Analyse des Zusammenhangs von Advanced Glycation End Products mit Faktoren des gesunden Alterns in der älteren Allgemeinbevölkerung

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Referat

Zielsetzung Advanced Glycation End Products (AGEs) sind durch Zucker modifizierte Proteine oder Aminosäuren und werden vermehrt bei altersassoziierten Erkrankungen gebildet und angesammelt. Ziel dieser Arbeit war die Untersuchung der Assoziationen zwischen AGEs, ihren löslichen Rezeptoren (sRAGE) und dem Quotient AGE/sRAGE mit aktuellen und chronischen Outcomes in der Allgemeinbevölkerung am Beispiel von aktueller körperlicher Funktionsfähigkeit und Mortalität.

Methoden Wir verwendeten die Daten der CARLA-Studie (CARdiovascular disease, Living and Ageing in Halle), einer Kohortenstudie der Allgemeinbevölkerung in Halle (Saale), Deutschland.

Für die Untersuchung der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE mit aktueller körperlicher Funktionsfähigkeit haben wir die Querschnittsdaten der Baselineuntersuchung von 967 Männern und 812 Frauen im Alter zwischen 45 und 83 Jahren genutzt. Die Untersuchung der Assoziationen mit Mortalität wurde an den Daten von 958 Männern und 802 Frauen im Alter zwischen 45 und 83 Jahren mit einem medianen Follow-up von 12 Jahren durchgeführt.

Wir nutzten ordinale logistische Regressionsmodelle zur Untersuchung der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE mit körperlicher Funktionsfähigkeit und Kaplan-Meyer-Kurven sowie multivariable und zeitabhängige Cox-Regressionsanalysen, um die Assoziationen mit Mortalität zu untersuchen.

Ergebnisse Höhere Serum-Konzentrationen von AGEs und dem AGEs/sRAGE Quotienten waren nur bei Frauen mit geringerer körperlicher Funktionsfähigkeit assoziiert, auch nach Adjustierung für Gesundheits- und Lifestyleparameter (Odds Ratio (OR) =0.86, 95% Konfidenzintervall (KI) = 0.74- 0.98 und = 0.86, 95% KI= 0.75- 0.98 für AGEs und den AGE/sRAGE Quotient). Die Cox-Regressionsanalysen ergaben keine Assoziationen von AGEs, sRAGE oder AGE/sRAGE mit Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen oder Mortalität durch Krebserkrankungen.

Schlussfolgerungen Wir konnten eine stärkere und geschlechtsspezifische Assoziation des AGE-RAGE Systems mit aktuellen Outcomes im Vergleich zu chronischen Outcomes am Beispiel der aktuellen körperlichen Funktionsfähigkeit und Mortalität zeigen. Zur Nutzung von AGEs und sRAGE als Biomarker oder Therapieziele ist ein genaueres Verständnis der Mechanismen nötig, mit denen AGEs und sRAGE in die pathologischen Prozesse verschiedener Erkrankungen involviert sind.

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Abstract

Aims Advanced Glycation End Products (AGEs), sugar modified proteins or amino acids, are increasingly produced and accumulated with age-related diseases. We aimed to investigate the association between AGEs, their soluble receptors (sRAGE) and the AGE/sRAGE ratio with current and chronical outcomes in the general population using the examples of current physical activity and mortality.

Methods We analysed data from the CARLA-study (CARdiovascular disease, Living and Ageing in Halle), a population-based cohort of the general population of the city Halle (Saale), Germany.

For studying the association between AGEs, sRAGE and AGE/sRAGE with physical functioning, we used cross sectional data of the baseline examination of 967 men and 812 women, aged between 45 and 83 years. For the examination of the association with mortality, we used data of 958 men and 802 women, aged between 45 and 83 years, with a median follow-up of 12 years.

We used ordinal logistic regression to examine associations between AGEs, sRAGE, and AGE/sRAGE ratio with physical functioning as well as Kaplan-Meyer survival curves and multivariable and time-varying Cox-regression to assess the associations with mortality.

Results Higher levels of AGEs and AGE/sRAGE ratio were associated with lower physical functioning only in women, even after consideration of classical health-related and lifestyle factors (odds ratio (OR) = 0.86, 95%confidence interval = 0.74–0.98 and OR = 0.86, 95%CI = 0.75–0.98 for AGEs and AGE/sRAGE ratio respectively). The cox-regression showed no association of AGEs, sRAGE or AGE/sRAGE with all-cause mortality, cardiovascular disease mortality or cancer related mortality.

Conclusions We showed a stronger and sex-related association of the AGE-RAGE system with current outcomes than with chronical outcomes using the examples of current physical functioning and mortality. We need a more detailed understanding how AGEs and sRAGE influence the pathology of different diseases to use them as potential biomarker or therapy targets.

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1. Einleitung und Zielstellung

In einer zunehmend alternden Gesellschaft, hat Altersforschung eine steigende Bedeutung. Schon in den 1970er Jahren wurde die Hypothese aufgestellt, dass die Akkumulation von Advanced Glycation End Products (AGEs) ein wesentlicher Grund des Alterns ist [1]. Seitdem sind die AGEs und ihre Rezeptoren Gegenstand verschiedenster Forschungen und die Anzahl an Publikationen und Veröffentlichungen steigt kontinuierlich an.

1.1 AGEs, RAGE, sRAGE

AGEs und RAGE

Die Advanced Glykation End Products (AGEs) sind eine heterogene Gruppe von Makromolekülen, die Stoffe wie N-ε-Carboxymethyllysine (CML), N-ε -Carboxyethyllysine (CEL), Pyrraline oder Pentosidine umfasst [2-4]. Sie werden durch die nichtenzymatische Reaktion von reduzierenden Zuckern, zum Beispiel Glukose oder Fruktose, mit Aminogruppen von Proteinen, Lipiden (ALEs) oder Aminosäuren gebildet. Als Zwischenprodukte entstehen Amadori-Produkte und Schiffsche Basen [2, 5, 6]. Diese Reaktion wird nach dem französischen Chemiker Louis Camille Maillard als Maillardreaktion bezeichnet, der 1912 zum ersten Mal beschrieb, dass das Erhitzen von Aminosäuren mit reduzierenden Zuckern zum Bräunen von Nahrungsmitteln führt [7]. Neben der Maillardreaktion werden AGEs durch eine Vielzahl anderer Reaktionen gebildet (z.B. Polyol-Signalweg oder Oxidation von Glukose), was zur Vielfältigkeit ihrer chemischen Strukturen führt [3, 8]. Die endogene AGE-Entstehung wird durch Rauchen und erhöhte Blutzuckerspiegel verstärkt [2, 9]. Außerdem können AGEs exogen durch die Nahrung aufgenommen werden. Sie entstehen bei der Essenszubereitung bei hohen Temperaturen und geringer Feuchtigkeit (z.B. Braten und Grillen) und sind besonders in tierischen, fett- und proteinreichen Produkten enthalten, wie fettigem Käse oder rotem Fleisch [10]. Die Ausscheidung erfolgt durch die Niere, weshalb auch bei verminderter Nierenfunktion die AGE-Konzentrationen erhöht sind [6].

AGEs verändern durch Bindung an Proteine deren strukturellen und funktionellen Eigenschaften [3, 6]. Sie bilden Quervernetzungen zwischen Proteinen, wie Kollagenen, was zu veränderten funktionellen Eigenschaften von Organen und Geweben, z.B. Niere, Herz, Gefäßsystem, Linse oder Muskeln führt [11-15]. Darüber hinaus wirken AGEs indirekt durch Bindung an ihren Rezeptor (RAGE). RAGE ist ein Multiligandenrezeptor,

der neben AGEs proinflammatorische Liganden bindet, z.B. Amphoterin, Mitglieder der S100 Polypeptidfamilie, Amyloid ß und C3a [16-21]. RAGE gehört zur Familie der Immunoglobulin-Superfamilie und wird auf der Oberfläche von verschiedenen Zellen exprimiert, z.B. Endothelzellen, glatten Muskelzellen, Neuronen, Podozyten und Monozyten [22-24]. Die RAGE Expression ist unter homöostatischen Bedingungen gering, sie kann aber in ligandenreicher Umgebung gesteigert werden, was seine Bedeutung in pathologischen Situationen erhöht [25, 26]. Durch die Vielzahl an Liganden und seine Expression auf verschiedenen Zellen, ist RAGE an unterschiedlichen Signalwegen beteiligt, die zur Regulation von Entzündung, Zellbewegung und Zellmasse führen [21]. Über ihre direkten Effekte an Proteinen und die Beeinflussung von Signalwegen durch die Bindung an RAGE sind AGEs an der Entwicklung von alters-assoziierten Erkrankungen beteiligt, zum Beispiel Kardiovaskulären Erkrankungen und Nierenerkrankungen [14, 15, 21, 27, 28].

sRAGE

Neben dem Rezeptor RAGE, der eine extrazelluläre Domäne, eine Transmembranhelix und einen zytosolischen Teil besitzt [22, 29], werden AGEs auch von löslichen RAGE-Varianten (sRAGE= soluble RAGE) gebunden. Diese besitzen keinen intrazellulären und transmembranären Teil und können daher die AGE-Signale nicht in die Zelle weiterleiten (Abbildung 1) [3, 17, 30]. sRAGE ist ein Sammelbegriff für lösliche AGE-Rezeptoren.

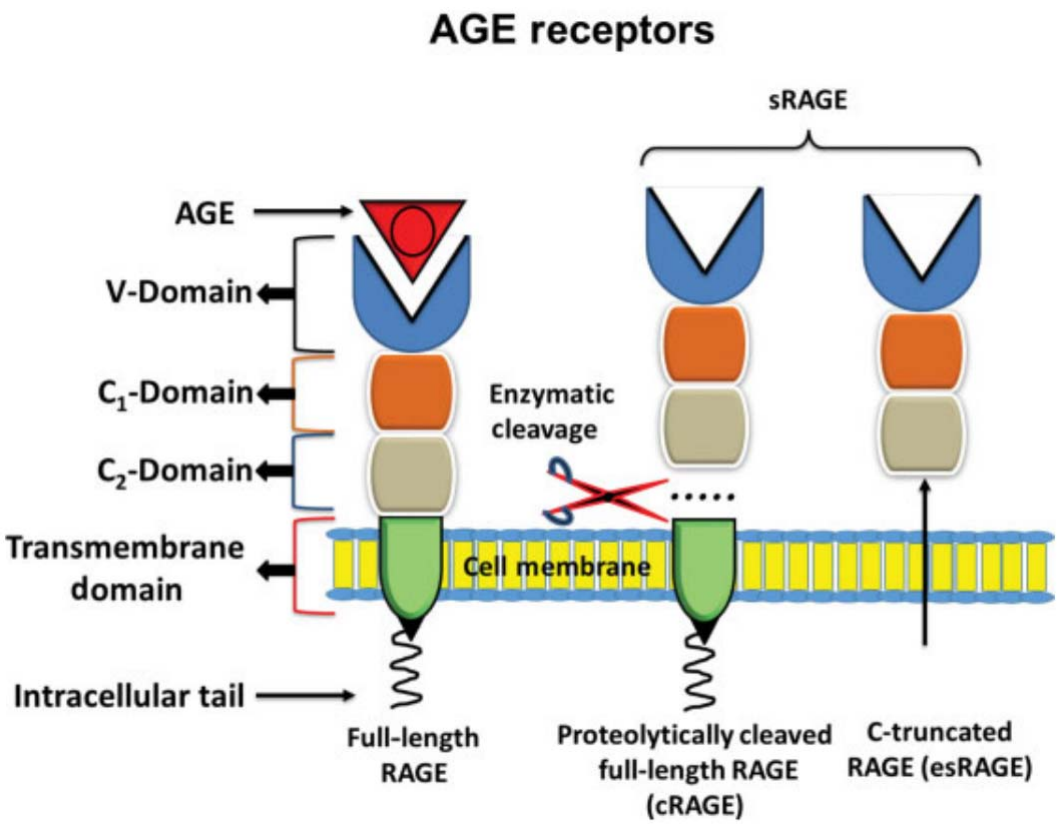

Abb. 1 Schematische Darstellung der Rezeptoren für Advanced Glycation End Products (RAGE) (Herausgeber dieses urheberrechtlich geschützten Materials ist die Georg Thieme Verlag KG, Nutzungslizenz für Dissertation erhalten) [3].

Er umfasst den hauptsächlich gebildeten cRAGE (cleaved RAGE), der durch proteolytisches Spalten von RAGE entsteht und esRAGE (endogenous secretory RAGE), der durch alternatives Spleißen von RAGE-mRNA gebildet wird [31, 32]. Die Funktion von sRAGE ist bis jetzt nicht vollständig geklärt. Einige Studien zeigen, dass Patienten mit Alzheimer, vaskulärer Demenz, Chronisch obstruktiver Lungenerkrankung (COPD), Hypercholesterinämie und Diabetes mellitus im Vergleich zu Kontrollen niedrigere sRAGE Konzentrationen haben, und niedrigere sRAGE Quartile prädiktiv für inzidenten Diabetes mellitus, koronare Herzerkrankungen und Gesamtmortalität nach 18 Jahren Beobachtungszeit sind [33-36]. Diese Studien und auch die Ergebnisse aus Tierstudien, die gezeigt haben, dass durch Verabreichung von sRAGE Entzündung vermieden und fortgeschrittene Artherosklerose unterdrückt werden kann, unterstützen die Hypothese, dass sRAGE einen protektiven Effekt vor altersassoziierten Erkrankungen und Entzündung besitzt [17, 37, 38]. Anderen Studien zeigen jedoch Assoziationen zwischen erhöhten sRAGE-Werten und altersassoziierten Outcomes, wie

inzidenten kardiovaskulären Erkrankungen, erhöhter 28-Tage Sterblichkeit in septischen Patienten, erhöhter kardiovaskulärer- und Gesamtmortalität, verminderter Nierenfunktion und prävalentem Diabetes mellitus [39-44]. Diese Studien weisen darauf hin, dass die sRAGE-Entstehung durch erhöhte Ligandenbindung an RAGE gesteigert wird, wodurch erhöhte sRAGE Konzentrationen ein Zeichen für eine erhöhte Expression und Aktivierung von RAGE sein könnten und damit ein Marker für Entzündung [31, 40, 44]. Da sRAGE in verschiedenen Situationen verschiedene Reaktionen zeigt und bis jetzt die Gründe dafür unverstanden sind, scheint seine Aussagekraft als biologischer Marker eingeschränkt zu sein [45].

AGE/sRAGE Quotient

Ein Grund für die Assoziation von erhöhten sRAGE-Konzentrationen mit altersassoziierten Erkrankungen, im Gegensatz zu den vermuteten protektiven Effekten, könnte eine noch größere Erhöhung der AGE-Konzentrationen über die sRAGE-Konzentrationen hinaus sein [45]. Es wird daher vermutet, dass man das Verhältnis zwischen schädigenden AGEs und möglicherweise protektivem sRAGE betrachten muss, um den Zustand des AGE-RAGE Systems richtig einzuschätzen [3, 9, 45]. Ein erhöhter AGE/sRAGE Quotient würde demnach für eine Verlagerung in Richtung der schädigenden AGEs sprechen [3]. Unterstützend für diese Hypothese wurde in Studien gezeigt, dass der AGE/sRAGE Quotient mit Atherosklerose, endothelialer Dysfunktion, Hyperthyreose, Restenosen nach Angioplastie und fortgeschrittenen Nierenerkrankungen assoziiert ist und diese Assoziation mitunter stärker war, als die Assoziation mit AGEs oder sRAGE allein [46-50].

1.2 Zielstellung dieser Arbeit

Das Ziel dieser Arbeit war die Untersuchung der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE mit aktueller körperlicher Funktionsfähigkeit und Mortalität in der Allgemeinbevölkerung. Diese Studien sollten einzeln und gemeinsam analysiert und interpretiert werden. Dabei wird zwischen den Assoziationen von AGEs, sRAGE und AGE/sRAGE mit aktuellen Outcomes (aktuelle körperliche Funktionsfähigkeit) und chronischen Outcomes (Mortalität) differenziert und diese Erkenntnisse bei der

Diskussion der Bedeutung des AGE-RAGE Systems für Klinik und Forschung einbezogen.

Forschungslücken

Die körperliche Funktionsfähigkeit zwischen Individuen der gleichen Altersgruppe unterliegt großen Schwankungen aufgrund des Unterschieds zwischen biologischem und chronologischem Alter [51]. Da AGEs als Biomarker für das biologische Alter diskutiert werden, haben wir untersucht, ob sie auch mit der aktuellen körperlichen Funktionsfähigkeit der Studienteilnehmer/innen assoziiert sind [52].

Vor dieser Studie existierten ausschließlich Studien, die die Assoziation zwischen AGEs und körperlicher Funktionsfähigkeit bei Personen über dem 65. Lebensjahr untersucht haben [53-55]. Es fehlten dadurch Informationen über die Assoziation bei jüngeren Personen. Außerdem wird angenommen, dass der AGE/sRAGE Quotient das AGE-RAGE System besser abbildet, als die einzelnen Komponenten [3]. Es gab jedoch keine Studie, die die Assoziation zwischen dem AGE/sRAGE Quotienten und körperlicher Funktionsfähigkeit untersucht hat.

Für die Untersuchung der Assoziationen von AGEs, sRAGE und AGE/sRAGE mit chronischen Outcomes, haben wir deren Assoziationen mit Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen und Mortalität durch Krebserkrankungen untersucht [56]. Vorherige Studien haben die Assoziationen zwischen AGEs und/oder sRAGE mit Mortalität in sehr selektiven Studienpopulationen (schwerstkranke Patienten, Dialysepatienten, Frauen der Allgemeinbevölkerung >65 Jahre, Typ 1 Diabetiker oder Patienten mit diabetischer Nephropathie, nichtdiabetische Allgemeinbevölkerung) mit jeweils sehr unterschiedlichen Beobachtungszeiträumen (28 Tage bis 18 Jahre) untersucht [39, 42, 44, 57-62]. Es gab außerdem keine Studie, die die Assoziation von AGE/sRAGE mit Mortalität untersucht hat. In den existierenden Studien wurden ausschließlich einzelne Hazard Ratios (HR) berichtet. Diese zeigen jedoch die gemittelte Assoziation über den gesamten Beobachtungszeitraum [63]. Es fehlten daher Erkenntnisse, ob sich die Assoziationen von AGEs, sRAGE und AGE/sRAGE und Mortalität über den Beobachtungszeitraum ändern. Daher haben wir den Zusammenhang zwischen AGEs, sRAGE und AGE/sRAGE und Mortalität (Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen, Mortalität durch Krebserkrankungen) in einem breiten Altersspektrum der Allgemeinbevölkerung über

einen langen Beobachtungszeitraum (Median 12 Jahre) untersucht und unter anderem zeitabhängige HR berichtet.

Methoden

Für die Untersuchung der Assoziationen von AGEs, sRAGE und AGE/sRAGE mit aktuellen und chronischen Outcomes haben wir die Daten der CARLA-Studie (CARdiovascular disease, Living and Ageing in Halle) verwendet [64]. Die CARLA-Studie ist eine Kohortenstudie der Allgemeinbevölkerung in Halle (Saale), Deutschland. Sie wurde durchgeführt, um Informationen über die Prävalenz, Inzidenz und Risikofaktoren von Kardiovaskulären Erkrankungen und die Bedeutung der Herzfrequenzvariabilität in der Allgemeinbevölkerung zu gewinnen [64].

Für die Untersuchung der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE mit aktueller körperlicher Funktionsfähigkeit haben wir die Querschnittsdaten der Baselineuntersuchung von 967 Männern und 812 Frauen im Alter zwischen 45 und 83 Jahren genutzt [65]. Die Untersuchung der Assoziationen mit Mortalität wurde an den Daten von 958 Männern und 802 Frauen im Alter zwischen 45 und 83 Jahren mit einem medianen Follow-up von 12 Jahren durchgeführt.

Die Messung von AGEs und sRAGE wurde im Plasma der Studienteilnehmer/innen (nicht nüchtern) durchgeführt. Die Messwerte der AGEs wurden durch Fluoreszenzmessung bei 370 +/- 10 nm Exzitation und 440 +/- 10 nm Emission mit dem FLUOstar OPTIMA reader (BMG Labtechnologies, Offenburg, Deutschland) ermittelt. sRAGE wurde durch ein standarisiertes ELISA (Enzyme-linked Immunosorbent Assay) gemessen (Quantikine; R&D Systems). Der AGE/sRAGE Quotient wurde aus den Rohmesswerten der AGEs und sRAGE berechnet. Die Publikationen enthalten weitere Angaben zu den Messmethoden [56, 65].

Angaben über die aktuelle körperliche Funktionsfähigkeit der Studienteilnehmer/innen wurden durch eine Skala des SF-12 Gesundheitsfragebogens (Short Form 12 Items) ermittelt [66]. Es wurden Angaben über gesundheitliche Einschränkungen bei moderaten Tätigkeiten des täglichen Lebens (zum Beispiel Staubsaugen oder Kegeln) und beim Treppensteigen gemacht. Aus den Angaben haben wir nach einem standarisierten Verfahren einen Gesamtscore für körperliche Funktionsfähigkeit berechnet, mit einer diskreten Skala von 0 bis 100 (0; 25; 50; 75; 100) [66].

Für die Informationen zur Mortalität wurden die Studienteilnehmer/innen, die an der Baselineuntersuchung (2002-2006) teilgenommen haben bis Mai 2016 nachverfolgt. Es

wurden Informationen zum Vitalstatus und zur Todesursache aus den Totenscheinen gesammelt. Die Todesursachen wurden nach der „International Classification of Diseases- 10th version“ (ICD-10) codiert [67]. Mortalität aufgrund kardiovaskulärer Erkrankungen wurde durch die ICD-10-Codes I00- I99 definiert, Mortalität durch Krebserkrankungen durch die Codes C00- D48.

Um die Odds Ratios (OR) und Konfidenzintervalle (KI) für die Assoziationen zwischen AGEs, sRAGE und AGEs/sRAGE mit aktueller körperlicher Funktionsfähigkeit zu ermitteln, haben wir ordinale logistische Regressionsanalysen mit einem rohen Modell und 2 adjustierten Modellen durchgeführt. Zusätzlich haben wir die Analysen stratifiziert für Personen <65 Jahren und ≥65 Jahren durchgeführt, um die Stabilität der Assoziationen im Laufe des Alterns zu sehen. Wir haben das 65. Lebensjahr als Grenze genommen, da bisherige Studien ausschließlich Studienteilnehmer/innen über/im Alter von 65 Jahren eingeschlossen haben. Weitere Untersuchungen mit dem Alter als Kontinuum und Alter in 10-Jahres Schritten wurden durchgeführt. Da Diabetes Mellitus und Erkrankungen mit einer verminderte Nierenfunktion Erkrankungen sind, die die AGE-Konzentration stark erhöhen, haben wir zusätzlich Sensitivitätsanalysen ohne Probanden, die an diesen Erkrankungen leiden durchgeführt [5]. Die Publikation enthält zusätzliche Analysen und weitere statistische Informationen [65].

Die Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE, und Mortalität haben wir durch Kaplan-Meyer-Kurven und multivariable Cox-Regression analysiert. Um die Stabilität der Assoziationen über die Zeit zu untersuchen, haben wir zusätzlich zeitabhängige, multivariable Cox-Regressionsanalysen durchgeführt [63]. Auch für diese Analysen haben wir Sensitivitätsanalysen ohne Teilnehmer/innen mit Diabetes Mellitus und/ oder verminderter Nierenfunktion, und altersstratifizierte Analysen für Teilnehmer/innen <65 Jahre und ≥65 Jahre betrachtet.

Aufgrund der Ergebnisse vorheriger Studien, die gezeigt haben, dass AGEs geschlechtsspezifisch unterschiedlich mit gesundheits- und altersbezogenen Outcomes assoziiert sind, haben wir alle Analysen geschlechtsstratifiziert durchgeführt [62, 68, 69].

2. Diskussion

2.1 Ergebnisse

In beiden Studien hatten Männer höhere AGE-Konzentrationen im Plasma, als Frauen und auch die Quotienten von AGE/sRAGE waren höher, als die der Frauen. Diese hatten hingegen in beiden Studien höhere Konzentrationen von sRAGE im Plasma (Tabelle 1 /Publikation 1, Tabelle 1/ Publikation 2).

Bei der Untersuchung der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE und körperlicher Funktionsfähigkeit gaben die Männer weniger gesundheitliche Einschränkungen der körperlichen Funktionsfähigkeit an, als die Frauen. 68,25% der Männer gaben an keine Einschränkungen bei moderaten Aktivitäten zu haben und 55,53% der Männer hatten keine Einschränkungen beim Treppensteigen. Bei den Frauen hingegen waren es nur 52,83% und 41,50% (Tabelle 1/ Publikation1).

Die ordinalen logistischen Regressionsanalysen haben gezeigt, dass nur bei Frauen höhere AGE-Konzentrationen und höhere AGE/sRAGE Quotienten mit verminderter aktueller körperlicher Funktionsfähigkeit assoziiert waren (OR=0.86, 95%KI=0.74-0.98 für AGEs und OR=0.86, 95%KI=0.75-0.98 für AGE/sRAGE) (Abbildung 1 und Zusatzmaterial Tabelle 2/ Publikation 1). sRAGE war bei Frauen nicht signifikant mit körperlicher Funktionsfähigkeit assoziiert, tendierte aber zur Assoziation zwischen höheren sRAGE-Konzentrationen und erhöhter körperlicher Funktionsfähigkeit (OR=1.06, 95%KI=0.93-1.21) (Abbildung 1 und Zusatzmaterial Tabelle 2/ Publikation 1). Bei Männern waren weder AGEs noch sRAGE oder AGE/sRAGE mit körperlicher Funktionsfähigkeit assoziiert (Abbildung 1 und Zusatzmaterial Tabelle 2/ Publikation 1). Die Sensitivitätsanalysen ohne Personen mit Diabetes mellitus oder verminderter Nierenfunktion ergaben ähnliche Ergebnisse (Zusatzmaterial Abbildung 6 und Tabelle 6/ Publikation 1). Zwischen Personen in verschiedenen Altersgruppen waren bei Männern und Frauen keine Unterschiede in den Assoziationen zu erkennen (Abbildung 2 und Zusatzmaterial Tabelle 2/ Publikation 1) [65].

Bei der Untersuchung der Assoziationen von AGEs, sRAGE und AGE/sRAGE und Mortalität bestand die Studienpopulation bei der Baseline-Untersuchung aus 958 Männern und 802 Frauen. Im Beobachtungszeitraum von 12 Jahren verstarben davon 284 Männer und 124 Frauen (Tabelle 1/ Publikation 2). Die Hauptursachen waren Kardiovaskuläre Erkrankungen (101 Männer und 53 Frauen) und Krebserkrankungen (101 Männer und 37 Frauen) (Tabelle 1/ Publikation 2). Die multivariablen Cox-

Regressionsanalysen ergaben keine Assoziationen von AGEs, sRAGE oder AGE/sRAGE mit Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen oder Mortalität durch Krebserkrankungen nach 12 Jahren Beobachtungszeit (Tabelle 2 und Zusatzmaterial Tabelle 2/ Publikation 2). Auch in den Kaplan-Meyer-Kurven waren keine Unterschiede der Assoziationen mit Gesamtmortalität zwischen den Quartilen von AGEs, sRAGE und AGE/sRAGE zu sehen (Zusatzmaterial Abbildung 2 /Publikation 2). Die Sensitivitätsanalysen und altersstratifizierten Analysen zeigten ebenfalls keine Assoziationen oder Unterschiede zwischen den Altersgruppen (Zusatzmaterial Tabelle 2/ Publikation 2). Bei der zeitabhängigen, multivariablen Cox-Regressionsanalyse wurden diese Ergebnisse bestätigt. Es waren zudem keine Schwankungen der Assoziationen während des 12jährigen Beobachtungszeitraums zu erkennen (Abbildung 1/ Publikation 2) [56].

2.2 Diskussion

Biomarker, die das biologische Alter eines Individuums besser wiedergeben, als das chronologische Alter selbst oder die das Auftreten einer Erkrankung voraussagen, haben eine steigende Bedeutung in der Medizin und es werden große Studien durchgeführt, um neue Biomarker zu identifizieren [52]. Auch die Bedeutung von AGEs und sRAGE als Biomarker wird vielfach untersucht und diskutiert [45, 52]. Wir haben die Assoziationen von AGEs und sRAGE mit aktuellen und chronischen Outcomes untersucht.

Wir haben nur bei Frauen eine Assoziation zwischen höheren AGE- Konzentrationen und höheren AGE/sRAGE Quotienten mit verminderter körperlicher Funktionsfähigkeit gesehen. Die unterschiedlichen Assoziationen von AGEs mit alters- und gesundheitsbezogenen Outcomes zwischen Männern und Frauen wurde auch schon in vorherigen Studien gezeigt [62, 68, 69] . In den Studien von Kilhovd, *et al.*, 2005 und 2007 waren höhere AGE-Konzentrationen im Serum mit erhöhter Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen und Mortalität durch koronare Herzerkrankungen assoziiert, jedoch nur in Frauen [62, 69]. Die Autoren vermuteten, dass hohe AGE-Konzentrationen möglicherweise nur bei Personen mit einem gesunden Lebensstil und geringem Risiko für kardiovaskuläre Erkrankungen ein zusätzliches Risiko für kardiovaskuläre Erkrankungen und damit Mortalität darstellen [62]. Wie in dieser Studie rauchten auch die Teilnehmerinnen der CARLA Studie seltener, als die

Teilnehmer und litten auch seltener an kardiovaskulären Erkrankungen, als die Männer (Tabelle 1/ Publikation 1) [65]. Aus diesem Grund könnten AGEs bei Frauen einen zusätzlichen schädigenden Effekt gehabt haben (zusätzlich zu den klassischen Risikofaktoren) und damit auch einen größeren Einfluss auf die körperliche Funktionsfähigkeit gehabt haben, als bei Männern (konkurrierende Risiken).

Eine weitere Erklärung für die unterschiedlichen Ergebnisse zwischen den Geschlechtern könnten genetische Unterschiede sein. Es wurde gezeigt, dass AGEs die Entstehung von Osteoporose durch ihre Effekte auf Knochenproteine begünstigen und die AGE-Konzentrationen in Frauen mit Osteoporose höher sind, als bei Frauen ohne Osteoporose [70, 71]. Außerdem wurde in Tierstudien gezeigt, dass eine AGE-reiche Ernährung einen geschlechtsspezifischen Effekt auf die Knochenstruktur und Knochenfunktion hat und besonders bei weiblichen Mäusen zu einer verminderten Knochenqualität und erhöhten Brüchigkeit führt [72]. Eine weitere Ursache für die Assoziation zwischen AGEs und verminderter körperlicher Funktionsfähigkeit ausschließlich bei Frauen könnte daher auch die Beeinflussung durch Osteoporose sein, die zu verminderter körperlicher Funktionsfähigkeit führt und für die besonders bei Frauen erhöhte AGE-Konzentrationen ein Risikofaktor sind. Um diese Hypothese zu prüfen, haben wir eine Zusatzanalyse der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE und körperlicher Funktionsfähigkeit gemacht und haben zusätzlich für Osteoporose als Confounder adjustiert (Zusatzmaterial Tabelle 7/ Publikation 1) [65]. Die zusätzliche Adjustierung für Osteoporose hat die Ergebnisse (Odds Ratios und Konfidenzintervalle) jedoch nur sehr gering oder nicht verändert. Gründe dafür könnten eine zu geringe Anzahl von Frauen mit Osteoporose sein oder zusätzliches Confounding durch vermehrte körperliche Aktivität der Frauen, zum Beispiel durch Hausarbeit.

Auch nach der Veröffentlichung unserer Ergebnisse wurden in neueren Arbeiten Hinweise auf geschlechtsspezifische Assoziationen von AGEs und sRAGE mit körperlicher Funktionsfähigkeit gefunden. Bei einer populationsbasierten Studie mit Männern und Frauen >65 Jahren konnte eine signifikante Abnahme der Handkraft mit steigender CML-Konzentration im Urin nur bei Frauen festgestellt werden [73]. Eine weitere Studie mit Personen der Allgemeinbevölkerung >65 Jahren zeigte eine positive Assoziation zwischen sRAGE mit geringerer Muskelmasse und Muskelkraft ebenfalls nur bei Frauen [74]. Die Autoren beider Arbeiten vermuten, dass Geschlechtshormone die Assoziation von AGEs und sRAGE mit körperlicher Funktionsfähigkeit beeinflussen könnten, indem unter anderem der wegfallende Östrogenschutz bei Frauen in der

Menopause eine zusätzliche Angriffsfläche an verschiedenen Geweben bieten könnte [73, 74].

Unsere Studie “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“ zeigte weiterhin, dass niedrigere sRAGE Konzentrationen tendenziell mit verminderter körperlicher Funktionsfähigkeit assoziiert waren. Das unterstützt die Hypothese, dass sRAGE einen protektiven Effekt vor altersassoziierten Erkrankungen besitzt [36-38].

Die Analyse der Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE und chronischen Outcomes ergab keine Assoziationen mit Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen oder Mortalität durch Krebserkrankungen nach 12 Jahren Beobachtungszeit. Außerdem schwankten die Assoziationen während des 12jährigen Beobachtungszeitraums nicht. Wir hatten im Vorfeld eine Abnahme der Assoziationen von AGEs, sRAGE und AGE/sRAGE mit steigender Beobachtungszeit durch veränderte Lebensgewohnheiten (veränderte Ernährung, verändertes Rauchverhalten) erwartet.
Die Ergebnisse zu den Assoziationen mit Mortalität passen dennoch in das Bild vorheriger Studien, bei denen uneinheitliche Ergebnisse berichtet wurden. Bei sehr unterschiedlichen Studienkollektiven und Beobachtungszeiten waren erhöhte AGE-Konzentrationen im Blut sowohl mit erhöhter Mortalität [42, 62], verminderter Mortalität [60] oder gar nicht mit Mortalität assoziiert [59].
Vergleicht man die Assoziationen von AGEs mit aktuellen (körperliche Funktionsfähigkeit) und chronischen Outcomes (Mortalität) erkennt man, dass im Blutplasma gemessene AGEs stärker mit aktuellen, als mit chronischen Outcomes assoziiert sind. Eine Erklärung dafür könnte sein, dass Plasma-AGEs den aktuellen AGE-Status des Körpers abbilden, da sie erst kürzlich gebildet wurden und damit nicht so stark mit chronischen Outcomes assoziiert sind. Durch Fluoreszenzmessung in der Haut gemessene AGEs wurden über längere Zeit an langlebige Proteine der Haut gebunden und reflektieren damit den chronischen AGE-Status des Körpers. Daher sind in der Haut gemessene AGE-Konzentrationen möglicherweise besser mit chronischen Outcomes wie Mortalität assoziiert, als Plasma- oder Serum-AGE-Konzentrationen [75]. Diese Hypothese wird durch die Ergebnisse einer aktuellen Studie gestützt, in der bei Dialysepatienten eine positive Assoziation zwischen AGEs und Gesamtmortalität sowie

Mortalität durch kardiovaskuläre Erkrankungen ausschließlich bei durch Hautfluoreszenz gemessenen AGEs, jedoch nicht bei im Serum gemessenen AGEs festgestellt wurde [76].

Sowohl für körperlicher Funktionsfähigkeit, als auch Mortalität konnte keine Assoziation mit im Plasma gemessenem sRAGE gefunden werden. Diese Ergebnisse stimmen mit Überlegungen überein, dass sRAGE allein kein universeller Biomarker sei, da es bei einigen Erkrankungen erhöht, bei anderen jedoch erniedrigt ist [45]. Es wird angenommen, dass man die sRAGE-Konzentrationen immer im Zusammenhang mit den AGE-Konzentrationen betrachten muss, um einen universellen Biomarker zu erhalten [45]. Daher haben wir zusätzlich die Assoziationen vom Quotienten aus AGEs und sRAGE (AGE/sRAGE) mit körperlicher Funktionsfähigkeit und Mortalität untersucht. Gemäß der Hypothese, dass die Bindung von AGEs an sRAGE die schädigenden Einflüsse der AGEs auf den Körper verhindert und der Quotient das Verhältnis zwischen protektiven und schädigenden Faktoren widerspiegelt, haben wir erwartet, dass die Assoziation von AGE/sRAGE mit den aktuellen und chronischen Outcomes größer ist, als die Assoziationen von AGEs und sRAGE allein [45]. Jedoch haben wir sowohl bei der Assoziation zu aktueller körperlicher Funktionsfähigkeit, als auch zu Mortalität keine Überlegenheit des AGE/sRAGE Quotienten gegenüber der AGE-Konzentration allein gefunden. AGE/sRAGE war wie die AGE-Konzentration ausschließlich bei Frauen negativ mit körperlicher Funktionsfähigkeit assoziiert und hat sich auch in der Stärke der Assoziation nicht wesentlich von der Assoziation der AGEs unterschieden (OR=0.86, 95%KI=0.74-0.98 für AGEs und OR=0.86, 95%KI=0.75-0.98 für AGE/sRAGE) (Abbildung 1 und Zusatzmaterial Tabelle 2/ Publikation 1). Mit Mortalität (Gesamtmortalität, Mortalität durch kardiovaskuläre Erkrankungen, Mortalität durch Krebserkrankungen) waren AGE/sRAGE wie AGEs allein nicht assoziiert (Tabelle 2 und Zusatzmaterial Tabelle 2/ Publikation 2). sRAGE scheint folglich keinen großen Einfluss auf die Assoziation zwischen AGEs und Outcome zu haben. Ein Grund könnte die zu geringe Konzentration von sRAGE im Körper sein. Zwar wurde in verschiedenen Studien gezeigt, dass exogen verabreichtes sRAGE die negativen Effekte der AGEs auf den Körper vermindern kann [17, 37, 38], es wurde jedoch auch festgestellt, dass die endogene Konzentration von sRAGE vielfach zu niedrig ist, als dass es AGEs suffizient abfangen könnte [47, 77, 78].

Trotz der möglicherweise geringen Bedeutung von endogenem sRAGE als Biomarker, hat es in einer exogen verabreichten Form bereits therapeutische Wirksamkeit gezeigt.

Exogen verabreichtes sRAGE führte unter anderem zur Unterdrückung von Entzündungsreaktionen und fortgeschrittener Artherosklerose [17, 37, 38]. Ein weiterer therapeutischer Ansatzpunkt im AGE-RAGE System ist die Therapie mit Alagebriumchlorid (ALT-711). ALT-711 bricht die durch AGEs entstandenen Quervernetzungen zwischen Proteinen und reduziert damit die Steifigkeit von Geweben [79]. Dadurch konnte bei Patienten mit diastolischer Herzinsuffizienz die diastolische Herzfunktion verbessert werden, die linksventrikuläre Hypertrophie vermindert werden und die Lebensqualität erhöht werden [79]. In diabetischen Ratten wurde durch die Verabreichung von ALT-711 die arterielle Steifigkeit und bei Hunden die altersassoziierte linksventrikuläre Steifigkeit vermindert und damit die Herzfunktion verbessert [80, 81]. Diese beispielhaft genannten Interventionsmöglichkeiten zeigen das große Potenzial von AGEs und sRAGE als mögliche Ziele für die Prävention und Therapie von AGE-assoziierten Erkrankungen [1].

Ein Problem bei der Nutzung von AGEs als therapeutisches Ziel oder Biomarker in der Klinik und Forschung stellen die uneinheitlichen Messmethoden der AGEs dar. Zum einen kann man die AGE-Konzentrationen in der Haut und im Blut messen, was wie bereits besprochen einen Unterschied macht. Zum anderen existieren jedoch auch mehrere Möglichkeiten der AGE-Messung im Plasma, zum Beispiel die Messung durch verschiedene ELISA oder die Fluoreszenzmessung [82]. AGEs sind eine sehr heterogene Gruppe mit großen strukturellen Unterschieden und noch nicht alle sind identifiziert [2, 83]. Durch die verschiedenen Messmethoden werden verschiedene AGEs gemessen, die möglicherweise unterschiedliche pathologische Eigenschaften haben und mit verschiedenen pathologischen Zuständen assoziiert sind [82]. Es wurde gezeigt, dass die Messung der AGEs durch ELISA mit der Fluoreszenzmessung der AGEs korreliert und die Bildungskinetik und auch Assoziationen mit Alter, Nierenfunktion, oxidativem Stress und Glykierung verschiedener AGEs untereinander korrelieren [82] [84]. Zwischen den ELISA-Messungen mit verschiedenen Antikörpern bestand jedoch keine Korrelation [84].

Unsere Studie hat einige Stärken. Die Daten für diese Studie stammen von einer großen Kohorte der Allgemeinbevölkerung in einem breiten Altersspektrum von 45 bis 83 Jahren. Dadurch sind unsere Ergebnisse nicht nur für eine kleine Unterpopulation (z.B. Patientenkollektiv) gültig, sondern für eine große Gruppe der Allgemeinbevölkerung. Ein weiterer Vorteil ist, dass wir alle Analysen neben AGEs und sRAGE zusätzlich mit ihrem

Quotienten AGE/sRAGE durchgeführt haben, was laut neuen Erkenntnissen das AGE-RAGE System besser abbildet und damit wissenschaftlich indiziert ist [3]. Außerdem haben wir das große Studienkollektiv über einen sehr langen Zeitraum beobachtet und unseres Wissens das erste Mal die Assoziation von AGEs, sRAGE und AGE/sRAGE und Mortalität nicht nur als einzelnes, über die Zeit gemitteltes Hazard Ratio berichtet, sondern als zeitabhängiges Hazard Ratio. Dadurch war erstmals der Verlauf des Hazard Ratios über die Zeit sichtbar. Durch die große Menge an ermittelten Informationen über die Studienteilnehmer/innen hatten wir zudem die Möglichkeit für eine Vielzahl an relevanten Confoundern zu korrigieren.

Jedoch hat diese Studie auch Schwächen. sRAGE, wie auch RAGE bindet hauptsächlich AGEs wie CML, CEL und Hydroimidazolone 1 (MG-H1) [85-87]. Durch die Fluoreszenzmessung wird CML jedoch nicht gemessen, sondern vor allem Pentosidine [82]. Daher könnte der von uns berechnete AGE/sRAGE Quotient möglicherweise das Verhältnis zwischen den AGEs als Liganden und sRAGE als Rezeptoren nicht richtig widerspiegeln. Jedoch wurde aber auch gezeigt, dass verschiedene AGEs, fluoreszierend und nicht fluoreszierend, speziell auch CML und Pentosidine, miteinander korrelieren, was die Berechnung des Quotienten auf diese Weise rechtfertigt [82]. Eine weitere Schwäche ist die Ermittlung der aktuellen körperlichen Funktionsfähigkeit der Studienteilnehmer/innen durch einen Fragebogen und nicht durch objektive Messmethoden wie Handkraftmessung oder Laufgeschwindigkeit. Der SF-12 Gesundheitsfragebogen ist jedoch ein sensitives Werkzeug, um Unterschiede der körperlichen Funktionsfähigkeit zu ermitteln, das häufig in Studien eingesetzt wird [66, 88-91]. Weiterhin wurden die zwei Unterformen von sRAGE, cRAGE (proteolytic cleaved RAGE) und esRAGE (endogenous secretory RAGE), gemeinsam gemessen. Obwohl sie stark miteinander korrelieren, könnten sie verschiedene pathologische Eigenschaften besitzen [3, 92, 93]. Eine potenzielle Schwäche bei der Messmethode der AGEs ist die Messung von Plasma-AGEs bei nicht-nüchternen Patienten. Die AGE-Konzentrationen im Plasma werden durch AGE-reiches Essen beeinflusst und sinken erst nach frühestens 18-20 Stunden wieder auf ihre Anfangskonzentration [94, 95]. Bei der Adjustierung für die Nüchternzeit der Patienten und bei der Stratifizierung nach der Nüchternzeit haben wir jedoch keine Unterschiede gesehen, weshalb wir diesen Einfluss als gering einschätzen.

In der vorliegenden Studie wurden die Assoziationen von AGEs, sRAGE und AGE/sRAGE mit aktuellen und chronischen Outcomes verglichen und diskutiert. Weiterhin wurde ihre Bedeutung für die Klinik und Forschung betrachtet.

Das AGE-RAGE System spielt eine Rolle in der Pathogenese von einer Vielzahl von altersassoziierten Erkrankungen [14, 15, 21, 27, 28] . Dadurch haben AGEs, sRAGE und AGE/sRAGE ein großes Potenzial als mögliche Biomarker oder Therapieziele.

In dieser Studie haben wir gezeigt, dass erhöhte Werte von AGEs und AGE/sRAGE mit einer verminderten körperlichen Funktionsfähigkeit bei Frauen assoziiert sind. Bei Männern fanden wir jedoch keine Assoziationen und auch sRAGE war nicht mit körperlicher Funktionsfähigkeit bei Männern oder Frauen assoziiert. Weiterhin haben wir keine Assoziationen zwischen AGEs, sRAGE und AGE/sRAGE und Mortalität in einem Beobachtungszeitraum von 12 Jahren gesehen. Auch innerhalb der 12 Jahre hat sich dieses Ergebnis nicht verändert.

Diese Ergebnisse zeigen, dass das AGE-RAGE System durchaus mit aktuellen Outcomes assoziiert ist, auch wenn wir diese Assoziationen nur für Frauen zeigen konnten. Wir konnten keine Assoziationen mit den chronischen Outcome Mortalität sehen, was möglicherweise mit der Messung der AGEs im Plasma und nicht in der Haut zu begründen ist.

Zum aktuellen Zeitpunkt haben AGEs und sRAGE noch keine große Relevanz als Therapiezeile oder Biomarker im klinischen Alltag. Dafür ist ein genaueres Verständnis der Mechanismen nötig, mit denen AGEs und sRAGE in die pathologischen Prozesse der verschiedenen Erkrankungen involviert sind. Außerdem ist die weitere Aufklärung über die chemischen Strukturen der verschiedenen AGEs und deren standarisierte Messung notwendig, um die Ergebnisse vergleichbar zu machen. Falls das gelingt und auch weitere Interventionsstudien erfolgreich sind, können AGEs und sRAGE in Zukunft wichtige Biomarker und Ziele bei der Behandlung und Prävention von altersbedingten Erkrankungen darstellen.

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4 Thesen

Erhöhte Konzentrationen von Advanced Glykation End Products (AGEs) und erhöhte Quotienten aus AGEs und ihren löslichen Rezeptoren sRAGE (AGE/sRAGE) sind mit verminderter körperlicher Funktionsfähigkeit nur bei Frauen assoziiert.

AGEs sind unterschiedlich mit altersbedingten Erkrankungen/ Outcomes bei Männern und Frauen assoziiert.

AGEs, sRAGE und AGE/sRAGE sind nicht mit Mortalität im Beobachtungszeitraum von 12 Jahren assoziiert.

Im Blutplasma gemessene AGEs sind bessere Biomarker für aktuelle Outcomes, als für chronische Outcomes.

sRAGE ist weder mit körperlicher Funktionsfähigkeit noch mit Mortalität assoziiert.

sRAGE allein ist kein universeller Biomarker.

Der AGE/sRAGE Quotient ist den AGEs und sRAGE allein als Biomarker für körperliche Funktionsfähigkeit und Mortalität nicht überlegen.

AGEs und sRAGE haben großes Potenzial als Biomarker oder Ziele für die Therapie oder Prävention von altersbedingten Erkrankungen.

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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

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Abstract

Background: Advanced glycation end products (AGEs), modifications of proteins or amino acids, are increasingly produced and accumulated with age-related diseases. Recent studies suggested that the ratio of AGEs and their soluble receptor (sRAGE) is a more accurate biomarker for age-related diseases than each separately. We aim to investigate whether this also applies for physical functioning in a broad age-spectrum.

Methods: AGE and sRAGE levels, and physical functioning (SF-12 questionnaire) of 967 men and 812 women (45–83 years) were measured in the CARLA study. We used ordinal logistic regression to examine associations between AGEs, sRAGE, and AGE/sRAGE ratio with physical functioning in sex- and age-stratified models.

Results: Higher levels of AGEs and AGE/sRAGE ratio were associated with lower physical functioning only in women, even after consideration of classical lifestyle and age-related factors (education, BMI, smoking, alcohol consumption, diet, creatinine clearance, diabetes mellitus, lipid lowering and antihypertensive drugs) (odds ratio (OR) =0.86, 95%confidence interval = 0.74–0.98 and OR = 0.86, 95%CI = 0.75–0.98 for AGEs and AGE/sRAGE ratio respectively). We could not demonstrate a significant difference across age.

Conclusions: We showed a sex-specific association between physical functioning and AGEs and AGE/sRAGE, but no stronger associations of the latter with physical functioning. Further investigation is needed in the pathophysiology of this association.

Keywords: Advanced glycosylation, Physical function, Biomarker, Disability

Background

There is a natural decline in physical functioning with increasing age. This leads to loss of autonomy and eventually need of long-term care. Decline of physical functioning is also associated with multi-morbidity, increased mortality [1], and cognitive impairment [2, 3]. However, the decline in physical functioning has a high inter-individual variability, related to the difference between "biological" and "chronological" age [4]. Advanced glycation end products (AGEs) are considered reliable biomarkers of biological age [5]. AGEs are the irreversible products of the Maillard reaction, a non-enzymatic reaction of reducing sugar with long living proteins and amino acids [6]. They accumulate during normal aging, but also with age-related diseases [7, 8]. A decline in physical functioning has been associated with higher concentrations of AGEs in several studies [9, 10]. Further studies showed the value of AGEs for the prediction of developing disability and severe walking disability [11].

The mechanism of action of AGEs is through non-receptor mediated alterations of protein properties and increase of inflammatory factors by binding to the receptor of AGEs (RAGE). The RAGE is a cell-bound, multi-ligand receptor, which leads to the

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transcription of pro-inflammatory genes [12]. The sRAGE is the soluble form of RAGE in the blood and has no transmembrane and intracellular domain. sRAGE might be involved in the feedback regulation of the toxic effects of RAGE signaling, hence sRAGE is considered the natural decoy of AGEs [13]. Consistently, several studies suggested that the AGE/sRAGE ratio is stronger than AGEs alone associated with age-related diseases like atherosclerosis [14], endothelial dysfunction [15], hyperthyroidism [16], and end-stage renal disease [17]. Thus, it has been suggested that the ratio is more prone to decline with age than each of the components alone [18]. However, to our knowledge, no study has yet investigated whether this is also true for physical functioning. Moreover, existing studies about the association between AGEs and physical functioning exclusively included participants at advanced age (65 years and above) [9–11, 19]. Therefore, there is a lack of knowledge whether this relationship exists also in younger participants.

Our aim was to assess the association of AGEs, sRAGE, and AGE/sRAGE ratio with physical functioning using data from a population based study in a broad age range (45 to 83 years). We hypothesize that there is variation in the above association across the wider age range (particularly, that the associations might be stronger at older ages). Moreover, we await a stronger association between physical functioning and the AGE/sRAGE ratio than with each of the components alone.

Methods

Study population

Cross sectional data from the baseline examination of a total of 1779 participants (967 men and 812 women) of the CARLA study were included in the present analysis. The CARLA-study is a cohort recruited from the general population of the city Halle in Germany [20]. The data collection included a computer-assisted personal standardized interview and medical examinations.

Outcome: physical functioning (PF)

We used the physical functioning sub-scale of the SF-12 (Medical Outcomes Study Short-Form 12-items [21]) to assess limitations in daily life due to health problems. This subscale consists of two items: impairment in performance of moderate activities and impairment in climbing stairs. The two items had three response categories: “severe limitation”, “minor limitation” and “no limitation”. Following the standard procedures [21], both items were combined to create a physical functioning sub-scale with discrete values between 0 and 100 (0, 25, 50, 75, and 100), where 0 indicates severe limitations and 100 no limitations. Additionally, we conducted analyses with the continuous physical component score (PCS) of the SF-12 questionnaire, which includes information on general health situation, physical functioning, bodily pain, role functioning, mental health, vitality and social functioning [21]. It ranges from 0 to 100, where 0 indicates worst and 100 best health conditions.

Exposure: AGE and sRAGE measurement

The AGE -specific fluorescence and the sRAGE levels were determined as published before for non-fasting plasma samples of the CARLA cohort participants [22]. Human plasma was thawed, centrifuged at 20,000 g for 3 min at room temperature and diluted 1:20 in PBS (optimal dilution was tested before). One hundred microliter of each diluted sample was transferred to each well of a black 96-well microplate (Greiner, Frickenhausen, Germany). AGE-related fluorescence was measured at least three times on a FLUOstar OPTIMA reader (BMG Labtechnologies, Offenburg, Germany) at 370 +/− 10 nm excitation and 440 +/− 10 nm emission, and the results of the three measurements were averaged. Glucose-modified bovine serum albumin (AGE-BSA) was used for creating an internal standard curve and for plate-to-plate corrections. The results were provided in concentrations equivalent to AGE-BSA. Plasma sRAGE levels were determined using a commercially available enzyme-linked immunosorbent assay ELISA kit (Quantikine; R&D systems) according to the manufacturer’s protocol. Measurements were performed three times and the results were averaged.

Confounders

Covariates known to affect physical functioning, that were also associated with AGEs were identified from the literature [8, 23–25] and considered as confounders in the analyses. Information about age, years of formal education, smoking status, alcohol consumption, diabetes mellitus (self-reported physician-diagnosed diabetes mellitus or use of antidiabetic medication, ATC-code A10), and osteoporosis (osteoporosis or femoral neck fracture) was acquired by a computer-assisted interview. Information on diet was collected using the paper-version of the EPIC Food Frequency Questionnaire [26]. The use of medication in the previous 7-days was collected through the computer-based IDOM program of the KORA-study (study about the health status of the population in Augsburg, Germany) [27].

Anthropometrical measurements were measured according to standardized protocols. Height and weight were measured using the SECA 220 height measuring system and the SECA 701 digital scale and recorded to the nearest 0.1 cm and 100 g, respectively. BMI was defined as weight measured in kilograms divided by squared-height in meters. Creatinine was determined colorimetrically enzymatically

on the Modular system [20]. Creatinine clearance was estimated using the Cockroft-Gault-formula [28].

Statistical analysis

The AGE/sRAGE ratio was calculated from the raw AGE and sRAGE values. The AGE/sRAGE ratio, AGEs, and sRAGE were log-transformed. In order to facilitate comparison of estimates generated for AGEs, sRAGE and AGE/sRAGE ratio, we normalized the three measurements to units of standard deviation.

We used ordinal logistic regression models to estimate the odds ratio for the association of AGEs, sRAGE and AGE/sRAGE ratio (per one standardized unit increase), and physical functioning (25-points increase in the physical functioning-score). The proportional odds assumption was fulfilled (e.g. fully adjusted model AGEs-physical functioning men $\chi^2 = 45.9$, $p = 0.21$ and women $\chi^2 = 51.2$, $p = 0.09$) for the present data. Two models were used with an increasing number of confounders. Model 1 was age-adjusted. To test the additional influence of lifestyle and age-related factors, model 2 was additionally adjusted for years of education, BMI, smoking status, alcohol-consumption, diet, diabetes mellitus, creatinine-clearance, antihypertensive drugs and lipid lowering drugs. Analyses with separate models for lifestyle factors (years of education, BMI, smoking status, alcohol-consumption, diet) and age-related factors (diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs) showed similar results, thus we report only the results of the combined model.

As previous studies had shown sex-specific differences in the effects of AGEs [11, 29], we report the results sex-stratified. For the examination of the stability of the association between physical functioning and laboratory biomarkers across age, we stratified the results for people under the age of 65 and over/ at the age of 65. We used the age of 65 as the cut-point, as all existing studies about AGEs and physical functioning included participants over/at the age of 65. We additionally investigated whether there were differences in age as a continuum or in 10-year age-groups.

Seventy-five participants (3.2%) had missing data for exposure, outcome or confounding variables (see Additional file 1: Table S1). We used the Markov Chain Monte Carlo method with fully conditional specification in SAS for imputation of these data (as implemented in PROC MI) [30]. We generated a database containing 10 imputed datasets. Ordinal logistic regression models were estimated separately for each dataset and results were combined using the MIANALYZE procedure in SAS.

We conducted sensitivity analyses that were restricted to data from participants without missing values ($N = 930$ men/ 792 women) and to participants without extreme values of AGE- and sRAGE ($N = 951$ men; 797 women), defined as the highest 1% of the measurements. A third sensitivity analysis was performed on participants lacking diabetes mellitus or impaired renal function (creatinine clearance < 90 ml/ min) as these two diseases strongly increase AGE levels ($N = 488$ men/ 340 women) [7]. We used SAS version 9.4 (SAS Inc., Cary, North Carolina, USA), for the statistical analyses and R version 3.5.0 (R Core Team (2018) for the figures.

Results

Study population

Among the 1779 participants of the CARLA study, there were 54% men (967) and 46% women (812), with a mean age of 65 (standard deviation (SD) =10.23) and 64 (SD = 9.94), respectively. Table 1 shows that men had a higher alcohol-consumption, were more likely to smoke, and more often suffered from cardiovascular diseases (myocardial infarction, coronary bypass graft, percutaneously transluminal coronary angioplasty, stroke, and carotid-surgery), while women more often had osteoporosis. No sex differences could be observed for years of education, nutritional status, prevalence of diabetes mellitus, and kidney function (as measured by creatinine clearance).

Men showed significant higher median levels of plasma-AGEs and AGE/sRAGE ratios than women. In contrast, women had significant higher median levels of plasma-sRAGE. There was no association of AGEs, sRAGE and AGE/sRAGE with age among men and among women (see Additional file 1: Figure S1).

With respect to physical functioning, men reported fewer limitations than women. Almost a third of the men vs. almost half of the women felt at least minor limitations in moderate activities and 44% men vs. 58% women felt limited in climbing stairs. Participants aged 65 years or above were more often slightly or severe limited in moderate activities and climbing stairs than younger participants (Additional file 1: Figure S2).

Associations between AGEs, sRAGE, and AGE/sRAGE levels and physical functioning

Neither raw nor adjusted ordinal logistic regression models showed significant associations between AGEs, sRAGE, or the AGE/sRAGE ratio and physical functioning for men (Fig. 1 and Additional file 1: Table S2). The odds ratios (OR) ranged from 0.97 (95% confidence interval (CI) 0.85–1.10) for sRAGE to 1.05 (95%CI 0.92–1.19) for the AGE/sRAGE ratio. For women, higher AGE levels were associated with lower physical functioning in the fully adjusted model (OR = 0.86, 95%CI = 0.74–0.98). Similarly, in the fully adjusted model, higher AGE/sRAGE ratios were associated with lower physical functioning (OR = 0.86, 95%CI = 0.75–0.98). Higher levels of sRAGE

Table 1 Characteristics of the CARLA study population

	Men ($n = 967$)	Women ($n = 812$)
Age, mean ± SD (years)	65 ± 10.23	64 ± 9.94
< 65 years, N (%)	487 (50.36)	455 (56.03)
> = 65 years, N (%)	480 (49.64)	357 (43.97)
BMI, mean ± SD, (kg/m^2)	28.15 ± 4.08	28.54 ± 5.36
Smoker, N, yes (%)	225 (23.27)	119 (14.66)
Food index[a], mean ± SD	14.52 ± 3.20	16.44 ± 3.16
Diabetes mellitus, N (%)	154 (15.93)	120 (14.78)
Cardiovascular disease, N (%)	153 (15.82)	48 (5.91)
Osteoporosis, N (%)	64 (6.62)	142 (17.49)
Creatinine clearance, median (P25/P75) (ml/min)	97.22 (76.61/119.41)	88.95 (71.76/109.86)
AGE-levels, median (P25/P75) (relative units)	12,289 (9548/14796)	11,385 (8574/13712)
sRAGE-levels, median (P25/P75) (pg/ml)	827.62 (604.41/1107.88)	964.49 (706.93/1279.58)
AGE/sRAGE, median (P25/P75)	14.46 (10.01/21.09)	11.38 (8.19/16.37)
Limitations in moderate activities, N (%)		
Severe limitation	61 (6.31)	78 (9.61)
Minor limitation	246 (25.44)	305 (37.56)
No limitation	660 (68.25)	429 (52.83)
Limitations in climbing stairs, N (%)		
Severe limitation	69 (7.14)	90 (11.08)
Minor limitation	361 (37.33)	385 (47.41)
No limitation	537 (55.53)	337 (41.50)

Note. *CRP* High sensitive C-reactive protein, *BMI* Body Mass Index, *AGEs* Advanced glycation end products, *sRAGE* Soluble receptor of AGEs, *SD* Standard deviation, *P25/P75* 25th/75th percentile
[a]score 0–30, high score indicates healthier nutrition

tended to be associated with higher physical functioning for women (OR = 1.06, 95%CI = 0.93–1.21) (Fig. 1 and Additional file 1: Table S2). There were similar results for AGEs, sRAGE, and AGE/sRAGE in the fully adjusted models in complete case analysis and sensitivity analyses without extreme values (Additional file 1: Figure S3, Table S3, Figure S4 and Table S4), and with the PCS as outcome (Additional file 1: Figure S5 and Table S5). Even the analysis of the subsample without diabetes mellitus and impaired renal function provided similar results (Additional file 1: Figure S6 and Table S6).

Association between AGEs, sRAGE, and AGE/sRAGE levels and physical functioning in different age groups

Figure 2 and Additional file 1: Table S2 show the OR for men and women in the age groups: < 65 years and > =65 years. We saw no significant differences in the associations between the two age groups for men or women. An analysis with 10-years age-groups or age as a continuous variable showed similar results (Additional file 1: Figure S7).

Discussion

We confirmed results from previous studies that found that higher plasma-AGE values are associated with lower physical functioning [9–11, 19]. However, we found this association only in women, but not in men. Furthermore, we could not confirm the superiority of AGE/sRAGE ratio over AGEs that had been observed for other health outcomes with respect to their association with physical functioning. We could not demonstrate a difference across age groups (Fig. 2). The sex-specific result of our study is consistent with studies in diabetic [29] and non-diabetic [31] middle-aged participants (aged 45 to 64 years), which showed a stronger association of AGEs with mortality in women than in men. It was suggested that AGEs have stronger deleterious effects on participants with a "healthier" life-style and thus a lower risk of cardiovascular diseases, i.e. non-smokers, subjects with low alcohol consumption and higher

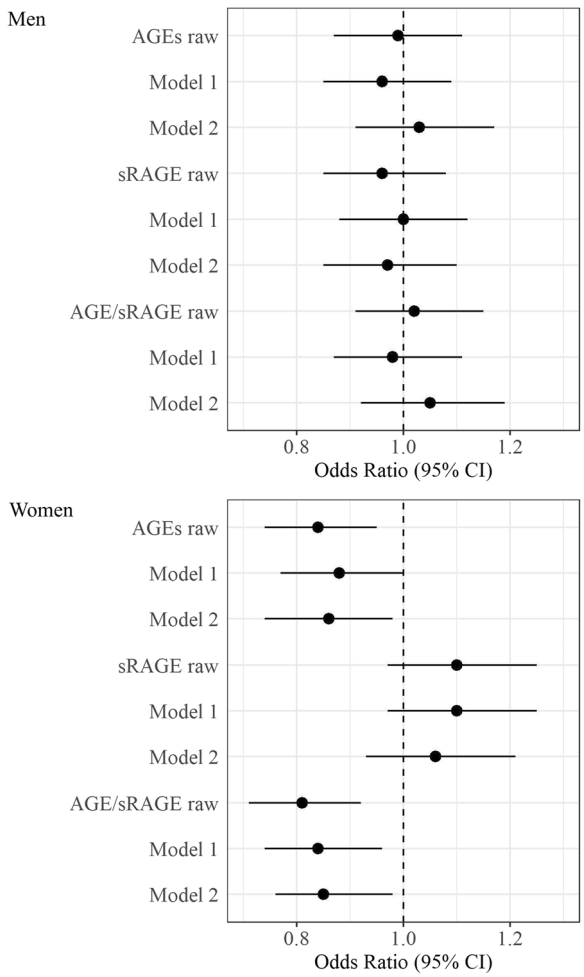

Fig. 1 Association between physical functioning and AGEs, sRAGE and AGE/sRAGE for men and women. Ref.: PF = 0 (severe limitations in physical functioning). Model 1: age; Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs. AGEs = advanced glycation end products; sRAGE = soluble receptor of advanced glycation end products; PF = physical functioning; CI = confidence interval

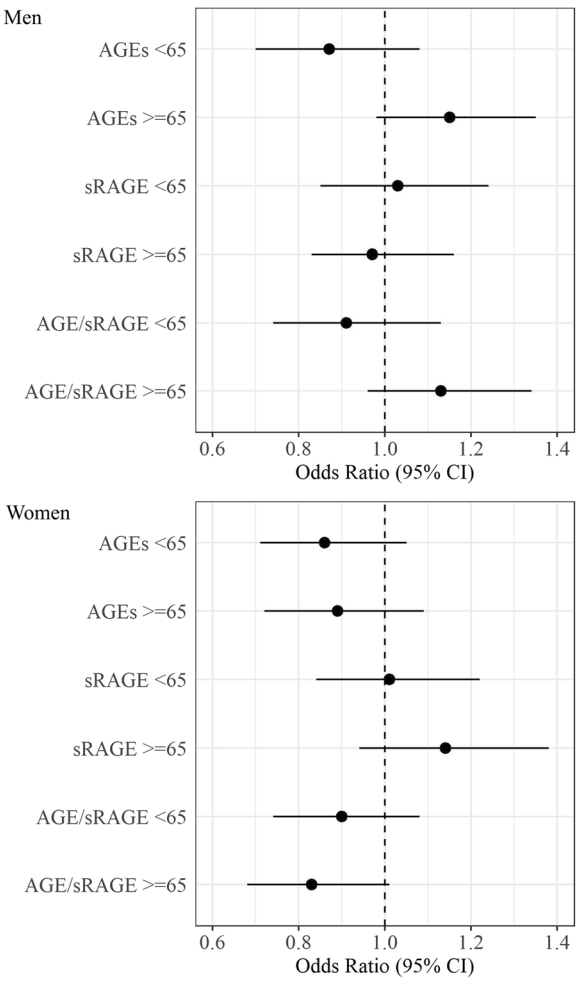

Fig. 2 Association between physical functioning and AGEs, sRAGE and AGE/sRAGE for men and women, age-stratified. Age-stratification for men: age < 65 *N* = 487, age > = 65 *N* = 480; and women: age < 65 *N* = 455, age > = 65 *N* = 357. Ref.: PF = 0 (severe limitations in physical functioning). Model 1: age; Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs. AGE = advanced glycation end products; sRAGE = soluble receptor of advanced glycation end products; PF = physical functioning; CI = confidence interval

HDL-cholesterol levels, whereas for participants with an "unhealthy" lifestyle there might be no additional negative effect of AGEs to the classical risk factors (competing risk factors) [29, 31]. This is also true for the CARLA study, where women had a lower risk of several chronic illnesses and a lower prevalence of cardiovascular disease. Although, we adjusted for a number of life-style factors, probably there is residual confounding, like physical activity or housework that cannot be considered and is different between the two sexes. Another explanation could be genetic differences between sexes. Some studies report the association of AGEs with the mechanical properties of bones and their formation process, which could also affect physical functioning [32, 33]. A further study showed a sex-dependent effect of AGEs on vertebral structures and function only in female mice [34]. In our study, we observed higher AGE levels in participants with osteoporosis and higher prevalence of osteoporosis in women (data not shown). However, in regression models, we saw only minor differences in OR and CI when additional adjusting for osteoporosis (Additional file 1: Table S7), possibly due to too low numbers of participants with osteoporosis or residual confounding due to physical activity.

Further, another study investigated the association of AGEs with frailty in an older population (65 years and older) and found an association only in men [11]. They concluded that women at higher risk for frailty and with

an unhealthier life-style or with poor health do not participate in cohort studies and thus do not contribute data [11]. Although this selection effect may occur in every cohort, the CARLA cohort is comparable in health-outcomes to the general population reflected in the micro census [35].

It has been hypothesized that the soluble form of the receptor (sRAGE), has a protective effect against the non-receptor and receptor mediated effects of AGEs, which lead to increased muscle stiffening, endothelial dysfunction and inflammation [8, 23, 36]. Therefore, it was supposed that the AGE/sRAGE ratio is more sensitive to age-related decline than each of the components alone [18, 23, 36]. Our results showed that a higher AGE/sRAGE ratio was associated with lower physical functioning in women. To our knowledge no previous study investigated the association between AGE/sRAGE-ratio and physical functioning. However, several studies showed that the AGE/sRAGE-ratio is higher in patients with age-related diseases than in healthy participants. Two studies with cardiac adult and older adult outpatients showed that the severity of atherosclerosis [14] and endothelial dysfunction [15] was associated with a higher AGE/sRAGE-ratio. Additionally, case-control comparisons showed that hyperthyroidism and end-stage renal disease patients had a higher AGE/sRAGE-ratio as compared to healthy adults, whereas there were no differences in levels of AGEs alone [16, 17]. However, in our study the association between the AGEs/sRAGE ratio and physical functioning showed a similar effect as the association of AGEs and physical functioning. Thus, the influence of sRAGE on AGEs doesn't change the association with physical functioning as much as for chronic diseases. This could be due to the too small effect of endogenous sRAGE on the effect of AGEs. It was hypothesized that the concentrations of endogenous sRAGE are 1000 times lower than needed to act as a sufficient decoy for AGEs [15]. However, in animal models, exogenous administration of sRAGE leads to the suppression of inflammation [37] and attenuation of early acceleration of artherosclerosis [12], showing its role as a possible therapeutic target. Probably higher, exogenous administrated sRAGE levels would influence the association of AGEs to physical functioning.

Further, we evaluated the associations between AGEs, sRAGE and AGE/sRAGE ratio and physical functioning at different life-stages and did not find any difference across age. This finding is interesting as previous studies addressed mainly older participants and extending the evidence to younger age groups appears useful. In women, AGEs are apparently associated with older age, but their association with physical functioning is stable and not restricted to older ages. This supports the notion of AGEs as a marker of biological age, being independent of chronological age – and a potential target of interventions [38].

Our study has several strengths. We included a large number of men and women at a broad age-range from the general population, also including younger age-groups which previously had not been studied before. Additionally, to our knowledge, it is the first study, which calculated the AGE/sRAGE ratio to investigate its association with physical functioning. Moreover, we adjusted our analyses for a variety of important confounders in the association between outcome and exposure. The main limitation of the current study is that we rely on self-reported impairment of physical functioning. Instead of using an objective measurement, such as walking speed or grip strength we measured physical functioning by questions of the SF-12 questionnaire. This might attenuate the strength of association observed in our study. Nevertheless, the SF-12 is a valid questionnaire for measuring physical functioning, reproducing more than 90% of the variance of the long form (SF-36 questionnaire), which sensitively measures physical functioning differences [21, 39, 40]. Moreover, the measurement of plasma-AGEs with fluorescence method could only assess fluorescent AGEs (e.g. pentosidine). However, fluorescence of AGEs correlates also with levels of non-fluorescent AGEs (e.g. N(6)-Carboxymethyllysine) [41] and the results of fluorescence measurement correlate with the results of ELISA measurement of AGEs [42]. Additionally, other fluorescent substances in the plasma could influence the measurement and make it inaccurate. Moreover, the plasma-AGE levels could be strongly influenced by the intake of AGE rich food before the measurement [43]. However, due to the small differences in AGE-levels we observed when stratifying for duration of fasting-time (data not shown), we considered this influence as low.

Conclusions

In conclusion, this study shows an association between AGEs and AGE/sRAGE ratio and physical functioning only in women. The reasons for the observed sex-differences in the associations still need to be elucidated in further studies. There was no considerable difference in terms of effect size between the association of AGEs, and AGE/sRAGE ratio and physical functioning in contrast to previously suggested better performance of the ratio. We also did not observe differences across age, which supports the notion, that AGEs are a marker of biological rather than chronological age.

Additional file

Additional file 1: Table S1. Number of missing values for variables of interest. **Figure S1.** Scatterplots showing the association between standardied and log-transformed AGEs, sRAGE and AGE/sRAGE with chronological age **Figure S2.** Physical functioning of the study

population stratified for sex and age. **Table S2.** Association between physical functioning and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio. **Figure S3.** Association between physical functioning with AGEs, sRAGE and AGE/sRAGE ratio for men and women in complete cases without missings in exposure, outcome or confounding variables. **Table S3.** Complete case analysis. **Figure S4.** Association between physical functioning with AGEs, sRAGE and AGE/sRAGEratio for men and women in a subsample without AGE or sRAGE extreme values (higher 1%). **Table S4.** Analysis without AGE and sRAGE extreme values (higher 1%). **Figure S5.** Association between physical component scale with AGEs, sRAGE and AGE/sRAGE ratio for men and women. **Table S5.** Association between physical component scale and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio. **Figure S6.** Association between physical functioning with AGEs, sRAGE and AGE/sRAGE ratio for men and women in a subsample without diabetes mellitus or impaired renal function. **Table S6.** Association between physical functioning and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio in a subsample without diabetes mellitus and impaired renal function. **Figure S7.** Association between physical functioning with AGEs, sRAGE and AGE/sRAGE ratio for men and women stratified for 10-year age-groups. **Table S7.** Association between physical functioning and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio with and without adjusting for osteoporosis.

Abbreviations

AGEs: Advanced glycation end products; CARLA: CARdiovascular disease, Living and Ageing in Halle; CI: Confidence interval; OR: Odds ratio; PCS: Physical component score of SF-12; PF: Physical functioning; SD: Standard deviation; SF-12: Medical Outcomes Study Short-Form 12 items; sRAGE: Soluble receptor for advanced glycation end products

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Authors' contributions

RM conceived the presented study. DT, KHG and AK were responsible for data collection. AS measured the biomarkers. HE, NK and MEL performed the data analysis and wrote the first draft. All authors contributed and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author (Rafael Mikolajczyk) on reasonable request.

Ethics approval and consent to participate

All CARLA participants gave their written informed consent. The local ethics committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and the State Data Privacy Commissioner of Saxony-Anhalt approved the study. The CARLA study conforms to the principles outlined in the declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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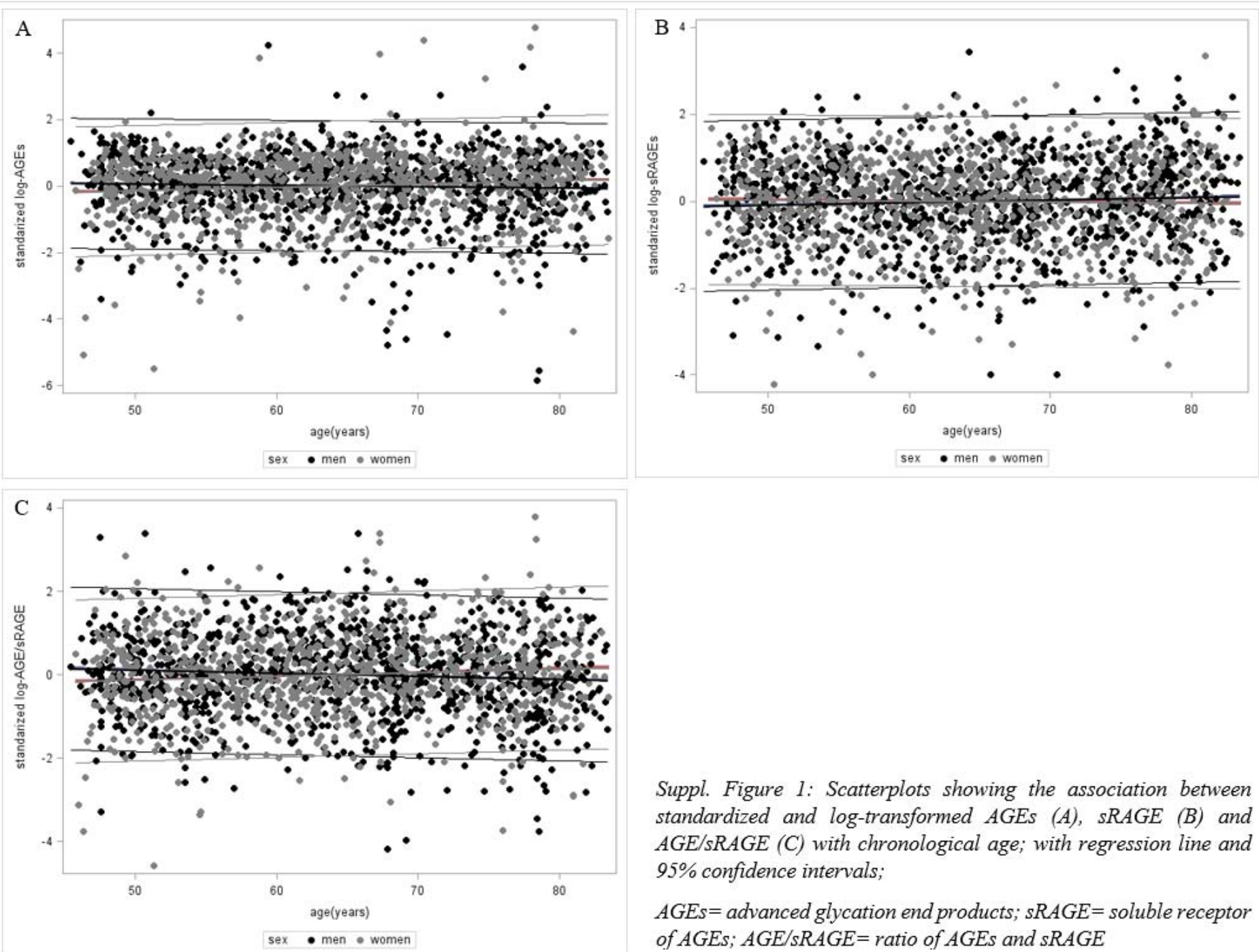
Publisher's Note

Zusatzmaterial Publikation 1

Suppl. Table 1. Number of missing values for variables of interest

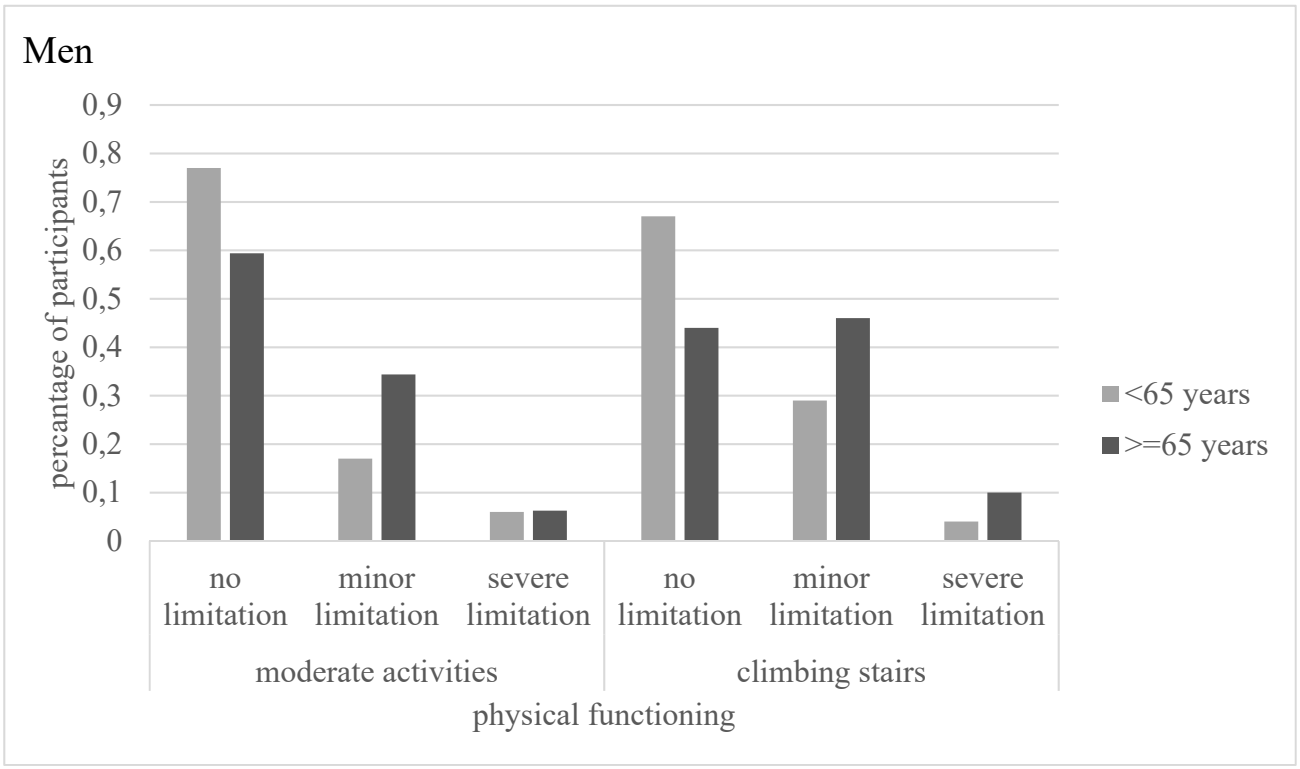
Variable	Number of missing values	
	men	women
AGEs	9	10
sRAGE	9	10
SF-12 Physical functioning item 1 (moderate activities)	23	9
SF-12 Physical functioning item 2 (climbing stairs)	14	7
SF-12 Physical summary scale (PCS)	55	45
Age	0	0
Years of education	0	0
BMI	0	0
Alcohol consumption	3	0
Smoking	1	0
Food-Frequency Index	2	0
Self-reported medical diagnose of diabetes mellitus	1	0
Self-reported medical diagnose of osteoporosis	6	14
Use of antihypertensive drugs	0	0
Use of lipid lowering drugs	0	0
Creatinine-clearance	7	5

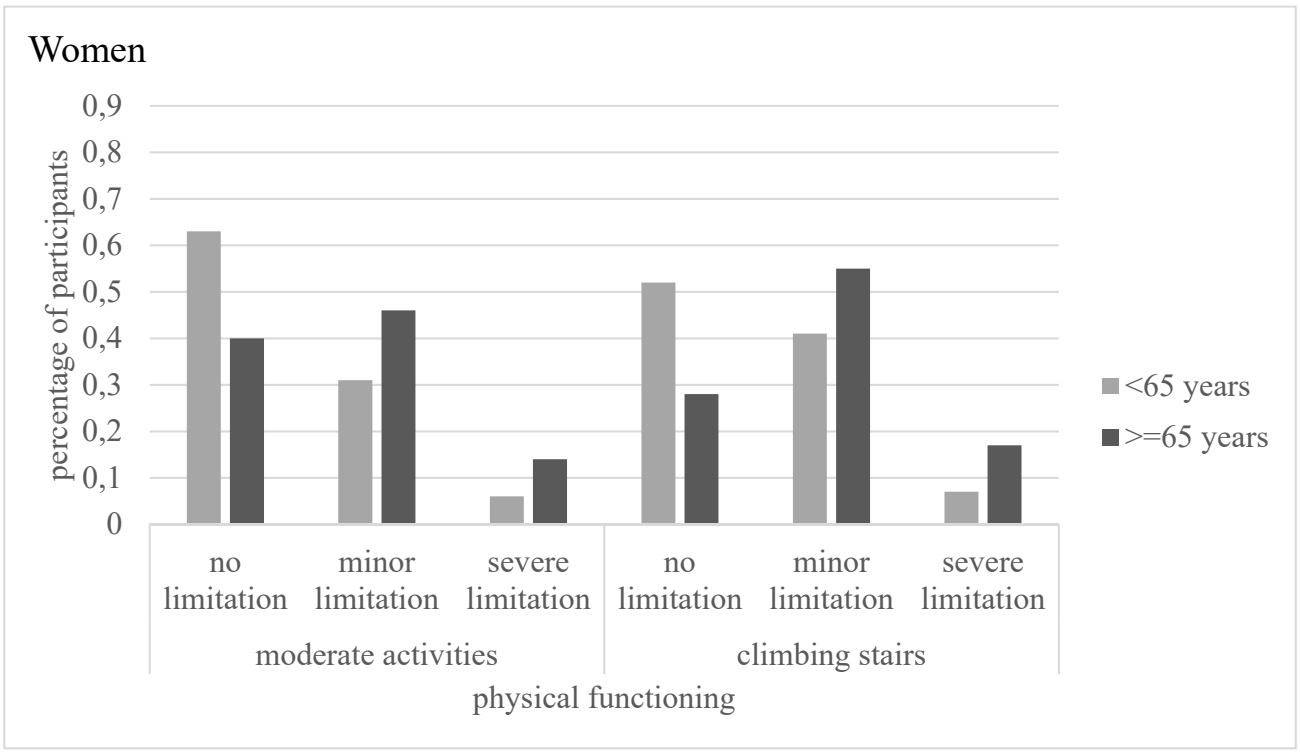
Notes. AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; BMI= Body mass index



Suppl. Figure 1: Scatterplots showing the association between standardized and log-transformed AGEs (A), sRAGE (B) and AGE/sRAGE (C) with chronological age; with regression line and 95% confidence intervals;

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE





Suppl. Figure 2: Physical functioning of the study population stratified for sex and age

N= men: 487 <65years; 480 >=65years; women: 455<65years; 357>= 65years

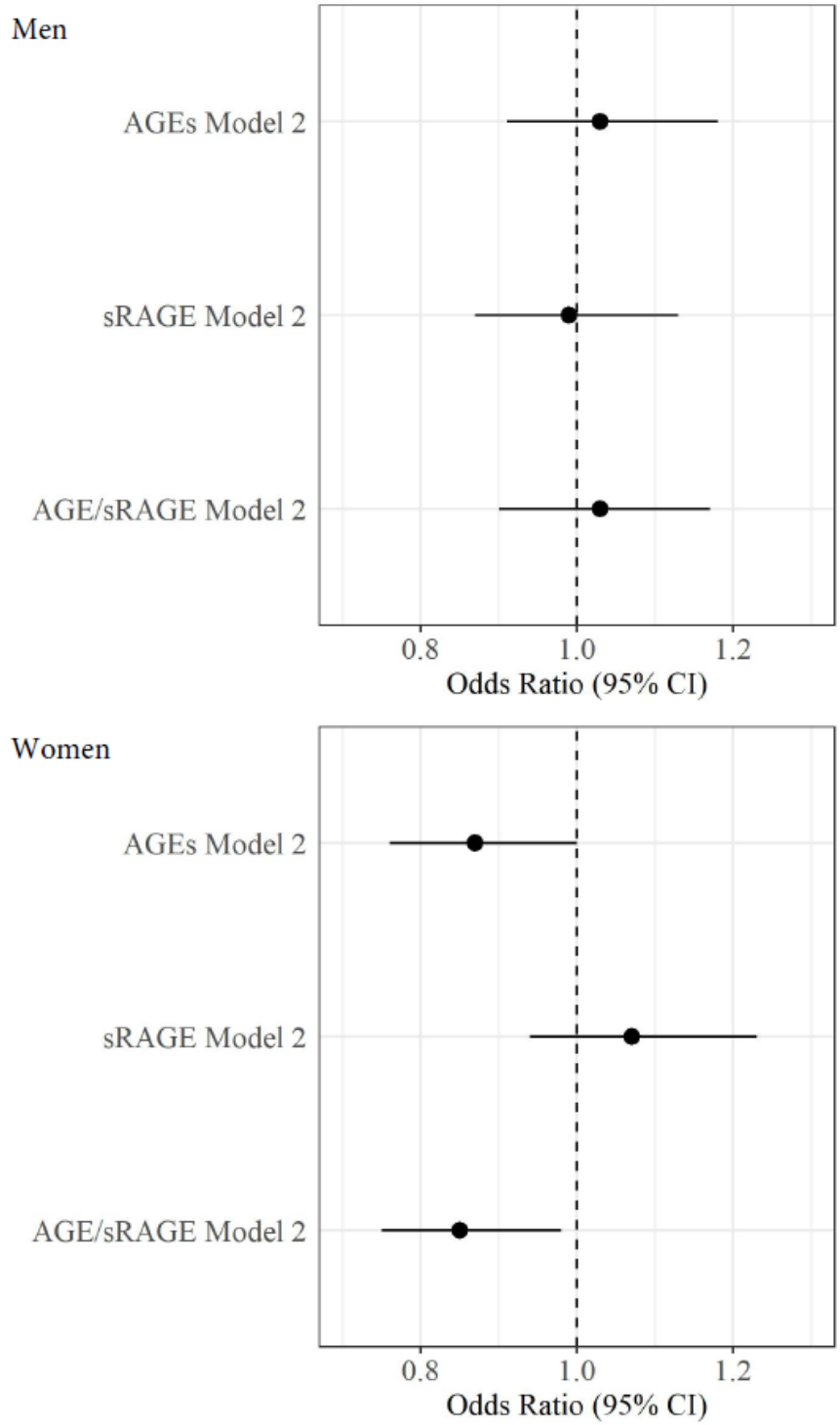
Suppl. Table 2. Association between physical functioning (PF) and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio, Odds ratios (95 % confidence interval)

	Raw		**Model 1***		**Model 2§**	
	Men	*Women*	*Men*	*Women*	*Men*	*Women*
	PF 0 = Ref.					
	Association between PF and AGEs					
All	0.99 (0.87; 1.11)	0.84 (0.74; 0.95)	0.96 (0.85; 1.09)	0.88 (0.77; 1.00)	1.03 (0.91; 1.17)	0.86 (0.74; 0.98)
<65	0.87 (0.71; 1.06)	0.93 (0.78; 1.11)	0.85 (0.69; 1.05)	0.95 (0.79; 1.13)	0.87 (0.70; 1.08)	0.86 (0.71; 1.05)
>= 65	1.03 (0.88; 1.20)	0.82 (0.67; 0.99)	1.04 (0.89; 1.21)	0.80 (0.66; 0.97)	1.15 (0.98; 1.35)	0.89 (0.72; 1.09)
	Association between PF and sRAGE					
All	0.96 (0.85; 1.08)	1.10 (0.97; 1.25)	1.00 (0.88; 1.12)	1.10 (0.97; 1.25)	0.97 (0.85; 1.10)	1.06 (0.93; 1.21)
<65	1.08 (0.90; 1.30)	1.09 (0.91;1.30)	1.05 (0.88; 1.27)	1.11 (0.93; 1.32)	1.03 (0.85; 1.24)	1.01 (0.84; 1.22)
>= 65	0.91 (0.78; 1.07)	1.12 (0.93; 1.35)	0.98 (0.83; 1.16)	1.09 (0.91; 1.32)	0.97 (0.82; 1.16)	1.14 (0.94; 1.38)
	Association between PF and AGE/ sRAGE					
All	1.02 (0.91; 1.15)	0.81 (0.71; 0.92)	0.98 (0.87; 1.11)	0.84 (0.74; 0.96)	1.05 (0.92; 1.19)	0.86 (0.75; 0.98)
<65	0.86 (0.71; 1.04)	0.89 (0.74; 1.06)	0.87 (0.72; 1.05)	0.88 (0.74; 1.06)	0.91 (0.74; 1.11)	0.90 (0.74; 1.08)
>= 65	1.09 (0.94; 1.28)	0.79 (0.65; 0.95)	1.05 (0.89; 1.23)	0.79 (0.65; 0.96)	1.13 (0.96; 1.34)	0.83 (0.68; 1.01)

*Notes: * Model 1: age; § Model 2: age, years of education ,BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;*

N= 967 men; 812 women;

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PF= total score physical functioning (SF-12)



Suppl. Figure 3: Association between physical functioning (PF) with advanced glycation end products (AGEs), soluble receptor of AGEs (sRAGE) and ratio of AGEs and sRAGE (AGE/sRAGE) for men (N=930) and women (N=792) in ***complete cases*** *without missings in exposure, outcome or confounding variables; Odds ratios (95 % confidence interval)*

Ref.: PF=0 (severe limitations in physical functioning)

Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;

CI= confidence interval

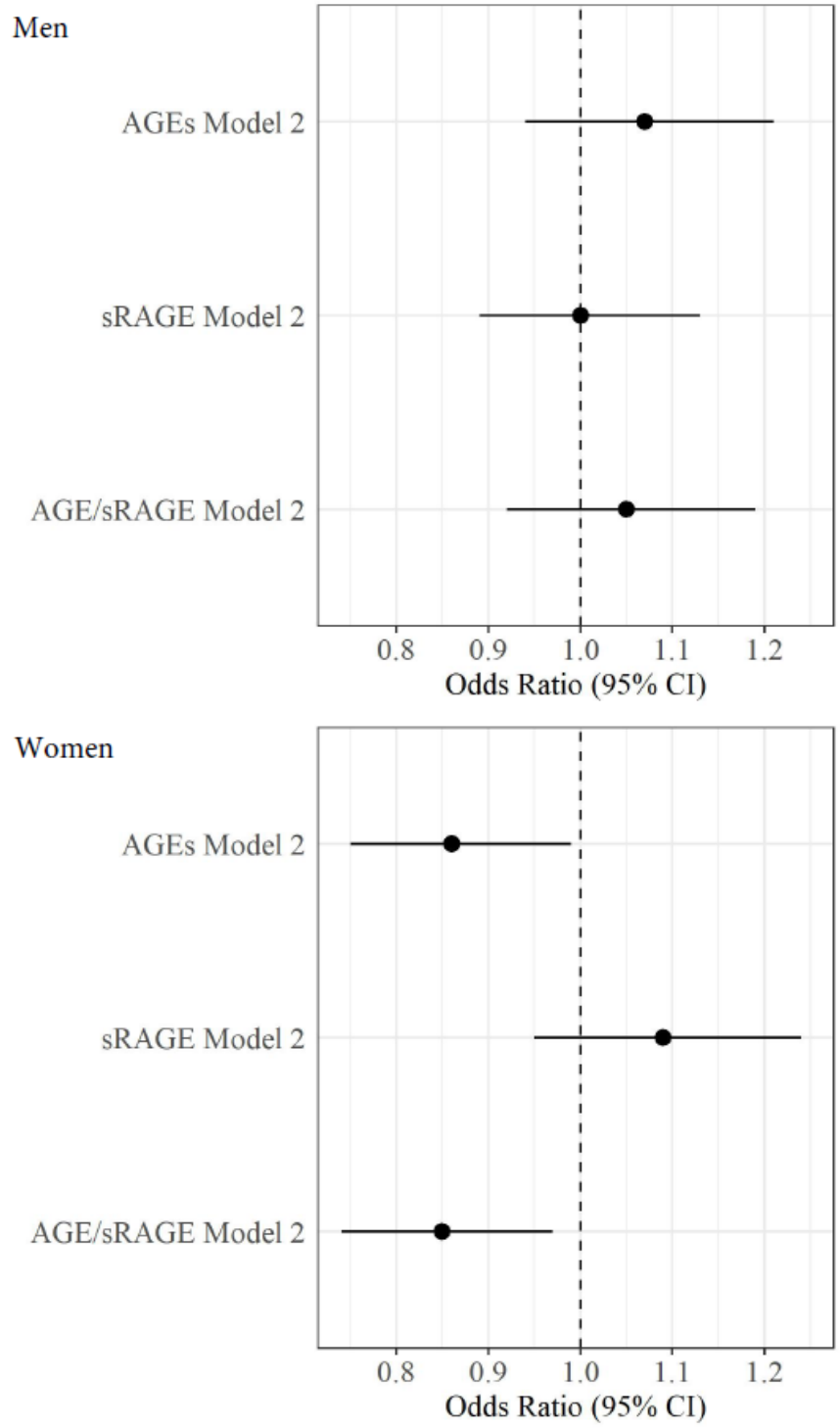
Suppl. Table 3. ***Complete case analysis****;*
Association between physical functioning (PF) and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio, Odds ratios (95 % confidence interval)

	Raw		Model 1*		Model 2§	
	Men	*Women*	*Men*	*Women*	*Men*	*Women*
	PF 0 = Ref.					
	Association between PF and AGEs					
All	0.98 (0.87; 1.11)	0.84 (0.74; 0.96)	0.96 (0.85; 1.09)	0.88 (0.77; 1.01)	1.03 (0.91; 1.18)	0.87 (0.76; 1.00)
	Association between PF and sRAGE					
All	0.97 (0.86; 1.10)	1.12 (0.98; 1.26)	1.01 (0.90; 1.15)	1.11 (0.97; 1.26)	0.99 (0.87; 1.13)	1.07 (0.94; 1.23)
	Association between PF and AGE/sRAGE					
All	1.01 (0.89; 1.14)	0.81 (0.71; 0.92)	0.96 (0.85; 1.09)	0.84 (0.74; 0.96)	1.03 (0.90; 1.17)	0.85 (0.75; 0.98)

*Notes: * Model 1: age; § Model 2: age, years of education ,BMI, alcohol consumption, smoking, diet , diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;*

N=930 men; 792 women

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PF= total score physical functioning (SF-12)



Suppl. Figure 4: Association between physical functioning (PF) with advanced glycation end products (AGEs), soluble receptor of AGEs (sRAGE) and ratio of AGEs and sRAGE (AGE/sRAGE) for men (N=951) and women (N=797) in a subsample ***without AGE or sRAGE extreme values*** *(higher 1%); Odds ratios (95 % confidence interval)*

Ref.: PF=0 (severe limitations in physical functioning)

Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;

CI= confidence interval

*Suppl. Table 4. Analysis **without AGE and sRAGE extreme values** (higher 1%)*

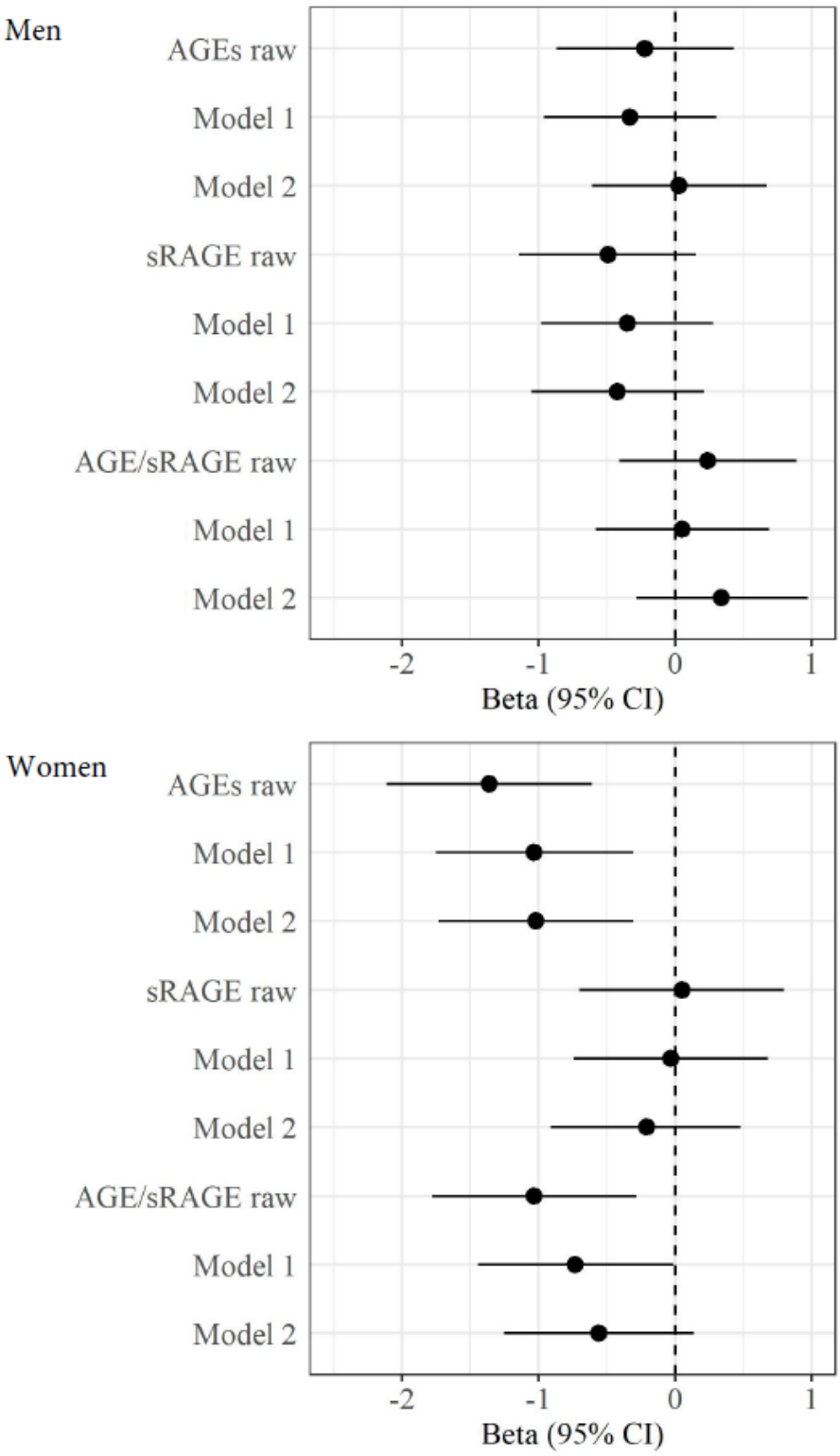
Association between physical functioning (PF) and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio, Odds ratios (95 % confidence interval)

Raw		Model 1*		Model 2§	
Men	*Women*	*Men*	*Women*	*Men*	*Women*
PF 0 = Ref.					
Association between PF and AGEs					
1.05 (0.93; 1.19)	0.86 (0.76; 0.98)	1.03 (0.91; 1.16)	0.89 (0.78; 1.01)	1.07 (0.94; 1.21)	0.86 (0.75; 0.99)
Association between PF and sRAGE					
1.02 (0.91; 1.15)	1.14 (1.00; 1.29)	1.06 (0.93; 1.19)	1.12 (0.98; 1.27)	1.00 (0.88; 1.13)	1.09 (0.95; 1.24)
Association between PF and AGE/ sRAGE					
1.02 (0.90; 1.15)	0.81 (0.71; 0.92)	0.98 (0.86; 1.10)	0.84 (0.73; 0.95)	1.05 (0.92; 1.19)	0.85 (0.74; 0.97)

*Notes: * Model 1: age; § Model 2: age, years of education ,BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;*

N=951 men; 797 women

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PF= total score physical functioning (SF-12)



Suppl. Figure 5: Association between ***physical component scale*** *(PCS) with advanced glycation end products (AGEs), soluble receptor of AGEs (sRAGE) and ratio of AGEs and sRAGE (AGE/sRAGE) for men (N=967) and women (N=812), Beta (β) (95 % confidence interval)*

Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;

CI= confidence interval

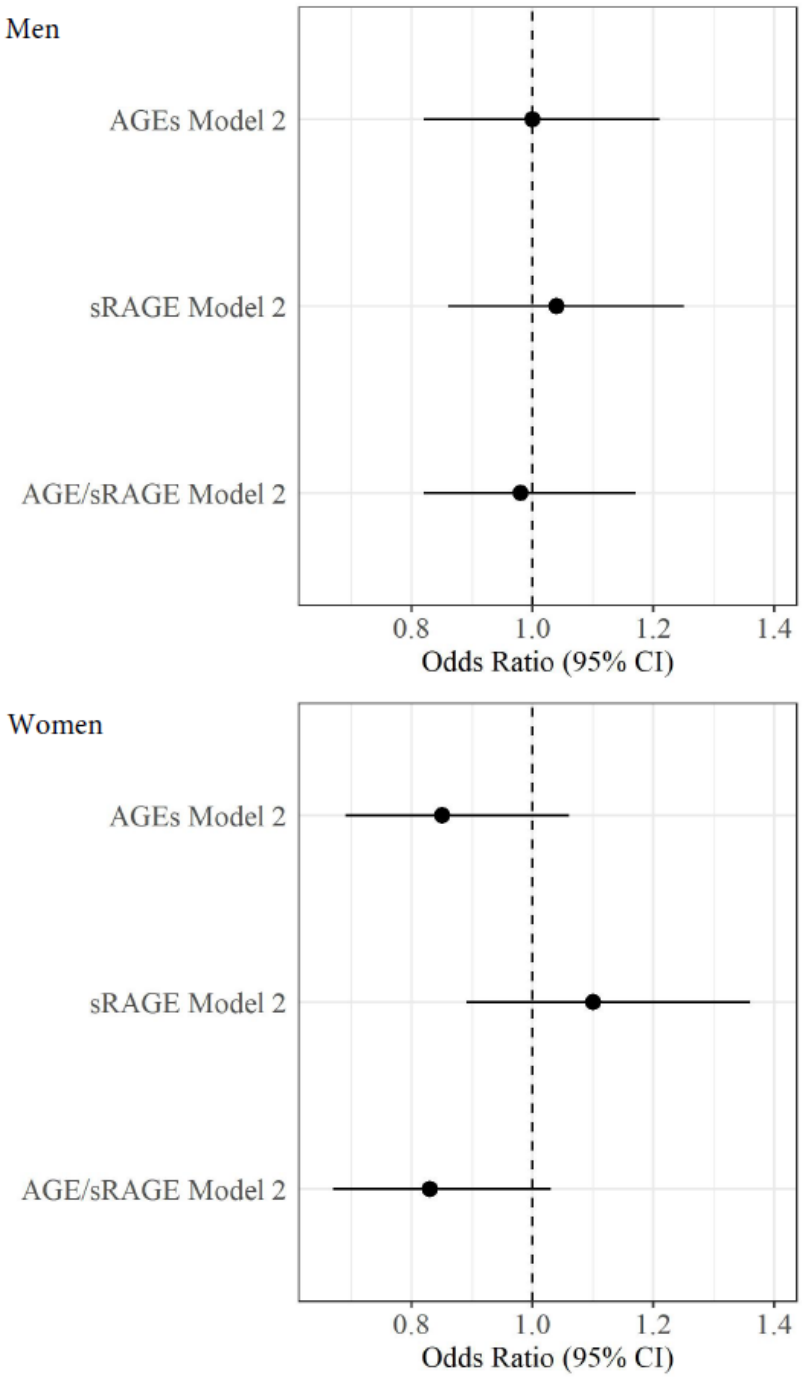
Suppl. Table 5. Association between ***physical component scale (PCS)*** *and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio, β (95 % confidence interval)*

Raw		Model 1*		Model 2§	
Men	*Women*	*Men*	*Women*	*Men*	*Women*
Association between PCS and AGEs					
0.22	-1.36	-0.33	-1.03	0.03	-1.02
(-0.87; 0.43)	(-2.11; -0.61)	(-0.96; 0.30)	(-1.75; 0.31)	(-0.61; 0.67)	(-1.73; -0.31)
Association between PCS and sRAGE					
-0.49	0.05	-0.35	-0.03	-0.42	-0.21
(-1.14; 0.15)	(-0.70; 0.80)	(-0.98; 0.28)	(-0.74; 0.68)	(-1.05; 0.21)	(-0.91; 0.48)
Association between PCS and AGE/ sRAGE					
0.24	-1.03	0.05	-0.73	0.34	-0.56
(-0.41; 0.89)	(-1.78; -0.28)	(-0.58; 0.69)	(-1.44; 0.01)	(-0.28; 0.97)	(-1.25; 0.14)

*Notes: * Model 1: age; § Model 2: age, years of education, BMI, alcohol consumption, smoking, diet , diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs;*

N= 967 men; 812 women

AGEs= advanced glycation end oroducts; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PCS = physical sum scale (SF-12).



Suppl. Figure 6: Association between physical functioning (PF) with advanced glycation end products (AGEs), soluble receptor of AGEs (sRAGE) and ratio of AGEs and sRAGE (AGE/sRAGE) for men (N=488) and women (N=340) in a ***subsample without diabetes mellitus or impaired renal function****, Odds ratios (95 % confidence interval)*

Ref.: PF=0 (severe limitations in physical functioning)

Model 2: age, years of education, BMI, alcohol consumption, smoking, diet, creatinine clearance, antihypertensive drugs, lipid lowering drugs;

CI= confidence interval

Suppl. Table 6. Association between physical functioning (PF) and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio in a ***subsample without diabetes mellitus and impaired renal function****, Odds ratios (95 % confidence interval)*

Raw		Model 1*		Model 2§	
Men	*Women*	*Men*	*Women*	*Men*	*Women*
PF 0 = Ref.					
Association between PF and AGEs					
0.98 (0.82; 1.17)	0.94 (0.77; 1.14)	0.96 (0.80; 1.15)	0.94 (0.77; 1.15)	1.00 (0.82; 1.21)	0.85 (0.69; 1.06)
Association between PF and sRAGE					
1.07 (0.89; 1.28)	1.16 (0.95; 1.41)	1.04 (0.87; 1.25)	1.15 (0.94; 1.40)	1.04 (0.86; 1.25)	1.10 (0.89; 1.36)
Association between PF and AGE/ sRAGE					
0.94 (0.79; 1.12)	0.85 (0.70; 1.04)	0.94 (0.79; 1.13)	0.86 (0.70; 1.05)	0.98 (0.82; 1.17)	0.83 (0.67; 1.03)

*Notes: * Model 1: age; § Model 2: age, years of education ,BMI, alcohol consumption, smoking, diet , creatinine clearance, antihypertensive drugs, lipid lowering drugs;*

N=488 men; 340 women

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PF= total score physical functioning (SF-12).

Suppl. Table 7. Association between physical functioning (PF) and log-transformed, standardized AGEs, sRAGE and AGE/sRAGE ratio ***with and without adjusting for osteoporosis****, Odds ratios (95 % confidence interval)*

	Model 2§		Model 2a	
	Men	*Women*	*Men*	*Women*
PF 0 = Ref.				
Association between PF and AGEs				
All	1.03 (0.91; 1.17)	0.86 (0.74; 0.98)	1.04 (0.92; 1.18)	0.87 (0.75; 1.00)
<65	0.87 (0.70; 1.08)	0.86 (0.71; 1.05)	0.85 (0.69; 1.06)	0.88 (0.72; 1.07)
>= 65	1.15 (0.98; 1.35)	0.89 (0.72; 1.09)	1.14 (0.97; 1.34)	0.88 (0.71; 1.08)
Association between PF and sRAGE				
All	0.97 (0.85; 1.10)	1.06 (0.93; 1.21)	0.97 (0.85; 1.10)	1.06 (0.93; 1.22)
<65	1.03 (0.85; 1.24)	1.01 (0.84; 1.22)	1.01 (0.83; 1.22)	1.01 (0.81; 1.19)
>= 65	0.97 (0.82; 1.16)	1.14 (0.94; 1.38)	0.97 (0.82; 1.16)	1.14 (0.94; 1.39)
Association between PF and AGE/ sRAGE				
All	1.05 (0.92; 1.19)	0.86 (0.75; 0.98)	1.06 (0.93; 1.20)	0.86 (0.75; 0.99)
<65	0.91 (0.74; 1.11)	0.90 (0.74; 1.08)	0.91 (0.74; 1.12)	0.91 (0.75; 1.10)
>= 65	1.13 (0.96; 1.34)	0.83 (0.68; 1.01)	1.14 (0.97; 1.35)	0.83 (0.68; 1.01)

Notes: § Model 2: age, years of education ,BMI, alcohol consumption, smoking, diet , diabetes mellitus, creatinine clearance, antihypertensive drugs, lipid lowering drugs; Model 2a: Model 2 + osteoporosis as confounder

N= 967 men; 812 women

AGEs= advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE: ratio of AGE and sRAGE; PF= total score physical functioning (SF-12).

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Association between advanced glycation end products, their soluble receptor, and mortality in the general population: Results from the CARLA study

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ABSTRACT

Background: Advanced glycation end products (AGEs) in the plasma are associated with a number of age-related diseases that possibly lead to reduced longevity. However, previous studies showed large inconsistencies in the association between AGEs or their soluble receptor (sRAGE) and mortality. We studied this association in a cohort study of general population and assessed the potential changes in this association over time.

Methods: We used data of 958 men and 802 women from the general population in Halle, Germany with a follow up of 12 years. The associations were assessed by means of Kaplan-Meyer survival curves and multivariable and time-varying Cox-regression.

Results: AGEs and sRAGE were either not or only weakly (and in the other direction than expected) associated with all-cause mortality after 12 years follow-up in men and women (AGEs: Hazard ratio (HR) = 0.93, 95% confidence interval (95%CI) = 0.83–1.05 for men; HR = 0.88, 95%CI = 0.74–1.05 for women; sRAGE: HR = 1.08, 95%CI = 0.95–1.23 for men; HR = 1.10, 95%CI = 0.92–1.30 for women). There was no change of the predictive values over the follow up time. Sub-analyses with participants with and without AGEs-related conditions (diabetes mellitus and decreased renal function), with age stratified groups (younger (< 65 years) and older (≥65 years) participants), with cardiovascular disease mortality as the outcome and the AGE/sRAGE ratio as predictor provided similar results.

Conclusions: Our findings suggest a lack of the expected association with mortality and contribute to the inconsistent findings for plasma-measured AGEs, sRAGE, and AGE/sRAGE ratio.

1. Introduction

One source of age-related decline has been proposed to be the accumulation of damaged macromolecules and cells, including advanced glycation end products (AGEs), as a result of increased production or inadequate elimination (Franceschi et al., 2018; Franceschi and Campisi, 2014). AGEs are the products of non-enzymatic glycation of proteins, lipids and amino acids with reducing sugar like glucose or fructose (Bucala and Cerami, 1992; Singh et al., 2001). They can be built endogenously which is increased by high blood-sugar concentrations or cigarette smoking (Bucala and Cerami, 1992) (Prasad et al., 2015). Additionally, they can be taken exogenously by the intake of AGE-rich food like grilled food or food, which is high in animal fat and proteins (Uribarri et al., 2010). The accumulation of AGEs seems to contribute to the age-related multisystem decline through two non-exclusive mechanisms: the alteration of proteins through cross-link formation of AGEs with long-living proteins, leading to sclerosis of renal glomeruli, thickening of capillary basement membrane and atherosclerosis development (Singh et al., 2001), and the alteration of signalling cascades through binding of AGEs to its receptor (RAGE) leading to an increase in inflammation and oxidative stress (Cavero-Redondo et al., 2018; Prasad and Mishra, 2018; Maillard-Lefebvre et al.,

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2009). AGE levels measured in blood have been identified as a promising aging biomarker. Increased AGE concentrations have been found in age-related diseases like cardiovascular disease (CVD), renal disease or Alzheimer's disease, all of which are associated with reduced longevity (Semba et al., 2010; Hartog et al., 2007; Kizer et al., 2014; Arsov et al., 2014; Vitek et al., 1994).

The soluble form of RAGE (sRAGE) has the same binding specificity as RAGE, but no intracellular tail and transmembrane domain, thus cannot lead the signal into the cells and would have consequently a protective effect against AGEs (Prasad and Mishra, 2018; Maillard-Lefebvre et al., 2009; Santilli et al., 2007). Nevertheless, previous studies showed inconsistent associations between concentrations of sRAGE in blood and age-related diseases (Prasad and Mishra, 2018; Santilli et al., 2007; Emanuele et al., 2005; Miniati et al., 2011; Selvin et al., 2013; Fujisawa et al., 2013; Kalousova et al., 2006; Semba et al., 2009; Tan et al., 2006) and there are also studies suggesting that sRAGE itself reflects ongoing inflammation (Fujisawa et al., 2013; Raucci et al., 2008).

Despite postulated mechanisms and observed associations with diseases, findings of studies on AGEs or sRAGE as potential biomarker for mortality are inconsistent. This might be related to the very heterogeneous settings of these studies: They used different populations (patients vs. participants of community-based cohorts) and observation times (from 28-days in ICU-units to up to 18 years in general population) (Semba et al., 2009; Cheng et al., 2014; Roberts et al., 2006; Suliman et al., 2003; Schwedler et al., 2002; Nin et al., 2011; Kilhovd et al., 2005; Brodska et al., 2013; Nin et al., 2010).

While some studies reported contradictory results even in similar settings, there seems to be a pattern, with studies in patients showing short-term and medium-term/intermediate effects, and studies in the general population requiring longer follow-up periods. Since hazard ratio (HR) averages effects over time (Hernan, 2010), it is not clear if associations between AGEs, sRAGE, and mortality change over the time.

Also, while several studies were performed in older age groups, little is known about the predictive values of AGEs and sRAGE on all-cause mortality in a broad age-range of the adult general population. With the current study, we aimed to close these gaps. In addition to all-cause mortality, we analysed CVD mortality because previous studies showed a stronger association of AGEs with CVD than with all-cause mortality (Semba et al., 2009; Kilhovd et al., 2005). All analyses were repeated for the ratio AGE/sRAGE, as it has been shown that this is a better biomarker of age-related diseases than either AGEs or sRAGE separately (Tahara et al., 2012; Prasad et al., 2016; Kajikawa et al., 2015; Caspar-Bell et al., 2016). Additionally, we used cancer-related mortality as an outcome in our analyses.

2. Methods

2.1. Study sample

We analysed data from the CARLA study (Cardiovascular Disease, Living and Ageing in Halle), a population-based cohort of the general population of the city Halle (Saale) in Germany. The study design is explained in detail elsewhere (Greiser et al., 2005). In brief, the aim of the study was to obtain information about the prevalence and incidence of cardiovascular diseases in the local population, the role of heart rate variability, overall and the risk factors for cardiovascular diseases in the general population. Initially, 1779 participants aged 45 to 83 years were recruited for the study and participated in the baseline examination between July 2002 and January 2006. Nineteen participants were excluded due to missing laboratory values, thus data from 1760 participants were used for the analysis of the association of AGEs and sRAGE with mortality.

All participants gave their written informed consent. The local ethics committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and the State Data Privacy Commissioner of Saxony-Anhalt approved the study. The CARLA study conforms to the principles outlined in the declaration of Helsinki.

2.2. AGE and sRAGE measurements

The AGE- and sRAGE-values were determined as previously described in non-fasting plasma samples of the CARLA cohort participants (Bartling et al., 2011; Simm et al., 2014). In brief, the participants' plasma was thawed and centrifuged at 20,000*g* for 3 min at room temperature and diluted 1:20 in PBS (optimal dilution was tested before). Per well of a black 96-well microplate (Greiner, Frickenhausen, Germany), 100 µl of each diluted sample was transferred. The AGE-related fluorescence was measured at least three times (we report averaged values) on a FLUOstar OPTIMA reader (BMG Labtechnologies, Offenburg, Germany) at 370 ± 10 nm excitation and 440 ± 10 nm emission. An internal standard curve and plate-to-plate corrections were created by using glucose-modified bovine serum albumin (AGE-BSA). Measurement results are reported in concentrations equivalent to AGE-BSA.

A commercially available enzyme- linked immunosorbent assay ELISA kit (Quantikine; R&D systems) was used to determine plasma sRAGE levels according to the manufacturer's protocol. Mean values of three measurements are reported.

2.3. Outcomes

All participants were followed up for vital status and cause of death until May 2016 via the population registry. The death certificates were provided by the health department of the city of Halle. The cause of death was examined by a medical doctor and coded according to ICD-10 (International Classification of Diseases – 10th version) (World Health Organization, 1992) by a coder of the State Statistical Office of Saxony-Anhalt (Statistisches Landesamt Sachsen-Anhalt). Outcomes were all-cause mortality and CVD mortality. We defined CVD mortality by the death codes I00-I99 from ICD-10. The codes include all diseases of the cardiovascular system, for example hypertensive diseases, ischemic heart diseases, diseases of the pulmonary circulation or cerebrovascular diseases. We additionally repeated our analysis with cancer-related mortality as an outcome (death codes C00-D48).

2.4. Covariates

Variables affecting the association between the AGE-RAGE system and mortality were identified from the literature and included as confounders in the analyses: i.e. age, body mass index (BMI), smoking status, alcohol consumption, diet, diabetes mellitus, kidney function, lipid lowering drugs, and antihypertensive drugs (Prasad and Mishra, 2018; Semba et al., 2010; Kalousova et al., 2004; Kellow et al., 2017) (Supp. Fig. 1). In a computer-assisted interview, participants provided information on age, smoking status, alcohol consumption and diabetes mellitus (self-reported physician-diagnosed diabetes mellitus or use of antidiabetic medication, ATC-code A10). Dietary patterns were determined based on the self-administered EPIC food frequency questionnaire (Kroke et al., 1999). Medication use in the 7 days before the examination was collected with the computer-based IDOM program of the KORA study (Mühlberger et al., 2003). BMI was calculated as weight, in kilograms rounded to the nearest 100 g, divided by squared-height, in meters rounded to the nearest 0.1 cm. Creatinine concentration was determined on the Modular system (Greiser et al., 2005) and creatinine clearance was calculated using the Cockroft-Gault-formula (Cockcroft and Gault, 1976).

2.5. Statistical analysis

The AGE/sRAGE ratio was calculated from the raw AGE and sRAGE concentrations. The data showed consistent log-normality, so the

(natural) log-transformation was applied to all three values. Additionally, we normalized the parameters to unit-standard deviation to facilitate comparison among them. Analyses were sex-stratified, as previous studies showed sex-specific associations between AGEs and age-related outcomes (Kilhovd et al., 2005; Whitson et al., 2014; Kilhovd et al., 2007; Ebert et al., 2019).

To investigate the association of AGEs, sRAGE, and their ratio with all-cause and CVD mortality, we used Kaplan-Meyer survival curves and conducted multivariable Cox-regression analysis adjusted for the above-described confounders (see also directed acyclic graph (DAG) in Supplement file, which visualises the assumptions of the analysis). Adjusting for diseases and conditions known to affect AGEs and being associated with mortality at the same time implicates that we do not assess the total effect of AGEs, but the effect independent of the other diseases. The proportional hazards assumption was evaluated using the method of Lin, Wei and Ying with Martingale-residuals (Lin et al., 1993). No violations of the proportional hazards assumption or linearity assumption were found. Previous studies often contrasted quartiles of AGEs, we therefore created sex-specific Kaplan-Meyer survival curves for the quartiles of AGEs, sRAGE, and AGE/sRAGE. To investigate the stability of the associations over the follow-up period, we conducted time-varying multivariable Cox-regression analyses with the "*coxph*" command in R, adjusted for previously reported confounders (Therneau et al., 2018).

Sensitivity analyses were performed for: 1) participants with conditions strongly associated with increased AGE levels: participants suffering from diabetes mellitus and with decreased renal function (creatinine clearance < 60 ml/min/1.73 m^2) (Singh et al., 2001) and 2) age-stratified (45–64 years and ≥ 65 years).

Replication analyses were conducted to resemble the population-based samples from previous publications investigating the association between AGEs and sRAGE and mortality: Women 65 years or older at baseline with a restricted follow-up time to 4.5 years were selected for the replication of the study of Semba et al. (2009). We formed quartiles of the raw AGE values and calculated the HR for participants in the highest AGE quartile compared to those in the three lower AGE quartiles. We calculated the HR for 1 standard deviation (SD) (492.6 pg/ml) increase of the sRAGE concentration. In regression analyses, we adjusted for the same variables as Semba et al. (excluding Mini Mental State Examination (MMSE)) (Supp. Table 3).

For the replication of the study of Kilhovd et al. (2005), we selected non-diabetic participants, aged 45 to 65 years at baseline, and followed up for the maximum time of 12 years (as our study had a shorter follow up than the study by Kilhovd et al. with a follow up of 18 years). We calculated the HR for all-cause and CVD mortality for 1 SD increase of the raw AGE values (4589.77). We defined CVD mortality as the death codes I00-I99 from ICD-10, whereas Kilhovd et al. used death codes 390–459 based on the International Classification of Diseases, 9th Revision. However, both encode the "diseases of the circulatory system". In regression analyses, we adjusted for the same variables as Kilhovd et al. (Supp. Table 4). We used the statistical software R version 3.5.0 (R Core Team, 2018) for all analyses.

3. Results

3.1. Study population

The study population consisted of 958 (54%) men and 802 (46%) women at the baseline examination (Table 1). During the median 12 years of follow-up, 284 (30%) men and 124 (15%) women died. The leading causes of death were CVD (101 men and 53 women) and cancer-related mortality (101 men and 37 women). Men were more likely smokers and suffered more often from CVD than women. Further, men had higher levels of AGEs and AGE/sRAGE ratio, but lower levels of sRAGE than women.

3.2. Association of AGEs, sRAGE, and AGE/sRAGE ratio with all-cause or CVD mortality

In the sex-specific analysis in the total sample, we found no or only weak associations between AGEs, sRAGE, or AGE/sRAGE and all-cause mortality in the opposite direction to the expected effects (estimates within 14% increase/decrease per 1 SD and with 95% confidence intervals including 1) (Table 2). These were the adjusted findings, thus describing the effects of AGEs, sRAGE, and AGE/sRAGE independent of the factors also known to increase them. However, also the survival curves, which provide information about crude effects, showed no differences in the associations with all-cause mortality across the quartiles of AGEs, or sRAGE, or AGE/sRAGE (Supp. Fig. 2). These results were similar for CVD mortality, cancer-related mortality, for age-stratified groups (participants younger and older than 65 years) and for those with or without AGE related conditions (Supp. Table 2).

3.3. Replication analyses

In replication analyses, our estimates for AGEs disagreed with those from Semba et al. (Semba et al., 2009), but the estimates for sRAGE were similar (for all-cause mortality only; our sample was too small for validation of CVD mortality – Supp. Table 3).

The replication analysis for the younger (45–65 years) non-diabetic subsample (Kilhovd et al., 2005) revealed no association for all-cause mortality. Kilhovd et al. (2005) observed no association between AGEs and CVD-mortality in men, while our results indicate an association in men in the expected direction ((1.35 per 1 SD (not logarithmized to reach comparability to the analyses of Kilhovd et al.), 95% CI 1.05–1.73), Suppl. Table 4). In our study, CVD mortality was a smaller fraction of overall mortality than in the study by Kilhovd et al., which could explain why we had discordant findings for CVD and all-cause mortality. In women, a too low number of events prohibited an analysis of CVD-mortality.

3.4. Stability of the association with all-cause mortality

The period-specific Cox models showed no differences in association between AGEs, sRAGE, and AGE/sRAGE with all-cause mortality over the complete follow-up time for men and women in the whole sample (Fig. 1) and in all sub-analyses: with/without AGEs-related conditions, age-stratified, for CVD mortality and for cancer-related mortality (data not shown).

4. Discussion

Our study showed either no or only weak associations between AGEs, sRAGE and the AGE/sRAGE ratio, and all-cause, CVD or cancer-related mortality after a median of 12 years follow-up time in a German cohort of the general adult population. Further, there was no indication that this association changes over time.

Our findings of only at best a weak association between AGEs and mortality contribute to the picture of inconsistent findings in previous research. While some of our analyses were hampered by a too small sample size, when compared to previous studies (Semba et al., 2009; Kilhovd et al., 2005), the estimated required sample size to achieve 80% power to detect a 50% or a 90% increase in the mortality hazard of the participants in the higher AGEs´ quartile by using a 0.05-level log-rank test would be 255 or 102 events respectively. Thus, we had enough observations for detecting a 90% increase for men ($N = 284$) and women ($N = 124$), and at least for men there were enough events to detect a 50% increase.

The fact that physiological mechanisms do not translate into epidemiological associations could be potentially explained by the instability of blood-measured AGE levels due to their change by food intake. It was shown that it takes at least 18–20 h until the plasma-AGE

Table 1
Characteristics of CARLA study population at baseline (2002–2006).

	Men (n = 958)	Women (n = 802)
Age, mean ± SD (years)	65 ± 10.23	64 ± 9.95
BMI, mean ± SD (kg/m^2)	28.15 ± 4.07	28.50 ± 5.35
Current smoker, N (%)	224 (23.38%)	118 (14.71%)
Score Food Frequency Questionnaire,[a] mean ± SD	14.51 ± 3.21	16.43 ± 3.16
Diabetes mellitus, N (%)	153 (15.97%)	115 (14.34%)
CVD, N (%)	151 (15.76%)	47 (5.86%)
Creatinine clearance, median (P25/P75) (ml/min)	97.15 (76.78/119.57)	88.84 (71.76/109.57)
AGE-levels [rel. units], median (P25/P75)	12,288.39 (9548.35/14796.00)	11,385.56 (8574.87/13,710.42)
sRAGE-levels [pg/ml], median (P25/P75)	827.62 (604.41/1104.11)	963.79 (706.93/1277.99)
AGE/sRAGE, median (P25/P75)	14.49 (10.07/21.09)	11.37 (8.19/16.37)
All-cause mortality, N (%)	284 (29.65%)	124 (15.46%)
CVD mortality, N (% all-cause)	101 (35.56%)	53 (42.74%)
Cancer-related mortality, N (% all-cause)	101 (35.56%)	37 (29.84%)

Note. BMI = body mass index; CVD = cardiovascular disease; AGEs = advanced glycation end products; sRAGE = soluble receptor of AGEs; SD = standard deviation; P25/P75 = 25th/75th percentile.
[a] Score 0–30, higher score indicates healthier nutrition.

Table 2
Multivariable Cox regression for AGE, sRAGE, and AGE/sRAGE (per 1 SD increase in logarithmied values) and all-cause mortality after median 12 years follow-up.

	Age-adjusted model N = 1760 (958 men/802 women)[b]	Fully adjusted model[a] N = 1756 (954 men/802 women)[b]
	HR (95% CI)	HR (95% CI)
AGEs		
Men	0.98 (0.87–1.09)	0.93 (0.83–1.05)
Women	0.95 (0.80–1.13)	0.88 (0.74–1.05)
sRAGE		
Men	1.02 (0.91–1.15)	1.08 (0.95–1.23)
Women	1.12 (0.95–1.33)	1.10 (0.92–1.30)
AGE/sRAGE		
Men	0.97 (0.86–1.09)	0.90 (0.80–1.01)
Women	0.88 (0.74–1.04)	0.86 (0.73–1.02)

Note. AGEs = Advanced Glycation End Products; sRAGE: soluble receptor of AGEs; AGE/sRAGE = ratio of AGEs and sRAGE; BMI = body mass index; 95%CI = 95% confidence interval.
[a] Model adjusted for: age, smoking status, alcohol consumption, BMI, score of food frequency questionnaire, diabetes mellitus, creatinine clearance, medication.
[b] Total mortality: 284 men; 124 women.

levels are at the baseline level after an AGE-rich meal (Koschinsky et al., 1997). Additionally, it was suggested that blood-measured AGE-levels reflect only a minor part of the whole-body AGE-pool (Schwedler et al., 2002). Other assumptions are that AGEs are only recently produced and therefore blood AGE levels are not as related to chronic outcomes as tissue-AGEs, which are bound on long-living proteins over time (Cavero-Redondo et al., 2018). Therefore, skin-measured AGEs might be better predictors for mortality.

To our knowledge, this is the first study that investigated the stability of the association between AGEs, sRAGE and AGE/sRAGE ratio, and mortality over time. On the one hand we expected, as implied by previous studies with different follow-up times, a stronger association between AGEs, sRAGE and AGE/sRAGE, and mortality with increasing follow-up time. On the other hand we thought that the predictive values of AGEs, sRAGE and AGE/sRAGE ratio might decrease over time due to changing lifestyle behaviours (e.g. smoking, diet) or incident diseases, which influence their levels and lower the predictive value of the baseline levels. However, we could not confirm any of the proposed explanations. It could be thought that AGEs in people of the general population have only a very long-term effect, possibly longer than our follow-up of 12 years. Some indication of this association was possibly observed in our replication of the study by Kilhovd et al. (2005).

Our study has a number of strengths as we have participants of the general adult population in a broad age spectrum between 45 and 83 years. Moreover, we considered many relevant confounders in the analysis. We addressed also the question of stability of the studied association over follow-up time. However, there are also some limitations. First, non-fasting plasma was used to measure the AGE levels, although AGE levels can be influenced by fasting status (Koschinsky et al., 1997). Though, adjustment for fasting time generated analogous results (data not shown). Second, two subtypes of sRAGE: cRAGE (proteolytic cleaved RAGE) and esRAGE (endogenous secretory RAGE) were measured together, although they might have different binding patterns to AGEs and different pathological properties (Prasad and Mishra, 2018; Reichert et al., 2017).

Our findings that the association between plasma-measured AGEs, sRAGE, and their AGE/sRAGE ratio, and all-cause, CVD or cancer-related mortality in the general adult population during 12 years of follow-up was either non-existent or weaker than in previous studies, adds to the inconsistent results on this topic. We could not explain the differences in previous studies by assessing variation in effects depending on the time horizon at which they occur. Given the reported higher stability of AGE levels in skin, we suggest to asses epidemiological correlates of assumed physiological mechanisms based on measurements of skin AGE levels.

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CRediT authorship contribution statement

Helen Ebert: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization.**Maria Elena Lacruz:** Conceptualization, Methodology, Writing - review & editing.**Alexander Kluttig:** Conceptualization, Investigation, Data curation, Visualization.**Andreas Simm:** Investigation, Resources, Writing - review & editing.**Karin Halina Greiser:** Investigation, Resources.**Daniel Tiller:** Investigation, Writing - review & editing.**Nadja Kartschmit:**

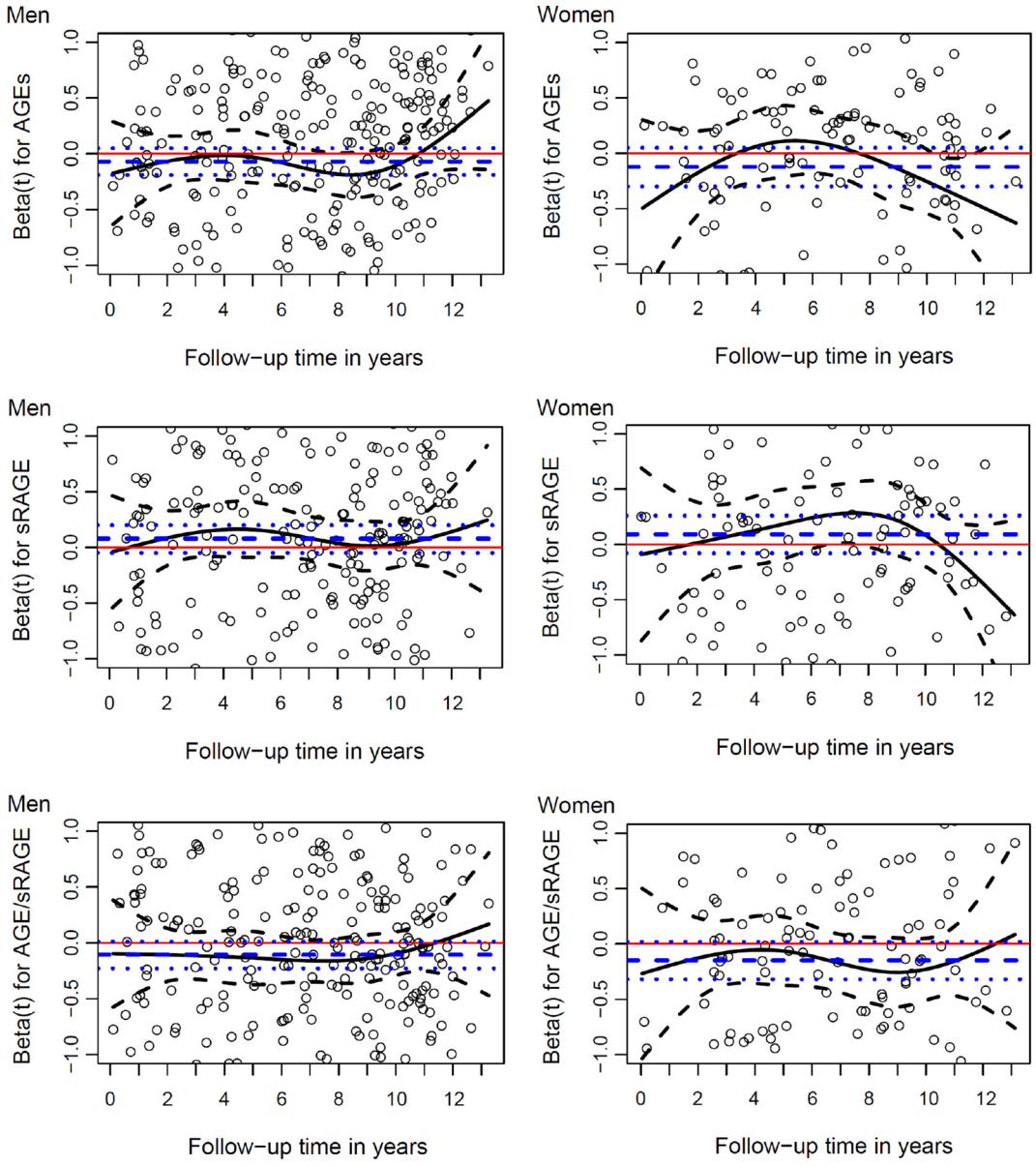

Fig. 1. Time varying Betas (black line) with 95%CI (dashed black lines) and averaged Betas (dashed blue line) with 95% CI (pointed blue lines) for the association between AGEs, sRAGE and AGE/sRAGE and all-cause mortality, points represent events; adjusted for age, smoking status, alcohol consumption, BMI, score of food frequency questionnaire, diabetes mellitus, creatinine clearance, medication. Red line shows lack of association.
Note. AGEs = advanced glycation end products; sRAGE = soluble receptor of AGEs; AGE/sRAGE = ratio of AGEs and sRAGE; BMI = body mass index; N = 956 men (†284), 802 women (†124). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Formal analysis, Writing - review & editing.**Rafael Mikolajczyk:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2019.110815.

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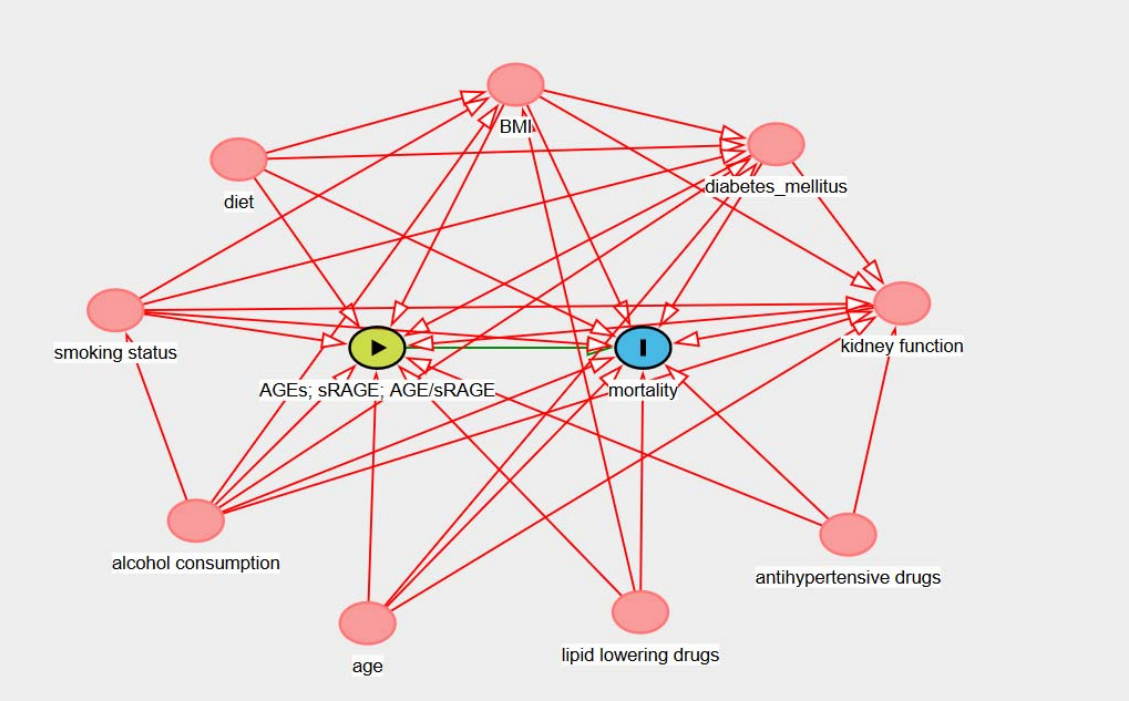
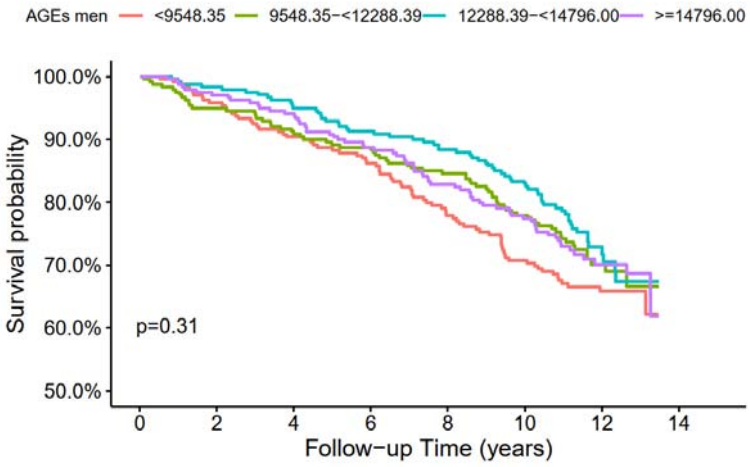
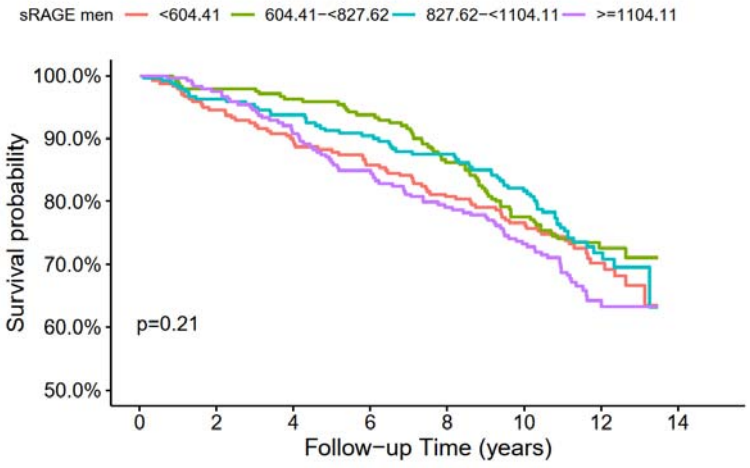
Zusatzmaterial Publikation 2

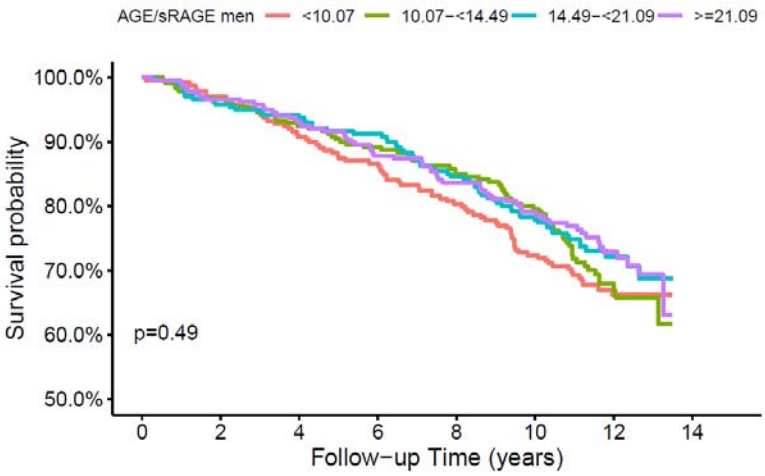


Supp. Figure 1. Directed acyclic graph (DAG) for the chosen covariates
Note. AGEs=advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE; BMI= Body Mass Index

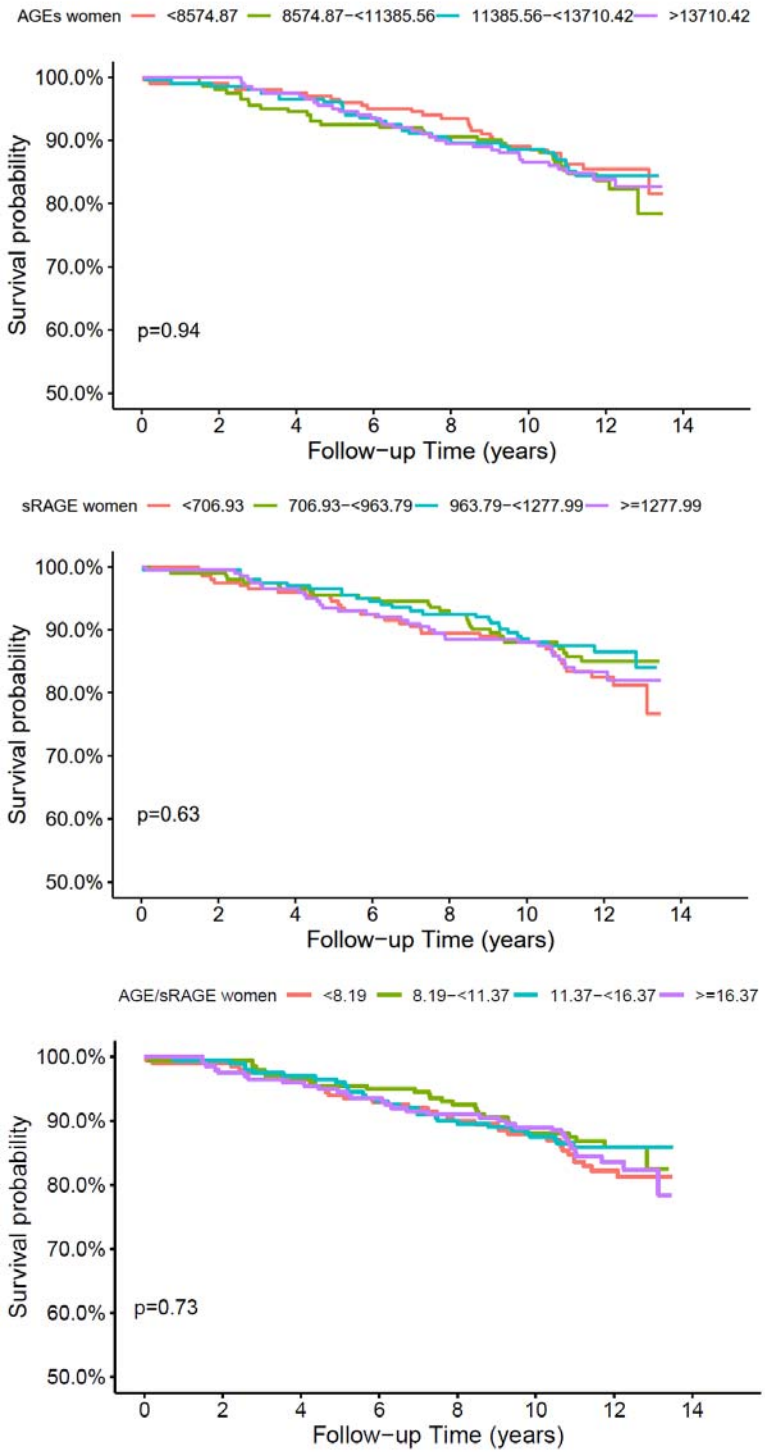
Men


AGEs men
<9548.35
9548.35–<12288.39
12288.39–<14796.00
>=14796.00
Survival probability
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
p=0.31
0
2
4
6
8
10
12
14
Follow–up Time (years)


sRAGE men
<604.41
604.41–<827.62
827.62–<1104.11
>=1104.11
Survival probability
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
p=0.21
0
2
4
6
8
10
12
14
Follow–up Time (years)


AGE/sRAGE men
<10.07
10.07–<14.49
14.49–<21.09
>=21.09
Survival probability
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
p=0.49
0
2
4
6
8
10
12
14
Follow–up Time (years)

Women



Supp. Figure 2. Survival curves during a median of 12 years follow-up for all-cause mortality according to quartiles of AGEs [numbers correspond to rel. units] and sRAGE [pg/ml], sex-stratified. P values are from log rank test
Note. AGEs=advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE;

Supp. Table 2. Multivariable Cox Regression for AGEs, sRAGE and AGE/sRAGE (per 1 standard deviation increase in logarithmied values) and mortality after median 12 years follow-up

		HR (95% CI)	
CVD Mortality[1]		Men *(N= 954; †=101)*	Women *(N= 802; †=53)*
	AGEs	0.92 (0.75-1.12)	0.91 (0.69-1.19)
	sRAGE	0.91 (0.73-1.12)	1.23 (0.94-1.60)
	AGE/sRAGE	1.02 (0.82-1.26)	0.80 (0.62-1.04)
Cancer-related Mortality[1]		Men *(N= 954; †=100)*	Women *(N= 802; †= 37)*
	AGEs	0.90 (0.75-1.08)	1.00 (0.71-1.41)
	sRAGE	1.09 (0.88-1.34)	0.93 (0.68-1.28)
	AGE/sRAGE	0.87 (0.72-1.06)	1.06 (0.77-1.46)
All-cause mortality[1]		Men <65 years *(N= 482; †=60)*	Women < 65 years *(N= 449 †= 17)*
	AGEs	1.05 (0.78-1.41)	0.81 (0.50-1.30)
	sRAGE	1.14 (0.87-1.49)	1.18 (0.68-2.06)
	AGE/sRAGE	0.92 (0.71-1.21)	0.77 (0.47-1.25)
All-cause mortality[1]		Men >= 65 years *(N= 472; †= 223)*	Women >= 65 years *(N= 353 †= 107)*
	AGEs	0.92 (0.81-1.05)	0.88 (0.73-1.06)
	sRAGE	1.04 (0.90-1.20)	1.03 (0.86-1.24)
	AGE/sRAGE	0.92 (0.80-1.05)	0.90 (0.75-1.07)
All-cause mortality[2]		Men without diabetes mellitus or decreased renal function *(N= 736 †= 171)*	Women without diabetes mellitus or decreased renal function (N= 614 *†= 56)*
	AGEs	0.91 (0.79-1.04)	0.84 (0.62-1.13)
	sRAGE	1.06 (0.91-1.24)	1.18 (0.88-1.57)
	AGE/sRAGE	0.89 (0.77-1.04)	0.79 (0.60-1.05)

All-cause mortality[2]		Men with diabetes mellitus or decreased renal function *(N= 218 †= 112)*	Women with diabetes mellitus or decreased renal function (N= 188 *†= 68)*
	AGEs	0.95 (0.77-1.16)	0.95 (0.76-1.18)
	sRAGE	0.96 (0.77-1.19)	1.05 (0.85-1.31)
	AGE/sRAGE	0.99 (0.80- 1.23)	0.93 (0.75-1.15)

[1]*adjusted for age, smoking status, alcohol consumption, BMI, score of food frequency questionnaire , diabetes mellitus, creatinine clearance, medication*
[2]*adjusted for age, smoking status, alcohol consumption, BMI, score of food frequency questionnaire , creatinine clearance, medication*
† number of events
Note. AGEs=advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE; HR= hazard ratio; 95% CI= 95% confidence interval; CVD= cardiovascular disease; BMI= Body Mass Index

Supp. Table 3. Replication of the analyses of Semba et al. [42] for the Cox-regression between AGEs (highest quartile vs. lower three quartiles) and sRAGE (for 1SD increase) with all-cause mortality for community-dwelling elderly women (aged 65 years or above) after 4.5 years follow up

		Raw model		Age-adjusted model		Fully adjusted model *	
		HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
AGEs	WHASI-I	1.71	1.16-2.55	1.50	1.01-2.24	1.47	0.97-2.22
	CARLA	0.73	0.30-1.79	0.73	0.30-1.80	0.59	0.23-1.50
sRAGE	WHASI-I	1.33	1.12-1.57	1.26	1.06-1.50	1.19	0.98-1.44
	CARLA	1.14	0.83-1.56	1.10	0.82-1.47	1.17	0.80-1.69

Semba: N=559; events (†): 123; CARLA: N=353; events (†): 30 (fully adjusted model: N=344; †:30)

** Adjustment: age, BMI (underweight (<18.5 kg/m²), normal range (18.5-24.9 kg/m²), overweight (≥25-29.9 kg/m²), obese (≥30 kg/ m²)), renal insufficiency (yes= eGFR<60ml/min/1.73m²/ no), depression (yes/no) (Semba adjusted additionally for MMSE (cognitive impairment= MMSE<24)*

Note. AGEs=advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE; HR= hazard ratio; 95% CI= 95% confidence interval; CVD= cardiovascular disease; BMI= Body Mass Index; MMSE= Mini Mental State Examination; eGFR= estimated glomerular filtration rate; WHASI-I=Women's Health and Aging Study I; CARLA= Study of Cardiovascular Disease, Living and Ageing in Halle

Supp. Table 4. Replication of the analysis of Kilhovd et al. [62] for the Cox-regression between AGEs and total/ CVD mortality (per 1standard deviation increase) in non-diabetic participants (aged 45-64 years) after 18 years in the Finnish nondiabetic cohort and 12 years follow-up in the younger nondiabetic subsample of the CARLA cohort

		Raw model		Fully adjusted model*	
Total Mortality		**HR**	**95% CI**	**HR**	**95% CI**
Finnish	All	1.03	0.99–1.06	1.01	0.98–1.05
	Men	0.98	0.93–1.02	0.98	0.94–1.03
	Women	1.07	1.02–1.13	**1.08**	**1.02–1.14**
CARLA	All	1.13	0.93-1.38	1.03	0.79-1.33
	Men	1.06	0.83-1.35	1.03	0.77-1.37
	Women	0.97	0.52-1.83	0.95	0.51-1.76
CVD-mortality		**HR**	**95% CI**	**HR**	**95% CI**
Finnish	All	1.05	1.00–1.10	1.03	0.98–1.09
	Men	0.98	0.92–1.05	1.00	0.94–1.06
	Women	1.13	1.05–1.20	**1.10**	**1.02–1.20**
CARLA	All	1.36	1.12-1.65	**1.38**	**1.10-1.72**
	Men	1.30	1.04-1.63	**1.35**	**1.05-1.73**
	Women	*(too low number of events)*			

**adjusted for age, sex (only men and women together), BMI, smoking status (current/ no), hypertension (systolic blood pressure≥ 160mmHg or diastolic blood pressure≥ 95mmHg) , cholesterol, triglycerides, HDL, menopausal status (only in women)(period/ irregular period/ no period)*

Total Mortality:

Finnish nondiabetic cohort: N=535 men, 606 women; events (†): 170 men, 83 women

CARLA: N=427 men, 410 women; events (†): 49 men, 12 women

CVD-mortality:
Finnish nondiabetic cohort: N=535 men, 606 women; events (†): 92 men, 22 women
CARLA: N=427 men, 410 women; events (†): 14 men, 3 women

Note. AGEs=advanced glycation end products; sRAGE= soluble receptor of AGEs; AGE/sRAGE= ratio of AGEs and sRAGE; HR= hazard ratio; 95% CI= 95% confidence interval; CVD= cardiovascular disease; BMI= Body Mass Index; HDL= High Density Lipoprotein; CARLA= Study of Cardiovascular Disease, Living and Ageing in Halle

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Halle, 30.12.2022

Helen Ebert

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