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Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter

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Cigarette smoking doubles the risk of dementia and Alzheimer's disease. Various pathophysiological pathways have been proposed to cause such a cognitive decline, but the exact mechanisms remain unclear. Smoking may affect the microstructural integrity of cerebral white matter. Diffusion tensor imaging is known to be sensitive for microstructural changes in cerebral white matter. We therefore cross-sectionally studied the relation between smoking behaviour (never, former, current) and diffusion tensor imaging parameters in both normal-appearing white matter and white matter lesions as well as the relation between smoking behaviour and cognitive performance. A structured questionnaire was used to ascertain the amount and duration of smoking in 503 subjects with small-vessel disease, aged between 50 and 85 years. Cognitive function was assessed with a neuropsychological test battery. All subjects underwent 1.5 Tesla magnetic resonance imaging. Using diffusion tensor imaging, fractional anisotropy and mean diffusivity were calculated in both normal-appearing white matter and white matter lesions. A history of smoking was associated with significant higher values of mean diffusivity in normal-appearing white matter and white matter lesions (P-trend for smoking status = 0.02) and with poorer cognitive functioning compared with those who never smoked. Associations with smoking and loss of structural integrity appeared to be strongest in normal-appearing white matter. Furthermore, the duration of smoking cessation was positively related to lower values of mean diffusivity and higher values of fractional anisotropy in normal-appearing white matter [$\beta = -0.004$ (95% confidence interval -0.007 to 0.000; $P = 0.03$) and $\beta = 0.019$ (95% confidence interval 0.001–0.038; $P = 0.04$)]. Fractional anisotropy and mean diffusivity values in normal-appearing white matter of subjects who had quit smoking for >20 years were comparable with subjects who had never smoked. These data suggest that smoking affects the microstructural integrity of cerebral white matter and support previous data that smoking is associated with impaired cognition. Importantly, they suggest that quitting smoking may reverse the impaired structural integrity.

Keywords: smoking; smoking status; smoking cessation; cognition; DTI; normal-appearing white matter; white matter lesions
Abbreviations: DTI = diffusion tensor imaging; NAWM = normal-appearing white matter

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Introduction

Smoking affects cognitive performance (Richards *et al.,* 2003) and prospective studies have shown a doubled risk of Alzheimer's disease and dementia not otherwise specified (Ott *et al.,* 1998). The underlying mechanism remains unclear but cerebral small-vessel disease, including white matter lesions and lacunar infarcts (Roman *et al.*, 2002), may play a role as it is both related with the risk factor (smoking) and the outcome. Previous aetiological studies mainly focused on these macrostructural changes of the brain (Liao *et al.*, 1997; Howard *et al.*, 1998; Longstreth *et al.*, 2000), whereas it seems biologically plausible that there are additional effects of cigarette smoking on cognitive decline that may go beyond the detection limit of conventional MRI sequences.

Assessment of the microstructural integrity of the cerebral white matter has become available with the introduction of diffusion tensor imaging (DTI). DTI is sensitive to subtle changes in cerebral white matter and gives quantitative information about damage to white matter structures (Basser *et al.*, 1994*b*; Basser and Pierpaoli, 1996). The most frequently used parameters to quantify this structural integrity include the fractional anisotropy and mean diffusivity. Fractional anisotropy is believed to decrease, conversely mean diffusivity is thought to increase with impaired structural integrity (Pierpaoli *et al.*, 1996; Le Bihan *et al.*, 2001).

The use of DTI also enables us to investigate the effects of smoking on the microstructural integrity of the white matter and cognition. In addition, the structural integrity of the white matter and the cognitive performance of those who stopped smoking can be assessed. This may yield insight into the (repair) mechanisms related to possible recovery of cognitive performance and the attendant structural correlates. Recent findings from the Nurses' Health Study indicated that smoking cessation was associated with a strong decrease in excess risk of vascular mortality and lung diseases (Kenfield *et al.*, 2008). In line with this observation, the risk of cerebrovascular disease, and its related cognitive consequences, can probably be reversed by quitting smoking. However, virtually nothing is known about this possible risk reduction in cognitive decline and the underlying anatomical substrate after smoking cessation. We therefore wanted to investigate the association between smoking, smoking cessation and DTI parameters in both normal-appearing white matter (NAWM) and white matter lesions. We hypothesized that smoking would be negatively related with both the degree of structural integrity in both NAWM and white matter lesions, and cognitive function. We did so at the cross-sectional level among 503 subjects with small-vessel disease from the Radboud University Nijmegen DTI and MRI cohort study.

Materials and methods

Study population

This study is embedded within the Radboud University Nijmegen DTI and MRI cohort study, a prospective cohort study that was designed to investigate risk factors and cognitive, motor and mood consequences of functional and structural brain changes as assessed by MRI among elderly with cerebral small-vessel disease. The primary outcome of the longitudinal part of this study is the development of dementia or parkinsonism.

Cerebral small-vessel disease is characterized on neuroimaging by either white matter lesions or lacunar infarcts. Symptoms of small-vessel disease include acute symptoms, such as transient ischaemic attacks or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) and/or mood disturbances (Roman *et al.*, 2002). As the onset of cerebral small-vessel disease is often insidious, clinically heterogeneous, and typically with mild symptoms, it has been suggested that the selection of subjects with cerebral small-vessel disease in clinical studies should be based on these more consistent brain imaging features (Erkinjuntti, 2002). Accordingly, consecutive patients referred to the Department of Neurology between October 2002 and November 2006, were selected for participation.

Inclusion criteria were: (i) age between 50 and 85 years; and (ii) cerebral small-vessel disease on neuroimaging [white matter lesions and/or lacunar infarct(s)]. Subsequently, the above-mentioned acute or subacute clinical symptoms of small-vessel disease were assessed by standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes. Exclusion criteria included: (i) dementia (American Psychiatric Association, 2000); (ii) parkinson(ism) (Gelb *et al.*, 1999; Louis and Luchsinger, 2006); (iii) intracranial haemorrhage; (iv) life expectancy of <6 months; (v) intracranial space-occupying lesion; (vi) (psychiatric) disease interfering with cognitive testing or follow-up; (vii) recent or current use of acetylcholine esterase inhibitors, neuroleptic agents, L-dopa or dopa-a(nta)gonists; (viii) non-small-vessel disease-related white matter lesions mimics (e.g. multiple sclerosis); (ix) prominent visual or hearing impairment; (x) language barrier; or (xi) MRI contraindications or known claustrophobia.

From 1004 invited individuals, 727 were eligible and 525 agreed to participate. Complete information, including a cerebral MRI scan was obtained from 503 individuals. Exclusion criteria were found in 22 individuals during their visit to our research centre (14 with unexpected claustrophobia, one died before MRI scanning, one was diagnosed with multiple sclerosis, in one there was a language barrier, one subject fulfilled the criteria for Parkinson's disease and four met the dementia criteria), yielding a response of 71.3% (503/705) for the original cohort of the study. These 503 individuals had symptoms of transient ischaemic attack or lacunar syndrome ($n = 219$), cognitive disturbances ($n = 245$), motor disturbances ($n = 97$), depressive symptoms ($n = 100$) or a combination thereof. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem–Nijmegen approved the study.

Cigarette smoking assessment

A structured questionnaire was used to ascertain smoking behaviour. Information on smoking behaviour (current, former and never) was obtained; current smokers were asked their age at which they started smoking, periods (years) during which they did not smoke and the average number of cigarettes smoked per day. Former smokers were asked about starting age, periods (years) during which they did not smoke, the age at which they stopped smoking and the average daily number of cigarettes smoked. 'Pack-years' were calculated as the number of cigarettes smoked per day divided by 20, multiplied by the number of years they had smoked. Complete data were available for all subjects.

Cognitive assessment

Cognitive function was measured with a neuropsychological test battery that proved to be sensitive and suitable for this purpose in other, large epidemiological studies (de Groot *et al.*, 2000). The tests included the Mini-Mental State Examination (Folstein *et al.*, 1975) and the Rey Auditory Verbal Learning Test (Van der Elst *et al.*, 2005). To evaluate speed of mental processes, four tests were used: (i) the Stroop test (Houx *et al.*, 1993); (ii) the Paper–Pencil Memory Scanning Task (Sternberg, 1969); (iii) the Symbol–Digit Substitution Task (Lezak, 1995); and (iv) a Verbal Fluency Task in which as many animals, followed by as many professions as possible, had to be named within 60 s. Attention was measured by the Verbal Series Attention Test (Mahurin and Cooke, 1996). To evaluate visuo-spatial memory we used the Rey's Complex Figure Test (Osterrieth, 1944). Performance across tests was made comparable by transforming the raw test scores into *z*-scores as described elsewhere (de Groot *et al.*, 2000). Due to the expected systemic effect of smoking on the brain (and thus on cognition) we chose to construct compound scores of cognition that would cover most cognitive domains that have been validated and proven its value in other large epidemiological studies (de Groot *et al.*, 2000). To evaluate global function, the cognitive index was calculated as the mean of the *z*-scores of the one-letter subtask of the Paper–Pencil Memory Scanning Task, the reading subtask of the Stroop test, the Symbol–Digit Substitution Task and the added score on the three learning trials of the Rey Auditory Verbal Learning Test, and the delayed recall of this last test. To construct an executive function test, the compound score for psychomotor speed was calculated as the mean of the *z*-scores of the one-letter subtask of the Paper–Pencil Memory Scanning Task, the reading subtask of the Stroop test and the Symbol–Digit Substitution Task (de Groot *et al.*, 2000).

Magnetic resonance imaging scanning protocol

MRI scans of all subjects were acquired on a single 1.5 Tesla scanner (Magneton Sonata, Siemens Medical Solutions). The protocol included a 3D T_1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence (repetition time/echo time/inversion time = 2250/3.68/850 ms; flip angle 15°; voxel size 1.0 × 1.0 × 1.0 mm), a fluid-attenuated inversion recovery (FLAIR) sequence (repetition time/echo time/inversion time = 9000/84/2200 ms; voxel size 1.0 × 1.2 × 5.0 mm, plus an inter-slice gap of 1 mm) and a DTI sequence (repetition time/echo time = 10100/93 ms, voxel size 2.5 × 2.5 × 2.5 mm, four unweighted scans, 30 diffusion weighted scans with *b*-value of 900 s/mm^2).

Magnetic resonance imaging data analysis

Conventional magnetic resonance imaging

All images were evaluated without prior notice of any clinical parameter. White matter signal hyperintensities on FLAIR scans, which were not, or only faintly, hypointense on T_1-weighted images, were considered white matter lesions, except for gliosis surrounding infarcts (Fisher, 1965; Pullicino *et al.*, 1995; Wahlund *et al.*, 2001; Herve *et al.*, 2005). White matter lesions were manually segmented on the FLAIR image on a Intuos3® graphics tablet (Wacom Co.), by two experienced raters, with a high inter-rater agreement after log-transformation, given the right-skewedness of the white matter lesions distribution (intraclass correlation coefficient for total volume = 0.97). To express overlap between the segmentations of the two raters we calculated the Dice coefficient (Dice score on average = 0.71). Total white matter lesion volume was calculated as the sum of all segmented areas multiplied by slice thickness.

Normalization parameters to the ICBM152 template brain (as provided with SPM5; Wellcome Department of Cognitive Neurology, University College London, London, UK) and grey and white matter tissue probability maps were computed by using SPM5 unified segmentation routines on the T_1 MPRAGE images (Ashburner and Friston, 2005). Total grey and white matter volumes were calculated by summing all voxel volumes that had a $P > 0.5$ for belonging to the tissue class. Co-registration parameters of the FLAIR image to the T_1 image were computed (SPM5 mutual information co-registration) and used to bring both the FLAIR and white matter lesion segmentation images into the subject's (anatomical) reference frame. Transformed images were visually checked for co-registration errors. Subsequently, the white matter lesion segmentations were resampled to and combined with the white matter maps to yield a white matter lesions map (the intersection of white matter lesions and white matter) and NAWM map (the complement of white matter lesions in white matter) in the T_1 reference space.

Diffusion tensor imaging

The diffusion weighted images of each subject were realigned on the unweighted image using mutual information routines from SPM5. Then the diffusion tensor (Basser *et al.*, 1994*a*) and its eigenvalues were computed using an SPM5 add-on (http://sourceforge.net/projects/spmtools). Unphysical spurious negative eigenvalues of the diffusion tensor were set to zero, after which the tensor derivatives of the fractional anisotropy and mean diffusivity were calculated (Basser and Jones, 2002). The mean unweighted image was used to compute the co-registration parameters to the anatomical reference image (SPM5 mutual information co-registration), which were then applied to all diffusion weighted images and results. The volume averaged fractional anisotropy and mean diffusivity were calculated in the NAWM and white matter lesions. All images were visually checked for motion artefacts and co-registration errors, which resulted in a final sample of 499 subjects because of the exclusion of four due to excessive motion artefacts.

Covariates

The variables age, sex, blood pressure, body mass index, diabetes, the use of lipid lowering drugs and alcohol consumption were considered to be possible confounders. Blood pressure was measured three times supine after 5 min of rest and after each 15 s by two experienced research nurses. The average of the three measurements of systolic and diastolic blood pressure was used. Hypertension was defined as systolic blood pressure $\geqslant$140 mmHg or diastolic blood pressure $\geqslant$90 mmHg and/or the use of blood pressure-lowering agents (Rosendorff *et al.*, 2007). Information on blood pressure-lowering medication was collected by means of a structured, computerized questionnaire, which was checked by a resident in neurology. Height and weight were measured in light clothing without shoes and body mass index was calculated as weight divided by height (in metres) squared. Diabetes mellitus was considered to be present if the participant was taking oral glucose-lowering drugs or insulin. Information on alcohol consumption was obtained from standard questions on the use of alcohol (never, former, current), amount and duration of alcohol intake. For the analyses, the number of alcohol units per week was calculated. Level of education and depressive symptoms were

considered additional confounders in all analyses. Depressive symptoms were present if a subject had either a score ⩾16 on the Centre of Epidemiologic Studies on Depression Scale (Radloff, 1977) or was on current anti-depressive medication (de Groot *et al.*, 2000).

Statistical analysis

Chi-square tests and ANOVA were used to compare subject characteristics by smoking status. The relation between white matter lesions volume and smoking status (never, former, current) was assessed by ANOVA, adjusted for age, sex, alcohol intake, education and cardiovascular risk factors including hypertension and/or the use of anti-hypertensive drugs, body mass index, diabetes mellitus and the use of lipid-lowering drugs. Since the white matter lesions were not normally distributed, we corrected for right-skewedness by using natural log transformation. Mean differences in fractional anisotropy and mean diffusivity for the different (never/former/current smokers) groups (never smokers as the reference group) were calculated in both NAWM and white matter lesions by means of ANOVA adjusted for age, sex, alcohol intake, education and cardiovascular risk factors. Test for trends were calculated by placing the categorical smoking variable (never/former/current) as a continuous variable in a linear analysis with fractional anisotropy and mean diffusivity in NAWM and white matter lesions as the dependent variable. Linear regression analysis was used to investigate the relation between the duration of years subjects had quit smoking and DTI parameters in NAWM and white matter lesions.

The relation between smoking and cognition was assessed by means of ANOVA, adjusted for age, sex, cardiovascular risk factors, pack-years, alcohol intake, education and the presence of depressive symptoms. Finally, we assessed the relation between fractional anisotropy and mean diffusivity in NAWM and global cognitive function as measured by the cognitive index, adjusted for age, sex, cardiovascular risk factors, alcohol intake and education with additional adjustments for white matter lesions volume and brain atrophy.

All analyses were performed using the software package Statistical Package for the Social Sciences (version 17.0; SPSS).

Results

Table 1 presents the characteristics of the study population ($n = 499$) by smoking status. Of the total cohort, 350 (70.1%) were either currently smokers or had previously smoked. The mean age was 66.4 (SD 8.8), 65.8 (8.8) and 63.6 (8.8) years for never, former and current smokers, respectively. Of the 'never' smokers, 67.8% were females. The mean number of pack-years for former smokers was 22.6 years (SD 23.5) and for current smokers 23.5 years (SD 20.4). The mean period between cessation of smoking and the current assessment was 21.9 years (SD 12.8). Alcohol intake was significantly associated with smoking status ($P < 0.05$) and current smokers had a significant lower body mass index compared with former and never smokers ($P < 0.05$).

Figure 1 shows the relation between smoking status and white matter lesion volume. Former and current smokers had a significantly higher mean white matter lesion volume compared with the reference group (never smokers) (P-trend = 0.045).

Table 2 shows the cross-sectional relation between smoking status and DTI parameters in both NAWM and white matter lesions. There were significant relations between mean diffusivity in NAWM and white matter lesions and smoking status (P-trend = 0.02 for both). There was a borderline significant relation between the fractional anisotropy in NAWM and smoking status (P-trend = 0.09).

Table 3 presents the linear relation between cessation of smoking (years) and DTI parameters in NAWM and white matter lesions. For NAWM, there was a linear relation between

Table 1 Subject characteristics by smoking status

Characteristics[a]	Overall	Smoking status		
	$n = 499$	Never ($n = 149$)	Former ($n = 275$)	Current ($n = 75$)
Age (years)	65.6 (8.8)	66.4 (8.8)	65.8 (8.8)	63.6 (8.8)
Females* (%)	43.5	67.8	32.7	34.7
Education[b] (%)	90.2	90.6	90.9	86.7
Mini-Mental State Examination	28.1 (1.6)	28.2 (1.5)	28.2 (1.6)	28.0 (1.8)
Systolic blood pressure	140.7 (20.7)	141.2 (18.8)	140.5 (21.3)	140.6 (22.1)
Diastolic blood pressure	78.1 (9.5)	76.9 (8.2)	78.4 (9.6)	79.7 (10.9)
Use of anti-hypertensive drugs** (%)	54.3	51.7	58.9	42.7
Use of lipid-lowering drugs** (%)	46.9	37.6	53.1	42.7
Body mass index** (kg/m^2)	27.1 (4.1)	27.3 (4.1)	27.4 (4.1)	25.7 (3.7)
Diabetes** (%)	14.6	8.1	18.9	12.0
Alcohol intake** (U/week)	7.9 (9.3)	5.4 (7.9)	9.1 (9.4)	8.6 (10.6)
Pack-years	16.0 (21.8)	–	22.6 (23.5)	23.5 (20.4)

Association with smoking status (*$P < 0.001$, **$P < 0.05$).
a Means and standard errors unless indicated as per cent.
b Beyond primary school.

the length of cessation and lower mean diffusivity and higher fractional anisotropy values, $\beta = -0.004$ [95% confidence interval (CI) −0.007 to 0.000; $P = 0.03$) and $\beta = 0.019$ (95% CI 0.001–0.038; $P = 0.04$), respectively.

The association between mean diffusivity in NAWM and duration of smoking cessation (years) is presented in Fig. 2A. Mean diffusivity was lower with increasing number of cessation years (P-trend = 0.001). Participants that had stopped smoking for >20 years had identical mean diffusivity and fractional anisotropy values compared with those who had never smoked. A similar relation was found within white matter lesions (Fig. 2B).

A history of smoking was significantly negatively related to cognitive performance. There was a significant trend towards better cognitive performance in which each increase of years during which subjects had stopped smoking was positively related to higher mean z-scores (Fig. 3). Finally, we found a significant linear relationship between mean diffusivity in NAWM and global cognition function as measured by the cognitive index, $\beta = -0.256$ (95% CI −0.457 to −0.056; $P = 0.001$). A similar relation was found for fractional anisotropy in NAWM, $\beta = 0.040$ (95% CI 0.004–0.076; $P = 0.003$).

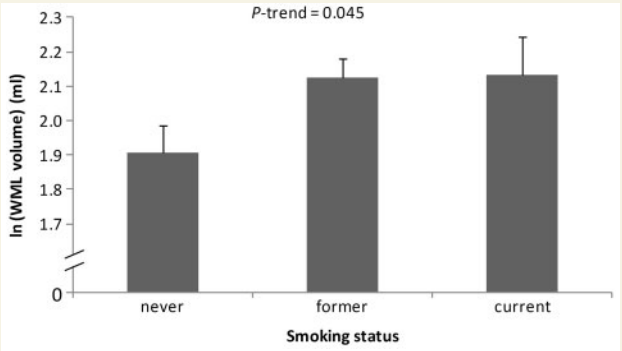

Figure 1 The relation between smoking status and mean ln(WML volume), adjusted for age, sex, cardiovascular risk factors, alcohol intake and education (beyond primary school). WML = white matter lesions.

Discussion

The present study shows that current and former smokers, who stopped <20 years ago, have a lower degree of microstructural integrity of the cerebral white matter. This was true for total NAWM and white matter lesions. These associations were strongest in NAWM. Additionally, current smoking was associated with impaired cognitive function, as expressed by compound mean z-scores of both global cognitive and executive function testing.

Most strikingly, we found that fractional anisotropy and mean diffusivity values in NAWM of subjects who had quit smoking for >20 years were comparable with those who had never smoked, as was their cognitive performance. There is accumulating evidence that smoking negatively affects cognitive performance (Richards *et al.*, 2003) and is associated with Alzheimer's disease (Ott *et al.*, 1998). Conversely, the cessation of smoking may then inhibit or postpone the development of cognitive impairment or dementia when our findings are confirmed in prospective studies. We are not aware of a larger study that has investigated the effect of cigarette smoking on the microstructural integrity of cerebral white matter. However, some methodological limitations have to be considered. Although self-report of smoking is generally reliable, especially smoking history, both under- and over-reporting may have occurred (Fendrich *et al.*, 2005). Nevertheless, it seems

Table 3 The cross-sectional relation between duration of smoking cessation (years) and DTI parameters in both NAWM and white matter lesions[a]

DTI parameters	Smoking cessation (years)	
	β (95% CI)	P-value
NAWM		
Mean diffusivity	−0.004 (−0.007 to 0.000)	0.03
Fractional anisotropy	0.019 (0.001 to 0.038)	0.04
White matter lesions		
Mean diffusivity	−0.004 (−0.010 to 0.001)	0.14
Fractional anisotropy	0.011 (−0.19 to 0.042)	0.46

a Adjusted for age, sex, cardiovascular risk factors, number of pack-years, alcohol intake and education (beyond primary school).

Table 2 The association between smoking status and mean DTI parameters (standard errors) in both NAWM and white matter lesions[a]

DTI parameters[b]	Smoking status			P-trend
	Never	Former	Current	
NAWM				
Mean diffusivity	8.84 (0.03)	8.88 (0.02)	8.95 (0.04)	0.02*
FA	32.96 (0.15)	32.71 (0.11)	32.53 (0.20)	0.09
White matter lesions				
Mean diffusivity	9.90 (0.05)	10.01 (0.03)	10.07 (0.06)	0.02*
FA	33.80 (0.25)	33.85 (0.18)	33.28 (0.33)	0.32

a Estimated means, adjusted for age, sex, cardiovascular risk factors, alcohol intake and education (beyond primary school).
b Fractional anisotropy (FA) ($\times 10^{-2}$); mean diffusivity ($mm^2/s \times 10^{-7}$).
*$P < 0.05$.

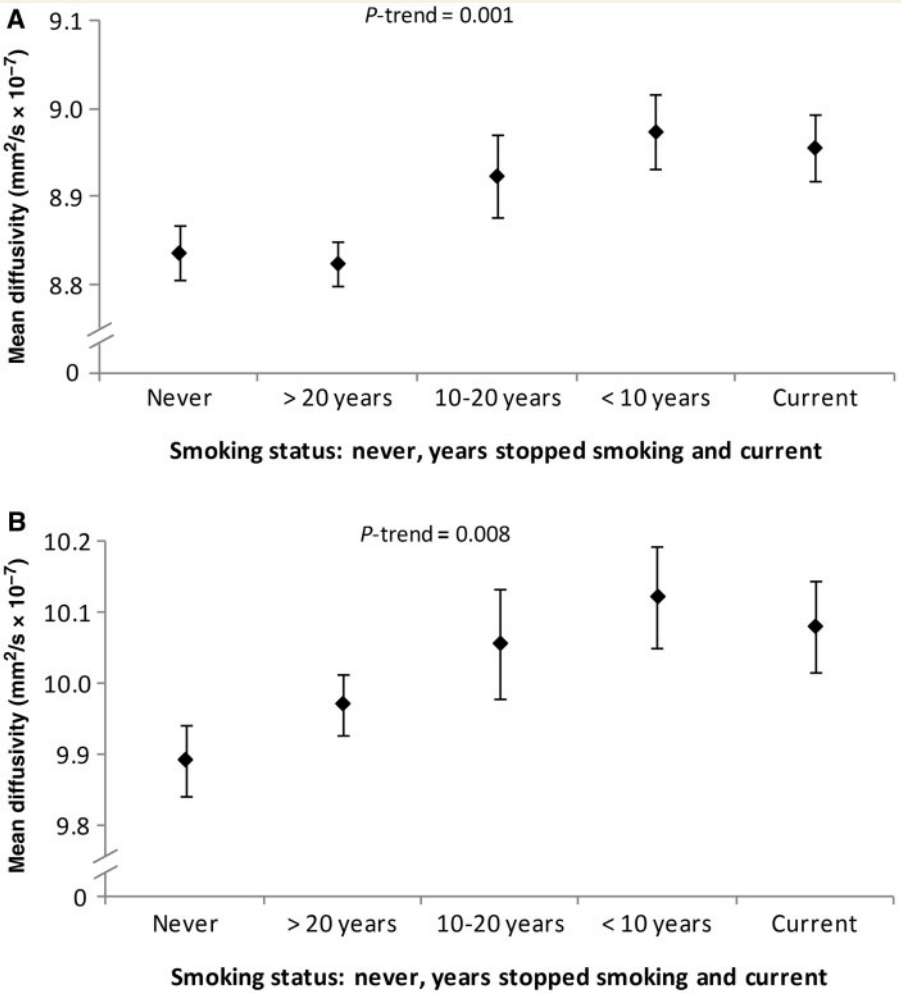

Figure 2 The relation between cessation of smoking (years) and mean diffusivity in (**A**) NAWM and (**B**) white matter lesions, adjusted for age, sex, cardiovascular risk factors, number of pack-years, alcohol intake and education (beyond primary school). 'Never' smokers as reference group.

unlikely that this would have influenced our findings (differences for white matter lesions, structural integrity of the white matter and cognitive performance in current, former and never smokers), given the large number of subjects included in this study. It could also be that our results are influenced by other factors not directly evaluated in this study, such as physical activity, nutrition, exposure to environmental tobacco smoke or genetic predispositions. Unfortunately we did not assess social class and, especially among elderly, the socioeconomic status may be high, despite a relatively low education. We think that, if anything, the adjustment for education rather than for socioeconomic status might have underestimated the strength of the association between smoking and microstructural integrity.

All of our results are cross-sectional associations, preventing one from drawing conclusions in terms of directionality and causality, although the dose dependency suggests causality. Strong elements of our study include its large sample size and the fact that our study is single centre with a high response (71.3%). Additionally, a structured assessment of both the risk factor and smoking status was used. All white matter lesions were manually segmented by two experienced researchers, blinded to clinical data, with high inter-rater agreement. The Dice score was on average 0.71, which is in line with Dice scores on manual white matter lesion segmentations reported in the literature (de Boer *et al.,* 2009). All associations were corrected for possible confounders, strongly related to small-vessel disease, brain structural deficits or cognitive impairment.

Most of the previous neuroimaging studies on the effect of cigarette smoking on the brain focused on macrostructural alterations and those of grey matter in particular. In those studies, smokers with or without alcohol dependence, as compared with non-smokers exhibited reduced grey matter volumes in frontal regions (anterior cingulated, prefrontal and orbitofrontal cortex), parietal cortex, temporal lobe and cerebellum (Brody *et al.*, 2004; Gallinat *et al.*, 2006). Apart from volumetric changes or atrophy, cigarette smoking is also associated with the presence of white matter lesions (Liao *et al.*, 1997; Longstreth *et al.*, 2000) and both silent (Howard *et al.*, 1998; Longstreth, 1998) and

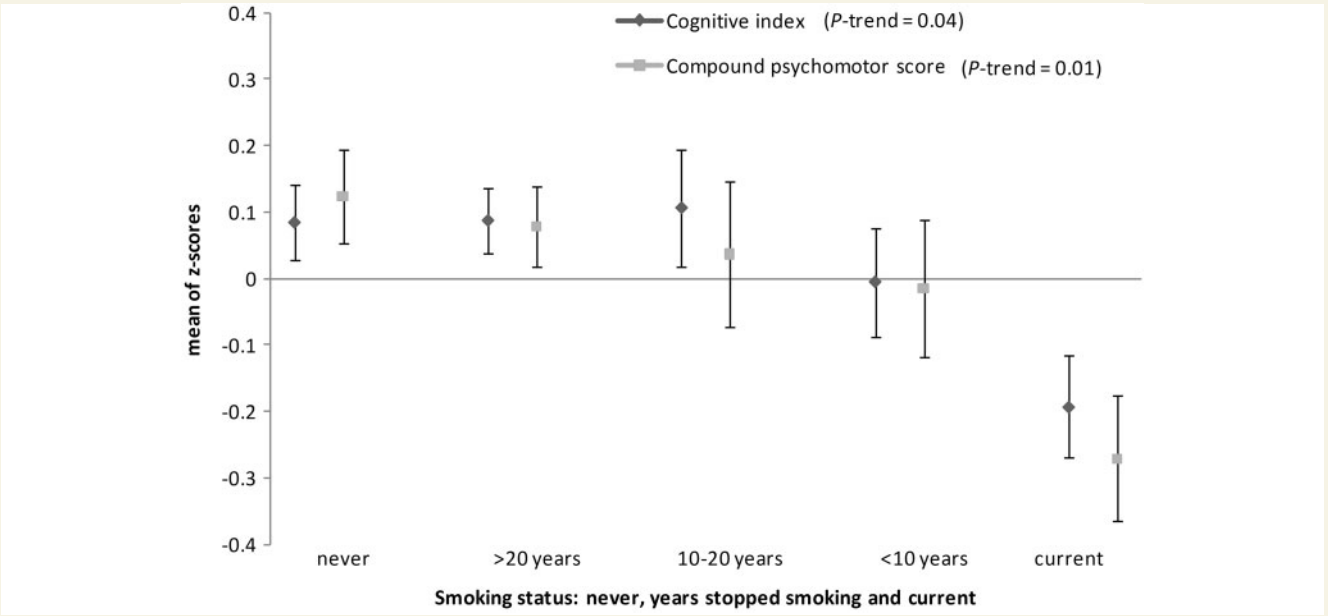

Figure 3 The association between cessation of smoking (years) and cognition, adjusted for age, sex, cardiovascular risk factors, pack-years, alcohol, education (beyond primary school) and depressive symptoms (Centre of Epidemiologic Studies on Depression Scale score $\geqslant$16 and/or the use of anti-depressive medication).

symptomatic stroke (Longstreth, 1998; Longstreth *et al.*, 2001), which may explain the frequently reported cognitive dysfunctions or dementia in chronic cigarette consumers (Ott *et al.*, 1998, 2004; Tyas *et al.*, 2003). However, less is known about the effect of cigarette smoking on the microstructural integrity of cerebral white matter. There is one study on 10 healthy chronic smokers (mean age 38.6 years) versus 10 healthy non-smokers (mean age 38.5 years), in which the authors showed that smokers had a higher fractional anisotropy in the corpus callosum than non-smokers (Paul *et al.*, 2008), which is remarkable since a higher fractional anisotropy is generally indicative of a more intact microstructural integrity. As one possible explanation, the authors addressed the stimulating effect of nicotine on nicotine receptors, expressed in oligodendrocyte precursor cells, which could result in better microstructural white matter integrity in cigarette smokers. However, as the authors rightly point out themselves, the total numbers of subjects in this study was relatively small, therefore the results should be interpreted as preliminary (Paul *et al.*, 2008). It could also be that the deleterious effects of chronic cigarette smoking, negatively influencing fractional anisotropy, are yet not noted, given the relatively low mean age of the participants. In addition it could be that the reported differences in fractional anisotropy are due to other differences than smoking status alone on which there is no information available, like the presence of hypertension, which is known to influence fractional anisotropy (Gons *et al.*, 2010). More recently, significant white matter integrity recovery was found to be dependent on the smoking status, in individuals with abstinence from alcohol (Gazdzinski *et al.*, 2010).

To summarize, most (methodologically sound) larger studies indicate the deleterious effects of smoking on brain structure and the possible beneficial effects of smoking cessation. Although there is ample evidence that cigarette smoking is clearly associated with preclinical changes in the brain (including atrophy, silent infarcts and white matter lesions), the underlying mechanisms, however, are poorly understood. There is evidence from autopsy studies of a significant association between smoking and Alzheimer type of neuropathology (Tyas *et al.*, 2003), but there is not much data on the underlying neuropathology of lower microstructural integrity among smokers, as assessed by DTI. This lower microstructural integrity may be responsible for the cognitive consequences (Vernooij *et al.*, 2009), and is in line with those we found in our study.

Another interesting finding of our study was that the duration of smoking cessation was linearly related to higher fractional anisotropy and lower mean diffusivity, reflecting a higher integrity of the cerebral white matter with increasing periods of smoking cessation. This relation was significant in NAWM but not in white matter lesions, although a trend towards more microstructural integrity was present. Our findings may suggest that a lower structural integrity of cerebral white matter, not visible on conventional MRI, may be reversible after smoking cessation, especially in NAWM. It could be that tissue in white matter lesions may reflect more severe stages of damage, not amenable to recovery (anymore). In line with these results, a similar association was found with cognition; mean *z*-scores on cognitive tasks were positively related to the duration of smoking cessation. It is noteworthy that there seemed to be a disconnect between the findings regarding microstructural integrity of the NAWM and white matter lesions by smoking status and cognition by smoking status: cognition appeared worse in current smokers versus the rest and yet the microstructural findings showed more similarities between current

and former smoking versus those who had never smoked. A pathophysiological explanation could be that differences in microstructural integrity do not completely explain differences in cognitive performance between the several groups of smokers. It also seems plausible that other, perhaps direct, toxic effects of smoke, influence cognitive performance. Therefore, current smokers (who still experience these toxic effects) perform worse on the cognitive index, while those who stop, even those for <10 years, have a better cognitive performance, albeit that there is (yet) no microstructural integrity at the level of those who have stopped for >20 years or as those who have never smoked. However, due to the cross-sectional design of our study we do not have information on the microstructural integrity of the NAWM and white matter lesions during the earlier smoking period, though prior research in other research areas supports our results. In a prospective observational study of 104 519 female participants in the Nurses' Health Study, the excess risk of vascular mortality due to smoking reduced rapidly upon cessation and within 20 years for lung diseases, in which damaging effects are greatest (Kenfield *et al.*, 2008).

In conclusion, DTI is considered a potential tool in detecting early changes in cerebral white matter at the microstructural level. It may therefore add to our understanding of the development of white matter lesions in relation to risk factors and clinical outcome, such as cognitive decline. This study shows that cigarette smoking is associated with impaired microstructural integrity of the white matter and with impaired cognitive function, independent of other cardiovascular risk factors, alcohol consumption and depressive symptoms. More importantly, cessation of smoking may lead to reversal of the harmful effects of smoking on the brain. Future studies are needed to study whether areas of reduced microstructural integrity ultimately develop into FLAIR visible white matter lesions. Finally, since tobacco is the second major cause of death and the fourth most common risk factor for disease worldwide (WHO), our data underline the potential benefits of quitting.

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References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Washington, DC, 2000.

Ashburner J, Friston KJ. Unified segmentation. Neuroimage 2005; 26: 839–51.

Basser PJ, Jones DK. Diffusion-tensor MRI: theory, experimental design and data analysis - a technical review. NMR Biomed 2002; 15: 456–67.

Basser PJ, Mattiello J, Le Bihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B 1994a; 103: 247–54.

Basser PJ, Mattiello J, Le Bihan D. MR diffusion tensor spectroscopy and imaging. Biophys J 1994b; 66: 259–67.

Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J Magn Reson B 1996; 111: 209–19.

de Boer R, Vrooman HA, van der Lijn F, Vernooij MW, Ikram MA, van der Lugt A, et al. White matter lesion extension to automatic brain tissue segmentation on MRI. Neuroimage 2009; 45: 1151–61.

Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC, et al. Differences between smokers and nonsmokers in regional gray matter volumes and densities. Biol Psychiatry 2004; 55: 77–84.

de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol 2000; 47: 145–51.

Erkinjuntti T. Subcortical vascular dementia. Cerebrovasc Dis 2002; 13 (Suppl 2): 58–60.

Fendrich M, kesy-Amiti ME, Johnson TP, Hubbell A, Wislar JS. Tobacco-reporting validity in an epidemiological drug-use survey. Addict Behav 2005; 30: 175–81.

Fisher CM. Lacunes: small, deep cerebral infarcts. Neurology 1965; 15: 774–84.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189–98.

Gallinat J, Meisenzahl E, Jacobsen LK, Kalus P, Bierbrauer J, Kienast T, et al. Smoking and structural brain deficits: a volumetric MR investigation. Eur J Neurosci 2006; 24: 1744–50.

Gazdzinski S, Durazzo TC, Mon A, Yeh PH, Meyerhoff DJ. Cerebral white matter recovery in abstinent alcoholics—a multimodality magnetic resonance study. Brain 2010; 133: 1043–53.

Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol 1999; 56: 33–9.

Gons RA, de Laat KF, van Norden AG, van Oudheusden LJ, van Uden IW, Norris DG, et al. Hypertension and cerebral diffusion tensor imaging in small vessel disease. Stroke 2010; 41: 2801–6.

Herve D, Mangin JF, Molko N, Bousser MG, Chabriat H. Shape and volume of lacunar infarcts: a 3D MRI study in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Stroke 2005; 36: 2384–8.

Houx PJ, Jolles J, Vreeling FW. Stroop interference: aging effects assessed with the Stroop Color-Word Test. Exp Aging Res 1993; 19: 209–24.

Howard G, Wagenknecht LE, Cai J, Cooper L, Kraut MA, Toole JF. Cigarette smoking and other risk factors for silent cerebral infarction in the general population. Stroke 1998; 29: 913–7.

Kenfield SA, Stampfer MJ, Rosner BA, Colditz GA. Smoking and smoking cessation in relation to mortality in women. JAMA 2008; 299: 2037–47.

Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. J Magn Reson Imaging 2001; 13: 534–46.

Lezak MD. Neuropsychological assessment. New York: Oxford University Press; 1995.

Liao D, Cooper L, Cai J, Toole J, Bryan N, Burke G, et al. The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: the ARIC Study. Neuroepidemiology 1997; 16: 149–62.

Longstreth WT Jr. Brain abnormalities in the elderly: frequency and predictors in the United States (the Cardiovascular Health Study). Cardiovascular Health Study Collaborative Research Group. J Neural Transm Suppl 1998; 53: 9–16.

Longstreth WT Jr, Arnold AM, Manolio TA, Burke GL, Bryan N, Jungreis CA, et al. Clinical correlates of ventricular and sulcal size on cranial magnetic resonance imaging of 3,301 elderly people. The Cardiovascular Health Study. Collaborative Research Group. Neuroepidemiology 2000; 19: 30–42.

Longstreth WT Jr, Bernick C, Fitzpatrick A, Cushman M, Knepper L, Lima J, et al. Frequency and predictors of stroke death in 5,888

participants in the Cardiovascular Health Study. Neurology 2001; 56: 368–75.

Louis ED, Luchsinger JA. History of vascular disease and mild parkinsonian signs in community-dwelling elderly individuals. Arch Neurol 2006; 63: 717–22.

Mahurin K, Cooke N. Verbal series attention test: clinical utility in the assessment of dementia. The Clinical Neuropsychologist 1996; 10: 43–53.

Osterrieth PA. Le test de copie d'une figure complexe: contribution a l'etude de la perception et de la memoire [The test of copying a complex figure: a contribution to the study of perception and memory]. Archives Psychologie 1944; 30: 205–353.

Ott A, Andersen K, Dewey ME, Letenneur L, Brayne C, Copeland JR, et al. Effect of smoking on global cognitive function in nondemented elderly. Neurology 2004; 62: 920–4.

Ott A, Slooter AJ, Hofman A, van Harskamp F, Witteman JC, van Broeckhoven C, et al. Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. Lancet 1998; 351: 1840–3.

Paul RH, Grieve SM, Niaura R, David SP, Laidlaw DH, Cohen R, et al. Chronic cigarette smoking and the microstructural integrity of white matter in healthy adults: a diffusion tensor imaging study. Nicotine Tob Res 2008; 10: 137–47.

Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di CG. Diffusion tensor MR imaging of the human brain. Radiology 1996; 201: 637–48.

Pullicino PM, Miller LL, Alexandrov AV, Ostrow PT. Infraputaminal 'lacunes'. Clinical and pathological correlations. Stroke 1995; 26: 1598–602.

Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Measure 1977; 1: 385–401.

Richards M, Jarvis MJ, Thompson N, Wadsworth ME. Cigarette smoking and cognitive decline in midlife: evidence from a prospective birth cohort study. Am J Public Health 2003; 93: 994–8.

Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol 2002; 1: 426–36.

Rosendorff C, Black HR, Cannon CP, Gersh BJ, Gore J, Izzo JL, et al. Treatment of hypertension in the prevention and management of ischemic heart disease: a scientific statement from the American Heart Association Council for High Blood Pressure Research and the Councils on Clinical Cardiology and Epidemiology and Prevention. Circulation 2007; 115: 2761–88.

Sternberg S. Memory-scanning: mental processes revealed by reaction-time experiments. Am Sci 1969; 57: 421–57.

Tyas SL, White LR, Petrovitch H, Webster Ross G, Foley DJ, Heimovitz HK, et al. Mid-life smoking and late-life dementia: the Honolulu-Asia Aging Study. Neurobiol Aging 2003; 24: 589–96.

Van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. J Int Neuropsychol Soc 2005; 11: 290–302.

Vernooij MW, Ikram MA, Vrooman HA, Wielopolski PA, Krestin GP, Hofman A, et al. White matter microstructural integrity and cognitive function in a general elderly population. Arch Gen Psychiatry 2009; 66: 545–53.

Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke 2001; 32: 1318–22.