Associations of urinary 8-iso-prostaglandin F\(_{2\alpha}\) levels with all-cause dementia, Alzheimer’s disease, and vascular dementia incidence: results from a prospective cohort study

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### Abstract

**Introduction:** Prospective studies on a potential association of 8-iso-prostaglandin F\(_{2\alpha}\) (8-iso-PGF\(_{2\alpha}\)) levels, a biomarker of lipid peroxidation, with dementia are limited.

**Methods:** Multivariate Cox regression models were used to assess potential associations of urinary 8-iso-PGF\(_{2\alpha}\) levels with all-cause, Alzheimer’s disease (AD), and vascular dementia (VD) incidence in 5853 older adults from a German, population-based cohort.

**Results:** Over 14 years of follow-up, 365 all-cause dementia cases including 127 VD and 109 AD cases were diagnosed. Participants in the top compared to the bottom 8-iso-PGF\(_{2\alpha}\) tertile had a 45% increased risk of all-cause dementia incidence (hazard ratio [95% confidence interval]: 1.45 [1.12 to 1.88]). Interaction with the apolipoprotein E (APOE) \(\varepsilon4/\varepsilon4\) genotype was detected (\(P=0.02\)). Furthermore, continuously modeled, logarithmized 8-iso-PGF\(_{2\alpha}\) levels were statistically significantly associated with all-cause dementia and AD incidence.

**Discussion:** Oxidative stress may be involved in the pathogenesis of dementia. Individuals with increased 8-iso-PGF\(_{2\alpha}\) levels and the APOE \(\varepsilon4/\varepsilon4\) genotype showed a considerably increased dementia risk.

### Keywords
8-iso-prostaglandin F\(_{2\alpha}\), Alzheimer’s disease, cohort study, dementia, oxidative stress, vascular dementia

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**1 | BACKGROUND**

Growing evidence shows that oxidative stress (OS) plays an important role in the development of dementia, especially of Alzheimer’s disease (AD) and vascular dementia (VD).\(^1\) OS occurs if the production of oxidants exceeds antioxidant defenses, causing a disruption of redox signaling and control.\(^2\) Reactive oxygen species (ROS), the most abundantly produced oxidants, are increasingly produced and damage proteins and lipids in the brain. Consequently, neurodegeneration and cell death occur.\(^1,3\) However, it has been observed that OS is not only associated with dementia, but also with its risk factors, including hypertension, diabetes, hypercholesterolemia, obesity, depression,
smoking, and low physical activity. Therefore, OS could be a mediator.

F₂-isoprostanes are considered to be the gold standard of OS biomarkers because they are chemically stable in biological fluids like blood, urine, and cerebrospinal fluid. F₂-isoprostanes, a family of prostaglandin isomers, are produced in vivo by ROS induced oxidation of polyunsaturated fatty acids. The 8-iso-prostaglandin F₂α molecule (8-iso-PGF₂α; synonym: 15-F₂t-isoprostane) is the most frequently measured member of the F₂-isoprostane family because, unlike some other F₂-isoprostanes, it has been shown to be biologically active (it can bind to the prostanoid thromboxane A₂ receptor and can mediate vasoconstriction and bronchoconstriction). The levels of 8-iso-PGF₂α and other F₂-isoprostanes have previously been shown to be increased in plasma and/or urine samples of patients with diabetes, obesity, hypercholesterolemia, asthma, cardiovascular disease, stroke, or cancer. Moreover, F₂-isoprostane levels were found to be increased in neurodegenerative diseases like AD (in cerebrospinal fluid) and Parkinson's disease (in plasma). However, because most studies utilized a cross-sectional study design in which reverse causation cannot be excluded, the predictive value of 8-iso-PGF₂α levels remains unexplored. To date, only a small longitudinal study of elderly men examined associations of urinary 8-iso-PGF₂α levels and AD, as well as all-cause dementia, and found no association.

The present study examined the association of urinary 8-iso-PGF₂α levels with all-cause dementia, AD, and VD incidence in a large, prospective cohort study and potential interactions with established dementia risk factors were investigated. Apart from this, the associations between known dementia risk factors with dementia incidence and 8-iso-PGF₂α levels were examined to indicate whether oxidative stress might be a mediator between other dementia risk factors and dementia development.

2 | METHODS

2.1 | Study population

The ESTHER study (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung [German]) is a prospective cohort study established in Saarland, a German federal state. A total of 9940 study participants between the age of 50 and 75 years were recruited during a general health checkup from 2000 to 2002. Participants were followed up 2, 5, 8, 11, and 14 years after baseline so far. Details have been described elsewhere. The distribution of sociodemographic baseline characteristics and common prevalent chronic diseases were similar to the distribution in the respective age categories in the German National Health Survey, which is a representative sample of the German population. The study was approved by the ethics committees of the Heidelberg University and the state medical board of Saarland, Germany.

2.2 | Dementia ascertainment

Dementia information was collected at the 14-year-follow-up via questionnaires sent to the study participants' general practitioners (GPs). Details have been published elsewhere. In brief, the dementia ascertainment included the sending of standardized questionnaires to the GPs of all study participants, including those who dropped out during follow-up due to ill health or had died. The GPs were asked several dementia-related questions, including whether they were aware of a dementia diagnosis for their patients. If so, they were asked to provide all available medical records of neurologists, psychiatrists, memory, or other specialized providers that documented the diagnosis of dementia. The current guidelines in Germany for AD diagnosis follow the National Institute on Aging and the Alzheimer's Association or the International Working Group (IWG)-2 criteria.

2.3 | Measurement of 8-iso-prostaglandin F₂α (8-iso-PGF₂α) levels

Urine 8-iso-PGF₂α levels were measured from spot urine samples collected during the health checkup at baseline. Almost all urine samples were collected in the morning (98%) and there was no rule for a time distance to the last urination. Urine samples were shipped...
to the study center and were stored at −80°C for 14 to 16 years until 8-iso-PGF$_{2\alpha}$ levels were measured in summer/autumn 2016. Urinary levels of F$_2$-isoprostanes are generally considered stable in frozen samples but long-term storage studies are still lacking in the literature. The 8iso1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Detroit R&D (Detroit, MI, USA) to determine 8-iso-PGF$_{2\alpha}$ levels in urine samples, which were not purified by high-performance liquid chromatography (HPLC) before analysis. The assay was performed according to the manufacturer’s protocol as described previously. In brief, this assay is based on the competition between 8-iso-PGF$_{2\alpha}$ in the sample and an 8-iso-PGF$_{2\alpha}$-horseradish peroxidase conjugate for a limited number of 8-iso-PGF$_{2\alpha}$-specific rabbit anti-serum binding sites. According to the manufacturer, measurement of authentic 8-iso-PGF$_{2\alpha}$ and a panel of eicosanoids structurally similar to 8-iso-PGF$_{2\alpha}$ showed a specificity of this assay for 8-iso-PGF$_{2\alpha}$ of 100% with cross-reactivity to other compounds <0.1%. We are not aware of a manufacturer independent study that checked these claims. Usually, results from ELISAs are not comparable with those from more precise gas chromatography (GC) or liquid chromatography tandem mass-spectrometry (LC-MS/MS) because they produce higher absolute values due to cross-reactivity. Generally, ELISA and LC-MS/MS results correlate better when measured in urine than in plasma samples but still no correlation coefficients >0.610 should be expected.

To correct for variability in dilution of 8-iso-PGF$_{2\alpha}$ molecules in the urine samples, they were standardized by urinary creatinine levels. Thus, 8-iso-PGF$_{2\alpha}$ levels are expressed in nmol/mmol creatinine. The creatinine concentration was determined by the kinetic Jaffe method.

### 2.4 Covariate assessment

Information on age, sex, education, smoking status, alcohol consumption, physical activity, body mass index (BMI), and lifetime history of depression were obtained from a standardized self-administered questionnaire. History of diabetes and cardiovascular disease (CVD) were physician-reported diagnoses. The apolipoprotein E (APOE) ε4 genotypes were measured using TaqMan single-nucleotide polymorphism (SNP) genotyping assays with genotypes analyzed in an endpoint allelic discrimination test using a PRISM 7000 Sequence detection system (Applied Biosystems). Total cholesterol was measured from serum samples by an enzymatic colorimetric test with the Synchron LX multicalibrator system (Beckman Coulter, Galway, Ireland). Serum C-reactive protein (CRP) levels were determined by immunoturbidimetry with the wrCRP antibody (Bayer, Leverkusen, Germany) on the ADVIA 2400.

### 2.5 In- and exclusion criteria

Participation in the ESTHER study (baseline age range 50 to 75 years) was the only inclusion criterion. Exclusion criteria were unavailability of information or uncertainty about a dementia diagnosis during follow-up and unavailability of an 8-iso-PGF$_{2\alpha}$ measurement. Dementia information could not be collected for participants who withdrew consent to contact their GP (n = 1121) or whose GPs withdrew consent to be contacted (n = 304) during follow-up (see flow-chart in Figure S1 in supporting information). Furthermore, dementia information was not available if GPs could not be contacted, for example, due to closure of practice, retirement or death (n = 930), or due to other reasons like address changes (n = 105). In total, the dementia questionnaire was repeatedly sent to the GPs of n = 7480 study participants. Information was received from the GPs of n = 6422 study participants (response rate: 85.9%). Participants were excluded if GPs did not have information about whether dementia was diagnosed (n = 288), or if dementia diagnosis was suspected (n = 108), which resulted in suitable dementia information for n = 6026 study participants. A few of these study participants (n = 173) did not donate a urine sample or the 8-iso-PGF$_{2\alpha}$ biomarker could not be measured. Therefore, in total, n = 5853 study participants could be included in the present analysis.

Baseline characteristics of the included n = 5853 and excluded n = 4087 ESTHER study participants were reasonably comparable, supporting the absence of selection bias, although many factors showed statistically significant differences given the large sample size (Table S1 in supporting information).

### 2.6 Statistical analyses

The associations of baseline characteristics with levels of 8-iso-PGF$_{2\alpha}$ in the top tertile (>0.242 nmol/mmol creatinine) were determined by a multivariate logistic regression model. A Cox proportional hazards regression model was used to identify baseline characteristics statistically significantly associated with all-cause dementia, AD, and VD incidence. Age and sex were pre-selected covariates adjusted for in the main Cox proportional hazards regression model, used to determine hazard ratios (HR) and 95% confidence intervals (95%CI) for the associations of 8-iso-PGF$_{2\alpha}$, levels with all-cause dementia, AD, and VD incidences. Baseline characteristics that were statistically significantly (P < .05) associated with both 8-iso-PGF$_{2\alpha}$ levels and all-cause dementia were considered to be potential confounders and adjusted for in the main Cox model in addition to age and sex. In a sensitivity analysis, we adjusted for all baseline characteristics shown in Table 1. In a further sensitivity analysis, we considered the competing risk of death by estimating cause-specific hazards and Fine-Gray subdistribution hazards. As all results of the main analysis were confirmed in competing risk models, these results are not shown.

To assess the dose-response relationship with total dementia incidence, 8-iso-PGF$_{2\alpha}$ levels were first modeled with restricted cubic splines. As this analysis suggested a non-linear relationship, fractional polynomials were used to discover the best fitting first-order term for 8-iso-PGF$_{2\alpha}$ levels. The natural logarithm had significantly better model fit than the linear term (P = .002) and therefore logarithmized isoprostane levels were used in all analyses in addition to 8-iso-PGF$_{2\alpha}$ tertiles. Analyses for dementia endpoints were carried out for the total population and stratified by sex and age. Potential interactions
### TABLE 1  
Baseline characteristics of included study participants (n = 5853) and their associations with 8-iso-prostaglandin F$_{2\alpha}$ levels in the top tertile (>0.242 nmol/mmol creatinine)

| Baseline characteristics | n (%) | 8-iso-PGF$_{2\alpha}$ levels (nmol/mmol creatinine) | Association with 8-iso-PGF$_{2\alpha}$ levels >0.242 nmol/mmol creatinine |
|--------------------------|-------|----------------------------------------------------|-------------------------------------------------------------------------|
|                          |       | Median (IQR)                                      | Odds ratio (95%CI)$^a$ P-value                                          |
| **Age (years)**          |       |                                                   |                                                                         |
| 50–64                    | 3740 (63.9) | 0.20 (0.16-0.27) | 1.00 (Ref.) Ref.                                                       |
| 65–69                    | 1309 (22.4) | 0.20 (0.15-0.26) | 0.97 (0.88-1.07) 0.606                                                |
| 70–75                    | 804 (13.7)  | 0.20 (0.16-0.27) | 0.98 (0.88-1.10) 0.741                                                |
| **Sex**                  |       |                                                   |                                                                         |
| Female                   | 3200 (54.7) | 0.21 (0.16-0.28) | 1.00 (Ref.) Ref.                                                       |
| Male                     | 2653 (45.3) | 0.19 (0.15-0.25) | 0.60 (0.53-0.69) <0.001                                               |
| **Education (years)**    |       |                                                   |                                                                         |
| 74 (1)                   | 4236 (74.1) | 0.21 (0.16-0.27) | 1.00 (Ref.) Ref.                                                       |
| 9–11                     | 819 (14.3)  | 0.19 (0.15-0.26) | 0.84 (0.71-0.99) 0.048                                                |
| ≥12                      | 661 (11.6)  | 0.19 (0.14-0.25) | 0.85 (0.70-1.03) 0.089                                                |
| **Smoking status**       |       |                                                   |                                                                         |
| Never smoker             | 2909 (50.8) | 0.20 (0.15-0.26) | 1.00 (Ref.) Ref.                                                       |
| Former smoker            | 1939 (33.9) | 0.19 (0.15-0.26) | 1.13 (0.98-1.29) 0.101                                                |
| Current smoker           | 874 (15.3)  | 0.26 (0.19-0.35) | 3.12 (2.65-3.68) <0.001                                               |
| **Alcohol consumption**  |       |                                                   |                                                                         |
| None                     | 1611 (30.3) | 0.21 (0.16-0.27) | 1.00 (Ref.) Ref.                                                       |
| Low or moderate          | 3333 (62.6) | 0.20 (0.15-0.26) | 1.17 (1.01-1.35) 0.032                                                |
| High                     | 381 (7.2)   | 0.22 (0.16-0.30) | 1.50 (1.17-1.93) 0.002                                               |
| **Physical activity**    |       |                                                   |                                                                         |
| Inactive                 | 1133 (19.4) | 0.22 (0.16-0.29) | 1.00 (Ref.) Ref.                                                       |
| Low                      | 2645 (45.3) | 0.20 (0.15-0.27) | 0.83 (0.71-0.96) 0.015                                                |
| Medium or high           | 2061 (35.3) | 0.19 (0.15-0.26) | 0.76 (0.64-0.90) 0.001                                               |
| **BMI (kg/m$^2$)**       |       |                                                   |                                                                         |
| <25                      | 1632 (27.9) | 0.20 (0.15-0.27) | 1.00 (Ref.) Ref.                                                       |
| 25–<30                   | 2738 (46.9) | 0.20 (0.15-0.26) | 0.94 (0.87-1.02) 0.136                                                |
| ≥30                      | 1473 (25.2) | 0.21 (0.16-0.28) | 1.15 (1.05-1.26) 0.003                                               |
| **CVD**                  |       |                                                   |                                                                         |
| No                       | 4709 (80.5) | 0.20 (0.16-0.27) | 1.00 (Ref.) Ref.                                                       |
| Yes                      | 1143 (19.5) | 0.20 (0.15-0.27) | 1.01 (0.87-1.18) 0.865                                                |
| **Diabetes**             |       |                                                   |                                                                         |
| No                       | 4951 (85.8) | 0.20 (0.15-0.27) | 1.00 (Ref.) Ref.                                                       |
| Yes                      | 821 (14.2)  | 0.22 (0.16-0.29) | 1.25 (1.06-1.48) 0.007                                                |
| **Life-time history of depression** |       |                                                   |                                                                         |
| No                       | 4997 (85.5) | 0.20 (0.16-0.27) | 1.00 (Ref.) Ref.                                                       |
| Yes, without current pharmacotherapy | 660 (11.3) | 0.20 (0.15-0.27) | 0.90 (0.75-1.07) 0.235                                                |
| Yes, with current pharmacotherapy | 187 (3.2)  | 0.20 (0.16-0.27) | 1.00 (0.73-1.38) 0.977                                                |
| **Total cholesterol levels (mg/dL)** |       |                                                   |                                                                         |
| <200                     | 1913 (32.7) | 0.20 (0.15-0.27) | 1.00 (Ref.) Ref.                                                       |
| 200–<240                 | 1983 (33.9) | 0.20 (0.16-0.27) | 0.98 (0.90-1.06) 0.529                                                |
| ≥240                     | 1957 (33.4) | 0.21 (0.16-0.27) | 1.07 (0.99-1.16) 0.101                                               |

(Continues)
of logarithmized 8-iso-PGF$_{2\alpha}$ levels with the baseline characteristics selected for the main model were explored by adding interaction terms to the main model. In a further sensitivity analysis, patients diagnosed with dementia in the first 7 years of follow-up were excluded to check for potential reverse causality. All analyses described in this chapter were carried out for all-cause dementia incidence because of the low case numbers for dementia subtypes.

To our knowledge, missing values of covariates were missing at random. The highest proportion of missing values for a covariate was 9.5% (APOE polymorphism). Therefore, multiple imputation could be applied to impute missing values for all study participants. Five data sets were imputed with the Markov chain Monte Carlo (MCMC) method separately by sex with the SAS procedure PROC MI. The variables for the imputation model were those shown in Table 1. All analyses were performed in the five imputed data sets and results were combined by the SAS procedure PROC MIANALYZE.

Statistical tests were two-sided using an alpha level of 0.05. All statistical analyses were conducted with the Statistical Analysis System (SAS, version 9.4, Cary, North Carolina, USA).

### RESULTS

Table 1 shows the baseline characteristics of the study population and their associations with increased 8-iso-PGF$_{2\alpha}$ levels. Approximately two thirds of the participants were between 50 and 64 years old, while one third of the participants were aged 65 to 75. Slightly more females (55%) than males (45%) were included in the sample. The median (interquartile range [IQR]) 8-iso-PGF$_{2\alpha}$ level was 0.20 [0.15 to 0.27] nmol/mmol creatinine. The median (IQR) 8-iso-PGF$_{2\alpha}$ level was statistically significantly higher (P = .02, Wilcoxon rank-sum test) among study participants that developed all-cause dementia during follow-up (0.25 [0.16-0.28] nmol/mmol creatinine) than among those who did not (0.20 [0.15 to 0.27] nmol/mmol creatinine).

Among the baseline characteristics, current smoking, high alcohol consumption, obesity (BMI $\geq$ 30 kg/m$^2$), diabetes, increased CRP levels, and the APOE $\epsilon 4$ genotype were positively associated (P <.05) with increased levels of 8-iso-PGF$_{2\alpha}$. Moreover, 8-iso-PGF$_{2\alpha}$ levels were statistically significantly lower in males, study participants with longer school education, and individuals with higher physical activity.

Among all included n = 5853 study participants, 365 cases of all-cause dementia were diagnosed during a median follow-up of 13.7 years. Thereof, 109 study participants were diagnosed with AD and 127 with VD. Increasing age, male sex, low school education, physical inactivity, an increased BMI, diabetes, medically treated depression, and the APOE $\epsilon 3/4$, as well as $\epsilon 4/4$ genotype were statistically significantly associated with an increased all-cause dementia incidence (Table S2 in supporting information). From this list of baseline characteristics, education, physical activity, BMI, diabetes, and the APOE $\epsilon 4$ polymorphism were selected for the main model in addition to age and sex because they were statistically significantly associated with both 8-iso-PGF$_{2\alpha}$ levels (Table 1) and dementia and therefore could be confounders.

Table 2 shows the associations for 8-iso-PGF$_{2\alpha}$ levels with all-cause dementia, AD, and VD. Continuously modeled, logarithmized

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**TABLE 1** (Continued)

| Baseline characteristics | n (%) | 8-iso-PGF$_{2\alpha}$ levels (nmol/mmol creatinine) | Association with 8-iso-PGF$_{2\alpha}$ levels $>0.242$ nmol/mmol creatinine |
|--------------------------|-------|---------------------------------------------------|--------------------------------------------------------------------------|
| CRP levels (mg/L)        |       | Median (IQR)                                      | Odds ratio (95% CI)$^a$ | P-value |
| $<1$                     | 1563  (26.7) | 0.19 (0.15-0.25) | 1.00 (Ref.) | Ref. |
| 1:3                      | 2202  (37.6) | 0.20 (0.15-0.27) | 1.07 (0.99-1.16) | 0.093 |
| $\geq$3                  | 2088  (35.7) | 0.21 (0.16-0.28) | 1.09 (1.00-1.19) | 0.041 |

**Note:** Numbers printed in bold are statistically significant (P < .05).

**Abbreviations:** 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$; APOE, apolipoprotein E; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; IQR, interquartile range; NSAIDs, nonsteroidal anti-inflammatory drugs.

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$^a$ Multivariate logistic regression model including all variables shown in this table.

$^b$ Definition of low or moderate alcohol consumption: women 0 to 19.99 g ethanol/day (g/d) or men 0 to 39.99 g/d; definition of high alcohol consumption: women $\geq$ 20 to 39.99 g/d or men $\geq$ 40 g/d.

$^c$ ‘Inactive’ was defined by $<1$ hour of vigorous or $<1$ hour light physical activity per week. “Medium or high” was defined by $\geq$ 2 hours of vigorous and $\geq$ 2 hours of light physical activity/week. All other amounts of physical activity were grouped into the category ‘Low.’

$^d$ CVD was defined as coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries.
8-iso-PGF\(_{2\alpha}\) levels were statistically significantly associated with all-cause dementia incidence (HR [95%CI] per 1 standard deviation [SD]: 1.47 [1.19 to 1.82]) and AD incidence (HR [95%CI] per 1 SD: 1.55 [1.05 to 2.29]), whereas the association with VD was not statistically significant although the HR point estimate was increased (HR [95%CI] per 1 SD: 1.20 [0.83 to 1.73]). When 8-iso-PGF\(_{2\alpha}\) levels were modeled in tertiles, which reduces the statistical power, only the effect estimate for all-cause dementia remained statistically significant when comparing top to bottom tertile (HR [95%CI]: 1.45 [1.12 to 1.88]). In sensitivity analyses adjusting for all assessed baseline characteristics, effect estimates were very similar and associations found to be statistically significant in the main model remained statistically significant (Table S3 in supporting information). In a further sensitivity analysis excluding patients diagnosed with dementia in the first 7 years of follow-up, the HR point estimates for all-cause dementia and AD were somewhat attenuated but the association of logarithmized 8-iso-PGF\(_{2\alpha}\) levels and all-cause dementia incidence remained statistically significant (Table S4 in supporting information).

The dose-response curve showed a steady increasing dementia risk with increasing 8-iso-PGF\(_{2\alpha}\) levels until the 75th percentile (0.268 nmol/mmol creatinine) and plateaued thereafter (Figure 1), which is typical for logarithmic relationships. The interaction term of logarithmized 8-iso-PGF\(_{2\alpha}\) levels and the APOE \(\varepsilon4/\varepsilon4\) genotype was statistically significantly associated with all-cause dementia incidence on the \(P<.05\) significance level (\(\beta = 1.95, P = .02\)) but not after correction for multiple testing (Bonferroni-corrected threshold for statistical significance: \(P < .007\)). Table 3 shows the additive risks of the APOE \(\varepsilon4/\varepsilon4\) genotype and increased 8-iso-PGF\(_{2\alpha}\) levels (defined by top tertile: >0.242 nmol/mmol creatine) for dementia development. If both risk factors were present, the dementia risk was almost ninefold increased (HR [95%CI]: 8.63 [4.55 to 16.39]). In contrast, if only increased 8-iso-PGF\(_{2\alpha}\) levels were present, the dementia risk was 1.3-fold increased (HR [95%CI]: 1.30 [1.04 to 1.61]) and if only the APOE \(\varepsilon4/\varepsilon4\) genotype was present, the dementia risk was approximately twofold increased (HR [95%CI]: 2.10 [0.93 to 4.75]).

Last, we show results for all-cause dementia stratified by sex and age groups in Tables S5 and S6 in supporting information, respectively. The association of logarithmized 8-iso-PGF\(_{2\alpha}\) levels and dementia incidence was much weaker in younger (age 50 to 64 years) than in older (age 65 to 75 years) study participants and only statistically significant in the older age groups. In contrast, the association was comparably strong and statistically significant in both men and women.

### Table 2: Associations of 8-iso-prostaglandin F\(_{2\alpha}\) levels with all-cause and common subtype dementia incidences

| 8-iso-prostaglandin F\(_{2\alpha}\) [nmol/mmol creatinine] | All-cause dementia | Alzheimer’s disease | Vascular dementia |
|---------------------------------------------------------|-------------------|-------------------|-----------------|
| n\(_{\text{total}}\) | n\(_{\text{cases}}\) | HR (95%CI)a | n\(_{\text{cases}}\) | HR (95%CI)a | n\(_{\text{cases}}\) | HR (95%CI)a |
| Per 1 SD\(^b\) | 5853 | 365 | 1.47 (1.19-1.82) | 109 | 1.55 (1.05-2.29) | 127 | 1.20 (0.83-1.73) |
| Tertile 1 (<0.169) | 1951 | 105 | 1.00 (Ref.) | 33 | 1.00 (Ref.) | 37 | 1.00 (Ref.) |
| Tertile 2 (>0.169-0.242) | 1952 | 123 | 1.16 (0.89-1.51) | 27 | 0.80 (0.48-1.34) | 48 | 1.30 (0.85-2.01) |
| Tertile 3 (>0.242) | 1950 | 137 | 1.45 (1.12-1.88) | 49 | 1.54 (0.98-2.41) | 42 | 1.28 (0.82-2.00) |

Note: Numbers printed in bold are statistically significant (\(P < .05\)).

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

\(^{a}\)The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and APOE \(\varepsilon4\) polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin F\(_{2\alpha}\) levels

\(^{b}\)1 SD of 8-iso-prostaglandin F\(_{2\alpha}\) levels = 0.278 nmol/mmol creatinine

4 | Discussion

In this prospective cohort study, logarithmized 8-iso-PGF\(_{2\alpha}\) levels were significantly associated with all-cause dementia. An interaction test revealed that the simultaneous presence of the APOE \(\varepsilon4/\varepsilon4\) genotype and increased 8-iso-PGF\(_{2\alpha}\) levels substantially increased the risk of dementia. Regarding dementia subtypes, logarithmized 8-iso-PGF\(_{2\alpha}\) levels were statistically significantly associated with AD but not with VD. However, case numbers were limited for VD and future, larger studies may also establish an association of 8-iso-PGF\(_{2\alpha}\) levels with VD.
TABLE 3 Interaction of APOE ε4/ε4 genotype and 8-iso-prostaglandin F₂α levels for all-cause dementia incidence

| APOE ε4/ε4 | 8-iso-PGF₂α > 0.242 nmol/mmol creatinine | All-cause dementia | | | |
|---|---|---|---|---|---|
| No | No | 3853 | 222 | Ref. |
| No | Yes | 1916 | 127 | 1.30 (1.04-1.61) |
| Yes | No | 50 | 6 | 2.10 (0.93-4.75) |
| Yes | Yes | 34 | 10 | 8.63 (4.55-16.39) |

Abbreviations: APOE, apolipoprotein E; 8-iso-PGF₂α, 8-iso-prostaglandin F₂α; CI, confidence interval; HR, hazard ratio.

NOTE: Numbers printed in bold are statistically significant (P < .05). The P value for the interaction term of APOE ε4/ε4 genotype and 8-iso-prostaglandin F₂α levels (continuous) was P = .0002.

*The model was adjusted for age (continuously), sex, education, physical activity, body mass index (categorical), and diabetes.

4.2 Interpretation of the findings

The association of a biomarker of lipid peroxidation with all-cause dementia incidence and especially AD incidence observed in this study could be potentially explained by oxidative damages to neural cells. F₂-isoprostanes are produced by ROS induced peroxidation of arachidonic acid. In the brain, neurons are prone to ROS and oxidative damage because of high oxygen consumption, high energy production, and an impaired antioxidant defense mechanism. If redox homeostasis fails and ROS are excessively generated, F₂-isoprostanes can be formed. Oxidative damage then becomes apparent through alteration of integrity, fluidity, and permeability of neuronal membranes.

However, OS is related to other dementia risk factors and could also be a mediator. The potential pathways to dementia, involving lipid oxidation, are summarized in Figure 2. Low physical activity and diabetes, which have been recognized as risk factors for dementia also by other studies, were associated with dementia incidence and 8-iso-PGF₂α levels in our study. Moreover, it is well known that low physical activity promotes type 2 diabetes and that OS is also a risk factor for type 2 diabetes. Therefore, there is a cluster of three important risk factors for dementia that influence each other. In addition, 8-iso-PGF₂α levels were statistically significantly increased in subjects with the APOE ε4/ε4 genotype. However, physical activity, diabetes, the APOE ε4/ε4 genotype, and 8-iso-PGF₂α levels were also independently associated with all-cause dementia incidence in our study. HR point estimates for the associations of physical activity, diabetes, and the APOE ε4/ε4 genotype with dementia outcomes changed only slightly when the model was additionally adjusted for 8-iso-PGF₂α levels (Table S7 in supporting information). These results suggest that physical activity, diabetes, the APOE ε4/ε4 genotype, and increased 8-iso-PGF₂α levels are independent risk factors for all-cause dementia although they are interrelated.
Interestingly, an interaction between the APOE ε4/ε4 genotype and increased 8-iso-PGF\textsubscript{2α} levels was observed for all-cause dementia incidence. A possible mechanism for the interaction could be that individuals with the APOE ε4/ε4 genotype are more susceptible to the neurotoxic effects of OS and amyloid pathology.\textsuperscript{45} Normally, in the unimpaired lipid metabolism, apoE transfers toxic peroxidized lipids to astrocytes, preventing neuronal degradation.\textsuperscript{46} However, during the state of OS, microglias are activated and induce A1-reactive astrocytes. Instead of promoting neuronal survival, these cause neurodegeneration.\textsuperscript{47}

### 4.3 Strengths and limitations

The strengths of this study include the prospective cohort design, a representative sample of an older adult population (study participants with and without dementia information had similar baseline characteristics, Table S1), the large sample size (n = 5835), and a long follow-up period (14 years).

A general limitation is the observational study design. Although results were controlled for important potential confounders, residual confounding cannot be excluded. Another limitation is that the latency period from the onset of AD pathogenesis until a clinical dementia diagnosis can be even longer than 10 years.\textsuperscript{48} Hence, the long follow-up period of 14 years cannot totally exclude reverse causality. However, the observation of a statistically significant association of logarithmized 8-iso-PGF\textsubscript{2α} levels and all-cause dementia even after exclusion of events in the first 7 years refute a strong impact of reverse causality on the main results.

The dementia diagnoses collected in the ESTHER study reflect the community-based clinic setting. No screening for dementia was performed at baseline and no specific diagnostic procedures were used for the study. Therefore, the specific dementia subtype was often not determined with certainty, which may explain the low proportion of diagnosed AD among the all-cause dementia cases. However, other studies showed that most dementia patients have a mixed dementia type anyway.\textsuperscript{49,50} Another limitation is that 8-iso-PGF\textsubscript{2α} levels were only measured once at baseline (not in duplicates or triplicates at baseline and not repeatedly measured during follow-up) and with an ELISA (instead of more precise GC- or LC-MS/MS methods). These limitations were necessary to enable measuring the large number of samples in this cohort study in a cost-efficient manner. Potentially resulting imprecision of the measurements can be expected to be non-differential with respect to dementia outcomes and might have led to some underestimation of associations. Finally, a limitation of our study is that it can only be generalized to white populations aged 50 to 75 years.

### 5 CONCLUSION

In conclusion, this prospective cohort study showed that 8-iso-PGF\textsubscript{2α} levels were statistically significantly associated with all-cause dementia and AD incidence. Due to the relatively low case numbers for VD in our study, the non-significant finding for this outcome should not be interpreted as the absence of an association. Larger studies are needed for dementia subtypes that are less frequent than AD. Furthermore, future studies should corroborate the observed interaction of the APOE ε4/ε4 genotype and 8-iso-PGF\textsubscript{2α} levels, which was not statistically significant after correction for multiple testing but biologically plausible. In addition, the role of lipid peroxidation in dementia development should be explored by further basic research studies.

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### CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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