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INVITED SPOTLIGHT REVIEW

The RAGE/DIAPH1 axis: mediator of obesity and proposed biomarker of human cardiometabolic disease

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Abstract

Overweight and obesity are leading causes of cardiometabolic dysfunction. Despite extensive investigation, the mechanisms mediating the increase in these conditions are yet to be fully understood. Beyond the endogenous formation of advanced glycation endproducts (AGEs) in overweight and obesity, exogenous sources of AGEs accrue through the heating, production, and consumption of highly processed foods. Evidence from cellular and mouse model systems indicates that the interaction of AGEs with their central cell surface receptor for AGE (RAGE) in adipocytes suppresses energy expenditure and that AGE/RAGE contributes to increased adipose inflammation and processes linked to insulin resistance. In human subjects, the circulating soluble forms of RAGE, which are mutable, may serve as biomarkers of obesity and weight loss. Antagonists of RAGE signalling, through blockade of the interaction of the RAGE cytoplasmic domain with the formin, Diaphanous-1 (DIAPH1), target aberrant RAGE activities in metabolic tissues. This review focuses on the potential roles for AGEs and other RAGE ligands and RAGE/DIAPH1 in the pathogenesis of overweight and obesity and their metabolic consequences.

Keywords Diabetes • Obesity • Metabolism • RAGE/DIAPH1 • Soluble RAGE

This article is part of the Spotlight Issue on Obesity, Metabolism, and Diabetes.

1. Introduction

1.1 Scope of the problem

Data reported by the National Institute of Diabetes and Digestive and Kidney Diseases from the 2017–2018 National Health and Nutrition Examination Survey using body mass index (BMI)[1] measures indicate that approximately one in three adults is overweight; two in five adults have obesity; and one in 11 adults has severe obesity.[2] Data from the same source indicate that during ages 2–19, one in six children and adolescents is overweight; one in five children and adolescents has obesity; and one in 16 children and adolescents suffers with severe obesity.[3]

Yet, despite the burden of overweight and obesity, durable solutions and treatments are not fully available. With regard to personalized approaches to weight loss, there remain hurdles in terms of optimal behavioural interventions and suitable biomarkers to predict and track the efficacy of potential interventions. In this context, advanced glycation endproducts (AGEs) are formed during normal metabolism and aging[4,5] and to increased degrees during obesity and hyperglycemia.[6] AGEs stimulate various cellular pathways by interacting with their central cell surface receptor, receptor for advanced glycation endproduct (RAGE). Recent research has uncovered novel roles for the RAGE pathway in adipocyte physiology that may directly relate to the pathogenesis of obesity; furthermore, research has identified the potential utility of tracking the AGE/RAGE axis in human obesity and cardiometabolic disease. This review will focus on the biology of AGE/RAGE pathway in overweight and obesity and their cardiometabolic complications.

2. RAGE/Diaphanous-1 (DIAPH1) axis

2.1 RAGE is a multi-ligand receptor

RAGE was discovered on account of its ability to bind and to transduce AGE signals; in the years after its discovery, reports uncovered the multi-ligand nature of RAGE, in that multiple members of the S100/calgranulin family; amphoterin [more recently known as high mobility group box 1 (HMGB1)]; and amyloid beta peptide (Aß) were identified as ligands of RAGE.[7–10] Later

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studies revealed that RAGE also binds species such as phosphatidylserine, C1q, and lysophosphatidic acid (LPA).[11–13] RAGE, an immunoglobulin (Ig) superfamily receptor whose extracellular domains are composed of one Variable (V)-type domain followed by two Constant (C)-type domains, is expressed by multiple types of cells, such as vascular and immune cells, as well as adipocytes.[14] The complexity of these extracellular domains was further supported by the findings that while most of the RAGE ligands bind at the V-type Ig domain or to the V-C1 Ig domains, other ligands, such as certain members of the S100/calgranulin family, bind to the C-type Ig domains.[15] For these reasons, it was postulated that effective pharmacological targeting of RAGE through antagonism of intracellular RAGE signal transduction might be superior to targeting the extracellular domains.

2.2 The cytoplasmic domain of RAGE binds to DIAPH1: implications for signal transduction

To discover the mechanisms of RAGE signal transduction, a yeast-two-hybrid assay was employed to reveal molecules that might bind to the cytoplasmic domain of RAGE; through this approach, it was discovered that the intracellular domain of RAGE binds to the formin molecule, Diaphanous-1 (DIAPH1).[16] Specifically, the cytoplasmic domain of RAGE binds to the formin homology 1 (FH1) domain of DIAPH1; mutational analyses and consequent nuclear magnetic resonance (NMR) studies identified the precise amino acids in the RAGE cytoplasmic domain which are responsible for binding to DIAPH1.[17] When mutations of the RAGE cytoplasmic domain that mitigated interaction with DIAPH1 were introduced into cultured cells, RAGE ligand-mediated signalling, proliferation and migration responses were abrogated.[17] Notably, among the functions of the formins are activation of pathways such as RHOA, CDC42, and RAC1; studies revealed that RAGE ligand-mediated activation of CDC42 and RAC1 in cultured cells was blocked when *Diaph1* expression was silenced.[16] A detailed depiction of RAGE–DIAPH1 signal transduction was chronicled in a recent review.[18]

2.3 The discovery of small molecule antagonists that block RAGE–DIAPH1 interaction

These discoveries, particularly the identification of the precise amino acids in the RAGE cytoplasmic domain that bound DIAPH1 led to the discovery of small molecule antagonists that block the interaction of the RAGE cytoplasmic domain with DIAPH1.[19,20] After the screening of a > 59 000 compound library, followed by extensive structure–activity–relationship refinements to the basic scaffold molecules, a novel chemical probe, called RAGE229, was recently identified.[20] In a multi-disciplinary approach, RAGE229 was tested in binding assays, NMR spectroscopy, and in Förster resonance energy transfer experiments. RAGE229 was shown to block the interaction of the cytoplasmic domain of RAGE with DIAPH1. In cellular studies, RAGE229 antagonized RAGE ligand-mediated cellular migration, signalling and production of inflammatory mediators.[20] Furthermore, in *in vivo* models of inflammation, cardiac ischemia/reperfusion injury, impaired wound healing, and diabetic kidney disease in mice with type 1- or type 2-like diabetes, RAGE229 significantly attenuated RAGE–DIAPH1 binding and molecular/pathological consequences of stimulation of the RAGE/DIAPH1 axis in murine models.[20] These studies support the premise that the ligand/RAGE/DIAPH1 axis may be targeted for testing in clinical trials.

In the context of obesity, what is known about the ligand/RAGE/DIAPH1 axis and what are the implications for human cardiometabolic disease? The sections to follow address these points and present evidence to support key roles for the RAGE axis in the pathogenesis of obesity.

3. The RAGE/DIAPH1 axis, adipose tissue, and obesity

Important clues to potential roles for RAGE in obesity were reported in numerous studies illustrating the expression of multiple classes of RAGE ligands in adipocytes or in adipose tissues in obesity. In those studies, as will be detailed below, it was suggested that the production of RAGE ligands caused metabolic dysfunctions. 3T3-L1 adipocytes in culture produced RAGE ligand S100B and S100B interaction with mouse macrophage-like cells (RAW264.7) produced cytokines in a RAGE-dependent manner.[21] In human preadipocyte SW872 cells, expression of HMGB1 resulted in release of IL6 in a manner blocked by RAGE antagonism, but not by blockade of toll-like receptor 2 or 4 (TLR2 or TLR4).[22] Pathological roles for LPA in mice consuming a high-fat diet were illustrated in studies in which the gene encoding autotaxin (enzyme which produces LPA), *Enpp2*, was deleted. Compared with control mice, those mice devoid of *Enpp2* were protected from high-fat diet-induced obesity and displayed reduced hepatic steatosis.[23] Other studies indicated that compared with lean individuals, adipose tissue from human subjects with obesity displayed higher concentrations of carboxymethyl lysine (CML)-AGE, in parallel with higher expression of RAGE.[6] Interestingly, in that study, the authors suggested that CML-AGEs were trapped in the obese adipose tissue, which might account for the lower concentrations of circulating CML-AGE identified in subjects with obesity vs. the lean state.[6] It is noteworthy that not all studies report lower circulating concentrations of CML or general AGEs in subjects with obesity vs. lean state; this is not surprising because AGEs are a heterogeneous group of structures and it is established that beyond endogenous lipoxidation-mediated production of AGEs in the tissues, dietary AGEs are key contributors to the circulating pools of AGEs.[24–27] Thus, factors such as the quality and composition of a subject's diet, dietary intake, and the specific conditions during which blood samples were obtained (such as fasted vs. fed state or the time of day) may affect the final AGE concentrations reported among studies.

It is notable that there is further support for AGEs in the pathogenesis of obesity and its consequences; one of the endogenous mechanisms through which the AGEs may be generated is through the polyol pathway and the roles of its lead enzyme, aldose reductase, in these processes. Previous studies implicated aldose reductase in the production of AGEs, which led to their pathological actions on endothelial aging via the RAGE pathway.[28] Recently, it was shown that the expression of aldose reductase (gene and protein) was increased in human and mouse adipose tissue in obesity and that the global genetic deletion or pharmacological antagonism of aldose reductase in mice reduced high-fat diet-induced obesity.[29]

Hence, the ligand/RAGE axis plays a role in obese adipose tissue physiology and the ligands of RAGE may mediate pathological effects in metabolic cells. These studies raised the question, therefore, of whether the RAGE pathway contributed to the development of obesity. In the section to follow, studies testing the ligand/RAGE axis in animal models will be reviewed.

4. Studies in animal models established roles for the RAGE axis in obesity

Numerous studies have used *Ager* (the gene encoding RAGE)-modified mice to probe roles for RAGE in high-fat diet-induced obesity. Studies have employed both global and adipocyte-specific deletion of *Ager;* these studies will be detailed in the sections to follow.

4.1 Global deletion of *Ager*

The first studies implicating roles for RAGE in diet-induced obesity were reported in male mice devoid of *Apoe* (Apolipoprotein E) either expressing or globally devoid of *Ager* and fed a diet in which 20% of total calories was provided by cocoa butter and 1.5% of total calories was provided from cholesterol.[30] In that study, despite comparable food intake, *Apoe* null mice devoid of *Ager* displayed less body weight gain on the atherogenic diet. Epididymal adipose tissue weight was significantly lower along with smaller adipocytes and reduced adipose tissue inflammation in the *Apoe* null mice devoid of *Ager* vs. the controls and adipose tissue inflammation was also reduced, in parallel with reduced adipocyte size.[30] The expression

of mRNA encoding Uncoupling Protein 1 (*Ucp1*) in the interscapular brown adipose tissue (iBAT) did not differ between the *Ager*-expressing or *Ager*-deleted *Apoe* null mice and energy expenditure was not determined in that study.[30]

The above diet was also fed to male wild-type C57BL/6 mice and mice devoid of *Ager*. Compared with wild-type mice, those mice devoid of *Ager* demonstrated less body weight gain, lower epididymal fat weight, smaller adipocyte size, and significantly higher circulating adiponectin concentrations.[31] Energy expenditure and adipose tissue expression of UCP1 were not reported in that work. This work showed that RAGE-dependent mechanisms in the adipose tissue were accounted for, at least in part, by RAGE-dependent regulation of TLR2 and that mice devoid of *Ager* and fed the cocoa butter/cholesterol diet displayed less adipose inflammation and improved insulin sensitivity compared with the *Ager*-expressing control mice.[31]

In a distinct study, C57BL/6N Chr mice were fed a diet in which 60% of calories was supplied from fat. Male *Ager* null mice gained more weight and displayed higher concentrations of insulin and cholesterol compared with the wild-type mice fed this diet.[32] It was noted in that study that the mice were pair-fed but the specific details were not provided.

In contrast, others tested the role of RAGE in obesity in male C57BL/6 mice by feeding the animals a high-fat diet in which 60% of calories was supplied from fat.[33] Compared with wild-type littermate mice, mice devoid of *Ager* were protected from diet-induced obesity and displayed reduced adiposity and smaller adipocyte size. Hyperinsulinemic euglycemic clamps revealed that the high-fat diet-fed *Ager* null mice exhibited greater insulin sensitivity and superior hepatic insulin action compared with the wild-type mice fed the high-fat diet.[33] Despite no differences in caloric intake, energy expenditure was significantly higher in the *Ager* null vs. wild-type mice. Furthermore, gene expression of *Ucp1* was significantly higher in the iBAT of high-fat diet-fed *Ager* null vs. wild-type mice, and multiple indices of adipose inflammation were reduced in the mice devoid of *Ager*.[33] Pharmacological antagonism of the ligand/RAGE axis using soluble RAGE (sRAGE) demonstrated that compared with vehicle, sRAGE administration reduced weight gain in wild-type mice fed a high-fat diet.[33]

Collectively, in the studies presented above, the differences observed demonstrating protection vs. exacerbation of weight gain in high-fat feeding in *Ager* null mice may have multiple explanations, such as genetic background, manner of feeding, and temperature and conditions in the vivarium. It is also possible that the pathogen-free vs. pathogen-positive status of the vivarium may contribute to the overall findings. Irrespective of these issues, these various studies illustrated that a comprehensive metabolic phenotyping programme is essential to fully characterize the role of RAGE in diet-induced obesity.

Hence, homozygous deletion of *Ager* might cause confounding and complex effects because of the numerous cell types expressing *Ager*, and given the reports that *Ager* null mice fed the high-fat diet displayed protection from diet-induced obesity, reduced adiposity, higher expression of *Ucp1* in brown adipose tissue and higher energy expenditure than wild-type mice fed the diet, a logical next study was to probe the impact of high-fat feeding in mice bearing an adipocyte-specific deletion of *Ager*.

4.2 Adipocyte RAGE regulates thermogenesis but not adipocyte differentiation

Adipocyte-specific deletion of *Ager* in mice was a specific test of the role of RAGE in these cells in the high-fat diet feeding environment, as in *Ager* floxed mice bred into the *Adipoq* (Adiponectin) Cre recombinase background, expression of *Ager* in immune and vascular cells, as key examples, would be intact.[34] As a first step, primary adipocytes differentiated from the stromal vascular fraction of iBAT, inguinal white adipose tissue (iWAT) (subcutaneous), and epididymal white adipose tissue (eWAT) were shown to express, but not require, RAGE during the differentiation process; of note, even in homeostatic conditions, clues to key roles for RAGE in thermogenesis and mitochondrial activity emerged. For example, in iBAT-derived primary adipocytes bearing *Ager* deletion, basal respiratory rates and ATP production were higher than those observed in wild-type adipocytes. Furthermore, mRNA expression of multiple genes that regulate thermogenesis, lipolysis, and mitochondrial biogenesis was significantly higher in the adipose tissue depots of *Ager* null vs. control wild-type mice, especially in iBAT and iWAT.[34]

4.3 Deletion of adipocyte *Ager* protects from diet-induced obesity and cold intolerance in mice

As RAGE expression in adipocytes suggested roles for the receptor in the regulation of thermogenesis, these concepts were tested *in vivo*. Despite no differences in food intake or physical activity, mice bearing an adipocyte-specific deletion of *Ager* displayed less weight gain when fed a high-fat diet and demonstrated reduced insulin and glucose intolerance compared with *Ager*-expressing mice. Critically, adipocyte-specific deletion of *Ager* resulted in higher energy expenditure compared with *Ager*-expressing control mice fed the high-fat diet.[34]

Further evidence for regulatory roles for RAGE in adipocyte thermogenesis were uncovered in studies in which mice with adipocyte-specific deletion of *Ager* displayed superior core body temperature control when challenged with exposure to 4°C environment. Consistent with RAGE-dependent downregulation of genes linked to thermogenesis, in the iWAT of the mice lacking adipocyte *Ager,* mRNA expression of *Ucp1*, *Dio2*, *Ppargc1a*, and *Cidea* was significantly higher compared with that noted in the adipocyte *Ager*-expressing mice.[34]

Additional support for RAGE-dependent regulation of thermogenesis pathways was found in wild-type mice subjected to surgical transplantation of iBAT or iWAT from chow-fed mice bearing an adipocyte-specific deletion of *Ager* vs. the *Ager*-expressing controls. In both cases, the deletion of *Ager* in the iBAT or iWAT protected wild-type recipient mice from high-fat diet-induced obesity, and resulted in increased energy expenditure and enhanced expression of UCP1 in the iBAT or iWAT of the recipient mice.[34]

4.4 RAGE and ß3-adrenergic stimulation, protein kinase A, and lipolysis

An established mechanism underlying the regulation of thermogenic genes is through ß-adrenergic stimulation of lipolysis and the generation of fatty acid products; one consequence of which is the regulation of UCP1 expression and activity.[35–37] In adipocytes, RAGE ligands, via RAGE, suppressed ß-adrenergic-mediated lipolysis (production of glycerol and non-esterified fatty acids), oxygen consumption rates, and the expression of *Ucp1*.[34] Specifically, treatment of wild-type *Ager*-expressing adipocytes from iBAT or iWAT with norepinephrine and the RAGE ligand CML-AGE resulted in significant suppression of *Ucp1* and *Ppargc1a* mRNA expression vs. treatment with norepinephrine alone.[34] In both cultured adipocytes (C3H10T1/2 cells) and primary adipocytes, RAGE ligands reduced protein kinase A (PKA) activity and phosphorylation of hormone-sensitive lipase (Ser563) and p38 mitogen-activated protein kinase,[34] all of which play key roles in lipolysis.

4.5 Small molecule antagonism of RAGE/DIAPH1 interaction and adipocyte properties

In addition to genetic approaches targeting the RAGE/DIAPH1 axis in adipocytes, experiments were also performed with small molecule antagonists of RAGE/DIAPH1 interaction.[19,20] In C3H10T1/2 cells treated with the ß3-adrenergic agonist, CL316,243, incubation with an early stage RAGE/DIAPH1 antagonist[19] resulted in significantly higher glycerol release vs. cells treated with CL316,243 alone.[34] Of note, the RAGE/DIAPH1 antagonist exerted no independent effects on the numbers of lipid droplets assessed by BODIPY staining and did not affect the relative mitochondria content.[34] These findings suggest that small molecule antagonism of RAGE/

DIAPH1 might affect adipocyte physiology; however, the implications for the *in vivo* setting are not fully elucidated. Studies testing these concepts are currently underway.

As the small molecule antagonists target the binding of the RAGE cytoplasmic domain with DIAPH1, a key question arises: what is known about DIAPH1 in obesity and in adipose tissues? Indeed, studies are now reporting links of DIAPH1 to metabolic perturbations; these reports will be discussed in the section to follow.

5. DIAPH1 and emerging roles in adipocyte biology and obesity

The extracellular matrix (ECM) plays critical roles in adipocyte metabolism and adipocyte crosstalk with immune cells.[38–40] As AGEs and the RAGE pathway have been implicated in the modification of the ECM and the downstream consequences,[41–43] and as AGE/RAGE/DIAPH1 and the ECM signal, in part through the Rho GTPases,[44–46] it was of interest to study this pathway in the context of ECM-adipocyte biology. Visceral adipocytes from human subjects with obesity and with or without diabetes were studied in two-dimensional (2D) and three-dimensional (3D) systems in culture. Using adipocyte glucose uptake to monitor adipocyte metabolism, it was found that glycated ECM reduced adipocyte insulin-stimulated glucose uptake, which was particularly pronounced in diabetic vs. non-diabetic ECM.[47] To test the role of AGE receptors in these processes, antibodies to CD36 or RAGE were used, and to test the role of DIAPH1, the inhibitor SMIFH2 was employed.[48] Although antibodies to CD36 or RAGE had no effect on AGE-ECM-mediated inhibition of adipocyte glucose uptake, the DIAPH1 inhibitor reduced the AGE-ECM-mediated inhibition of adipocyte glucose uptake.[47] Although the ability of the CD36- or RAGE-directed antibodies to inhibit other AGE effects was not illustrated in that work, this report highlighted for the first time roles for DIAPH1 as a mediator of AGE-ECM metabolic crosstalk in adipocytes.

Others studied human adipose tissue from subjects with obesity or morbid obesity; two different adipose tissue depots were probed, subcutaneous adipose tissue (SAT) and omental adipose tissue (OAT).[49] The AGE/RAGE/DIAPH1 axis was addressed by monitoring the mRNA expression of *AGER* and *DIAPH1*; the AGE pathway was tested by measurement of *GLO1* mRNA. *GLO1* encodes Glyoxalase-1; GLO1 is a chief AGE-detoxifying enzyme in the tissues and, therefore, it regulates, in part, AGE content.[50,51] Although BMI and body weight differed between the two groups of subjects with obesity vs. morbid obesity, there were no significant differences noted in Homeostatic Model for Assessment of Insulin Resistance (HOMA-IR), a marker of insulin resistance. It was reported that in SAT, but not OAT, expression of *AGER* strongly and positively correlated with *DIAPH1* and *GLO1* mRNA.[49] With respect to markers of inflammation, in SAT, expression of *AGER* significantly correlated with *CD68;* expression of *DIAPH1* correlated with *TNF;* and increased *GLO1* expression in SAT correlated positively with *CD68* and *TNF*. In contrast, surprisingly, no such associations of *AGER/DIAPH1/GLO1* with inflammation were noted in OAT.[49]

In that work, the mRNA expression of *AGER/DIAPH1/GLO1* and the correlations with genes related to the regulation of metabolism were also probed; in SAT, expression of *AGER* and *GLO1* significantly and positively correlated with *PPARG* and expression of *DIAPH1* showed significant and positive correlations with *PPARG, PPARGC1A,* and *CIDEA*.[49] In contrast, in OAT, there were no significant associations between *AGER* or *DIAPH1* and metabolic genes; however, expression of *GLO1* correlated significantly and positively with *PPARGC1A* and *CIDEA*.[49]

Finally, potential associations between *AGER/DIAPH1/GLO1* and HOMA-IR were tested in that study. Strikingly, it was shown that in SAT, only *AGER* expression, but not an expression of *PPARG, DIAPH1, UCP1, GLO1,* or *CD68,* was significantly and positively correlated with HOMA-IR. In OAT, no associations between any of these AGE/RAGE/DIAPH1 markers with adipogenic or inflammatory factors or HOMA-IR were observed.[49] Whereas the RAGE axis might have been expected to be more associated with OAT and inflammation, this axis's chief associations in obesity and morbid obesity with inflammatory and metabolic factors were found in SAT. These surprising findings lay the foundation for studies probing the homeostatic and pathobiological effects of AGE/RAGE/DIAPH1 in human browning/beiging and overall adaptations to thermogenic stresses.

Collectively, these findings further implicate the AGE/RAGE/DIAPH1 pathway in human obesity and its complications. In the sections to follow, this review considers the biology of the AGE–RAGE–DIAPH1 pathway in the distinct adipose tissue depots, such as epicardial adipose tissue (EAT).

6. AGE, RAGE, and DIAPH1: implications for adipocyte biology in the epicardial depot: new insights to links to this pathway in cardiovascular disease—studies in rats and humans

6.1 EAT, metabolic functions, and cardiovascular disease

In addition to the adipose tissue depots typically considered to be relevant in obesity, metabolic dysfunctions, and cardiovascular disease, it is important to also consider the EAT depot and its links to the AGE–RAGE–DIAPH1 axis. EAT is visceral fat that is situated between the myocardium and the inner pericardium and, as such, it surrounds the coronary vessels.[52] On account of these proximities, the epicardial fat depot shares the same microcirculation with the proximal myocardium. Numerous investigations have shed light on multiple key functions of EAT, such as (1) EAT supplies the heart with free fatty acids to meet energy demands; (2) EAT may display brown adipose tissue-like characteristics, thereby aiding in protection against cold; (3) EAT provides a mechanical cushion for the heart, protecting it from arterial pulse wave and cardiac contractions; and (4) EAT may modulate inflammatory signals, which may yield cardioprotective properties.[52] However, it is also established that disruption in these pathways in EAT may contribute to the development of coronary artery/cardiovascular disease. In this context, a number of studies have shown direct correlations between coronary artery disease and dysfunctional EAT.[53] It was in this context that researchers sought to probe potential links between the AGE–RAGE–DIAPH1 pathway and the EAT depot in health and disease. In the section to follow, this review details evidence emerging from human studies suggesting roles for this pathway in EAT.

6.2 EAT and the AGE/RAGE/DIAPH1 pathway

To explore RAGE expression in adipose tissue depots, EAT and SAT were retrieved from humans undergoing cardiac surgery. Comparing patients with vs. without coronary artery disease, the mRNA expression of *AGER* and RAGE protein amounts were lower in the SAT, but not in EAT. Markers of oxidative stress, the mRNA expression of p22-phox (a component of NADPH oxidase), and *AGER* were higher in EAT vs. SAT. However, expression of markers of oxidative stress did not differ in patients with vs. without coronary artery disease.[54] Hence, although expression data such as that discussed here do not provide mechanistic insights into disease pathways, they nevertheless illustrate that human EAT expressed RAGE.

In a distinct study, samples from 33 patients undergoing open-heart surgery were obtained for tissue analyses and microarrays were used to detect expression of the genes encoding RAGE, GLUT4, adiponectin, GLO1, HMGB1, TLR4, and MyD88 and the thickness of EAT was measured using echocardiography. It was reported that increasing expression of RAGE was linked to the increased thickness of EAT, as well as reduced expression of GLUT4, adiponectin, and GLO1, in parallel with increased

expression of HMGB1, TLR4, and MyD88. Based on anthropomorphic measurements, the lipid accumulation product was found to significantly correlate with RAGE expression and the thickness of EAT.[55]

In other studies, EAT and SAT were retrieved from humans undergoing elective cardiac surgery in which five of the patients had type 2 diabetes and coronary artery disease and three of the patients had coronary artery disease without type 2 diabetes. RNA sequencing analysis was performed on both EAT and SAT. Interestingly, the authors reported that there were no significant differences in the gene expression between diabetic vs. non-diabetic SAT samples; in contrast, 592 differentially expressed genes were identified when comparing diabetic vs. non-diabetic EAT. In the diabetic patient-derived EAT, enrichment for inflammatory genes with respect to the innate immune response was identified and the associated KEGG pathways included the TNF family, NF-kB family, and the AGE–RAGE pathways. Furthermore, endothelium-related genes, such as those encoding for Pentraxin3 and endothelial lipase G, were enriched in the diabetic EAT.[56] The authors concluded that these findings might highlight a potential atherogenic pathway in EAT in diabetes.

Finally, an additional study examined if the presence of diabetes influenced the biology of epicardial fat and paracardial fat in 66 patients (33 of whom had diabetes) with multivessel coronary artery disease. The authors reported that the volume of epicardial fat was higher in patients with diabetes vs. without diabetes and that the EAT of diabetic patients displayed higher expression of RAGE. In addition to RAGE expression, it was shown that in epicardial fat and paracardial fat of diabetic patients, higher expression of adrenomedullin and lower expression of the FGF21 were observed.[57] The authors concluded that diabetes resulted in higher expression of potentially inflammatory factors as well as reduced expression of cardioprotective FGF21.

In summary, these studies place RAGE in EAT and provide associations between the degree of RAGE expression and the presence or not of metabolic dysfunctions and cardiovascular disease. Irrespective of these considerations, these important studies may provide further support for the well-described link between RAGE and the pathogenesis of atherosclerosis and cardiovascular complications.[27,58–60] As extensive evidence in animals and in humans identifies roles for the RAGE pathway in these disorders, the studies cited herein suggest that one component of RAGE's roles in cardiovascular disease may be through dysfunction in EAT in diabetes and non-diabetes as well.

As the procurement of serial adipose tissue biopsies in human subjects is largely impractical, distinct means to track the RAGE/DIAPH1 axis *in vivo* need to be considered and discovered. For this reason, efforts in the field have sought to interrogate the potential roles for tracking the concentrations of the soluble forms of RAGE and its ligands in cardiometabolic disorders. The sections to follow detail the sources of sRAGE isoforms and the implications for tracking sRAGE isoforms in obesity and weight loss.

7. Soluble RAGEs: proposed biomarkers of the RAGE axis *in vivo* in human subjects

7.1 sRAGE forms and detection *in vivo*

There are two main forms of sRAGE detected *in vivo*.[61] The first is the full-length form of soluble or sRAGE generated from cell surface cleavage of the receptor through the actions of matrix metalloproteinases or A Disintegrin And Metalloprotease (ADAM)-10. Total sRAGE is composed of the V, C1, and C2-type domains of the receptor.[62,63] A second form of sRAGE is called endogenous secretory or esRAGE; esRAGE represents about 20% of the overall total sRAGE and is derived from a pre-mRNA alternative splicing mechanism.[64] It is hypothesized that the circulating sRAGEs may function as decoys by binding RAGE ligands and preventing their interaction with and activation of the cell surface receptor. Consistent with this notion, the earliest studies testing the administration of sRAGE in mice to quell RAGE-dependent cellular stress showed that recombinant sRAGE suppressed diabetic atherosclerosis and facilitated wound healing, as examples.[65–67]

In human subjects, specific enzyme-linked immunosorbent assays detect total sRAGE and esRAGE; the cell surface-cleaved sRAGE or 'cRAGE' is calculated by subtracting esRAGE from total sRAGE.[61] As will be discussed below, numerous and recent studies are reporting the concentrations of all the known forms of sRAGEs, including total sRAGE, cRAGE, and esRAGE, and not just a single form. However, a key question that needed to be addressed in order to support the use of measurements of the sRAGEs as biomarkers of metabolic health or stress was if concentrations of sRAGEs were mutable. In the sections to follow, the mutability of sRAGEs in response to various dietary/metabolic measures was demonstrated.

7.2 Soluble RAGEs and the response to acute dietary interventions in human subjects

The effects of a high-fat meal without prior aerobic exercise and then on a different occasion, a high-fat meal preceded by aerobic exercise, on sRAGEs and on the expression of RAGE and other inflammatory/metabolic markers (TLR4, MYD88, and ADAM10) in peripheral blood mononuclear cells (PBMCs) was tested 4 h after meal consumption in $n = 12$ participants. The consumption of the high-fat meal significantly reduced the plasma concentrations of sRAGE, esRAGE, and cRAGE by 9.7, 6.9, and 10.5%, respectively, and there was no additional effect of aerobic exercise. Of note, the consumption of the high-fat meal (without any effect of exercise) resulted in higher expression of RAGE protein expression on PBMCs by 10.3%; however, there was no effect of the meal with/without exercise on the expression of TLR4, MYD88, or ADAM10 protein expression, nor on ADAM10 activity in the PBMCs.[68]

In a distinct study, the effect of the mixed meal challenge (20 g protein, 59 g carbohydrate, and 26 g fat) was tested in control subjects vs. patients with maintenance haemodialysis ($n = 8$/group). The concentrations of the plasma sRAGEs were tested at baseline and at 240 min post-meal and the last dialysis treatment was 24 h prior to the meal.[69] Baseline concentrations of plasma AGEs were significantly higher in the maintenance haemodialysis vs. control subjects; there were no basal differences or post-meal differences identified in the concentrations of total sRAGE, esRAGE, or cRAGE.[69] Overall, there was a group effect in that the concentrations of sRAGE and esRAGE were significantly higher in the maintenance haemodialysis vs. control subjects, with a trend towards higher concentrations of cRAGE as well ($P = 0.09$).[69] Key points in the interpretation of the findings include considerations such as there may be an independent and overriding effect of renal failure on sRAGEs that may be greater than that to be expected with a single mixed meal; the study may be underpowered; and in the maintenance haemodialysis subjects, use of medications would have distinguished those patients from the subjects in the control group.[69]

Others tested the effects of three different breakfasts (Mediterranean vs. Western with or without grilling in the latter case) in 20 healthy volunteers, without obesity or overweight, and aged 18–30 years.[70] The meals were consumed in 15 min and blood was collected at serial time points post-completion of the meal for up to 120 min. Only total sRAGE was measured and numerous AGE products were detected. Numerous observations were made; fasting concentrations of sRAGE in individual participants were highly consistent over a three-week period and carboxyethyl lysine AGE and free lysine concentrations were found to be higher in general among male vs. female participants.[70] In addition, irrespective of the specific breakfast or the dietary AGE content consumed, plasma CML concentrations increased and plasma total sRAGE concentrations decreased in the post-prandial state. Hence, although differences in esRAGE and cRAGE concentrations were not detected in that study, the work did demonstrate an inverse relationship between an AGE product (CML) and sRAGE after breakfast meals.[70]

In addition to the innate factors noted above that affect concentrations of AGEs and sRAGEs, numerous reports indicated that the concentrations of sRAGEs decline with age[71] and that AGEs increase with aging,[72] thereby supporting the concept of inverse relationships between the AGE ligands

Table 1 Examples of studies in varied conditions reporting AGEs/sRAGEs ratio in humans

Condition	Subjects	Major findings	Ref.
Type 2 diabetes	362 T2D, 125 controls	(1) AGEs/sRAGE & AGEs/cRAGE positively associated with chronological age ($P = 0.003$) (2) 15 years follow-up (4982 person-years) Increase in AGEs/cRAGE associated with higher risk of all-cause mortality in T2D subjects; the HR per each SD segment is 1.30, 95% CI 1.15–1.47; $P < 0.001$	71
Aging, renal disease	64 subjects, 70% male, 63% with diabetes and eGFR = 27 ± 10 mL/min/1.73 m^2 Measured at baseline & at 12 months	AGEs/sRAGE over 12 months was significantly higher (1.77 ± 0.92 vs. 2.24 ± 1.34, $P = 0.004$); AGEs/sRAGE inversely correlated with eGFR-however basal values and their variations did not show a significant change with eGFR changes	74
ESRD	88 ESRD patients, 20 healthy controls	AGEs/sRAGE, AGEs/cRAGE and AGEs/esRAGE all significantly higher in ESRD patients vs. controls ($P < 0.05$)	75
NSTEMI	46 male with NSTEMI; 28 age/sex-matched controls	AGEs/sRAGE ratio higher in NSTEMI patients vs. healthy controls (1.72 ± 0.14 vs. 0.54 ± 0.06; $P < 0.05$)	76
ISR	48 patients without ISR; 12 patients with ISR	AGEs/sRAGE was significantly higher at baseline and at 6 months follow-up in patients with ISR vs. without ISR	90
Cholesterol disorder	45 normal cholesterol; 55 high cholesterol	AGEs/sRAGE showed positive correlation with total cholesterol ($r = 0.73$, $P < 0.001$; LDL-c) ($r = 0.74$, $P = 0.001$); and triglycerides ($r = 0.77$, $P = 0.001$)	91
Health examinations	110 subjects undergoing health examination (14 were healthy)	AGEs/sRAGE was negatively and significantly correlated with flow-mediated dilation by four different models	92
Aging cohort	958 men, 802 women	No association between AGEs/sRAGE and mortality	77
Physical activity	967 men, 812 women	Higher AGEs/sRAGE associated with lower physical Activity function only in women even after correction For lifestyle and age-related factors OR = 0.86, 95% CI = 0.75–0.98	78
Embryology	21 with poor-morphology embryos and 23 with good morphology embryos	AGEs/sRAGE significantly higher in follicular fluid of poor- vs. good morphology embryos 2.6 ± 0.38 vs. 1.2 ± 0.29; $P = 0.0048$	79
Obesity	41 obese/overweight and 36 lean children	AGEs/sRAGE significantly higher in obese/overweight vs. lean control children ($r = 0.421$, $P = 0.000$)	80
NAFLD	58 with NAFLD 58 controls	AGEs/sRAGE significantly higher in NAFLD cases vs. controls ($P < 0.001$)	81
Essential hypertension	104 patients	AGEs/sRAGE independently associated with albuminuria (OR = 1.131, 95% CI = 1.001–1.278; $P = 0.048$)	82
Lung disease	62 IPF, 22 cHP 22 fNSIP and 12 healthy controls	AGEs/sRAGE higher in IPF vs. fNSIP and control; $P < 0.01$ AGEs/sRAGE higher in IPF vs. cHP; $P < 0.05$ AGEs/sRAGE higher in cHP vs. fNSIP; $P < 0.01$ AGEs/sRAGE in fNSIP similar to control	84
Cigarette smoking	Review article	Serum AGEs increase with cigarette smoking vs. without serum sRAGEs decrease with cigarette smoking suggests that AGEs/sRAGE is increased in cigarette smoking vs. without	86
Cystic fibrosis and diabetes	5 CF, 5 CFRD, 7 diabetes, 10 healthy controls	AGEs/sRAGE serum: higher in diabetes vs. control, $P < 0.05$ AGEs/sRAGE sputum: no significant differences	87

eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; NSTEMI, non-ST segment elevation myocardial infarction; ISR, in stent restenosis; NAFLD, non-alcoholic fatty liver disease; IPF, idiopathic pulmonary fibrosis; cHP, chronic hypersensitivity pneumonitis; fNSIP, fibrotic nonspecific interstitial pneumonia; CF, cystic fibrosis; CFRD, cystic fibrosis-related diabetes.

and sRAGEs concentrations. These considerations indicate that in studies testing AGEs and sRAGEs, subjects' chronological age must also be considered in the overall statistical models. In addition, it is notable that recent studies have advocated testing AGEs/sRAGE ratios,[71,73–92] as it was found in some cases that the ratio of AGEs/sRAGEs may be a more reliable predictor of pathologies than either measure alone (*Table 1*). In the context of these considerations, others have probed the value of sRAGEs as markers of metabolism and obesity in human subjects; examples of such studies will be presented in the sections to follow.

7.3 Obesity, cardiometabolic disease, and sRAGEs

Although multiple studies have tested sRAGEs as biomarkers in obesity, very few have considered how one of the single nucleotide polymorphisms of *AGER*, G82S, might relate to anthropomorphic measures and AGE/RAGE. One such study showed that patients with obesity demonstrated lower concentrations of sRAGE vs. non-obese subjects and that subjects with obesity who bore S82S had the highest C-reactive protein (CRP) and AGEs vs. G allele carriers.[93] In subjects without obesity, there were no significant *AGER* G82S-related differences in AGEs or CRP, therefore indicating a potential proclivity for the S alleles to be more inflammatory in obesity vs. lean state.[93] In adult females, it was shown that concentrations of esRAGE and adiponectin were significantly lower in women with obesity vs. lean state and that plasma esRAGE concentrations were associated with markers of oxidative stress and platelet activation.[94] Other studies performed in healthy women revealed that plasma concentrations of sRAGE were lower in women with obesity vs. normal weight and, as deduced from magnetic resonance imaging studies, sRAGE concentrations inversely correlated with EAT depot.[95] In healthy, young adults

with normal body weight, overweight, and obesity ($n = 69$), body weight, BMI, and waist circumference were negatively correlated with serum sRAGE and high molecular weight adiponectin positively correlated with sRAGE.[96] With respect to the prediabetes state, in 42 affected patients, significant negative correlations between plasma concentrations of sRAGE were identified with body weight, BMI, waist and hip circumferences, waist-to-hip ratios, and concentrations of LDL cholesterol; however, there were no associations with fasting plasma glucose, haemoglobin A1C or 2 h post-glucose challenge glucose concentrations.[97]

AGEs are independently related to cardiovascular disease and AGE accumulation favours the accumulation of cholesterol and oxysterol in macrophage foam cells.[80,98,99] Hence, it was logical to test the association between concentrations of plasma AGEs and sRAGE with the carotid plaque content of cholesterol and oxysterols in humans undergoing carotid endarterectomy.[100] In this cohort, 23 patients were symptomatic and 40 patients were asymptomatic. Lipids and sterols, including oxysterols, cholesterol, desmosterol, lathosterol, sitosterol, and campesterol were extracted from the plaques and quantified. In the plaques retrieved from symptomatic patients, an increased content of cholesterol and oxysterols was found compared with that noted in asymptomatic individuals. Plasma total AGEs and pentosidine (a specific AGE) concentrations were significantly and positively correlated to sterols accumulated in the plaques, including cholesterol, desmosterol, campesterol, sitosterol, and oxysterols. Concentrations of sRAGE were inversely correlated with total AGEs and pentosidine concentrations in plasma, and with the major forms of oxysterols, cholesterol, and markers of cholesterol synthesis and absorption in the plaques. Through multiple regression analyses, a significant inverse correlation was identified between the concentration of plasma sRAGE and 24-hydroxycholesterol and desmosterol, and a positive significant correlation was found between the concentrations of pentosidine and 24-hydroxycholesterol, 27-hydroxycholesterol, and campesterol.[100] The authors concluded that the plasma concentrations of AGEs and sRAGE may predict the accumulation of sterols in atherosclerotic lesions in both asymptomatic and symptomatic individuals. In that study, however, the authors did not report the AGEs/sRAGE ratios, only the individual values.

In children and adolescents, the concentrations of sRAGE and esRAGE were significantly lower in children with obesity vs. control children and these sRAGEs concentrations were independently correlated with carotid intima-media thickness measures.[101] Other studies addressed vascular damage as well in adolescents, aged 15–19 years, in which 33 had obesity and 33 were normal weight; in that study, the group of adolescents with obesity demonstrated higher cardiometabolic risk as shown by lower sRAGE and higher concentrations of triglycerides and markers of endothelial dysfunction.[102] In that study, sRAGE concentrations negatively correlated with flow-mediated dilation and positively correlated with arterial stiffness index.[102] In other studies in 522 male and 561 female adolescents, after correction for age and sex, the concentrations of sRAGE were inversely associated with obesity, and sRAGE was significantly and inversely correlated with an increasing number of components of the metabolic syndrome.[103]

Collectively, these examples of studies testing concentrations of sRAGEs in obesity, in adults and in children and adolescents, appear to suggest that lower concentrations of sRAGEs are associated with obesity and, at least in some studies, with cardiometabolic damage. Although few to any of these studies examined RAGE ligands or, perhaps more appropriately, AGEs/sRAGEs ratios, these considerations indicated that future work might address these points. In any case, these early studies raise the question—what happens to concentrations of sRAGEs after weight loss? In the section to follow, examples of studies addressing this point are presented in both surgical and non-surgical weight loss settings.

7.4 Bariatric surgery, weight loss, and sRAGEs

In surgical weight loss, 57 patients with type 2 diabetes and BMI (30–35 kg/m^2) underwent gastric bypass, gastric sleeve, or lap-band per patient preference. It was reported that higher baseline concentrations of sRAGE were associated with superior weight loss outcomes at 6 months post-surgery.[104] The same group of patients was followed up at three years post-surgery and higher baseline concentrations of sRAGE were associated with greater change in HbA1c and greater percent weight loss after surgery.[105] However, when this group of patients was followed up at five years post-surgery, the concentrations of sRAGE at baseline were no longer associated with long-term weight loss and metabolic outcomes.[106] In that series of studies, beyond the baseline, repeated measurements of sRAGE concentrations were not reported; hence, it remains possible that specific differences in sRAGE concentrations at three or five years post-bariatric surgery might have demonstrated associations with body weight, weight loss, and metabolic recovery.

In a distinct study of 85 patients with morbid obesity (mean BMI, 45.4 kg/m^2), the concentrations of sRAGE along with a host of other factors were tested at two years post-surgery. It was reported that mean concentrations of plasma sRAGE increased significantly at the two-year point.[107] The changes in the concentrations of sRAGE (from pre-surgery to two years post-surgery) were associated with changes in 1 and 2 h post-prandial glucose concentrations; the change in fasting insulin concentration; the change in the 2 h post-prandial concentrations of insulin; the change in HOMA-IR; and the change in triglyceride concentrations. In a multivariate model, changes in 1 and 2 h post-prandial glucose concentrations; the change in the 2 h post-prandial concentrations of insulin; and the change in HOMA-IR predicted the change in concentrations of sRAGE.[107] These interesting findings highlighted the potential value of both baseline and changes in concentrations of sRAGEs from pre-surgery to the post-surgery state and raise the question of the potential predictive value of measurement of sRAGE concentrations in medical/behaviour-induced weight loss as well.

7.5 Medical/behaviour-induced weight loss and sRAGEs

In 22 patients with severe obesity (mean BMI 44.5 kg/m^2), a dietary intervention was instituted with a very low-calorie formula diet for 12 weeks (800 kcal/day) followed by a 12-week weight maintenance programme. Overall, the patients experienced a mean weight loss of about 21.7 kg along with improvement in insulin resistance measures.[108] The baseline concentrations of serum sRAGE were inversely related to BMI and to HOMA-IR such that the lower the baseline concentrations of sRAGE, the greater the reduction in BMI.[108] Importantly, there was an inverse correlation between the change in BMI and changes in serum sRAGE concentrations after weight loss induction interventions.[108]

In a study of 42 patients with obesity, patients were randomized to control feeding or alternate-day fasting. For 24 weeks, although the control group did not change their diet, the patients on alternate-day fasting had consumed 25% or 125% of their caloric requirements on alternating days.[109] The control group did not display any change in weight but the group on alternate-day fasting lost approximately 6.8 kg and it was reported that sRAGE and esRAGE concentrations increased with weight loss.[109]

In eight adult patients with chronic kidney disease, a 12-week-supervised weight loss (lifestyle) was instituted consisting of low-fat dietary counselling with a prescribed amount of aerobic exercise (60 min/day and five days/week).[110] In those patients, weight loss resulted in a reduction in plasma concentrations of sRAGE and the decrease in sRAGE was associated with lower 2 h blood glucose concentrations in a glucose tolerance test and with increased insulin sensitivity.[110] These studies illustrate that weight loss, here associated with a reduction in plasma sRAGE, was obtained in the setting of chronic kidney disease, itself a condition that modulates the concentrations of the sRAGEs. In that study and in many of the weight loss studies, it is notable that RAGE ligands or AGEs/sRAGE ratios were not routinely measured.

Finally, a recent study addressed potential relationships between weight loss, body composition, and changes in energy expenditure and adaptive thermogenesis with baseline and changes in concentrations of sRAGEs (total sRAGE, esRAGE, and cRAGE) in 41 adults (70% female) undergoing a

three-month weight loss programme focused on precision nutrition approached designed to reduce post-prandial glycemic response vs. a standard low-fat diet.[111] At three months, a mean body weight change of −1.7% was noted. In the statistical models, the baseline concentrations of sRAGEs did not predict change in fat mass at 3 months, but baseline sRAGEs (sRAGE, esRAGE, and cRAGE) were significantly associated with the change in resting energy expenditure (REE) at three months vs. baseline REE after correction for age and sex.[111] With respect to adaptive thermogenesis, multiple algorithms were used to calculate this measure,[112] baseline concentrations of esRAGE were associated with adaptive thermogenesis in some but not all of the models employed. Overall, the associations of sRAGEs concentrations with changes in REE were independent of HbA1c, a measure of a reversible glycation process, but AGEs were not measured in the study.[111] These intriguing findings suggest the potential links between the RAGE pathway and energy expenditure; in the murine studies of either global or adipocyte deletion of *Ager,* these mice were protected from high-fat diet-induced obesity, at least in part through the release of a RAGE-dependent brake on energy expenditure.[33,34]

In summary, it is important to query how the results of these studies in obesity, weight loss, and adaptive thermogenesis might be affected by the dynamics of potential changes in AGEs over the course of these diseases and interventions. Certainly, together with the summary of studies in *Table 1*, these considerations strongly support the testing of AGEs/sRAGE ratios in obesity and weight loss interventions.

8. Perspectives & future directions

In summary, these considerations identify roles for the RAGE/DIAPH1 axis in obesity and key metabolic complications such as insulin resistance and type 2 diabetes and highlight that the measurement of the circulating concentrations of the sRAGEs (and/or AGEs/sRAGE ratios) in human subjects might provide a new biomarker profile to track obesity and the success, or not, of weight loss measures (*Figure 1*). Collectively, however, these concepts render it logical to query, if a principal function of RAGE is the suppression of adaptive thermogenesis and energy expenditure, why should such a molecule survive evolutionary forces to support the development of obesity and its complications, particularly insulin resistance and type 2 diabetes? Clues that the RAGE biology is relatively unique are embedded in the finding that the promiscuous ligands of RAGE, such as AGEs, S100/calgranulins, and HMGB1, interact with receptors beyond AGEs, such as the TLRs. It is fascinating to consider that although the appearance of TLRs traces to both vertebrates and invertebrates,[113] *AGER* appeared considerably later in evolution.

8.1 *AGER* and evolution

The gene *AGER*, by comparison with the TLRs, first appeared in Laurasiatheria,[114] a superorder of placental mammals and part of the larger group of mammals classified as Eutheria. The oldest Eutherian species is believed to be *Juramaia sinensis*, which appeared over 160 million years ago.[115] Eutherians, characterized by their ability to express UCP1 in BAT, are imbued with the capacity for thermogenesis.[116] Such considerations provide insight into possible forces that contributed to the appearance of *AGER*. *AGER*, by virtue of its ability to conserve energy within adipocytes, may have evolved to forestall the organismal consequences of insufficient/intermittent nutrition or wide swings in ambient temperature. Perhaps, in such settings, the production and accumulation of AGEs and the extracellular release and appearance of S100/calgranulins and HMGB1 were relatively uncommon.

In more recent times, however, advanced aging, well beyond the reproductive years and other factors, such as nutritional excess and reduced physical activity, trigger the overproduction and accumulation of these families of RAGE ligands. Indeed, as a group, these ligands have been termed Damage Associated Molecular Patterns, or DAMPs, as an acknowledgement of their derivation from milieus characterized by cellular stress and damage.[117] Together with the recent evidence that highly and ultra-processed foods contain copious exogenous sources of AGEs,[118–120] it is plausible that *AGER* functions devolved from protective roles to those co-opted by these endogenous and exogenous biochemical species and stresses to mediate obesity and its consequences. In this context, a question arises, is insulin resistance in obesity mediated through RAGE directly or, do RAGE-dependent roles in obesity and adipocyte perturbation lead to insulin resistance through indirect routes?[121]

8.2 RAGE and insulin resistance

To date, the experimental evidence does not yet definitively distinguish direct causal and/or consequence roles for RAGE in insulin resistance. In mice bearing global- or adipocyte-specific deletion of *Ager*, the reduction in obesity and adiposity was accompanied by an improvement in insulin sensitivity.[31,33,34] In parallel, in those studies, adipose tissue inflammation was also reduced. Yet, clues to primary roles for the RAGE pathway in insulin sensitivity emerge from the closer observation of the data from the hyperinsulinemic, euglycemic clamp studies reported by Song and colleagues.[33] Surprisingly, in the clamp studies, even in mice fed a low-fat, chow diet, glucose infusion rates, and glucose turnover were significantly higher in *Ager* null vs. wild-type mice and during the clamp, hepatic glucose production was significantly lower in the *Ager* null vs. the wild-type mice.[33] These intriguing findings suggest that even on a low-fat diet, RAGE plays role in metabolism and insulin sensitivity. However, these studies were performed in mice globally devoid of *Ager* and, therefore, do not provide any insight into the cell(s)-specific mechanisms underlying these findings. Studies are underway to address this precise question.

What about evidence linking RAGE to insulin sensitivity measures in human subjects? In fact, evidence is mounting to support this connection. First, a recent study by Popp and colleagues employed a three months precision nutrition intervention aimed to reduce post-prandial glycemic response to diet vs. a standard low-fat diet in subjects with obesity and overweight. In addition to determining that there were significant associations by a linear regression model between the differences in REE from three months vs. baseline with the baseline concentrations of sRAGE, esRAGE, and cRAGE, it was also reported that there were significant associations between the differences in fasting insulin concentrations from three months vs. baseline with the baseline concentrations of sRAGE, esRAGE, and cRAGE. No significant differences were reported in the differences in HOMA-IR over three months vs. baseline in that study.[111] However, others reported that the rises in sRAGE post-bariatric surgery were significantly associated with changes in HOMA-IR post-surgery.[107]

Clues to links of the RAGE pathway to insulin resistance also emerged from work reporting on adipose tissue expression of *AGER* and correlations with insulin resistance. Ruiz and colleagues reported that in SAT but not in OAT, *AGER* expression was significantly and positively correlated with HOMA-IR.[49] This surprising result implies that subcutaneous, but not OAT expression of *AGER*, was associated with a key index of insulin resistance. Does this finding suggest that potential roles for RAGE in insulin sensitivity reflect impact in adipose tissue depots traditionally more associated with energy expenditure vs. inflammation? Future studies might employ adipose tissue depot-specific (e.g. brown, white, beige) Cre recombinase strategies in *Ager* floxed mice, for example, to address this critical question. With the possibility that such adipose depot-specific reagents may be developed in the future, such an experimental paradigm might also permit the discovery of the RAGE-dependent depots that underlie obesity and adiposity in high-fat feeding, as well.

8.3 Tracking sRAGEs—biomarkers of cardiometabolic disease?

As discussed above, the prospects for pharmacological antagonism of RAGE as a therapeutic strategy may lie with the potential to interrupt the interaction of the RAGE cytoplasmic domain with DIAPH1. Although the reported chemical probe, RAGE229, exerted beneficial effects in multiple mouse models of inflammation, ischemia/reperfusion injury (heart), wound healing, and diabetic kidney disease,[20] it has yet to

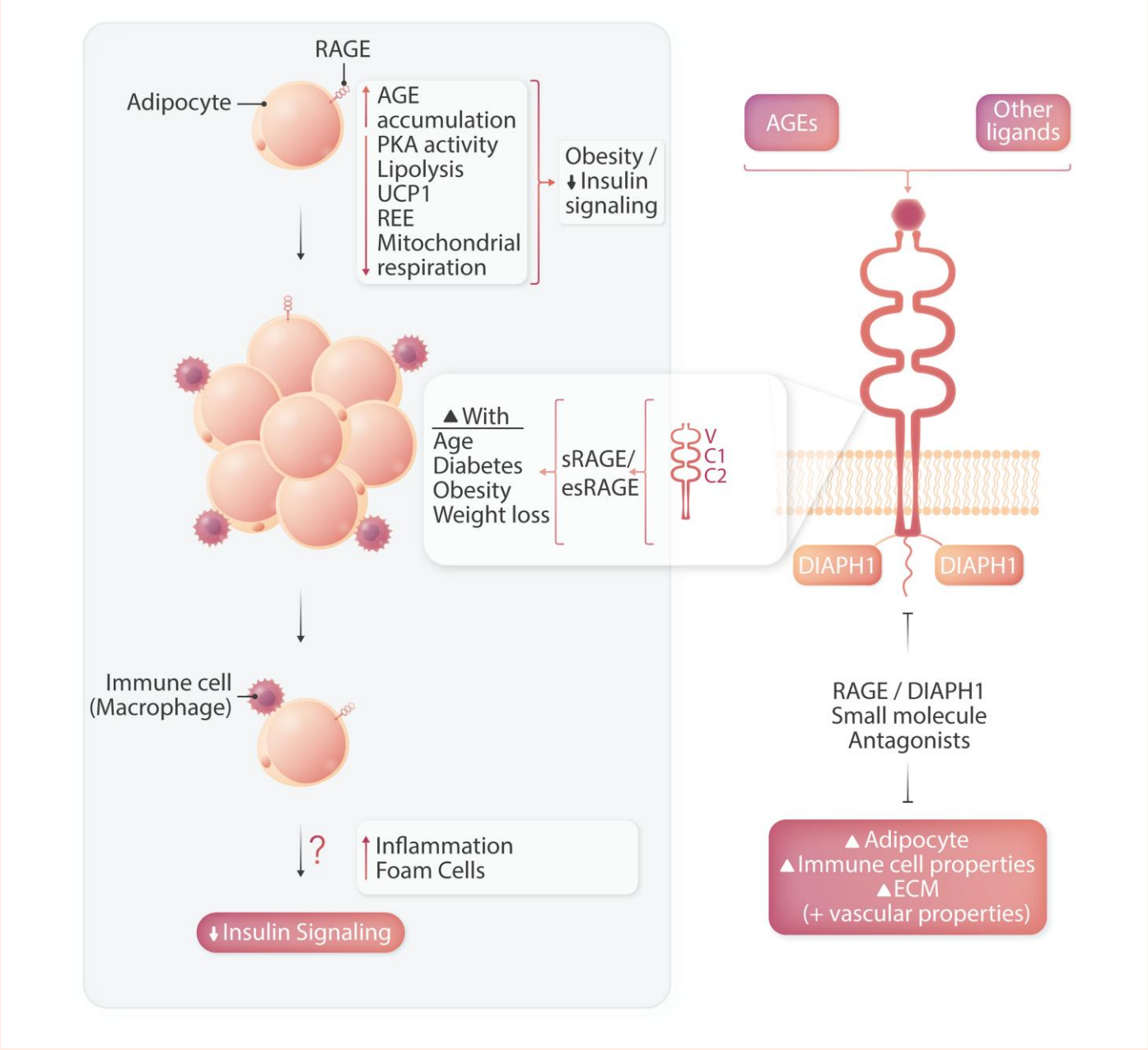

Figure 1 The ligand/RAGE/DIAPH1 axis in obesity and insulin resistance: mediator and proposed biomarker. Studies are accruing to suggest that ligand/RAGE interaction in adipose tissue in obesity exerts multiple pathological effects, such as increased accumulation of AGEs, and reduced adipocyte PKA activity, lipolysis, expression of Uncoupling Protein 1 (UCP1), REE, and mitochondrial respiration. Upon the development of obesity, immune cell perturbation, at least in part via RAGE/DIAPH1, contributes to inflammation and foam cell formation; processes associated with insulin resistance. In human subjects, circulating sRAGEs [cRAGE (cleaved RAGE), esRAGE (endogenous secretory RAGE), and/or total sRAGE] correlate with obesity, weight loss, changes in REE, and concentrations of insulin. Recent research has highlighted therapeutic opportunities in mice for novel small molecule antagonists of RAGE/DIAPH1 interaction to interrupt RAGE/DIAPH1 signalling. Antagonists of RAGE and/or DIAPH1 have been shown to modulate properties of adipocytes, immune cells, and the ECM. Studies are underway to test if small molecule antagonism of RAGE/DIAPH1 may enhance weight loss and diminish metabolic complications in the states of obesity and overweight in animal models.

be tested in the context of energy expenditure, adiposity, and weight loss. Positive results from such studies might suggest that among the potential benefits of RAGE/DIAPH1 antagonism would also be improvements in body mass, adiposity, and metabolic complications. Studies are underway to address these possibilities. Of note, such an approach inherently requires the means to track the effectiveness of these therapeutic interventions *in vivo*.

As noted above, numerous studies have illustrated that tracking baseline vs. post-weight loss concentrations of the sRAGEs may hold promise for predicting weight loss and metabolic outcomes after either surgical or behavioural weight loss. Of note, however, evidence is emerging to suggest that the ratio of AGEs/sRAGEs may be more predictive; this is entirely logical. By tracking the mutability of both a key RAGE ligand and the sRAGEs, it is possible that better predictive results may emerge, as it is very likely

that modulation of AGEs might well affect the concentrations of the sRAGEs. In the weight loss studies discussed above, only sRAGEs (and, sometimes only one form of sRAGE) were detected. Key next steps require the measurement of both baseline and post-weight loss intervention AGEs and sRAGEs and the reporting of the AGEs/sRAGE ratio as well.

The recent and striking findings reported by Sabbatinelli and colleagues, in which they showed that circulating AGEs and sRAGEs (and the ratios of AGEs to specific forms of sRAGEs) were associated with all-cause mortality and the development of major cardiovascular complications in patients with type 2 diabetes,[71] provide strong support for key roles for the RAGE pathway in diabetes and cardiovascular disease. In the context of cardiometabolic disease triggered by obesity and its consequences, it is proposed that eventual clinical trials testing the efficacy of RAGE/DIAPH1 antagonists may benefit from tracking this key index of the ligand–RAGE axis *in vivo*.

Author contributions

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References

1. https://www.niddk.nih.gov/health-information/health-statistics/overweight-obesity.
2. Fryar CD, Carroll MD, Afful J. Prevalence of overweight, obesity, and severe obesity among adults aged 20 and over: United States, 1960–1962 through 2017–2018. NCHS Health E-Stats, 2020. https://www.cdc.gov/nchs/data/hestat/obesity-adult-17-18/obesity-adult.htm.
3. Fryar CD, Carroll MD, Afful J. Prevalence of overweight, obesity, and severe obesity among children and adolescents aged 2–19 years: United States, 1963–1965 through 2017–2018. NCHS Health E-Stats, 2020. https://www.cdc.gov/nchs/data/hestat/obesity-child-17-18/obesity-child.htm.
4. Ruiz HH, Ramasamy R, Schmidt AM. Advanced glycation end products: building on the concept of the "common soil" in metabolic disease. *Endocrinology* 2020;**161**:1–10.
5. Kremers SHM, Remmelzwaal S, Schalkwijk CG, Elders PJM, Stehouwer CDA, van Ballegooijen AJ, Beulens JWJ. The role of serum and dietary advanced glycation endproducts in relation to cardiac function and structure: the hoorn study. *Nutr Metab Cardiovasc Dis* 2021;**31**:3167–3175.
6. Gaens KH, Goossens GH, Niessen PM, van Greevenbroek MM, van der Kallen CJ, Niessen HW, Rensen SS, Buurman WA, Greve JW, Blaak EE, van Zandvoort MA, Bierhaus A, Stehouwer CD, Schalkwijk CG. Nε-(carboxymethyl)lysine-receptor for advanced glycation end product axis is a key modulator of obesity-induced dysregulation of adipokine expression and insulin resistance. *Arterioscler Thromb Vasc Biol* 2014;**34**:1199–1208.
7. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM. RAGE Mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 1999;**97**:889–901.
8. Hori O, Brett J, Slattery T, Cao R, Zhang J, Chen JX, Nagashima M, Lundh ER, Vijay S, Nitecki D. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. *J Biol Chem* 1995;**270**:25752–25761.
9. Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM, Schmidt AM. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 2000;**405**:354–360.
10. Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* 1996;**382**:685–691.
11. He M, Kubo H, Morimoto K, Fujino N, Suzuki T, Takahasi T, Yamada M, Yamaya M, Maekawa T, Yamamoto Y, Yamamoto H. Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells. *EMBO Rep* 2011;**12**:358–364.
12. Ma W, Rai V, Hudson BI, Song F, Schmidt AM, Barile GR. RAGE Binds C1q and enhances C1q-mediated phagocytosis. *Cell Immunol* 2012;**274**:72–82.
13. Rai V, Touré F, Chitayat S, Pei R, Song F, Li Q, Zhang J, Rosario R, Ramasamy R, Chazin WJ, Schmidt AM. Lysophosphatidic acid targets vascular and oncogenic pathways via RAGE signaling. *J Exp Med* 2012;**209**:2339–2350.
14. Bucciarelli LG, Wendt T, Rong L, Lalla E, Hofmann MA, Goova MT, Taguchi A, Yan SF, Yan SD, Stern DM, Schmidt AM. RAGE Is a multiligand receptor of the immunoglobulin superfamily: implications for homeostasis and chronic disease. *Cell Mol Life Sci* 2002;**59**:1117–1128.
15. Yatime L, Betzer C, Jensen RK, Mortensen S, Jensen PH, Andersen GR. The structure of the RAGE:S100A6 Complex reveals a unique mode of homodimerization for S100 proteins. *Structure* 2016;**24**:2043–2052.
16. Hudson BI, Kalea AZ, Del Mar Arriero M, Harja E, Boulanger E, D'Agati V, Schmidt AM. Interaction of the RAGE cytoplasmic domain with diaphanous-1 is required for ligand-stimulated cellular migration through activation of Rac1 and Cdc42. *J Biol Chem* 2008;**283**:34457–34468.
17. Rai V, Maldonado AY, Burz DS, Reverdatto S, Yan SF, Schmidt AM, Shekhtman A. Signal transduction in receptor for advanced glycation end products (RAGE): solution structure of C-terminal rage (ctRAGE) and its binding to mDia1. *J Biol Chem* 2012;**287**:5133–5144.
18. Egaña-Gorroño L, López-Díez R, Yepuri G, Ramirez LS, Reverdatto S, Gugger PF, Shekhtman A, Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and mechanisms and therapeutic opportunities in diabetes and cardiovascular disease: insights from human subjects and animal models. *Front Cardiovasc Med* 2020;**7**:37.
19. Manigrasso MB, Pan J, Rai V, Zhang J, Reverdatto S, Quadri N, DeVita RJ, Ramasamy R, Shekhtman A, Schmidt AM. Small molecule inhibition of ligand-stimulated RAGE–DIAPH1 signal transduction. *Sci Rep* 2016;**6**:22450.
20. Manigrasso MB, Rabbani P, Egaña-Gorroño L, Quadri N, Frye L, Zhou B, Reverdatto S, Ramirez LS, Dansereau S, Pan J, Li H, D'Agati VD, Ramasamy R, DeVita RJ, Shekhtman A, Schmidt AM. Small-molecule antagonism of the interaction of the RAGE cytoplasmic domain with DIAPH1 reduces diabetic complications in mice. *Sci Transl Med* 2021;**13**:eabf7084.
21. Fujiya A, Nagasaki H, Seino Y, Okawa T, Kato J, Fukami A, Himeno T, Uenishi E, Tsunekawa S, Kamiya H, Nakamura J, Oiso Y, Hamada Y. The role of S100B in the interaction between adipocytes and macrophages. *Obesity (Silver Spring)* 2014;**22**:371–379.
22. Nativel B, Marimoutou M, Thon-Hon VG, Gunasekaran MK, Andries J, Stanislas G, Planesse C, Da Silva CR, Césari M, Iwema T, Gasque P, Viranaicken W. Soluble HMGB1 is a novel adipokine stimulating IL-6 secretion through RAGE receptor in SW872 preadipocyte cell line: contribution to chronic inflammation in fat tissue. *PLoS One* 2013;**8**:e76039.
23. Brandon JA, Kraemer M, Vandra J, Halder S, Ubele M, Morris AJ, Smyth SS. Adipose-derived autotaxin regulates inflammation and steatosis associated with diet-induced obesity. *PLoS One* 2019;**14**:e0208099.
24. de Courten B, de Courten MP, Soldatos G, Dougherty SL, Straznicky N, Schlaich M, Sourris KC, Chand V, Scheijen JL, Kingwell BA, Cooper ME, Schalkwijk CG, Walker KZ, Forbes JM. Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial. *Am J Clin Nutr* 2016;**103**:1426–1433.
25. Clarke RE, Dordevic AL, Tan SM, Ryan L, Coughlan MT. Dietary advanced glycation end products and risk factors for chronic disease: a systematic review of randomised controlled trials. *Nutrients* 2016;**8**:125.
26. Uribarri J, Cai W, Woodward M, Tripp E, Goldberg L, Pyzik R, Yee K, Tansman L, Chen X, Mani V, Fayad ZA, Vlassara H. Elevated serum advanced glycation endproducts in obese indicate risk for the metabolic syndrome: a link between healthy and unhealthy obesity? *J Clin Endocrinol Metab* 2015;**100**:1957–1966.
27. Arivazhagan L, López-Díez R, Shekhtman A, Ramasamy R, Schmidt AM. Glycation and a spark of ALEs (advanced lipoxidation end products)—igniting RAGE/diaphanous-1 and cardiometabolic disease. *Front Cardiovasc Med* 2022;**9**:937071.
28. Hallam KM, Li Q, Ananthakrishnan R, Kalea A, Zou YS, Vedantham S, Schmidt AM, Yan SF, Ramasamy R. Aldose reductase and AGE–RAGE pathways: central roles in the pathogenesis of vascular dysfunction in aging rats. *Aging Cell* 2010;**9**:776–784.
29. Thiagarajan D, Quadri N, Jawahar S, Zirpoli H, Hurtado del Pozo C, López-Díez R, Hasan SN, Yepuri G, Gugger PF, Finlin BS, Kern PA, Gabbay K, Schmidt AM, Ramasamy R. Aldose reductase promotes diet-induced obesity via induction of senescence in subcutaneous adipose tissue. *Obesity.* 2022;**30**:1647–1658.
30. Ueno H, Koyama H, Shoji T, Monden M, Fukumoto S, Tanaka S, Otsuka Y, Mima Y, Morioka T, Mori K, Shioi A, Yamamoto H, Inaba M, Nishizawa Y. Receptor for advanced glycation end-products (RAGE) regulation of adiposity and adiponectin is associated with atherogenesis in apoE-deficient mouse. *Atherosclerosis* 2010;**211**:431–436.

31. Monden M, Koyama H, Otsuka Y, Morioka T, Mori K, Shoji T, Mima Y, Motoyama K, Fukumoto S, Shioi A, Emoto M, Yamamoto Y, Yamamoto H, Nishizawa Y, Kurajoh M, Yamamoto T, Inaba M. Receptor for advanced glycation end products regulates adipocyte hypertrophy and insulin sensitivity in mice: involvement of toll-like receptor 2. *Diabetes* 2013;**62**:478–489.
32. Leuner B, Max M, Thamm K, Kausler C, Yakobus Y, Bierhaus A, Sel S, Hofmann B, Silber RE, Simm A, Nass N. RAGE Influences obesity in mice. Effects of the presence of RAGE on weight gain, AGE accumulation, and insulin levels in mice on a high fat diet. *Z Gerontol Geriatr* 2012;**45**:102–108.
33. Song F, Hurtado del Pozo C, Rosario R, Zou YS, Ananthakrishnan R, Xu X, Patel PR, Benoit VM, Yan SF, Li H, Friedman RA, Kim JK, Ramasamy R, Ferrante AW Jr, Schmidt AM. RAGE Regulates the metabolic and inflammatory response to high-fat feeding in mice. *Diabetes* 2014;**63**:1948–1965.
34. Hurtado del Pozo C, Ruiz HH, Arivazhagan L, Aranda JF, Shim C, Daya P, Derk J, MacLean M, He M, Frye L, Friedline RH, Noh HL, Kim JK, Friedman RA, Ramasamy R, Schmidt AM. A receptor of the immunoglobulin superfamily regulates adaptive thermogenesis. *Cell Reports* 2019;**28**:773–791.e777.
35. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;**84**:277–359.
36. Collins S, Surwit RS. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog Horm Res* 2001;**56**:309–328.
37. Rial E, Poustie A, Nicholls DG. Brown-adipose-tissue mitochondria: the regulation of the 32000-Mr uncoupling protein by fatty acids and purine nucleotides. *Eur J Biochem* 1983; **137**:197–203.
38. Lackey DE, Burk DH, Ali MR, Mostaedi R, Smith WH, Park J, Scherer PE, Seay SA, McCoin CS, Bonaldo P, Adams SH. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. *Am J Physiol Endocrinol Metab* 2014; **306**:E233–E246.
39. Muir LA, Neeley CK, Meyer KA, Baker NA, Brosius AM, Washabaugh AR, Varban OA, Finks JF, Zamarron BF, Flesher CG, Chang JS, DelProposto JB, Geletka L, Martinez-Santibanez G, Kaciroti N, Lumeng CN, O'Rourke RW. Adipose tissue fibrosis, hypertrophy, and hyperplasia: correlations with diabetes in human obesity. *Obesity (Silver Spring)* 2016;**24**:597–605.
40. Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B, Peterson CA, Kern PA. Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. *Am J Physiol Endocrinol Metab* 2010;**299**: E1016–E1027.
41. Bansode SB, Gacche RN. Glycation-induced modification of tissue-specific ECM proteins: a pathophysiological mechanism in degenerative diseases. *Biochim Biophys Acta Gen Subj* 2019;**1863**:129411.
42. Rojas A, Añazco C, González I, Araya P. Extracellular matrix glycation and receptor for advanced glycation end-products activation: a missing piece in the puzzle of the association between diabetes and cancer. *Carcinogenesis* 2018;**39**:515–521.
43. Simm A. Protein glycation during aging and in cardiovascular disease. *J Proteomics* 2013;**92**: 248–259.
44. Guan X, Guan X, Dong C, Jiao Z. Rho GTPases and related signaling complexes in cell migration and invasion. *Exp Cell Res* 2020;**388**:111824.
45. Zent J, Guo LW. Signaling mechanisms of myofibroblastic activation: outside-in and inside-out. *Cell Physiol Biochem* 2018;**49**:848–868.
46. Bouchet BP, Akhmanova A. Microtubules in 3D cell motility. *J Cell Sci* 2017;**130**:39–50.
47. Strieder-Barboza C, Baker NA, Flesher CG, Karmakar M, Neeley CK, Polsinelli D, Dimick JB, Finks JF, Ghaferi AA, Varban OA, Lumeng CN, O'Rourke RW. Advanced glycation end-products regulate extracellular matrix-adipocyte metabolic crosstalk in diabetes. *Sci Rep* 2019;**9**:19748.
48. Becker KN, Pettee KM, Sugrue A, Reinard KA, Schroeder JL, Eisenmann KM. The cytoskeleton effectors rho-kinase (ROCK) and mammalian diaphanous-related (mDia) formin have dynamic roles in tumor microtube formation in invasive glioblastoma cells. *Cells* 2022;**11**: 1559.
49. Ruiz HH, Nguyen A, Wang C, He L, Li H, Hallowell P, McNamara C, Schmidt AM. AGE/RAGE/DIAPH1 axis is associated with immunometabolic markers and risk of insulin resistance in subcutaneous but not omental adipose tissue in human obesity. *Int J Obes (Lond)* 2021;**45**:2083–2094.
50. Saeed M, Kausar MA, Singh R, Siddiqui AJ, Akhter A. The role of glyoxalase in glycation and carbonyl stress induced metabolic disorders. *Curr Protein Pept Sci* 2020;**21**:846–859.
51. Rabbani N, Thornalley PJ. Emerging glycation-based therapeutics-glyoxalase 1 inducers and glyoxalase 1 inhibitors. *Int J Mol Sci* 2022;**23**:2453.
52. Karampetsou N, Alexopoulos L, Minia A, Pliaka V, Tsolakos N, Kontzoglou K, Perrea DN, Patapis P. Epicardial adipose tissue as an independent cardiometabolic risk factor for coronary artery disease. *Cureus* 2022;**14**:e25578.
53. Ansaldo AM, Montecucco F, Sahebkar A, Dallegri F, Carbone F. Epicardial adipose tissue and cardiovascular diseases. *Int J Cardiol* 2019;**278**:254–260.
54. Rodiño-Janeiro BK, Salgado-Somoza A, Teijeira-Fernández E, González-Juanatey JR, Alvarez E, Eiras S. Receptor for advanced glycation end-products expression in subcutaneous adipose tissue is related to coronary artery disease. *Eur J Endocrinol* 2011;**164**:529–537.
55. Dozio E, Vianello E, Briganti S, Lamont J, Tacchini L, Schmitz G, Corsi Romanelli MM. Expression of the receptor for advanced glycation end products in epicardial fat: link with tissue thickness and local insulin resistance in coronary artery disease. *J Diabetes Res* 2016;**2016**:2327341.
56. Camarena V, Sant D, Mohseni M, Salerno T, Zaleski ML, Wang G, Iacobellis G. Novel atherogenic pathways from the differential transcriptome analysis of diabetic epicardial adipose tissue. *Nutr Metab Cardiovasc Dis* 2017;**27**:739–750.
57. Haberka M, Machnik G, Kowalówka A, Biedroń M, Skudrzyk E, Regulska-Ilow B, Gajos G, Manka R, Deja M, Okopień B, Gąsior Z. Epicardial, paracardial, and perivascular fat quantity, gene expressions, and serum cytokines in patients with coronary artery disease and diabetes. *Pol Arch Intern Med* 2019;**129**:738–746.
58. Singh S, Siva BV, Ravichandiran V. Advanced glycation end products: key player of the pathogenesis of atherosclerosis. *Glycoconj J* 2022;**39**:547–563.
59. Senatus L, MacLean M, Arivazhagan L, Egaña-Gorroño L, López-Díez R, Manigrasso MB, Ruiz HH, Vasquez C, Wilson R, Shekhtman A, Gugger PF, Ramasamy R, Schmidt AM. Inflammation meets metabolism: roles for the receptor for advanced glycation end products axis in cardiovascular disease. *Immunometabolism* 2021;**3**:e210024.
60. Wang X, Wang Q, Wei D, Chang X. Association between soluble receptor for advanced glycation end product and endogenous secretory soluble receptor for advanced glycation end product levels and carotid atherosclerosis in diabetes: a systematic review and meta-analysis. *Can J Diabetes* 2021;**45**:634–640.
61. Schmidt AM. Soluble RAGEs—prospects for treating & tracking metabolic and inflammatory disease. *Vascul Pharmacol* 2015;**72**:1–8.
62. Raucci A, Cugusi S, Antonelli A, Barabino SM, Monti L, Bierhaus A, Reiss K, Saftig P, Bianchi ME. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). *FASEB J* 2008;**22**:3716–3727.
63. Zhang L, Bukulin M, Kojro E, Roth A, Metz VV, Fahrenholz F, Nawroth PP, Bierhaus A, Postina R. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J Biol Chem* 2008;**283**:35507–35516.
64. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, Yamamoto H. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J* 2003;**370**:1097–1109.
65. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 1998;**4**:1025–1031.
66. Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM. RAGE Blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation* 2002;**106**:2827–2835.
67. Goova MT, Li J, Kislinger T, Qu W, Lu Y, Bucciarelli LG, Nowygrod S, Wolf BM, Caliste X, Yan SF, Stern DM, Schmidt AM. Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. *Am J Pathol* 2001;**159**:513–525.
68. Fuller KNZ, Valentine RJ, Miranda ER, Kumar P, Prabhakar BS, Haus JM. A single high-fat meal alters human soluble RAGE profiles and PBMC RAGE expression with no effect of prior aerobic exercise. *Physiol Rep* 2018;**6**:e13811.
69. Perkins RK, van Vliet S, Miranda ER, Fuller KNZ, Beisswenger PJ, Wilund KR, Paluska SA, Burd NA, Haus JM. Advanced glycation end products and inflammatory cytokine profiles in maintenance hemodialysis patients after the ingestion of a protein-dense meal. *J Ren Nutr* 2021;**S1051-2276**:00295-8.
70. Helou C, Nogueira Silva Lima MT, Niquet-Leridon C, Jacolot P, Boulanger E, Delguste F, Guilbaud A, Genin M, Anton PM, Delayre-Orthez C, Papazian T, Howsam M, Tessier FJ. Plasma levels of free N(ε)-carboxymethyllysine (CML) after different oral doses of CML in rats and after the intake of different breakfasts in humans: postprandial plasma level of sRAGE in humans. *Nutrients* 2022;**14**:1890.
71. Sabbatinelli J, Castiglione S, Macrì F, Giuliani A, Ramini D, Vinci MC, Tortato E, Bonfigli AR, Olivieri F, Raucci A. Circulating levels of AGEs and soluble RAGE isoforms are associated with all-cause mortality and development of cardiovascular complications in type 2 diabetes: a retrospective cohort study. *Cardiovasc Diabetol* 2022;**21**:95.
72. Simm A, Müller B, Nass N, Hofmann B, Bushnaq H, Silber RE, Bartling B. Protein glycation—between tissue aging and protection. *Exp Gerontol* 2015;**68**:71–75.
73. Steenbeke M, Speeckaert R, Desmedt S, Glorieux G, Delanghe JR, Speeckaert MM. The role of advanced glycation end products and its soluble receptor in kidney diseases. *Int J Mol Sci* 2022;**23**:3439.
74. Molinari P, Caldiroli L, Dozio E, Rigolini R, Giubbilini P, Romanelli MMC, Messa P, Vettoretti S. AGEs and sRAGE variations at different timepoints in patients with chronic kidney disease. *Antioxidants (Basel)* 2021;**10**:1994.
75. Prasad K, Dhar I, Zhou Q, Elmoselhi H, Shoker M, Shoker A. AGEs/sRAGE, a novel risk factor in the pathogenesis of end-stage renal disease. *Mol Cell Biochem* 2016;**423**:105–114.
76. McNair ED, Wells CR, Qureshi AM, Basran RS, Pearce C, Orvold J, Devilliers J, Prasad K. Low levels of soluble receptor for advanced glycation end products in non-ST elevation myocardial infarction patients. *Int J Angiol* 2009;**18**:187–192.
77. Ebert H, Lacruz ME, Kluttig A, Simm A, Greiser KH, Tiller D, Kartschmit N, Mikolajczyk R. Association between advanced glycation end products, their soluble receptor, and mortality in the general population: results from the CARLA study. *Exp Gerontol* 2020;**131**: 110815.
78. Ebert H, Lacruz ME, Kluttig A, Simm A, Greiser KH, Tiller D, Kartschmit N, Mikolajczyk R. Advanced glycation end products and their ratio to soluble receptor are associated with limitations in physical functioning only in women: results from the CARLA cohort. *BMC Geriatr* 2019;**19**:299.

79. Takahashi N, Harada M, Azhary JMK, Kunitomi C, Nose E, Terao H, Koike H, Wada-Hiraike O, Hirata T, Hirota Y, Koga K, Fujii T, Osuga Y. Accumulation of advanced glycation end products in follicles is associated with poor oocyte developmental competence. *Mol Hum Reprod* 2019;**25**:684–694.
80. Corica D, Aversa T, Ruggeri RM, Cristani M, Alibrandi A, Pepe G, De Luca F, Wasniewska M. Could AGE/RAGE-related oxidative homeostasis dysregulation enhance susceptibility to pathogenesis of cardio-metabolic complications in childhood obesity? *Front Endocrinol (Lausanne)* 2019;**10**:426.
81. Palma-Duran SA, Kontogianni MD, Vlassopoulos A, Zhao S, Margariti A, Georgoulis M, Papatheodoridis G, Combet E. Serum levels of advanced glycation end-products (AGEs) and the decoy soluble receptor for AGEs (sRAGE) can identify non-alcoholic fatty liver disease in age-, sex- and BMI-matched normo-glycemic adults. *Metabolism* 2018;**83**:120–127.
82. Chiang KH, Chen JW, Huang SS, Leu HB, Lin SJ, Huang PH. The ratio of AGE to sRAGE independently associated with albuminuria in hypertensive patients. *BMC Endocr Disord* 2018;**18**:84.
83. Prakash J, Pichchadze G, Trofimov S, Livshits G. Age and genetic determinants of variation of circulating levels of the receptor for advanced glycation end products (RAGE) in the general human population. *Mech Ageing Dev* 2015;**145**:18–25.
84. Machahua C, Montes-Worboys A, Planas-Cerezales L, Buendia-Flores R, Molina-Molina M, Vicens-Zygmunt V. Serum AGE/RAGEs as potential biomarker in idiopathic pulmonary fibrosis. *Respir Res* 2018;**19**:215.
85. Prasad K. Is there any evidence that AGE/sRAGE is a universal biomarker/risk marker for diseases? *Mol Cell Biochem* 2019;**451**:139–144.
86. Prasad K, Dhar I, Caspar-Bell G. Role of advanced glycation end products and its receptors in the pathogenesis of cigarette smoke-induced cardiovascular disease. *Int J Angiol* 2015;**24**: 75–80.
87. Mulrennan S, Baltic S, Aggarwal S, Wood J, Miranda A, Frost F, Kaye J, Thompson PJ. The role of receptor for advanced glycation end products in airway inflammation in CF and CF related diabetes. *Sci Rep* 2015;**5**:8931.
88. Skrha J Jr, Soupal J, Loni Ekali G, Prázný M, Kalousová M, Kvasnička J, Landová L, Zima T, Skrha J. Skin autofluorescence relates to soluble receptor for advanced glycation end-products and albuminuria in diabetes mellitus. *J Diabetes Res* 2013;**2013**:650694.
89. McNair ED, Wells CR, Mabood Qureshi A, Basran R, Pearce C, Orvold J, Devilliers J, Prasad K. Soluble receptors for advanced glycation end products (sRAGE) as a predictor of restenosis following percutaneous coronary intervention. *Clin Cardiol* 2010;**33**:678–685.
90. Pearce C, Islam N, Bryce R, McNair ED. Advanced glycation end products: receptors for advanced glycation end products axis in coronary stent restenosis: a prospective study. *Int J Angiol* 2018;**27**:213–222.
91. McNair E, Qureshi M, Prasad K, Pearce C. Atherosclerosis and the hypercholesterolemic AGE–RAGE axis. *Int J Angiol* 2016;**25**:110–116.
92. Kajikawa M, Nakashima A, Fujimura N, Maruhashi T, Iwamoto Y, Iwamoto A, Matsumoto T, Oda N, Hidaka T, Kihara Y, Chayama K, Goto C, Aibara Y, Noma K, Takeuchi M, Matsui T, Yamagishi S, Higashi Y. Ratio of serum levels of AGEs to soluble form of RAGE is a predictor of endothelial function. *Diabetes Care* 2015;**38**:119–125.
93. Kim OY, Jo SH, Jang Y, Chae JS, Kim JY, Hyun YJ, Lee JH. G allele at RAGE SNP82 is associated with proinflammatory markers in obese subjects. *Nutr Res* 2009;**29**:106–113.
94. Vazzana N, Guagnano MT, Cuccurullo C, Ferrante E, Lattanzio S, Liani R, Romano M, Davì G. Endogenous secretory RAGE in obese women: association with platelet activation and oxidative stress. *J Clin Endocrinol Metab* 2012;**97**:E1726–E1730.
95. Dozio E, Briganti S, Delnevo A, Vianello E, Ermetici F, Secchi F, Sardanelli F, Morricone L, Malavazos AE, Corsi Romanelli MM. Relationship between soluble receptor for advanced glycation end products (sRAGE), body composition and fat distribution in healthy women. *Eur J Nutr* 2017;**56**:2557–2564.
96. Davis KE, Prasad C, Vijayagopal P, Juma S, Imrhan V. Serum soluble receptor for advanced glycation end products correlates inversely with measures of adiposity in young adults. *Nutr Res* 2014;**34**:478–485.
97. Guclu M, Ali A, Eroglu DU, Büyükuysal SO, Cander S, Ocak N. Serum levels of sRAGE are associated with body measurements, but not glycemic parameters in patients with prediabetes. *Metab Syndr Relat Disord* 2016;**14**:33–39.
98. Kosmopoulos M, Drekolias D, Zavras PD, Piperi C, Papavassiliou AG. Impact of advanced glycation end products (AGEs) signaling in coronary artery disease. *Biochim Biophys Acta Mol Basis Dis* 2019;**1865**:611–619.
99. Fishman SL, Sonmez H, Basman C, Singh V, Poretsky L. The role of advanced glycation end-products in the development of coronary artery disease in patients with and without diabetes mellitus: a review. *Mol Med* 2018;**24**:59.
100. Pinto RS, Ferreira GS, Silvestre GCR, Santana MFM, Nunes VS, Ledesma L, Pinto PR, de Assis SIS, Machado UF, da Silva ES, Passarelli M. Plasma advanced glycation end products and soluble receptor for advanced glycation end products as indicators of sterol content in human carotid atherosclerotic plaques. *Diab Vasc Dis Res* 2022;**19**:14791641221085269.
101. de Giorgis T, D'Adamo E, Giannini C, Chiavaroli V, Scarinci A, Verrotti A, Chiarelli F, Mohn A. Could receptors for advanced glycation end products be considered cardiovascular risk markers in obese children? *Antioxid Redox Signal* 2012;**17**:187–191.
102. Rodríguez-Mortera R, Luevano-Contreras C, Solorio-Meza S, Gómez-Ojeda A, Caccavello R, Bains Y, Gugliucci A, Garay-Sevilla ME. Soluble receptor for advanced glycation end products and its correlation with vascular damage in adolescents with obesity. *Horm Res Paediatr* 2019;**92**:28–35.
103. He CT, Lee CH, Hsieh CH, Hsiao FC, Kuo P, Chu NF, Hung YJ. Soluble form of receptor for advanced glycation end products is associated with obesity and metabolic syndrome in adolescents. *Int J Endocrinol* 2014;**2014**:657607.
104. Parikh M, Chung M, Sheth S, McMacken M, Zahra T, Saunders JK, Ude-Welcome A, Dunn V, Ogedegbe G, Schmidt AM, Pachter HL. Randomized pilot trial of bariatric surgery versus intensive medical weight management on diabetes remission in type 2 diabetic patients who do NOT meet NIH criteria for surgery and the role of soluble RAGE as a novel biomarker of success. *Ann Surg* 2014;**260**:617–622; discussion 622–614.
105. Horwitz D, Saunders JK, Ude-Welcome A, Marie Schmidt A, Dunn V, Leon Pachter H, Parikh M. Three-year follow-up comparing metabolic surgery versus medical weight management in patients with type 2 diabetes and BMI 30–35. The role of sRAGE biomarker as predictor of satisfactory outcomes. *Surg Obes Relat Dis* 2016;**12**:1337–1341.
106. Horwitz D, Padron C, Kelly T, Saunders JK, Ude-Welcome A, Schmidt AM, Parikh M. Long-term outcomes comparing metabolic surgery to no surgery in patients with type 2 diabetes and body mass index 30–35. *Surg Obes Relat Dis* 2020;**16**:503–508.
107. Brix JM, Höllerl F, Kopp HP, Schernthaner GH, Schernthaner G. The soluble form of the receptor of advanced glycation endproducts increases after bariatric surgery in morbid obesity. *Int J Obes (Lond)* 2012;**36**:1412–1417.
108. Hagen I, Schulte DM, Müller N, Martinsen J, Türk K, Hedderich J, Schreiber S, Laudes M. Soluble receptor for advanced glycation end products as a potential biomarker to predict weight loss and improvement of insulin sensitivity by a very low calorie diet of obese human subjects. *Cytokine* 2015;**73**:265–269.
109. Miranda ER, Fuller KNZ, Perkins RK, Kroeger CM, Trepanowski JF, Varady KA, Haus JM. Endogenous secretory RAGE increases with improvements in body composition and is associated with markers of adipocyte health. *Nutr Metab Cardiovasc Dis* 2018;**28**:1155–1165.
110. Malin SK, Navaneethan SD, Fealy CE, Scelsi A, Huang H, Rocco M, Kirwan JP. Exercise plus caloric restriction lowers soluble RAGE in adults with chronic kidney disease. *Obes Sci Pract* 2020;**6**:307–312.
111. Popp CJ, Zhou B, Manigrasso MB, Li H, Curran M, Hu L, St-Jules DE, Alemán JO, Vanegas SM, Jay M, Bergman M, Segal E, Sevick MA, Schmidt AM. Soluble receptor for advanced glycation end products (sRAGE) isoforms predict changes in resting energy expenditure in adults with obesity during weight loss. *Curr Dev Nutr* 2022;**6**:nzac046.
112. Nunes CL, Jesus F, Francisco R, Matias CN, Heo M, Heymsfield SB, Bosy-Westphal A, Sardinha LB, Martins P, Minderico CS, Silva AM. Adaptive thermogenesis after moderate weight loss: magnitude and methodological issues. *Eur J Nutr* 2022;**61**:1405–1416.
113. Jared CR, Glusman G, Rowen L, Kaur A, Purcell Maureen K, Smith Kelly D, Hood Leroy E, Aderem A. The evolution of vertebrate toll-like receptors. *Proc Natl Acad Sci U S A* 2005; **102**:9577–9582.
114. Sessa L, Gatti E, Zeni F, Antonelli A, Catucci A, Koch M, Pompilio G, Fritz G, Raucci A, Bianchi ME. The receptor for advanced glycation end-products (RAGE) is only present in mammals, and belongs to a family of cell adhesion molecules (CAMs). *PLoS One* 2014; **9**:e86903.
115. Gerkema MP, Davies WI, Foster RG, Menaker M, Hut RA. The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proc Biol Sci* 2013;**280**:20130508.
116. Hughes DA, Jastroch M, Stoneking M, Klingenspor M. Molecular evolution of UCP1 and the evolutionary history of mammalian non-shivering thermogenesis. *BMC Evol Biol* 2009;**9**:4.
117. Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 2007;**28**:429–436.
118. Yuan X, Nie C, Liu H, Ma Q, Peng B, Zhang M, Chen Z, Li J. Comparison of metabolic fate, target organs, and microbiota interactions of free and bound dietary advanced glycation end products. *Crit Rev Food Sci Nutr* 2021:1–22.
119. Maasen K, Eussen S, Scheijen J, van der Kallen CJH, Dagnelie PC, Opperhuizen A, Stehouwer CDA, van Greevenbroek MMJ, Schalkwijk CG. Higher habitual intake of dietary dicarbonyls is associated with higher corresponding plasma dicarbonyl concentrations and skin autofluorescence: the Maastricht study. *Am J Clin Nutr* 2022;**115**:34–44.
120. Li Y, Quan W, Jia X, He Z, Wang Z, Zeng M, Chen J. Profiles of initial, intermediate, and advanced stages of harmful maillard reaction products in whole-milk powders pre-treated with different heat loads during 18 months of storage. *Food Chem* 2021;**351**:129361.
121. Klein S, Gastaldelli A, Yki-Järvinen H, Scherer PE. Why does obesity cause diabetes? *Cell Metabolism* 2022;**34**:11–20.