metabolic disorders we evaluated the relationship ~~among~~ between sRAGE and indicators of adiposity, including abdominal subcutaneous (SAT), abdominal visceral (VAT) and epicardial visceral (EAT) adipose tissues, to explore the potential role of sRAGE as an early biomarker of cadiometabolic risk.

Materials and Methods

Study population

Forty-seven healthy women referred to the Service of Clinical Nutrition and Cardiometabolic Prevention at the I.R.C.C.S. Policlinico San Donato were enrolled in this study. Inclusion criteria were: female sex, age > 18 years, eumenorrhea and signed written informed consent. Individuals who met the following criteria were not eligible for the study: body mass index (BMI) <18.5, chronic illnesses (hematological and rheumatic diseases, inflammatory bowel disease, chronic renal failure, hypercortisolism, diabetes mellitus, hyper or hypothyroidism), history of cancer, smoking, alcohol and drug abuse, contraindication to magnetic resonance imaging (MRI). The study protocol, conducted in accordance with the declaration of Helsinki as revised in 2013, has been approved by the local ethics committee (ASL Milano 2, protocol 2732) and all participants signed an informed consent before enrollment.

Blood collection

Blood samples were collected after an overnight fasting into pyrogen-free tubes with ethylenediaminetetraacetic acid as anticoagulant. Plasma samples were separated after centrifugation at 1500 g for 15 min and stored at -20°C until analyses.

Biochemical assays

The quantitative determination of sRAGE concentrations ~~was~~ were performed by a commercial human sRAGE immunoassay kit (R&D System, Minneapolis, MN, USA) according to manufacturer's instructions. The minimum detectable dose ranged from 1.23-16.14 pg/mL. The maximum intra- and inter-assay coefficient of variations were 4.8 and 8.3%, respectively.

Other biochemical parameters were assayed as previously reported [23,29]. LDL-cholesterol was calculated with the Friedewald formula. Homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin [μU/mL] × fasting glucose [mmol/L] /22.5 [30]. The formula used for the lipid accumulation product (LAP) index was: (waist circumference [WC, cm] - 58) × (triglycerides [TG, mmol/L]) [31].

Anthropometric measures

All participant were measured in light clothing and without shoes. Weight and height were taken with standard scales and stadiometers and recorded to the nearest 0.1 kg and 0.5 cm, respectively. WC and hip circumference were taken with a flexible non-stretchable tape, according to WHO guidelines [32]. BMI and waist to hip ratio (WHR) were calculated.

Bioimpedance analysis

Whole body composition was estimated using the Tanita-BC-420-MA Body Composition Analyzer (BIA) (Tanita Europe B.V., Amsterdam, The Netherlands), in the morning, under standardized conditions (prohibition of: alcohol intake and intensive training during the twelve hours preceding the measurements, excessive food and drink intake the day before, food and drink intake during the four hours before the test, measurement during menstruation). Body composition, including body fat mass (FM), was estimated by BIA according to resistance and reactance values, which are measured by the system, and subject's height, sex and age.

Quantification of EAT, VAT and SAT volumes by MRI

MRI was used to determine epicardial fat volume, as previously reported [33,34]. All examinations were performed using a 1.5-T system (Magnetom Sonata, Siemens Medical Systems, Erlangen, Germany). Subjects remained positioned in the scanner during the entire examination and a dedicated 4-element phased-array cardiac coil was used. Images were acquired during repeated end-expiratory breath-holds. Electrocardiographically gated cine true fast imaging with steady-state precession sequences (true-FISP) were acquired (bright blood, time of repetition [TR]/time to echo [TE] 45/1.5 ms, slice thickness 8 mm, flip angle 65°, 1 excitation; no interslice gap) along the 4-chamber axis to cover the entire cardiac volume. EAT volume was measured using Argus VA50A (Leonardo, Siemens Medical Systems) on a remote workstation. Epicardial fat was measured as the fat deposition between the outer layer of the myocardium and the visceral layer of the pericardium. A radiologist manually segmented true-FISP images of the end-systolic cardiac cycle. The area corresponding to the epicardial fat of each slice was multiplied by slice thickness and then added each other to obtain the total volume [34]. MRI was also used to determine VAT and SAT [35]. During the same MRI examination, a true-FISP sequence was acquired with the following parameters: TE 4.3ms, TR 2.15 ms and acquisition time 70 s, slice thickness 5 mm. The field of view was set to encompass both VAT and the SAT. Slices were obtained at the level of L4 vertebral body. VAT and SAT areas were identified by an experienced trained radiologist with a manual segmentation.

Statistical analysis

Qualitative variables are summarized as numbers and percentages; quantitative variables are expressed as mean with standard deviation (SD) or median and interquartile range (IQR). The normality of data distribution was assessed by the Kolmogorov-Smirnoff test. ~~For group-wise comparison, t~~T-test ~~(two groups),~~ or Mann-Whitney test ~~(two groups)~~were used to compared sRAGE levels after women were classified according to the median value of EAT, VAT and SAT volumes or the WC cut off value of 80 cm. For group-wise comparison (three groups), ~~and~~ ANOVA or Kruskal-Wallis tests ~~(n groups)~~ followed by Bonferroni or Dunns tests were used as appropriate. To test the univariate association between sRAGE and

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the other variables, ~~P~~Pearson (for normal-distributes data) or ~~S~~Spearman (for non-normal distributed data) correlation tests were used, ~~to test the univariate correlation of sRAGE with variables~~as appropriate. Stepwise regression analysis was performed to test the independent association between sRAGE and indices of fat distribution. All statistical analyses were performed using STATISTIX 7.0 (Analitical Software, Tallahassee, FL) and GraphPAd Prism 5.0 biochemical statistical package (GraphPad Software, San Diego, CA). A p value < 0.05 was considered significant.

Results

The characteristics of the individuals included in the study are presented in Table 1. Of the 47 women, 16 (34%) were normal weight (BMI 18.5-24.9 kg/m^2), 10 (21.3%) were overweight (BMI 25-29.9 kg/m^2) and 21 (44.7%) were obese (BMI ≥ 30 kg/m^2). No women were taking drugs at time of enrollment. Obese wom~~e~~an had lower sRAGE levels (640.8 ng/mL, 423.2-1345.0 ng/mL) compared to normal-weight (1363.0 ± 693.2 ng/mL, p=0.022). No differences were observed between overweight (1389.0 ± 617.8 ng/mL) and normal weight ~~-~~women (Figure 1A and Table 1).

After ~~women~~ participants were classified~~cation~~ according to BMI, all the evaluated anthropometric parameters and indices of adipose tissue distribution, that is EAT, VAT and SAT, were statistically significantly higher in both overweight and obese groups compared to normal weight (Table 1). Fasting glucose and fasting insulin levels were higher whereas HDL cholesterol lower in obese than normal weight group, but all in the normal ranges (Table 1). We also observed an increase in HOMA-IR and LAP, two indicators of insulin resistance, according to the obesity status (Table 1).

Concerning parameters of body fat distribution, we explored sRAGE levels after women ~~classification~~ were classified according to the median value of EAT (9.2 mm), VAT (21.65 mm) and SAT (300 mm) volumes or the established WC cut off value of 80 cm, which is used to predict cardiovascular risk in European women [36]. sRAGE levels ~~was~~ were lower in all the groups with the highest EAT, VAT or SAT volumes compared to the lowest (p < 0.05 for all) (Figure 1B, C, D). Lower sRAGE levels ~~was~~ were also observed in the group with WC above the cut off value of 80 cm (n=29) (p=0.048) (Figure 1E).

The association of sRAGE with anthropometric and fat distribution parameters was then evaluated using Pearson or Spearman correlation coefficients. sRAGE inversely correlated with BMI, FM, WC and hip circumference (Table 2). We also observed an inverse association of sRAGE with EAT (r =-0.426, p=0.003) and VAT (-0.341, p=0.025), but not with SAT volume (r=- 0.197, p=0.206) (Table 2).

HOMA-IR and LAP were observed to be higher in women with depressed sRAGE levels but their correlations with sRAGE did not reach ~~the~~ statistical significance (HOMA-IR: r=-0.252, p=0.087; LAP: r=-0.252, p=0.085; Table 2).

To emphasize the role of fat distribution, a multiple stepwise regression analysis was then used to investigate the influence of different adipose tissue compartments on sRAGE levels. FM and parameters of fat distribution that were significantly correlated in the univariate analysis were included in the model. EAT volume most correlated with sRAGE, whereas the other indices did not enter the model (Table 3).

Discussion

Our study suggests that, in a group of healthy women, lower sRAGE levels mainly reflect~~s~~ visceral fat accumulation at the epicardial level rather than total body fat or fat accumulation in other adipose tissue depots. Due to the role of EAT as an important risk factor for CVDs and the fact that our study population is composed by adult women without known metabolic diseases, the existing association observed among sRAGE and EAT suggest a potential role of sRAGE as an early marker of cardiometabolic complications.

The inverse correlation among sRAGE and obesity has been suggested in previous studies performed using both *in vitro* models, RAGE knockout animals as well as in humans and confirmed also by our study [8,9,12,16,17,37-39]. Monden et al. [8] described the role of RAGE in promoting adipocyte hypertrophy. They observed lower weight, lower epididymal adipose tissue weight and adipocyte size in RAGE-/- mice. In addition, they also described a direct effect of adenoviral RAGE over-expression in promoting 3T3-L1 adipocyte hypertrophy. In the field of human studies, a recent paper from our group suggested that increasing expression of RAGE in EAT is associated with increased tissue thickness [40]. Concerning the circulating form, lower sRAGE levels ~~was~~ were associated with increasing BMI and metabolic syndrome, also in proportion to the number of metabolic components, including central obesity, hyperglycemia and blood

pressure [9,12,16,21,41-43]. Although these studies suggested the existence of an association between sRAGE and central obesity, they utilized WC as a marker of abdominal visceral fat accumulation, not the direct quantification of VAT volume. Differently, we were the first to explore the association among sRAGE levels and indicators of adiposity in a group of healthy premenopausal women, including indicators of visceral adiposity, such as VAT and EAT, directly quantified by MRI. It is well known that adipose tissue distribution, and not adipose tissue *per se*, is important in CVD risk estimation and in the last decade EAT received great consideration in this field mainly due to its anatomical proximity to the myocardium and its endocrine activity.

Currently, there is no consensus on the 'gold standard' for the *in vivo* quantification of EAT and both computed tomography (CT), ultrasound and MRI techniques have been used to quantify cardiac fat [44]. Ultrasound is the most widely available, fastest and the least expensive technique for estimating cardiac fat [44] but has the limitation of not being truly volumetric and cannot directly quantify the volume of cardiac fat [45]. Moreover, using ultrasound, it is difficult to distinguish between EAT and pericardial adipose tissue and often this last rather than EAT thickness is reported. In addition, ultrasound evaluation of EAT may be difficult in overweight patients. CT provides a true volumetric visualization and quantification of EAT and pericardial adipose tissue and may provide a more accurate evaluation of fat tissue due to its higher spatial resolution compared with ultrasound and MRI [44,46] but provides a ionizing radiation exposure. The gold standard for the evaluation of total body fat as well as for ventricular volume and mass is MRI and so the use of MRI is a reliable choice for the assessment of EAT [47]. Moreover, there is a high correlation between measurement of EAT with echocardiography and MRI. [46]. For these reasons, in our study we decided to evaluate EAT with MRI, considering this technique reliable also in overweight and obese patients, without a radiation exposure. For the evaluation of VAT and SAT, the most reliable tools are CT and MRI. MRI can produce high quality cross-sectional images without ionizing radiation exposure. Therefore, MRI is the first-choice exam for studies performed on young subjects . Total VAT volume of the abdominal compartment by MRI is the gold standard measurement for VAT but is expensive and a long-lasting exam [48]. Maislin et al. in a cohort of 826 subjects, including also overweight and obese subjects, demonstraed that VAT area estimated on a single axial MRI image is highly correlated with VAT volume and is an accurate surrogate for the total VAT volume.

Previous publications, also from our group, suggested the potential role of EAT in promoting / accelerating CAD and other CVDs [23,27,28,34]. To be noted, in the present study we just observed that EAT is the main fat depot influencing sRAGE levels. Considering that sRAGE has been previously suggested as a potential biomarker of metabolic complications and future cardiovascular events, our results prompted us to consider the increased accumulation of visceral fat at the epicardial level one potential explanation of the link between sRAGE and CVDs. To date, the mechanisms of interaction between sRAGE and obesity are still unclear and our results did not prove the causal-relationship between sRAGE and EAT. This remains an interesting area to explore in the future.

The "healthy" characteristic of our group is probably the reason of the lack of association between sRAGE and the biochemical markers of CVD risk observed in other studies [16,43]. Only HOMA-IR and LAP indices showed a trend of correlation with sRAGE. This finding is a further evidence that sRAGE may be an early marker of metabolic dysfunction. Especially, since the individuals included in the study were young, healthy adults without diabetes, the trend towards higher HOMA-IR and LAP indices with lower sRAGE means that sRAGE could be indicative of organ fat which may predispose to insulin resistance and metabolic complications before any disease.

Whether the same results are observable also in men need to be explored in future studies.

In conclusion, in ~~healthy~~ obese women without metabolic disease, lower sRAGE levels reflect~~s~~ accumulation of visceral fat mainly at the epicardial level and ~~is~~ are present in advance the appearing of metabolic complications. Due to the role of EAT as an important risk factor for CVDs and the consistent association herein observed with sRAGE in healthy women, sRAGE might be suggested as potential early marker of cardiometabolic risk. This needs to be further confirmed in large-scale population studies.

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Figure legends

Figure 1

Evaluation of levels of soluble receptor for advanced glycation end products (sRAGE) according to body mass index (BMI), epicardial adipose tissue (EAT), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) median values and waist circumference (WC) cut off of 80 cm.

A) After classification according to BMI, sRAGE levels ~~was~~ were lower in obese (OB) than normal weight (NW) women (OW). * = $p < 0.05$ (Kruskal-Wallis test).

B-C-D) Women were stratified into two groups on the basis of the median EAT, VAT and SAT volumes, respectively. sRAGE levels ~~was~~ were lower in all the groups with the highest volumes compared to the lowest. * = $p < 0.05$ (Mann-Whitney test for EAT and VAT; t-test for SAT).

E) After classification according to the WC cut off value of 80 cm, sRAGE levels ~~was~~ were lower in the highest WC group ($p < 0.05$). * = $p < 0.05$ (Mann-Whitney test).

Figure 1

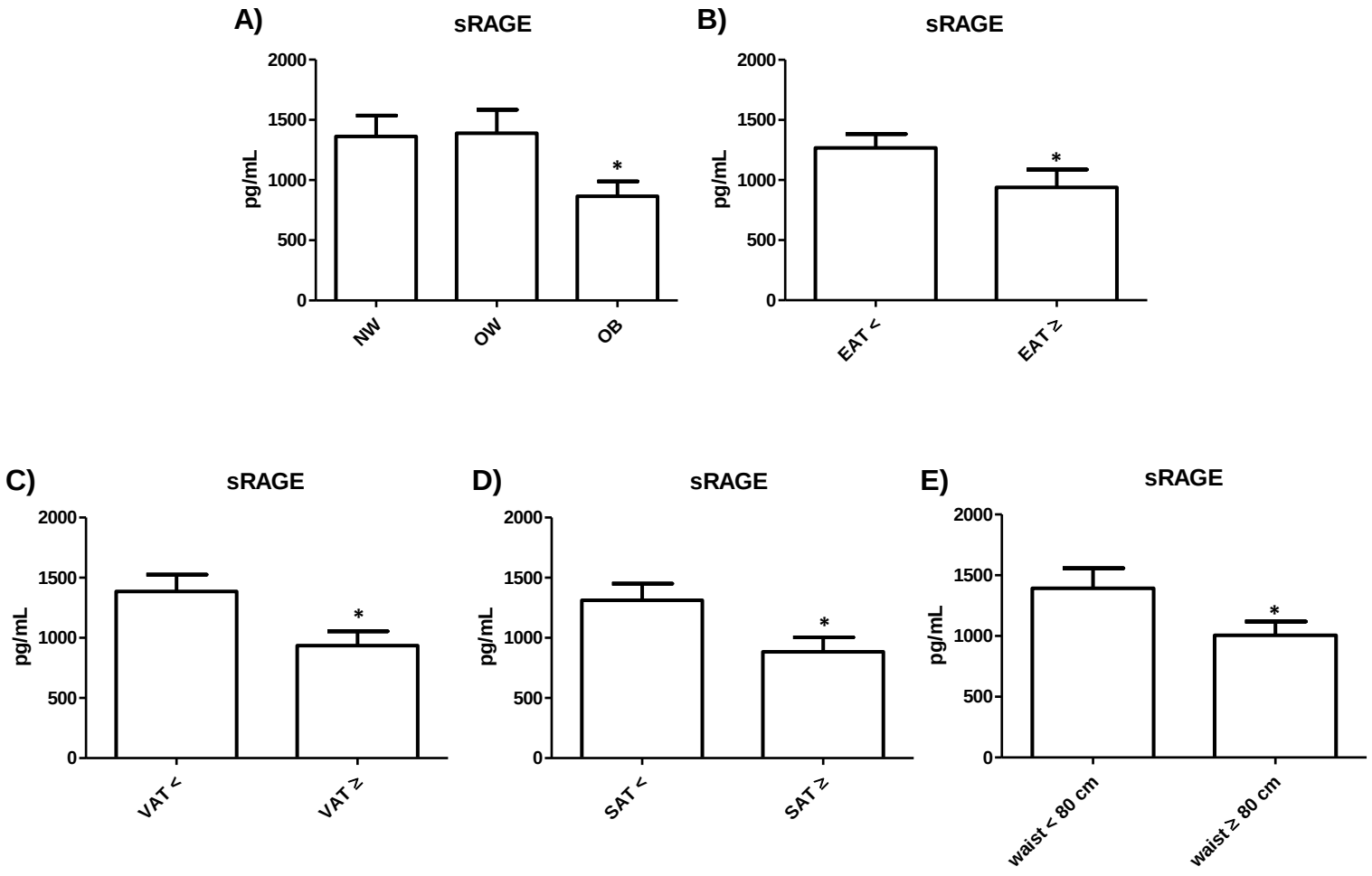
A)
sRAGE
pg/mL
2000
1500
1000
500
0
*
NW
OW
OB
B)
sRAGE
pg/mL
2000
1500
1000
500
0
*
EAT <
EAT ≥
C)
sRAGE
pg/mL
2000
1500
1000
500
0
*
VAT <
VAT ≥
D)
sRAGE
pg/mL
2000
1500
1000
500
0
*
SAT <
SAT ≥
E)
sRAGE
pg/mL
2000
1500
1000
500
0
*
waist < 80 cm
waist ≥ 80 cm

Table 1.
Demographic, anthropometric and biochemical characteristics of participants included in the study as a whole group and after classification according to body mass index.

	Healthy pre-menopausal women (n=47)	NW (n=16)	OW (n=10)	OB (n=21)
Age (years)	33.3 ± 8.4	30.1 ± 4.9	33.8 ± 6.4	34.3 ± 10.6
Obesity (n,%)				
NW	16, 34.0			
OW	10, 21.3			
OB	21, 44.7			
BMI (kg/m^2)	28.9 ± 6.6	22.0 ± 1.5	27.2 ± 1.1[c]	35.4 ± 3.5[e,f]
Waist (cm)	88.0 ± 2.4	71.4 ± 5.0	84.0 (82.3-91)[a]	102.4 ± 11.5[e]
Hip (cm)	104.0 (98.5-120.0)	97.2 ± 4.0	104.5 ± 5.5[a]	120.5 ± 7.4[e,f]
WHR	0.81 ± 0.1	0.7 ± 0.1	0.8 (0.8-0.9)[a]	0.9 ± 0.1[e]
Fatt mass (%)	35.3 ± 9.3	25.7 ± 5.6	35.0 ± 2.8[e]	44.1 ± 3.6[e,f]
EAT volume (mL)	10.0 ± 7.1	3.5 (1.9-7.8)	10.8 ± 4.2[a]	13.8 ± 7.4[e]
VAT volume (mL)	21.7 (12.9-40.0)	14.1 ± 10.1	23.2 (20.0-32.5)[a]	36.7 ± 18.5[e]
SAT volume (mL)	307.4 ± 88.2	226.9 ± 73.0	294.0 ±18.9[a]	377.9 ± 59.5[d,e]
Fasting glucose (mg/dL)	84.3 ± 8.1	77.0 (74.5-88.5)	85.3 ± 6.1	87.3 ± 7.9[a]
Fasting insulin (mU/mL)	9.8 (6.7-15.2)	7.9 ± 3.3	10.7 ± 3.0	14.0 (8.6-21.9)[c]
Total cholesterol (mg/dL)	189.1 ± 35.0	192.6 ± 33.6	202.3 ± 42.5	179.9 ± 31.3
HDL cholesterol (mg/dL)	60.8 ± 16.5	62.8 ± 17.2	61.5 ± 10.8	53.1 ± 14.7[c]
LDL cholesterol (mg/dL)	106.4 (89.3-124.7)	105.1 ± 29.7	116.9 ± 40.3	108.5 ± 25.7
Triglycerides (mg/dL)	81.0 (65.0-118.8)	88.4 ± 32.9	119.7 ± 64.5	82.0 (67.5-106.5)
HOMA-IR	2.1 (1.3-3.2)	1.6 ± 0.7	2.3 ± 0.7	3.8 ± 2.7[c]
LAP index	27.8 (13.2-43.8)	10.8 (6.8-19.3)	32.5 ±15.6[a]	42.9 (25.8-58.5)[e]
sRAGE	1020 (604.3-1633.0)	1363 ± 693.2	1389 ± 195.4	640.8 (423.2-1345)[a]

Data are expressed as mean ± SD, median (25th-75th percentiles) or number and proportions.
NW, normal weight; OW, overweight; OB, obese; BMI, body mass index; WHR, waist to hip ratio; EAT, epicardial adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulayion product; sRAGE, soluble receptor for advanced glycation end products.
Comparison between BMI categories was performed by ANOVA or Kruskal-Wallis tests followed by Bonferroni or Dunns tests.
a, p<0.05 *vs.* NW; b, p<0.05 *vs.* OW; c, p<0.01 *vs.* NW; d, p<0.01 *vs.* OW; e, p<0.001 *vs.* NW; f, p<0.001 *vs.* OW.

Table 2. Univariate association between plasma sRAGE, demographic, biochemical and body composition parameters in the study population

	r	p
Age	-0.123	0.411
BMI	-0.363	**0.012**
Waist	-0.391	**0.007**
Hip*	-0.416	**0.004**
WHR	-0.238	0.111
EAT volume	-0.426	**0.003**
VAT voume*	-0.341	**0.025**
SAT volume	-0.197	0.206
Fat mass	-0.312	**0.039**
Fasting glucose	-0.127	0.395
Fasting insulin*	-0.254	0.107
Total cholesterol	0.070	0.614
HDL cholesterol	0.017	0.908
LDL cholesterol*	-0.043	0.777
Triglycerides*	0.130	0.384
HOMA-IR*	-0.252	0.087
LAP index*	-0.254	0.085

Association between variables was explored using Pearson or Spearman (*) correlation coefficients.
BMI, body mass index; WHR, waist to hip ratio; EAT, epicardial adipose tissue;
VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue;
HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulayion product.

Table 3. Model of multiple regression analysis

VARIABLE	B	STD ERROR	t	p
EAT volume	-35,542	16,898	-2,103	**0.042**
VAT volume	4,203	7,683	0.547	0.588
Fat Mass	-8,626	14,590	-0.591	0.558

EAT, epicardial adipose tissue; VAT, visceral adipose tissue.