Chapter 4

Advanced Glycation End-Products are Associated with the Presence and Severity of Paranoia in Early Stage Alzheimer Disease

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ABSTRACT

Objective: Paratonia, a distinctive form of hypertonia in dementia patients, causes loss of functional mobility in early stage dementia to severe contractures and pain in the late stages. The pathogenesis of paratonia is not well understood. Patients in early stage dementia with Diabetes Mellitus (DM) showed a significantly higher risk for the development of paratonia. Both Alzheimer disease (AD) and DM are related to higher concentrations of advanced glycation end-products (AGEs). The purpose of this study is to explore the association of AGEs with the prevalence and severity of paratonia in patients with Alzheimer disease.

Design: Observational longitudinal, 1-year follow-up cohort study with 3 assessments.

Setting: Day care centers for patients with dementia.

Participants: A total of 144 community-dwelling patients with early stage Alzheimer or Alzheimer/vascular disease patients were recruited from 24 dementia day care centers in The Netherlands.

Measurements: The presence of paratonia (Paratonia Assessment Instrument), the severity of paratonia (Modified Ashworth Scale for paratonia), and AGE levels (AGE-reader).

Results: From the 144 participants (56.3% female and 43.7% male, with a mean [standard deviation] age of 80.7 [7.7] years), 118 participants were available for final follow-up. A significant association between AGE levels and the presence of paratonia (odds ratio 3.47, 95% confidence interval [CI]: 1.87 – 6.44, P < .001) and paratonia severity (β = 0.17, 95% CI: 0.11 - 0.23, P < .001) was determined. In participants who developed paratonia and those with persistent paratonia throughout the study the AGE levels (95% CI: -0.38 to -0.13, P < .001 and 95% CI: -0.46 to -0.06, P = .012, respectively) and the severity of paratonia (95% CI: -0.60 to -0.35, P < .001 and 95% CI: -0.38 to -0.12, P < .001, respectively) significantly increased, whereas the AGE levels remained stable in those participants without paratonia. Notwithstanding, change in AGE levels was not significantly (P = .062) related to change in paratonia severity, mixed model analyses provided evidence for both a significant time and between participant effect of AGEs on paratonia severity.

Conclusions: This study suggests that elevated AGE levels are a contributing factor to paratonia and its severity and could be the result of peripheral biomechanical changes reducing elasticity and increasing stiffness. These results provide a new perspective on paratonia and gives rise to further research whether paratonia could be postponed or movement stiffness can be improved by reducing AGE levels.
INTRODUCTION

A wide variety of movement disorders are reported in patients with dementia. Paratonia, a distinctive form of hypertonia, is one of these movement disorders and is characterized by an active unintentional resistance against passive movement. Paratonia has a prevalence of 10% in the early/mild stages, and up to 90-100% in later/severe stages of dementia. As dementia progresses, the severity of paratonia increases from actively assisting the passive movement (because of an inability to relax) and mild resistance towards severe high resistance and muscle tone which causes loss of mobility, severe contractures, and pain in the last stage of dementia. Resistance in paratonia is variable, in particular in the very early stages of dementia paratonia can fluctuate between no resistance, actively assisting, and active resistance against passive movement. In early stage dementia, paratonia hampers functional mobility such as raising from a chair, walking and turning. The pathogenesis of paratonia is not well understood, and no effective interventions are available to prevent, postpone or combat paratonia.

It has been shown that patients in early stage dementia with Diabetes Mellitus (DM) have a significantly higher risk for the development of paratonia in comparison with those with dementia but without DM. Previous research also reported that DM is a risk factor for muscle rigidity in patients without dementia. Interestingly, both Alzheimer disease (AD) and DM are related to higher concentrations of advanced glycation end-products (AGEs), suggesting that AGEs could possibly be involved in the development of paratonia. The occurrence of AGEs is mediated by non-enzymatic condensation of a reducing sugar with an amino group. With aging, there is an imbalance between the formation and natural clearance of AGEs, which results in an incremental accumulation in tissues.

As stated before, the pathogenesis of paratonia is not well understood. Obviously with dementia, central nervous system pathology plays a role; yet, peripheral biomechanical changes are also suggested. In this perspective, it is of interest to know that the cross-linking processes by AGEs are responsible for an increasing proportion of insoluble extracellular matrix and thickening of the tissues, thereby increasing mechanical stiffness and loss of elasticity. Non-cross-linking effects occur through the binding of AGEs to the receptor for AGEs (RAGE). RAGE is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules that is widely localized in a variety of cell lines, including endothelial, neuronal, smooth muscle, mesangial and monocytes. AGE/RAGE binding subsequently incites activation of intracellular signalling, gene expression, and production of pro-inflammatory cytokines and free radicals. At the peripheral (tissue) level, these inflammatory processes exhibit strong proteolytic activity by which the collagen becomes more vulnerable.
and tissue elasticity decreases\textsuperscript{12,13}. At the central level (central nervous system), AGE/RAGE interaction appears to affect neuronal function\textsuperscript{7,13}.

The role of AGEs in the development of paratonia is currently unknown. The purpose of this study is to explore the association of AGEs with the prevalence and severity of paratonia in patients with AD.

**METHODS**

**Study Design and Ethical Consideration**

This study was a multicenter, longitudinal, 1-year follow-up cohort study with 3 assessments; at baseline, after 6 months and after 12 months. The medical ethical committee of the University Medical Centre Groningen approved the study (NL43641.042.13).

**Study Population**

Participants, recruited from 24 dementia day care centers in The Netherlands, were considered to be eligible for inclusion in the study when they satisfied the following criteria: (1) an established diagnosis of AD or Alzheimer/vascular dementia (AD/VaD) according to DSM-IV criteria\textsuperscript{14}; (2) Early stage dementia; a score of stage 5 or lower on the global deterioration scale\textsuperscript{15} and (3) having a light colored (Caucasian) skin (skin pigmentation influence AGE measurements\textsuperscript{16}). Written informed consent was obtained from patients or their legal representatives. Participants were excluded if they had an established diagnosis of dementia other than type AD or AD/VaD or were using first generation psychotropic drugs as these drugs can possibly mimic paratonic rigidity.

**Outcome Measures**

**Paratonia**

The presence of paratonia was assessed by trained and experienced physiotherapists by employing the Paratonia Assessment Instrument (PAI), a reliable and valid measurement based on a successively passive mobilization of both shoulders towards ante-flexion/retro-flexion, elbows towards flexion/extension, and combined hips/knees towards extension/flexion\textsuperscript{17}. With the participant in a sitting position the examiner began with a slow movement of the limb, after which the movement was accelerated. Paratonia was diagnosed as being present when the following five criteria are all met: (1) an involuntary variable resistance; (2) a degree of resistance that varied depending on the speed of the movement (e.g., a low resistance to slow movements and a high resistance to fast movement); (3) resistance to
passive movement in any direction; (4) no clasp-knife phenomenon; and (5) resistance in two movement directions in the same limb or two different limbs.

We used a Modified Ashworth Scale, validated to assess paratonia severity (MAS-P) as described by Waardenburg et al. In the MAS-P each category contains a tone and passive movement feature, thereby creating a more consistent scale for paratonia. During the PAI assessment, the assessor quantified the muscle tone based on the resistance induced by the passive movements of the limbs into a total of 12 MAS-P scores (the corresponding movement directions from the PAI). The MAS-P is based on a 5-point scale ranging from 0 to 4, meaning 0 = no increase in muscle tone or no resistance during passive movement, 1 = slight increase in muscle tone or slight resistance during passive movement, 2 = more marked increase in muscle tone or more marked resistance during passive movement, 3 = considerable increase in muscle tone or considerable resistance during passive movement, and 4 = severe resistance such that passive movement is impossible. A score of 0.5 was assigned in the event of active assistance. For further analyses the 12 scores were summarized as the average MAS-P value. The MAS-P shows acceptable reliability.

AGE levels
AGE levels were assessed by the main researcher through skin autofluorescence (SAF) by using an AGE Reader device (Diagnoptics, Groningen, The Netherlands). The AGE reader measures fluorescent skin tissue AGEs which correlate with non-fluorescent AGEs. The AGE reader is a desktop device that has a light source which illuminates the skin of the forearm and uses the fluorescent properties of AGEs to measure tissue accumulation of AGEs. SAF is calculated (by the AGE reader software) as the ratio between the emission light and the excitation light, multiplied by 100 and expressed in arbitrary units. An elevated SAF (arbitrary units) score corresponds to a high tissue AGEs level. All AGE reader measurements were performed at room temperature in a standardized semi dark environment with the participants in a seated position and the volar side of the right forearm placed on top of the AGE reader. The measurements were performed on the skin without sweat, skin lotions, or visible skin abnormalities and with the assessor being blinded for PAI/MAS-P scores. The mean of 3 consecutive measurements was used. The AGE reader is reported to be a reliable and valid instrument for the quantification of AGEs accumulation.

Cognitive function and comorbidities
The diagnosis of dementia was established by the local physician or general practitioner based on the DSM-IV criteria. Dementia severity was staged by key staff personal using the global deterioration scale, which identifies 7 stages from no dementia to severe dementia. Cognitive function was measured by experienced psychologists or physicians with the Mini-Mental State Examination (MMSE). The MMSE is an 11-item questionnaire with a
maximum score of 30 which (30 = no cognitive decline; 0 = very severe cognitive decline). The comorbidities (International Classification of Diseases, Ninth Revision, Clinical Modification classification) and use of medication (Anatomical Therapeutic Chemical Classification System classification) were retrieved from the patients’ medical records and general practitioner files. If a participant used 5 or more medications, this was labeled as polypharmacy.

**Statistical Analyses**

*Sample size calculation*

Two *a priori* sample size calculations were undertaken. First, a mixed model sample size calculation was based upon a 0.5 correlation between repeated measurements, 0.3 variance of the random intercept, 0.3 residual variance, a true effect size of 0.5, a desired power of 80%, and a two-sided alpha of 0.05. This resulted in a total sample size of 152. Considering this sample size, an additional calculation for the binary PAI outcomes in a logistic regression model was performed to detect a true odds ratio (OR) = 1.65 with true binary probability 0.3 and a desired power of 80%. Accounting for a potential 10% withdrawal rate, the required total number of participants was approximately 165.

*Calculation of MAS-P scores*

The degree in which the 12 MAS-P scores can be reliably summarized as an average value was investigated by the two-way intra class correlation (ICC) coefficient (type: average, two-way, agreement). A cut-off ICC value of .80, which indicates a strong correlation, was desired for the average MAS-P score. Furthermore, to what degree the 12 MAS-P measurements have a dominating direction of variation was investigated by principal component analysis.

*Baseline characteristics and differences in characteristics between tertiles of AGE levels*

AGE levels were categorized in tertile groups and 1-way analysis of variance tests were performed for continuous data and $\chi^2$ tests for frequency data, with post hoc analysis (Tukey for 1-way analysis of variance), if relevant, to analyse differences in characteristics between the 3 AGE level groups.

*Association between AGE levels and paratonia presence and severity*

To investigate the association between AGE levels and the presence of paratonia, a generalized linear mixed model was used, estimated by restricted maximum likelihood, and taking the presence of paratonia (PAI) as a response at each of the 3 visits (baseline, 6 months, 12 months). To investigate the association between AGE levels and the severity of paratonia, a linear mixed model (LMM) was used, estimated by restricted maximum
likelihood, and taking the severity of paratonia measured by MAS-P as a response at each of the 3 visits (baseline, 6 months, 12 months). In both models, the association was statistically controlled for the (fixed) effects of sex, age, polypharmacy, dementia duration, cognition (MMSE), chronic kidney disease, cardiovascular disease, cerebral vascular disease and DM\(^8,27\) as well for random effects of the participants. Backward model selection was used for detecting statistically significant explanatory variables. During this process, the fixed effects of AGE levels and visits were always retained to explore for longitudinal effects.

To further investigate the within participant association of AGES with paratonia severity (MAS-P), the differences in paratonia severity (MAS-P) and in AGES, respectively, between Visit 3 and Visit 1 were computed separately for participants without paratonia, with incident paratonia, and persistent paratonia. To investigate changes in AGE levels and in paratonia severity (MAS-P), paired sample t-tests were performed. Change in paratonia proportion was analyzed by \(\chi^2\) tests. The association of these within-participant differences between AGES and paratonia severity (MAS-P) were further investigated by correlation and simple regression. Data were analyzed using R version v3.2.0 and SPSS version v 22 and taking a P value of < .05 as statistically significant.

**RESULTS**

A flowchart of the enrolment of participants is presented in figure 1. A total of 144 participants were finally included at baseline. After 1 year, 26 participants (18%) were lost to follow-up: 11 had deceased and 15 were transferred to unknown addresses or became too ill to be reassessed (Figure 1). For comorbidity and medication use of 11% of the patients \((n = 16)\), information was not accessible in the medical records.

**Baseline Characteristics and Differences in Characteristics Between Tertiles of AGE Levels**

Table 1 illustrates baseline characteristics of the participants according to the tertiles of AGE levels; 56.3% female and 43.7% male, with a mean (standard deviation [SD]) age of 80.7 [7.7] years. Participants had a mean (SD) dementia duration since diagnosis of 29.8 (35.9) months and had a mean (SD) of 1.18 (1.1) comorbidities. Paratonia was diagnosed in 60 participants (41.7%).

The ICC for paratonia severity (MAS-P) scores of visit 1 to 3 was high (> .90), and principal component analysis indicated strong evidence that the MAS-P has 1 underlying construct so that the 12 MAS-P item scores were allowed to be entered as individual averages in the analyses.
Association Between AGE levels and Paratonia Presence and Severity

The presence (PAI) and severity (MAS-P) of paratonia were both significantly more evident in the highest AGE level tertile compared to the lowest tertile. Participants in the highest AGE level tertile demonstrated a substantially greater number of comorbidities and showed a significantly higher prevalence of DM (table 1).

The mixed model analyses reveal a significant time effect as well as a significant regression type of association between AGEs and paratonia and AGES and paratonia severity (MAS-P). Table 2 shows that paratonia is significantly associated with the AGE levels (OR = 3.47, 95% CI: 1.87 - 6.44, P < .001) and with the progression over a time span of 1 year (OR = 2.89, 95% CI: 1.41 - 5.90, P = .004).
Table 1. Baseline Characteristics and Differences in Characteristics of the Participants According to the Tertiles of AGE Levels

<table>
<thead>
<tr>
<th>Tertiles of AGE levels</th>
<th>Participants, n (%)</th>
<th>Male, n (%)</th>
<th>Age, years</th>
<th>Dementia duration, months*</th>
<th>Co morbidity, n*</th>
<th>Cardio vascular disease, n (%)*</th>
<th>Cerebral vascular disease, n (%)*</th>
<th>Diabetes, n (%)*</th>
<th>Cancer, n (%)*</th>
<th>COPD, n (%)*</th>
<th>Chronic kidney disease, n (%)*</th>
<th>Systemic, n (%)*</th>
<th>Digestive tract, n (%)*</th>
<th>Polypharmacy (≥ 5 medications), n (%)*</th>
<th>MMSE, score 0-30</th>
<th>GDS, score 1-7</th>
<th>Paratonia, n (%)</th>
<th>MAS-P, score 0-4</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>144</td>
<td>63 (43.7%)</td>
<td>80.7 (7.7)</td>
<td>29.8 (35.9)</td>
<td>1.18 (1.1)</td>
<td>41 (32.0%)</td>
<td>22 (17.2%)</td>
<td>37 (28.9%)</td>
<td>14 (11.0%)</td>
<td>14 (11.0%)</td>
<td>14 (11.0%)</td>
<td>14 (11.0%)</td>
<td>6 (4.7%)</td>
<td>8 (6.3%)</td>
<td>71 (55.5%)</td>
<td>3.84 (1.0)</td>
<td>60 (41.7%)</td>
<td>0.40 (0.40)</td>
<td>.78</td>
</tr>
<tr>
<td>Low SAF ≤ 2.5 AU</td>
<td>52 (36.1%)</td>
<td>16 (30.8%)</td>
<td>79.9 (8.9)</td>
<td>35.9 (46.6)</td>
<td>0.9 (1.0)</td>
<td>14 (11.0%)</td>
<td>6 (4.7%)</td>
<td>11 (8.6%)</td>
<td>4 (3.1%)</td>
<td>4 (3.1%)</td>
<td>4 (3.1%)</td>
<td>6 (4.7%)</td>
<td>1 (0.8%)</td>
<td>6 (4.7%)</td>
<td>25 (19.5%)</td>
<td>3.85 (1.0)</td>
<td>13 (25%)</td>
<td>0.27 (0.26)</td>
<td>.51</td>
</tr>
<tr>
<td>Middle SAF 2.5 AU ~&gt; 3.1 AU</td>
<td>40 (27.8%)</td>
<td>19 (47.5%)</td>
<td>80.5 (7.3)</td>
<td>30.36 (29.4)</td>
<td>1.15 (1.0)</td>
<td>13 (10.0%)</td>
<td>5 (3.9%)</td>
<td>6 (4.7%)</td>
<td>2 (1.7%)</td>
<td>3 (2.3%)</td>
<td>3 (2.3%)</td>
<td>2 (1.7%)</td>
<td>-</td>
<td>1 (0.8%)</td>
<td>17 (13.3%)</td>
<td>3.9 (1.0)</td>
<td>18 (45%)</td>
<td>0.43 (0.35)</td>
<td>.56</td>
</tr>
<tr>
<td>High SAF ≥ 3.1 AU</td>
<td>52 (36.1%)</td>
<td>28 (53.8%)</td>
<td>81.5 (6.6)</td>
<td>22.90 (24.9)</td>
<td>1.51 (1.2)</td>
<td>14 (11.0%)</td>
<td>11 (8.6%)</td>
<td>20 (15.6%)</td>
<td>9 (7.0%)</td>
<td>4 (3.1%)</td>
<td>5 (3.9%)</td>
<td>2 (1.7%)</td>
<td>5 (3.9%)</td>
<td>-</td>
<td>29 (22.7%)</td>
<td>5 (1.0)</td>
<td>29 (55.8%)</td>
<td>0.52 (0.49)</td>
<td>.001†</td>
</tr>
</tbody>
</table>

AGEs = advanced glycation end-products; AU = arbitrary units (ie, the output units of the AGE reader); COPD = chronic obstructive pulmonary disease; GDS = Global Deterioration Scale; MAS-P = modified Ashworth scale for paratonia; MMSE = Mini Mental State Examination; SAF = skin autofluorescence (AGE reader).

Data represent mean values (SD) unless indicated otherwise.

*: Based on GP medical files n=128.
†: High vs low tertile.
‡: High vs middle tertile.

Table 2. Association between AGE levels and Paratonia Presence (PAI)

<table>
<thead>
<tr>
<th>Paratonia presence</th>
<th>Estimate</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-4.16</td>
<td>0.02</td>
<td>0.00</td>
<td>0.10</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>AGE levels</td>
<td>1.24</td>
<td>3.47</td>
<td>1.87</td>
<td>6.44</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Visit2</td>
<td>0.08</td>
<td>1.08</td>
<td>0.56</td>
<td>2.08</td>
<td>.819</td>
</tr>
<tr>
<td>Visit3</td>
<td>1.06</td>
<td>2.89</td>
<td>1.41</td>
<td>5.90</td>
<td>.004</td>
</tr>
</tbody>
</table>

GLMM, generalized linear mixed model.

GLMM was obtained after removing statistically insignificant variables. Response variable: PAI (the presence of paratonia “no/yes”). Explanatory variable: AGE levels.
Table 3 indicates that, after correction for MMSE, paratonia severity is significantly associated with the AGE levels (β = 0.17, 95% CI: 0.11 - 0.23, \( P < .001 \)) and with the progression over a time span of 1 year (β = 0.18, 95% CI: 0.12 - 0.25, \( P < .001 \)). To further investigate the degree in which the latter is due to within participant association, we computed respectively the difference in paratonia severity (MAS-P) and in AGEs between visit 3 and visit 1. These within participant difference were further investigated on degree of association and the regression of AGEs on paratonia severity (MAS-P).

**Table 3. Association between AGE levels and Paratonia Severity (MAS-P)**

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>95% CI lower limit</th>
<th>95% CI upper limit</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.30</td>
<td>0.04</td>
<td>0.56</td>
<td>.026</td>
</tr>
<tr>
<td>MMSE</td>
<td>-0.02</td>
<td>-0.03</td>
<td>-0.01</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>AGE levels</td>
<td>0.17</td>
<td>0.11</td>
<td>0.23</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Visit2</td>
<td>-0.01</td>
<td>-0.08</td>
<td>0.05</td>
<td>.576</td>
</tr>
<tr>
<td>Visit3</td>
<td>0.18</td>
<td>0.12</td>
<td>0.25</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

LMM, linear mixed model.
LMM was obtained after removing statistically insignificant variables. Response variable: MAS-P. Explanatory variable: AGE levels.

The 1-year development of AGE levels and paratonia presence (PAI) and severity (MAS-P) is presented in Table 4. One hundred and eighteen participants (82%) completed this study, of whom 84 showed no paratonia at baseline. After a 1-year follow-up, 32 participants developed paratonia, resulting in a 1-year incidence of 22.2%. Of the remaining participants, 40 exhibited no paratonia, 35 having persistent paratonia and 11 participants varied in paratonia status throughout the study.

In participants with paratonia throughout the study the AGE levels and severity (MAS-P) of paratonia increased. Participants who developed paratonia showed the highest increase in AGE levels and paratonia severity (MAS-P). AGE levels remained stable in participants without paratonia after 1-year follow up.

The difference in AGE levels change in 1 year between participants who developed paratonia and participants without paratonia was significant (95% CI: -0.40 to -0.04, \( P = .016 \)). There was no significant difference in AGE levels change between participants with persistent paratonia and without paratonia nor between participants with persistent paratonia and participants who developed paratonia (\( P = .052 \) and \( P = .99 \), respectively). Changes in AGE levels between visits 3 and 1 were not significantly correlated (\( r = .17, P = .062 \)) with changes
in paratonia severity (MAS-P) over this time period. In the group that developed paratonia the results were similar (r = .17, P = .344). In addition, the linear regression shows that change in AGE levels is not predictive for changes in paratonia severity (MAS-P) (β = 0.14, 95% CI: -0.007 to 0.296, P = .062) even after adjusting for baseline characteristics (β = 0.13, 95% CI: -0.44 to 0.299, P = .144) as shown in Table 5.

Table 4. One-Year Development of AGE Levels and Paratonia Presence (PAI) and Severity (MAS-P)

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Difference Visit 1-Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% CI upper limit</td>
</tr>
<tr>
<td>All participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paratonia (%)</td>
<td>41.7</td>
<td>43.8 §</td>
<td>56.8*</td>
<td></td>
</tr>
<tr>
<td>MAS-P</td>
<td>0.40 (0.40)</td>
<td>0.40 (0.38) †</td>
<td>0.58 (0.41) *</td>
<td>-0.29</td>
</tr>
<tr>
<td>AGE levels</td>
<td>2.8 (0.7)</td>
<td>2.9 (0.7) *</td>
<td>3.0 (0.7) *</td>
<td>-0.25</td>
</tr>
<tr>
<td>Participants with paratonia throughout the year (n=35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAS-P</td>
<td>0.64 (0.32)</td>
<td>0.60 (0.43) †</td>
<td>0.89 (0.42) *</td>
<td>-0.38</td>
</tr>
<tr>
<td>AGE levels</td>
<td>3.1 (0.8)</td>
<td>3.2 (0.7) *</td>
<td>3.4 (0.6) *</td>
<td>-0.46</td>
</tr>
<tr>
<td>Participants without paratonia throughout the year (n=40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAS-P</td>
<td>0.16 (0.20)</td>
<td>0.19 (0.22) †</td>
<td>0.28 (0.22) *</td>
<td>-0.19</td>
</tr>
<tr>
<td>AGE levels</td>
<td>2.7 (0.7)</td>
<td>2.7 (0.7) *</td>
<td>2.7 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Participants developing paratonia throughout the year (n=32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAS-P</td>
<td>0.18 (0.20)</td>
<td>0.29 (0.27) §</td>
<td>0.65 (0.37) *</td>
<td>-0.60</td>
</tr>
<tr>
<td>AGE levels</td>
<td>2.7 (0.6)</td>
<td>2.8 (0.7) §</td>
<td>3.0 (0.5) *</td>
<td>-0.38</td>
</tr>
<tr>
<td>AGE levels change (delta visit 3/ visit 1) ‡</td>
<td>-0.40</td>
<td>-0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AGE = advanced glycation end-product; MAS-P = modified Ashworth scale for paratonia.
Data represent mean values (SD) unless indicated otherwise.
§:  Significantly different from visit 1 and visit 3.
*:  Significantly different from visit 1.
†:  Significantly different from visit 3.
‡:  Significantly different from participants without paratonia.
DISCUSSION

It was ascertained that AGE levels are associated with the presence and severity of paratonia in patients in early stage AD and AD/VaD. During the course of 1 year, paratonia presence and severity as well as AGE levels increased.

This study reveals a statistically significant increase of paratonia severity (MAS-P) of 0.17 following a 1-year duration. The mixed model analyses revealed a significant association, but mixed models are an average of within participant differences over time and between participant differences at the different time (visit) points. Therefore, we decided to conduct more analyses (correlation and regression analyses on the change scores) to provide more clarity. The effect of time in the mixed model is a within effect. Data from the extra analysis provide some evidence for association between paratonia severity and AGEs within participants (correlation and simple regression show both borderline significance of .062). Therefore the significance of the effect (regression coefficient) from mixed models of AGEs on paratonia severity is caused by within participant association, and by between (cross-sectional per visit) participant association. The fact that the effect is small in size is most probably caused by a relative small time span of 1 year between visit 1 and visit 3. Changes in paratonia severity could be explained by the occurrence of new paratonia cases and due to slow aggravation of paratonia severity in those patients who already exhibited paratonia at baseline. Furthermore, because severe paratonia is related to profound

<table>
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<th>Estimate</th>
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<tr>
<td>AGE levels change</td>
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<td>-0.04</td>
<td>.144</td>
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Linear regression model. Corrected for possible explanatory variables.
Response variable: MAS-P change (delta visit 3/visit1). Explanatory variable: AGE levels change (delta visit 3/visit1).
limitations in functional mobility, as mentioned in the introduction, this relationship is of clinical importance. It has to be considered that our cohort existed of early stage dementia patients in which paratonia was less severe resulting in a limitation of range in MAS-P scores. The MAS has a tendency to cluster scores in the lower range, limiting the ability to discriminate between patients with low scores. The follow-up period of 1 year was probably too brief to detect a significant correlation between changes in AGE levels and changes in paratonia severity, however, it does provide an estimation on the progression speed on AGEs accumulation as well as the worsening of paratonia within patients with early stage dementia. In a range between 0 and 4 on the MAS-P, an increase of 0.17 in 1 year seems to be clinically moderate in size. However, with a typical clinical disease duration of 8 to 10 years, it becomes clinically relevant when these differences are extrapolated. Further research with a longer follow up is necessary to investigate how changes in AGE levels are related to changes in paratonia severity during the course of AD, AD/VaD or other dementias.

The speed by which collagen tissue is stretched, influences the degree to which the tissue elongates. The more rapid a movement occurs, the stiffer the connective tissue behaves. One of the criteria for diagnosing paratonia is the perceived increase in resistance during passive movement acceleration by the assessor and is exactly what can be expected from cross-linking AGE accumulation in peripheral tissue. This could imply that in early stage dementia, 2 phenomena are responsible for the development of paratonia: a central cerebral factor because of the AD pathology and a peripheral factor because of AGE accumulation. It could be hypothesised that, in early stage AD, AGE accumulation is a greater factor initiating movement stiffness and that the central cerebral factor builds up or even accelerates paratonia. Future research is necessary to study this more in depth.

It is of importance to investigate whether paratonia could be postponed or movement stiffness could be improved by reducing AGE levels. Excessive elevation of glucose concentration, such as in DM, most likely accelerates the glycation of proteins. Intensive glycaemic control may be a method for decreasing AGEs formation. For this study, we did not request permission to obtain blood samples from our participants as we expected that this would hamper ethical approval. Therefore, as a consequence, glycaemia could not be controlled in this study.

AGEs are not only produced endogenously; they can also be ingested via food. Specific circulating AGE levels correlate to dietary consumption, especially in food that has been processed at very high temperatures, such as frying, broiling, grilling and roasting. Dietary intake is a possible factor that can be influenced. However, evidence of the harmful
effects of long-term exposure to dietary AGEs are currently inconclusive. Regular physical exercise could also attenuate the formation and accumulation of AGEs, however, literature regarding the effects of physical exercise on AGEs formation is minimal, and the optimal exercise modalities remain ambiguous. Pharmacological approaches to prevent AGE formation or AGE accumulation can be divided into several classes as a function of their mechanism of action: AGE absorption inhibitors, AGE formation inhibitors, AGE cross-link breakers, RAGE antagonists, and AGE binders. Several of these pharmacological strategies with anti-AGEs effects are currently being studied, but results show conflicting evidence and additional research is necessary to obtain an optimal combination of possible strategies. Studies investigating the AGE targeted interventions on paratonia could be considered as a proof of concept underpinning the causal relationship of AGEs and paratonia.

**Strength and Limitations**

This is the first study investigating the association between AGEs and paratonia. The primary strength of this study is its longitudinal design with 3 assessments. A prolonged study over several years in a larger cohort could result in improved insight into the long-term effects and the within participant relation of AGEs on the development of paratonia. In fact the AGE formation, from a reversible to an irreversible end product takes weeks to months. The (collagen) tissue accumulation and its potential impact on paratonia, is a process that generally costs several months to years. Further studies involving dementia patients in very early stages over a longer time period (e.g., several years) could help to enlighten this aspect. In addition, a causal relationship cannot be inferred and further longitudinal studies over several years are needed to investigate this. Another strength of this study is that patients were included spread across The Netherlands (urban and rural), which created a study sample representative for this population. This study also has a number of limitations. First, the calculated number of 152 participants was not reached. However, the study did comprise a reasonable number of 144 participants for baseline and 118 for follow up analysis. Second, it was not feasible to indicate which type of AGEs (e.g., cross-linking or non-cross-linking) was responsible for the findings because of the limitations of the AGE reader. Future fundamental research is necessary to further explore this in order to enable targeted interventions to postpone the development or worsening of paratonia in patients with dementia.
CONCLUSION

This study suggests that elevated AGE levels are a contributing factor to the presence of paratonia as well as its severity in patients with early stage AD or AD/VaD. These results provide a new perspective on paratonia. Future research is necessary to investigate the effect of on AGE-targeted interventions to reduce or postpone the development of paratonia in dementia.
REFERENCES


