Original article

Anti-sRAGE autoimmunity in obesity: Downturn after bariatric surgery is independent of previous diabetic status

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Abstract

Aim. – Morbid obesity increases the risk of cardiovascular disease (CVD). The receptor for advanced glycation end-products (RAGE) is implicated in proinflammatory processes that underlie CVD. Its soluble form (sRAGE) has been proposed as a vascular biomarker. Recently, anti-sRAGE autoantibodies were described and found to be increased in diseases where RAGE is overexpressed. This study aimed to investigate serum levels of anti-sRAGE autoantibodies in morbidly obese patients.

Methods. – After exclusion based on specific criteria, 150 subjects (50 normoglycemics, 50 glucose-intolerants and 50 diabetics) were randomly recruited from a cohort of 750 obese patients (ABOS). Serum sRAGE and anti-sRAGE autoantibodies were measured before bariatric surgery. Sixty-nine patients were followed for up to 1 year after gastric bypass, and their levels of sRAGE and anti-sRAGE autoantibodies were measured. The control group consisted of healthy blood donors.

Results. – Compared with controls, baseline levels of sRAGE and anti-sRAGE autoantibodies were significantly higher in all obese patients independently of glucose regulation (P < 0.001). At 1 year after gastric bypass, sRAGE and anti-sRAGE were decreased (P < 0.001). The decrease in anti-sRAGE autoantibodies was correlated with an increase in high-density lipoprotein (HDL; P = 0.02).

Conclusion. – Independently of previous diabetic status, morbid obesity increases sRAGE and anti-sRAGE levels. Weight loss after gastric bypass is followed by a decrease in both titres. The decrease in anti-sRAGE correlates with an increase in HDL.

Keywords: Diabetes; Obesity; Anti-sRAGE; Bariatric surgery; Biomarker

1. Introduction

Obesity is one of the diagnostic components of the metabolic syndrome, and is associated with insulin resistance and increased vascular risk. Gastric bypass is one of the most efficient surgical procedures for weight loss and improvement of metabolic function [1].

Due to obesity-associated complications, several molecules have been studied as biomarkers. Adipokines, which often have deregulated secretion in obese subjects, are associated with cardiometabolic risk. The high levels of leptin and leptin resistance observed in the obese are known to contribute to their proinflammatory and prothrombotic status [2]. Adiponectin levels are decreased in obesity and associated with vascular dysfunction [3].

The receptor for advanced glycation end-products (RAGE) is a multiligand receptor involved in both proinflammatory and
pro-ageing processes. RAGE engagement increases reactive oxygen species (ROS), and induces the expression of adhesion molecules and vascular endothelial growth factor [4–6]. In adipocytes, RAGE activation induces an increase in ROS that mediates insulin resistance [7]. The soluble form of RAGE (sRAGE) acts as a decoy receptor, preventing RAGE activation [8], and has been extensively studied as a biomarker of vascular risk and metabolic dysfunction [9,10]. In the general population, sRAGE levels are inversely correlated with body mass index (BMI), waist-to-hip ratio and fasting glucose [11]. Levels of sRAGE are restored after bariatric surgery in morbidly obese patients [12], and the increase in sRAGE levels is associated with improvement of insulin resistance. Nevertheless, sRAGE levels across publications are controversial, a fact that demands rigorous selection of study populations to evaluate the true value of sRAGE as a biomarker.

Recently, autoantibodies directed against sRAGE were found to be increased in Alzheimer’s disease and rheumatoid arthritis [13,14]. Our group has also observed increased levels of anti-sRAGE autoantibodies in haemodialysis patients that might be associated with uraemic vascular dysfunction and with increased sRAGE levels [15].

The present work aimed to estimate the value of sRAGE and anti-sRAGE autoantibodies as markers of metabolic homeostasis. To minimize confounding factors, a well-defined population of morbidly obese patients (ABOS cohort) was investigated. The association of both titres with metabolic improvement after weight-loss surgery was also evaluated.

2. Methods

2.1. Study population

Patients included in this study were enrolled from the ABOS cohort (ClinicalGov NCT01129297) of 750 morbidly obese patients eligible for bariatric surgery [16]. Our exclusion criteria were chosen to avoid any factors that might influence sRAGE levels: hypertension, glomerular filtration rate (GFR) < 90 ml/min/1.73 m² (as per the Modification of Diet in Renal Disease Study), use of angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor antagonists (ARA2) and statin medications, and smokers.

Out of the entire cohort, 254 obese patients were enrolled. These subjects were further subclassified into three groups of those with diabetes or lesser degrees of impaired glucose regulation. Accordingly, oral glucose tolerance test criteria were: for normoglycaemia, fasting plasma glucose (FPG) < 5.5 mmol/L or 2-h plasma glucose (PG) < 7.7 mmol/L; for glucose intolerance, FPG > 5.5 mmol/L and < 6.9 mmol/L or 2-h PG > 7.7–10.9 mmol/L and for diabetes, FPG > 6.9 mmol/L or 2-h PG > 11 mmol/L. The number of subjects needed to detect a difference in anti-sRAGE antibodies between normoglycemic, glucose-intolerant and diabetic patients was estimated (Bonferroni correction) as per preliminary work by our group: the resulting number of patients to be included was 50 in each group (46 plus 10% for security). The control group included 46 healthy blood donors (French National Blood Service, Lille, France): gender ratio 0.79; mean age 33 ± 10.5 years; and mean BMI 23.79 ± 2.7 kg/m². After baseline analysis, patients who underwent gastric bypass (Roux-en-Y) were screened and followed up for 1 year after surgery (69 patients; Fig. 1). The Roux-en-Y procedure comprises an anastomosis between the jejunum and a gastric pouch near the oesophagus [17]. The procedure decreases nutrient absorption as food bypasses the main part of the stomach. Surgery is followed by weight loss (25–30% of total body weight) [18] and normalization of glucose levels in type 2 diabetes patients (40–80% diabetes remission) [19,20].

2.2. sRAGE quantification by ELISA

Serum sRAGE levels were quantified using the Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. Each sample was analyzed in duplicate, incubating 50 µL of serum for 2 h in 96-well microplates precoated with anti-sRAGE antibody.

2.3. Anti-sRAGE autoantibody quantification by ELISA

Quantification of anti-sRAGE autoantibodies (IgGs) was adapted from a previously reported protocol [13]. The 96-well microplates were coated with 0.5 µg/well of Fc fragment-free sRAGE (R&D Systems) in phosphate buffer saline (PBS) and kept overnight at 4 °C. After this, the wells were washed, then saturated with 10% fetal bovine serum (FBS) in PBS. Samples (100 µL/well) were incubated for 2 h at room temperature (RT). Following this step and after washing, 100 µL of alkaline phosphatase-conjugated anti-human IgG (Sigma-Aldrich, St Louis, MO, USA) were added to each well (1:2000 in saturation buffer) and incubated for 45 min at RT. Again, after this step
and after washing, 100 μL of 1 mg/mL para-nitrophenyl phosphate (Euromedex, Strasbourg, France) were added to each well and incubated for 1 h at 37 °C. Optical density was measured at 405 nm. Samples were measured in duplicate and anti-sRAGE IgG titres calculated by subtracting the blank control sample (wells not coated with sRAGE) from the duplicate mean.

2.4. Statistical analysis

Baseline characteristics are presented as means ± standard deviation (SD) for continuous variables, and as frequency and percentage for categorical variables. Comparisons were first done at baseline between the three subgroups for the entire cohort of 150 obese patients and control subjects. sRAGE levels were compared using one-way analysis of variance (ANOVA), followed by Tukey’s test. Levels of anti-sRAGE autoantibodies were compared using Dunn’s multiple comparison test.

Comparisons were then made between characteristics at baseline and at 1 year after bariatric surgery for the 69 obese patients included in the second part of the study. Paired Student’s t-test was used for all comparisons after verifying differences in the variance distributions. The percentage of diabetic patients was analyzed by a Chi-squared test. Finally, the associations between the evolution of anti-sRAGE antibodies and other characteristics were analyzed, using Pearson’s product-moment correlation coefficient to test the association between differences in anti-sRAGE antibodies (at baseline and at 1 year) and differences in other characteristics (at baseline and at 1 year). Significance level was 0.05 for all tests performed.

3. Results

3.1. Baseline sRAGE and anti-sRAGE autoantibodies are higher in obese patients

Clinical characteristics of the 150 obese patients in our initial study population are presented in Table 1. These initially included patients had higher levels of sRAGE and anti-sRAGE autoantibodies in comparison to the controls ($P < 0.0001$, except for $P < 0.05$ for sRAGE in the glucose-intolerant group; Fig. 2). Among obese patients, neither sRAGE nor anti-sRAGE levels differed between normoglycemic, glucose-intolerant and diabetic patients.

3.2. Gastric bypass is followed by decreases in sRAGE and anti-sRAGE autoantibodies

As sRAGE and anti-sRAGE autoantibodies did not differ according to glucose regulation, it was decided to investigate the role of obesity itself. Out of our original study population, we further selected 69 patients who underwent gastric bypass and a 1-year follow-up. Their clinical data are presented in Table 2. One year after weight-loss surgery, their percentage decrease in BMI was 31.9% (from 46.7 kg/m² to 31.8 kg/m²) along with an improvement in insulin. After gastric bypass, sRAGE and anti-sRAGE autoantibody levels decreased (Fig. 3). Serum sRAGE after surgery was different from levels before surgery only ($P < 0.0001$). Anti-sRAGE autoantibodies were significantly lower after surgery ($P < 0.0001$), but were still higher in comparison to controls ($P < 0.0001$).

3.3. Decreases in anti-sRAGE autoantibodies is associated with increases in HDL

Univariate analysis showed a correlation between increases in HDL levels and decreases in anti-sRAGE autoantibodies after surgery ($r^2 = 0.077$, $P = 0.02$). Other metabolic parameters
were not associated with either anti-sRAGE or sRAGE levels (Fig. 4).

4. Discussion

Our study has demonstrated that morbidly obese subjects eligible for weight-loss surgery present with higher serum levels of sRAGE and anti-sRAGE autoantibodies. One year after gastric bypass, both titres were significantly decreased. While sRAGE levels after surgery were similar to those of control subjects, anti-sRAGE titres remained higher in comparison. We also observed that the increase in HDL levels, a usual consequence of weight-loss surgery, was associated with a decrease in anti-sRAGE autoantibodies.

Serum sRAGE levels have been measured in patients with several diseases, and sRAGE has often been proposed as a biomarker of severity or prognosis. sRAGE levels in vivo appear to be modulated by several factors, such as ACEi and statin use, BMI, RAGE polymorphisms and kidney dysfunction [21]. The higher levels of sRAGE in our obese subjects were in contrast to the work of Brix and colleagues [12], who observed lower levels compared with controls and an increase after bariatric surgery. Regarding such controversial findings, we hypothesize it was mainly due to the inclusion/exclusion criteria applied. In our ABOs cohort, we specifically selected patients who were taking neither ACEi nor statins, and were non-smokers and without kidney dysfunction. The Brix et al. study did not include subjects with known diabetes, but we observed no influence of diabetes on either sRAGE or anti-sRAGE levels. In addition, our study (and 1-year follow-up) also included only patients who had undergone gastric bypass (Roux-en-Y) surgery, whereas the possible effects of this type of surgery on sRAGE levels were not presented in the Brix et al. work.

The majority of circulating sRAGE is formed by cleavage of membrane RAGE (cRAGE), by a disintegrin and metalloproteinase 10 (ADAM10) and matrix metalloproteinase 9 (MMP9) [22]. Although it has a putative protective role, studies showing RAGE blockade have used much higher doses of sRAGE than are found in circulation [23,24]. Furthermore, it remains unknown whether RAGE cleavage follows RAGE activation or is a simple preventative mechanism. sRAGE assay measures pooled cRAGE and endogenous secretory sRAGE (esRAGE) [25]. The latter, a splice variant of RAGE, represents only a small proportion of total circulating sRAGE. esRAGE has also been studied as a biomarker of vascular risk [26] although, in the present study, esRAGE was not specifically investigated as it has the same amino-acid sequence as cRAGE, and our aim was to focus on sRAGE and, in particular, anti-sRAGE antibodies.

In rheumatoid arthritis and Alzheimer’s disease [13,27], anti-sRAGE autoantibodies are believed to play a protective role even though they are associated with dementia. New autoantibody reactivity has been reported in relation to the overexpression of autoantigens, especially in a proinflammatory or proapoptotic context [28]. Such autoreactive specificity could contribute to regulation of the expression and clearance of overexpressed autoantigens [29,30]. The high levels of anti-sRAGE antibody
observed in our study population may have been due to the rise in sRAGE levels associated with increased inflammatory processes and endothelial dysfunction.

One year after gastric bypass, serum levels of sRAGE and anti-sRAGE autoantibodies were decreased in parallel with decreases in inflammation and C-reactive protein (CRP) levels. Nevertheless, anti-sRAGE autoantibodies showed a less prominent decrease than serum sRAGE, which was probably related to the different lifetimes of the two biological molecules. While the exact half-life of human sRAGE is still a matter of debate, animal studies have demonstrated that the elimination half-life of radiolabeled sRAGE is about 1–2.5 days after administration [31]. In contrast, the half-life of gamma globulins is in the order of 21 days [32]. This difference may have contributed to accumulation of the antibody, whereas the antigen (sRAGE) concentration was already decreased. It has also been shown that the sRAGE half-life is longer in diabetic than in healthy animals [31]. Reduction of the metabolic syndrome in humans by restoration of several physiological functions (associated with gastric bypass) could also have contributed to faster elimination of sRAGE compared with immunoglobulins. In another study, bariatric surgery promoted a decrease in oxidized low-density lipoprotein (LDL), while autoantibodies against this antigen either did not change or increased slightly 7 months after the intervention [33].

In addition to sRAGE and anti-sRAGE changes, our study observed that weight-loss surgery improved lipid profile (decreased LDL and increased HDL) and insulin sensitivity.
Acknowledgements

In obesity, the role of autoimmunity is still poorly understood. Some studies have proposed a role for leptin. Obesity favour the development of thyroid autoantibodies and these are associated with leptin levels [41].

Our present study is the first to demonstrate the presence of anti-sRAGE autoantibodies in morbidly obese patients. Our findings come from a large cohort selected by well-defined inclusion/exclusion criteria. Nevertheless, the role of RAGE in the pathophysiology of RAGE autoimmunity during obesity is yet to be investigated.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

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References


