

Vascular Risk Factors of Hippocampal Subfield Volumes in Persons without Dementia: The Medea 7T Study

Kim Blom^a, Huiberdina L. Koek^b, Maarten H.T. Zwartbol^c, Rashid Ghaznawi^c, Hugo J. Kuijff^d, Theo D. Witkamp^c, Jeroen Hendrikse^c, Geert Jan Biessels^e and Mirjam I. Geerlings^{a,*} on behalf of the UCC-SMART Study Group¹

^aJulius Center for Health Sciences and Primary Care, University Medical Center Utrecht and Utrecht University, Utrecht, the Netherlands

^bDepartment of Geriatrics, University Medical Center Utrecht and Utrecht University, Utrecht, the Netherlands

^cDepartment of Radiology, University Medical Center Utrecht and Utrecht University, Utrecht, the Netherlands

^dImage Sciences Institute, University Medical Center Utrecht and Utrecht University, Utrecht, the Netherlands

^eDepartment of Neurology, University Medical Center Utrecht and Utrecht University, Utrecht, the Netherlands

Accepted 9 July 2020

Abstract.

Background: Vascular risk factors have been associated with risk of Alzheimer's disease (AD) and volume loss of the hippocampus, but the associations with subfields of the hippocampus are understudied. Knowing if vascular risk factors contribute to hippocampal subfield atrophy may improve our understanding of vascular contributions to neurodegenerative diseases.

Objective: To investigate the associations between age, sex, and vascular risk factors with hippocampal subfields volumes on 7T MRI in older persons without dementia.

Methods: From the Medea 7T study, 283 participants (67 ± 9 years, 68% men) without dementia had 7T brain MRI and hippocampal subfield segmentation. Subfields were automatically segmented on the 3D T2-weighted 7T images with ASHS software. Using linear mixed models, we estimated adjusted associations of age, sex, and vascular risk factors with z-scores of volumes of the entorhinal cortex (ERC), subiculum (SUB), Cornu Ammonis (CA)1, CA2, CA3, CA4, and dentate gyrus (DG), and tail as multivariate correlated outcomes.

Results: Increasing age was associated with smaller volumes in all subfields, except CA4/DG. Current smoking was associated with smaller ERC and SUB volumes; moderate alcohol use with smaller CA1 and CA4/DG, obesity with smaller volumes of ERC, SUB, CA2, CA3, and tail; and diabetes mellitus with smaller SUB volume. Sex, former smoking, and hypertension were not associated with subfield volumes. When formally tested, no risk factor affected the subfield volumes differentially.

Conclusion: Several vascular risk factors were associated with smaller volumes of specific hippocampal subfields. However, no statistical evidence was found that subfields were differentially affected by these risk factors.

Keywords: Cornu ammonis, dentate gyrus, entorhinal cortex, hippocampal region, hippocampus, magnetic resonance imaging, risk factors

*Correspondence to: Mirjam I. Geerlings, PhD, University Medical Center Utrecht and Utrecht University, Julius Center for Health Sciences and Primary Care, Stratenum 6.131, P.O. Box

85500, 3508 GA Utrecht, The Netherlands. Tel.: +31 88750670; E-mail: m.geerlings@umcutrecht.nl

¹See Acknowledgments.

INTRODUCTION

Around 50 million people worldwide have dementia. An estimated 30 to 35 million (60–70%) of these people have Alzheimer's disease (AD). It is predicted that by 2050, 152 million people will have dementia [1]. Dementia has a huge impact on the physical, psychological, and emotional wellbeing of patients and their caregivers, and is also a major contributor of a rise in health care costs [2]. Understanding how risk factors contribute to AD etiology may aid in the prevention or treatment of AD. It has been established that vascular risk factors, of which most are modifiable by either lifestyle or medication, play an important role in AD risk [3], hippocampal calcifications [4], and hippocampal atrophy [5]. The hippocampus is one of the first structures to be affected in AD [6].

The hippocampus and adjacent areas consist of several subfields, including the entorhinal cortex, subiculum, Cornu Ammonis (CA)1, CA2, CA3, CA4, and dentate gyrus (DG) [7]. Evidence exists that hippocampal subfields are differentially affected by aging, by diseases such as diabetes, and by neurodegenerative disease as AD [8, 9]. A better understanding of how vascular risk factors contribute to hippocampal subfield atrophy may improve our understanding of the vascular contributions to the AD phenotype, vascular dementia, or other neurodegenerative diseases.

Studies on vascular risk factors—i.e., smoking, alcohol, obesity, hypertension, and diabetes mellitus—and hippocampal subfields *in vivo* in humans are rather scarce (overview in Table 1 of studies using manual or automated delineation methods). Further, these studies used relatively low MRI field strength, varying from 1.5 Tesla up to 4.7 Tesla, and usually looked at combined subfield volumes such as CA1 and 2 (CA1-2), or CA2, CA3, CA4, and DG (CA2-3-4-DG), rather than individual subfields, with variable results. Moreover, most studies did not examine all subfield volumes and none examined effects of risk factors on the different subfield volumes (see Table 1). The use of ultra-high field strength 7T MRI more than doubles the signal-to-noise ratio compared to 3T which enabled us to increase the spatial resolution while keeping scan duration within acceptable limits [10] and therefore allows for investigating associations between vascular risk factors and the different hippocampal subfield volumes more precisely.

We aimed to estimate the associations of age, sex, and vascular risk factors with hippocampal subfields volumes with 7T MRI in a large cohort of middle-aged and older adults without clinically established dementia. We also provide an overview of existing literature on vascular risk factors and hippocampal subfields volumes on MRI.

METHODS

Study population

Data were used from the Memory Depression and Aging (Medea)-7T study, a cohort study at the University Medical Center (UMC) Utrecht, with the objective to investigate risk factors and outcomes of brain changes defined on 7T MRI in a diverse population of individuals with and without dementia. For the Medea-7T study cohort, we originally recruited participants from four settings: 1) persons of 60 years or older registered in a general practice in Utrecht and without a diagnosis of mild cognitive impairment (MCI) or dementia, 2) participants of the PREDICT-MR study, 3) patients of the SMART-MR study, and 4) patients with MCI or early AD from the memory clinics of the UMC Utrecht and a general hospital in Utrecht. A total of 368 participants were included in these four settings between January 2010 and October 2017, 70 from the general practice, 50 from the PREDICT-MR study, 213 from the SMART-MR study, and 35 from the memory clinics. A more detailed description of the separate cohorts/settings is provided in the Supplementary Methods and Supplementary Figure 1. All participants underwent a 7T brain MRI using the same MRI sequences, and similar assessment of risk factors and outcomes and clinical examinations, all performed at the UMC Utrecht. For the present study we excluded 35 patients with MCI or AD and 8 persons with missing values on covariates, resulting in a sample of 325 participants with a 7T brain MRI. Another 42 participants were excluded from the analyses, due to incomplete hippocampal subfield segmentation (e.g., due to movement artefacts, see below for details), leaving 283 participants for this study sample. Approval of the medical ethics committee of the UMC Utrecht was obtained, and all participants provided written informed consent.

Demographics

Age and sex were assessed with questionnaires.

Table 1
Overview of studies researching vascular risk factors and hippocampal subfield volumes

Author	Risk factor(s)	Design and study population	N subjects	Age (years) ± S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Armstrong et al., 2019 [20]	All stratified by sex: age, hypertension, obesity, <i>APOE</i> ε4 carrier status, HDL cholesterol	Population-based cohort: Baltimore Longitudinal Study of Aging (BLSA)	<i>n</i> = 617	71.2 ± 8.7	Females <i>n</i> = 326 (53%) Males <i>n</i> = 291 (47%)	1.5 or 3.0 Tesla	ERC	Automated. Combined MUSE anatomical labeling approach with harmonized acquisition-specific atlases (Doshi et al., 2016 [45], Erus et al., 2018 [46]).	In males: Older age associated with decline in ERC, and higher HDL associated with less steep decline in ERC. No associations with hypertension, obesity or <i>APOE</i> ε4 status. In females: Older age, hypertension, obesity, <i>APOE</i> ε4 status, and HDL cholesterol not associated with ERC volume.
Daugherty et al., 2016 [14]	Age, sex	Participants (children, adults, and older adults) from Metro Detroit, part of ongoing longitudinal studies of neural correlates of memory development, cognitive and neural correlates of aging. The adult sample size has a 48% overlap with a previous publication (Raz et al. 2015 [25]; see below)	Adults <i>n</i> = 91 Older adults <i>n</i> = 74	Adults 29.96 ± 9.99 (18–49) Older adults 62.07 ± 7.74 (50–82)	Adults Females 63%, Males 37% Older adults Females 76% Males 24%	3.0 Tesla	ERC, CA1-2, CA3-DG, SUB	Manual, by two raters. Demarcation rules reported by Bender et al., 2013 [47] (rules were adapted from Shing et al. 2011 [26]; as modified from Mueller et al., 2007 [23]; Mueller and Weiner, 2009 [24])	Increasing age was negatively associated with volumes of ERC, CA1-2 and CA3-DG, but not with SUB. Sex, a covariate in all models, was unrelated to volume differences in any region.
De Flores et al., 2015 [17]	Age	All participants were included in the IMAP-study. Patients with MCI or Alzheimer were recruited via local memory clinics. Healthy community-dwelling participants were recruited via flyers and advertisements.	Alzheimer <i>n</i> = 18 MCI <i>n</i> = 17 Healthy individuals <i>n</i> = 98	Alzheimer 67.4 ± 9.9 MCI 71.7 ± 6 Healthy individuals 45.7 ± 19.2	Alzheimer Females 67% Males 33% MCI Females 53% Males 47% Healthy individuals Females 58% Males 42%	3.0 Tesla	SUB, CA1, CA2-CA3-CA4-DG (“OTHER”).	Both automated and manual. Manual, by one rater. Protocol was previously published by La Joie et al., 2010 [21]. Automated, Freesurfer 5.1	Increasing age was associated with smaller CA1 and SUB volumes when delineated manual, but not with the OTHER subfields. When automated delineation was used, increasing age was only associated with a smaller SUB volume.

Table 1
(continued)

Author	Risk factor(s)	Design and study population	N subjects	Age (years) \pm S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Durazzo et al., 2013 [29]	Smoking	Community-dwelling participants recruited via posters, bill-boards, and word-of-mouth	Non-smokers <i>n</i> = 43 Smokers <i>n</i> = 39	Non-smokers 46.5 \pm 11.0 (22.2–68.8) Smokers 43.3 \pm 12.6 (23.7–64.1)	Non-smokers Females 19% Males 81% Smokers Females 16% Males 84%.	4.0 Tesla	CA1, CA2-3, CA4-DG, fimbria, PreSUB, SUB, tail.	Automated. Freesurfer 5.1	Smokers had smaller volumes than non-smokers in the CA1, CA2-3, and CA4-DG. There were trends for smaller volumes in smokers compared to non-smokers with the PreSUB and SUB. Age-related volumes loss was greater in smokers than in non-smokers in CA1, CA2-3, CA4-DG, fimbria, and tail. Increasing age in non-smokers was only significantly associated with PreSUB.
La Joie et al., 2010 [21]	Age, sex	Healthy subjects, recruitment method unknown	<i>n</i> = 50	39.9 \pm 15.2	Females <i>n</i> = 31 (62%) Males <i>n</i> = 19 (38%)	3.0 Tesla	SUB, CA1, CA2-3-4-DG	Manual, by one rater. Demarcation rules based on an atlas of the human hippocampus (Harding et al., 1998[48])	Age was significantly associated with a decline in volume of SUB volume, not with CA1 or CA2-3-4-DG volume. There was no effect of gender or education on any volumes, nor any interaction between these variables and age.
Lee et al., 2016 [22]	Age, alcohol	Patients with alcohol dependence from a mental health hospital who were abstinent from alcohol for at least three months. Social drinkers (controls) from the community recruited via advertisements.	Alcohol dependence <i>n</i> = 26 Controls <i>n</i> = 26	Alcohol dependence 50.6 \pm 7.4 (40–63) Controls 50.3 \pm 6.0 (43–62)	Alcohol dependence Males <i>n</i> = 26 (100%) Controls Males <i>n</i> = 26 (100%)	3.0 Tesla	PreSUB, CA1, CA2-3, Fimbria, SUB, CA4-DG, Hippocampal sulcus	Automated. Freesurfer 5.3	Age was associated with a smaller fimbria, presubiculum and subiculum. In the group with alcohol dependence, there was a smaller left presubiculum, left fimbria, left subiculum and right subiculum compared to the control group.

Table 1
(continued)

Author	Risk factor(s)	Design and study population	N subjects	Age (years) \pm S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Malykhin et al., 2017 [16]	Age, sex	Healthy volunteers from the community, recruited via online advertisements, word-of-mouth, advertising in local community centers.	<i>n</i> = 129	47.6 \pm 18.9 (18–85.	Females <i>n</i> = 70 (54%) Males <i>n</i> = 59 (46%)	4.7 Tesla	CA1-3, CA4-DG, SUB	Manual, by one rater. Protocol followed was previously developed by Malykhin et al., 2007 [49], 2010 [50]	Age was associated with a smaller SUB and CA4-DG volume, but not with CA1-3 volume. When the interaction of age by sex was tested: the association with age and a smaller SUB volumes was only found in males and not in females, while the association with a smaller CA4-DG volumes was due to the association in females, not in males.
Mole et al., 2016 [31]	Alcohol, obesity	Subjects recruited from the community via advertisements. Patients with alcohol dependence who were abstinent for at least two weeks.	Alcohol dependence <i>n</i> = 32 Obese <i>n</i> = 42 Controls <i>n</i> = 44	Alcohol dependence 41.6 \pm 10.9 Obese 44.6 \pm 9.9 Controls 39.2 \pm 12.5	Alcohol dependence Females <i>n</i> = 14 (44%) Males <i>n</i> = 18 (56%) Obese Females <i>n</i> = 21 (50%) Males <i>n</i> = 21 (50%) Controls Females <i>n</i> = 18 (41%) Males <i>n</i> = 26 (59%).	3.0 Tesla	PreSUB, SUB, CA2-3, CA4-DG	Automated. A computational atlas built upon post-mortem brains was used. The atlas is now released as part of Freesurfer 6.0	Alcohol dependence was associated with smaller preSUB and smaller SUB, there was no association with CA2-3 or CA4-DG. Obesity was not associated with subfield volumes.
Mueller et al., 2007 [23]	Age, sex	Healthy volunteers from the community recruited via flyers and advertisements. Alzheimer patients from memory clinics.	Alzheimer <i>n</i> = 3 Controls <i>n</i> = 46 (<i>n</i> = 42 in text)	Alzheimer 82.3 (77–86) Controls 48.7 (21–85)	Alzheimer Females <i>n</i> = 1 (33%) Males <i>n</i> = 2 (67%) Controls Females <i>n</i> = 16 (35%) Males <i>n</i> = 30 (65%)	4.0 Tesla	ERC, SUB, CA1, CA2, CA3-4-DG	Manual, by one rater (and <i>n</i> = 10 by two raters to test reliability). Own protocol based on recognizable anatomical landmarks.	Age was associated with smaller CA1, and not with other subfields. <i>Post-hoc</i> showed that 60+ age had smaller CA1 than other age groups (decades). Male had a larger CA2 than females, no other subfields differed in volume.

Table 1
(continued)

Author	Risk factor(s)	Design and study population	N subjects	Age (years) \pm S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Mueller and Weiner, 2009 [24]	Age, <i>APOE</i> ϵ 4 carriers status	Subjects (including patients with aMCI and Alzheimer's disease) from different research projects undertaken by Center for Imaging of Neuro-degenerative Diseases and clinical collaborators.	Alzheimer <i>n</i> = 18 aMCI <i>n</i> = 20 Controls <i>n</i> = 119	Alzheimer 69.1 \pm 9.6 aMCI 73.5 \pm 7.1 Controls 53.4 \pm 17.2	Alzheimer Females <i>n</i> = 6 (33%) Males <i>n</i> = 12 (67%) aMCI Females <i>n</i> = 6 (30%) Males <i>n</i> = 14 (70%) Controls Females <i>n</i> = 64 (54%) Males <i>n</i> = 55 (46%)	4.0 Tesla	SUB, CA1, CA1-2 transition zone (CA2), CA3-DG.	Manual, by one rater Protocol as described by Mueller et al., 2007 [23], 2008 [51].	Age was associated with a smaller CA1 and CA3-DG, no association between age and the other subfields. From 50years of age CA3-DG started to decline, after 60years CA1 declined. Subjects without <i>APOE</i> ϵ 4 had a larger CA3-DG subfield than those with <i>APOE</i> ϵ 4
Nadal et al., 2020 [18]	Age, sex, educational level, ApoE genotype	Participants from the Bordeaux subset of the Three-city study, a longitudinal population-based cohort.	Alzheimer <i>n</i> = 35 Non-Alzheimer <i>n</i> = 269	Alzheimer 73.7 \pm 3.9 Non-Alzheimer 71.7 \pm 3.7	Alzheimer Females <i>n</i> = 66.7% Males <i>n</i> = 33.3% Non-Alzheimer Females <i>n</i> = 57.2% Males <i>n</i> = 42.8%	1.5 Tesla	SUB, CA1-3, CA4-DG.	Automated. volBrain system with HIPS pipeline	Increasing age showed most annual volume decline in CA4-DG, less in SUB and least in CA4-DG. Age, gender, educational level and ApoE genotype were not associated with annualized rate of atrophy of the subfields.
Raz et al., 2015 [25]	Age, sex hypertension, genetic polymorphisms of cytokines levels IL-1b C-511T and IL-6 C-174G, <i>APOE</i> ϵ 4	Adult volunteers recruited via local media as part of a longitudinal study on brain and cognitive aging.	<i>n</i> = 80 Of which <i>n</i> = 21 (26%) with hypertension	57.8 \pm 14.3 (22–82)	Females <i>n</i> = 60 (75%) Males <i>n</i> = 20 (25%)	3.0 Tesla	ERC, SUB, CA1-2, CA3-DG.	Manual, by two raters. Protocol adapted from Shing et al., 2011 [26]; Mueller et al., 2007 [23], and Mueller and Weiner 2009 [24].	Increased age was associated with a smaller CA1-2. There were no associations between age and volumes of SUB or CA3-DG. The T allele of IL-1b C-511T polymorphism was associated with smaller volumes of all hippocampal subfields than CC homozygotes did. There were no associations between sex, hypertension or the other genetic variants and subfield volumes.

Table 1
(continued)

Author	Risk factor(s)	Design and study population	N subjects	Age (years) \pm S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Shing et al., 2011 [26]	Age, hypertension	Younger and older adults Participants recruitment method unknown.	Older adults <i>n</i> = 19 Of which <i>n</i> = 10 (53%) with hypertension Younger adults <i>n</i> = 10 None with hypertension	Older adults 75.4 \pm 2.9 (70–78) Younger adults 23 \pm 1.7 (20–25)	Older adults Females <i>n</i> = 8 (42%) Males <i>n</i> = 11 (58%) Younger adults Females <i>n</i> = 5 (50%) Males <i>n</i> = 5 (50%)	3.0 Tesla	ERC, SUB, CA1-2, CA3-4-DG.	Manual, by two raters. Protocol adapted from Mueller et al., 2007 [23], 2008 [51].	In older adults CA1-2 smaller was smaller than in younger adults. Hypertension in the older adults was associated with a smaller CA1-2 volume compared to older adults without hypertension. Normotensive older adults did not have a different CA1-2 volume compared to younger adults.
Stoub et al., 2012 [19]	Age	Older adults via the community or from ongoing longitudinal (aging and AD) studies. Younger adults via Rush University Medical Center: students, employees, and their relatives / friends.	Older adults <i>n</i> = 51 Younger adults <i>n</i> = 40	Older adults 77 (65–89) Younger adults 27 (22–36)	Older adults Females <i>n</i> = 37 (73%) Males <i>n</i> = 14 (27%) Younger adults Females <i>n</i> = 18 (45%) Males <i>n</i> = 22 (55%)	1.5 Tesla	ERC	Manual, by an unknown number of raters. Own protocol, developed and validated previously (Goncharova et al., 2001 [52]).	In older adults the ERC was smaller.
Wisse et al., 2014 [10]	Age	Alzheimer and MCI patients via the memory clinic of the University Medical Center Utrecht. Controls from two study cohorts (UDES2 and PREDICT-MR), recruited via general practitioner.	Alzheimer <i>n</i> = 9 MCI <i>n</i> = 16 Controls <i>n</i> = 29	Alzheimer 70.8 \pm 8.4 MCI 74.4 \pm 9.0 Controls 70.2 \pm 3.5	Alzheimer Females <i>n</i> = 6 (67%) Males <i>n</i> = 3 (33%) MCI Females <i>n</i> = 9 (56%) Males <i>n</i> = 7 (44%) Controls Females <i>n</i> = 16 (55%) Males <i>n</i> = 13 (45%)	7.0 Tesla	ERC, SUB, CA1, CA2, CA3, CA4-DG	Manual segmentation by one blinded rater. Protocol and reliability described earlier (Wisse et al., 2012 [53])	Older age was associated with smaller CA1 and CA4-DG volume. There were no associations between age and ERC, SUB, CA2 or CA3 volumes.

Table 1
(continued)

Author	Risk factor(s)	Design and study population	N subjects	Age (years) \pm S.D. and/or (range)	Females <i>n</i> (%) and males <i>n</i> (%)	MRI field strength	Subfields	Segmentation method	Results of control / healthy participants
Zahr et al., 2019 [15]	Alcohol use disorder (AUD)	AUD patients from local outpatient and treatment centers, control participants via local community.	AUD <i>n</i> = 24 Controls <i>n</i> = 20	AUD 53.7 \pm 8.8 Controls 54.1 \pm 9.3	AUD Females <i>n</i> = 7 (29%) Males <i>n</i> = 17 (71%) Controls Females <i>n</i> = 7 (35%) Males <i>n</i> = 13 (65%)	GE MR750 system (probably 3.0 Tesla)	ParaSUB, PreSUB, SUB, CA1, CA2-3, CA4, GC-ML-DG, tail, HATA, fimbria, hippocampal fissure	Automated. Freesurfer 6.0	AUD was associated with smaller subfields of SUB, CA1, CA4, GC-ML-DG, HATA, and fimbria compared to controls. Patients with AUD had smaller CA2-3 volumes than expected by their age (diagnosis by age interaction).
Zhang et al., 2015 [33]	DM2	Patients with DM2 and healthy controls were recruited via Southwest hospital, Chongqing, China	DM2 <i>n</i> = 80 Controls <i>n</i> = 80	DM2 57.5 \pm 9.0 Controls 57.8 \pm 10.3	DM2 Females <i>n</i> = 45 (56%) Males <i>n</i> = 35 (44%) Controls Females <i>n</i> = 51 (64%) Males <i>n</i> = 29 (36%)	3.0 tesla	PreSUB, SUB, CA1, CA2-3, CA4-DG	Automated. Freesurfer 5.3	Patients with DM2 had a smaller SUB and CA1. There was no association between DM2 and volumes of CA2-3 or CA4-DG.

APOE ϵ 4, Apolipoprotein E ϵ 4; HDL cholesterol, high density lipoprotein cholesterol; IL, interleukin; AUD, alcohol use disorder; DM2, diabetes mellitus type 2; aMCI, amnesic mild cognitive impairment; MCI, mild cognitive impairment; paraSUB, parasubiculum; preSUB, presubiculum; SUB, subiculum; ERC, entorhinal cortex; CA, Cornu Ammonis; DG, dentate gyrus; GC-ML-DG, granule cell and molecular layer of the dentate gyrus, HATA, hippocampus-amygdala-transition-area.

Vascular risk factors

For this study, we defined the vascular risk factors as follows: smoking was determined as never, current, or former smoker. Alcohol use was expressed in units per week and was categorized as no alcohol use, up to 10 units per week, and more than 10 units per week. One unit of alcohol in the Netherlands is equal to 10 grams of alcohol or 12 mL of alcohol [11]. Both smoking history and alcohol use were assessed with questionnaires. Body mass index (BMI) was calculated as weight (kg)/height (m²) for which height and weight were measured without shoes or heavy clothing. Obesity was defined as having a BMI of 30.0 kg/m² or higher. Diabetes mellitus (DM) was defined as use of glucose-lowering agents or insulin, a known history of DM or a non-fasting plasma glucose ≥ 11.1 mmol/L. Blood pressure was measured three times in supine position and the average was calculated for both systolic and diastolic blood pressure. Hypertension was defined as mean systolic blood pressure >140 mmHg, mean diastolic blood pressure >90 mmHg or use of antihypertensive drugs.

7T MRI protocol

Brain images at 7 Tesla MRI were obtained using a 7.0 T MRI system (Philips Healthcare, Cleveland, OH, USA) with a 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). For the hippocampal subfield segmentation, 3D T1-weighted (3D T1-weighted (TI/TR/TE = 1225/4.8/2.2, acquired voxel size = 1.00 × 1.00 × 1.00 mm³, (reconstructed = 0.66 × 0.66 × 0.50 mm³)) and 3D T2-weighted (TR/TE = 3158/301, acquired voxel size = 0.70 × 0.70 × 0.70 mm³ (reconstructed = 0.35 × 0.35 × 0.35 mm³)) images were acquired.

1.5T and 3T MRI protocol

For the SMART-MR study and the PREDICT-MR study, MR images were obtained using a 1.5 Tesla whole-body system (Gyrosan ACS-NT, Philips Medical Systems, Best, the Netherlands). For segmentation of the intracranial volume (ICV), the protocol consisted of a sagittal 3D T1-weighted sequence (SMART-MR: repetition time (TR)/echo time (TE): 7.0/3.2 ms; voxel size = 0.94 × 0.94 × 1.00 mm³ isotropic; PREDICT-MR: TR/TE: 6.9/3.1 ms; voxel size = 0.98 × 0.98 × 1.10 mm³ isotropic). For the participants recruited via the general

practice MR images were obtained using a 3.0 Tesla MRI whole-body system (Philips Medical Systems, Best, the Netherlands). For segmentation of the ICV, the protocol consisted of a sagittal 3D T1-weighted sequence (TR/TE = 8.0/4.5, voxel size = 1.00 × 1.00 × 1.00 mm³ isotropic). Segmentation of ICV was performed on 1.5T and 3T images, respectively, as ICV segmentation by Automatic Segmentation of Hippocampal Subfields (ASHS, see Hippocampal segmentation below) on 7T has not been validated yet.

Segmentation of MR images

Brain segmentation: all studies

The 3D T1-weighted sequence of the 3T or 1.5T images was used for automatic brain segmentation by CAT 12 (version 1155), with use of SPM12 (version 6906) and MATLAB (version 8.6). Cat12 distinguishes gray matter, white matter, and cerebrospinal fluid (CSF). Total ICV was calculated by summing up total gray and white matter and total CSF volume.

Hippocampal segmentation at 7T MRI

Hippocampal subfields were automatically segmented on the 3D T2-weighted 7T images with the ASHS software (UPenn, PA, USA) [12]. ASHS segments the subfields CA1, CA2, CA3, CA4&DG, subiculum, the entorhinal cortex, and tail. Previously, a freely downloadable ASHS atlas (<https://www.nitrc.org/projects/ashs>) was validated for use on 7Tesla MRI [13]. For this study we used the 'UMC Utrecht 7T ASHS Atlas, compatible with original (slow) ASHS'. Generated hippocampal subfield volumes were checked for outliers using frequencies and histograms. Segmentations of outliers were then visually inspected and removed from the analysis if they were due to a segmentation error. Additionally, we visually inspected segmentations of a random sample of 5% of the total study sample. We did not manually adjust failed segmentations that were due to segmentation errors. Of the 325 participants without MCI or AD that underwent a 7T MRI and had no missing covariates, $n = 11$ had no T1 or T2 available, $n = 18$ were excluded due to movement or signal interference, and $n = 13$ had a segmentation error, leaving $n = 283$ with a complete hippocampal segmentation.

Data analysis

Baseline characteristics were calculated for the total study sample ($n = 283$). Hippocampal subfields

of the left and right hemisphere were summed and then Z-transformed to improve comparability between the results of the subfields. Using mixed effect regression models (PROC MIXED statement in SAS), we estimated associations between the vascular risk factors and the volume of the hippocampal subfields and tail. The mixed model was used as it takes the multiple correlated observations (volumes of the hippocampal subfields) per subject into account. Missing data on covariates were excluded from analyses. Missing values ranged from 0.4% for BMI to 14.8% for alcohol use. The vascular risk factors included age, sex, smoking (former, current versus never), alcohol use (up to 10 units per week, more than 10 units per week versus no alcohol use), BMI, obesity, diabetes mellitus, hypertension, and systolic and diastolic blood pressure. The volumes of the hippocampal subfields were included as dependent variables in one mixed model, while each vascular risk was included as the independent variable in separate models. All analyses were adjusted for age, sex, and ICV (calculated from the 1.5T or 3T scan), with exception of sex and age which were adjusted for age and ICV, and sex and ICV, respectively. If a risk factor was statistically significantly associated with one of the subfield volumes, we performed *post-hoc* analyses to explore to what extent this subfield was differentially affected by the risk factor by using estimate statements comparing the observed effect estimate with the effect estimate of the risk factor on CA1. We chose comparison with one subfield instead of comparison between all subfields to avoid multiple comparisons. Further, we chose CA1 as the reference subfield, because CA1 is relatively large in volume, which makes it more robust and less prone to measurement differences across methods, thus improving comparability with other (future) studies.

Data for statistical analyses were prepared using IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY: IBM Corp., and the analyses were conducted with SAS software, Version 9.4 of the SAS system for Windows, SAS Institute Inc., Cary, NC, USA.

RESULTS

An overview of existing literature on vascular risk factors and hippocampal subfield volumes using manual or automated delineation methods is displayed in Table 1. As can be seen, the majority of

in vivo studies with humans researching risk factors and hippocampal subfield volumes were published in the past 10 years and are relatively few in number. Risk factors studied differed between studies from only age or alcohol use disorder up to the inclusion of hypertension, obesity, *APOE* $\epsilon 4$ carrier status, and HDL-cholesterol. Studies used 1.5T to 4.7T MRI, except for one study that used 7T MRI. Following our inclusion criterion, subfields were segmented manually, or automatically by protocols developed by the authors or by freely available software (e.g., Freesurfer). Areas of the hippocampus that were studied ranged from one up to eleven, and number of subjects included in the studies widely varied with the majority having a study population less than hundred participants.

Baseline characteristics of the study population are displayed in Table 2. Participants were on average 67 years of age (SD 9 years) and 32% were female. Of all participants, 22.6% never smoked, 9.9% used no alcohol, 12.0% had diabetes, and 82.0% had hypertension.

Table 3 shows the results of the mixed models for the adjusted associations of age, sex, and other risk factors with z-transformed volumes of each hippocampal subfield. In Fig. 1, we also visualized the unadjusted volumes for each risk factor. As can be seen, with increasing age, all subfield volumes were significantly smaller, with the exception of CA4/DG. *Post-hoc* tests showed that with increasing age, the entorhinal cortex, subiculum, CA2, CA3, and the tail were similarly affected as CA1 (Table 4). Sex was not significantly associated with any of the subfield volumes.

Compared to never smokers, current smokers had statistically significantly smaller volumes of the entorhinal cortex and subiculum (entorhinal cortex: $B = -0.48$, 95% CI: $-0.85; -0.12$, and subiculum $B = -0.47$, 95% CI: $-0.83; -0.11$). This equals about half a standard deviation, and thus a 16% smaller volume than the mean volumes of these subfields. Former smokers also had smaller volume of the entorhinal cortex ($B = -0.27$, 95% CI: $-0.55; 0.01$) and subiculum ($B = -0.26$, 95% CI: $-0.53; 0.01$), although this association did not reach statistical significance (Table 3). When formally tested, *post-hoc* analyses did not show entorhinal cortex and subiculum to be significantly differentially affected by current smoking status compared to CA1 (Table 4).

Compared to no alcohol use, alcohol use up to 10 units/week showed statistically significantly smaller volumes of CA1 ($B = -0.39$, 95% CI: $-0.74; -0.04$)

Table 2
Baseline characteristics of the study sample

Characteristics		Study sample $n = 283$
Demographics		
Age (y)	Mean age of all patients \pm S.D.	67 \pm 9
Sex	Male	192 (67.8%)
	Female	91 (32.2%)
Mini-Mental State Examination score, mean \pm S.D.		
28.9 \pm 1.4		
Vascular risk factors		
Smoking	Never smoker	64 (22.6%)
	Current smoker	41 (14.5%)
	Former smoker	178 (62.9%)
	No alcohol use	28 (9.9%)
Alcohol use ¹	Up to 10 units per week	140 (49.5%)
	More than 10 units per week	73 (25.8%)
		26.7 \pm 3.9
Body mass index (kg/m ²) ²		52 (18.4%)
Obese (>30 kg/m ²) ³		34 (12.0%)
Diabetes mellitus present ⁴		232 (82.0%)
Hypertension present		141 \pm 18
Systolic blood pressure (mmHg), mean \pm S.D.		80 \pm 10
Diastolic blood pressure (mmHg), mean \pm S.D.		
Magnetic resonance imaging measurements		
Hippocampal subfield volumes (mm ³), mean \pm S.D. ⁵	Entorhinal cortex	841.4 \pm 151.3
	Subiculum	1168.3 \pm 167.1
	Cornu Ammonis 1	2983.2 \pm 349.0
	Cornu Ammonis 2	120.2 \pm 21.2
	Cornu Ammonis 3	198.2 \pm 46.1
	Cornu Ammonis 4/Dentate Gyrus	1587.8 \pm 217.2
	Tail	289.5 \pm 67.1
		1501.8 \pm 152.4
Intracranial volume (cm ³), mean \pm S.D.		

¹Data of 241 participants on alcohol use ($n = 42$ missing). ^{2,3}Data of 282 participants on body mass index and obesity ($n = 1$ missing). ⁴Data of 281 participants on diabetes mellitus ($n = 2$ missing). ⁵Sum of left and right hippocampus (subfield).

and CA4/DG ($B = -0.45$, 95% CI: -0.82 ; -0.08), and alcohol use of more than 10 units/week also showed a statistically significantly smaller CA1 volume ($B = -0.43$, 95% CI: -0.81 ; -0.05). CA1 was not significantly more affected, when formally tested, than the other subfield volumes as shown by *post-hoc* comparisons (Table 4).

Higher BMI was significantly associated with smaller volumes of the entorhinal cortex ($B = -0.04$, 95% CI: -0.06 ; -0.01), subiculum ($B = -0.04$, 95% CI: -0.07 ; -0.01), CA1 ($B = -0.03$, 95% CI: -0.05 ; -0.00), CA2 ($B = -0.03$, 95% CI: -0.06 ; -0.00), and CA3 ($B = -0.05$, 95% CI: -0.08 ; -0.02), but not CA4/DG or tail. These B values are similar to around 1–1.7% smaller volumes compared to the mean of these volumes. BMI affected entorhinal cortex, subiculum, CA2, and CA3 similarly to CA1 when we tested this formally with *post-hoc* analyses (Table 4).

Presence of obesity showed around 10 to 12% smaller subfield volumes compared to the mean volumes of the entorhinal cortex ($B = -0.33$, 95% CI: -0.62 ; -0.05), subiculum ($B = -0.33$, 95% CI: -0.61 ; -0.05), CA2 ($B = -0.35$, 95% CI: -0.63 ; -0.07),

CA3 ($B = -0.37$, 95% CI: -0.67 ; -0.08), and tail ($B = -0.30$, 95% CI: -0.59 ; -0.00), although not significantly different from CA1 as shown by *post-hoc* comparisons (Table 4).

Diabetes mellitus was associated with smaller volume of the subiculum ($B = -0.35$, 95% CI: -0.68 ; -0.02) but not with other subfields. Yet, the effect estimate for the subiculum was not statistically significantly different from the effect estimate for CA1 volume when tested formally (Table 4).

Hypertension was not statistically significantly associated with any of the hippocampal subfield volumes, nor was increasing systolic blood pressure (per 10 mmHg increase) or increasing diastolic blood pressure (per 10 mmHg increase) with the exception of CA4/DG volume, which was larger ($B = 0.13$, 95% CI: 0.02; 0.24). We repeated the analyses within participants without anti-hypertensive medication ($n = 41$ with hypertension based on elevated blood pressure, $n = 50$ without hypertension). There were no significant associations between hypertension, systolic or diastolic blood pressure and hippocampal subfield volumes (data not shown). When formally tested, *post-hoc* analyses showed no

Table 3
Associations between age, sex, vascular risk factors, and z-transformed hippocampal subfield volumes in the study sample

	Entorhinal cortex	Subiculum	Cornu Ammonis 1	Cornu Ammonis 2	Cornu Ammonis 3	Cornu Ammonis 4/ Dentate Gyrus	Tail
	Unstandardized B (95% CI)	Unstandardized B (95% CI)					
Age ¹	-0.03 (-0.05; -0.02)**	-0.03 (-0.04; -0.02)**	-0.02 (-0.03; -0.01)**	-0.02 (-0.03; -0.01)**	-0.02 (-0.03; -0.00)*	0.00 (-0.01; 0.01)	-0.02 (-0.03; -0.00)**
Sex (male; female) ²	0.04 (-0.25; 0.34)	0.11 (-0.17; 0.40)	-0.06 (-0.33; 0.22)	0.06 (-0.23; 0.35)	0.26 (-0.04; 0.56)	0.23 (-0.05; 0.51)	0.24 (-0.06; 0.54)
Vascular risk factors							
Current smoking ³	-0.48 (-0.85; -0.12)*	-0.47 (-0.83; -0.11)*	-0.13 (-0.48; 0.21)	-0.16 (-0.53; 0.21)	0.02 (-0.37; 0.41)	-0.16 (-0.51; 0.20)	-0.04 (-0.42; 0.34)
Former smoking ³	-0.27 (-0.55; 0.01)	-0.26 (-0.53; 0.01)	0.09 (-0.17; 0.35)	-0.07 (-0.34; 0.21)	0.15 (-0.14; 0.44)	0.03 (-0.23; 0.30)	0.06 (-0.23; 0.35)
Alcohol ≤10 units/week ⁴	0.06 (-0.34; 0.47)	0.20 (-0.19; 0.58)	-0.39 (-0.74; -0.04)*	-0.11 (-0.51; 0.29)	0.26 (-0.15; 0.67)	-0.45 (-0.82; -0.08)*	-0.26 (-0.67; 0.15)
Alcohol >10 units/week ⁴	0.15 (-0.29; 0.59)	0.11 (-0.31; 0.54)	-0.43 (-0.81; -0.05)*	-0.25 (-0.69; 0.18)	0.20 (-0.25; 0.65)	-0.35 (-0.75; 0.05)	-0.29 (-0.74; 0.15)
Body mass index ⁵	-0.04 (-0.06; -0.01)**	-0.04 (-0.07; -0.01)**	-0.03 (-0.05; -0.00)*	-0.03 (-0.06; -0.00)*	-0.05 (-0.08; -0.02)**	-0.02 (-0.05; 0.01)	-0.02 (-0.05; 0.01)
Obesity ⁵	-0.33 (-0.62; -0.05)*	-0.33 (-0.61; -0.05)*	-0.19 (-0.46; 0.08)	-0.35 (-0.63; -0.07)*	-0.37 (-0.67; -0.08)*	-0.16 (-0.44; 0.11)	-0.30 (-0.59; -0.00)*
Diabetes Mellitus ⁶	-0.29 (-0.63; 0.05)	-0.35 (-0.68; -0.02)*	-0.14 (-0.46; 0.18)	-0.29 (-0.62; 0.05)	0.03 (-0.32; 0.39)	-0.07 (-0.39; 0.26)	-0.06 (-0.41; 0.29)
Hypertension	0.15 (-0.16; 0.45)	0.02 (-0.28; 0.32)	0.07 (-0.21; 0.36)	0.05 (-0.25; 0.36)	-0.08 (-0.39; 0.24)	0.11 (-0.18; 0.40)	-0.09 (-0.40; 0.22)
Systolic blood pressure ⁷	0.01 (-0.05; 0.08)	0.01 (-0.05; 0.08)	0.04 (-0.02; 0.10)	-0.00 (-0.07; 0.06)	-0.01 (-0.08; 0.05)	0.06 (-0.00; 0.12)	0.02 (-0.04; 0.09)
Diastolic blood pressure ⁷	0.01 (-0.11; 0.13)	0.08 (-0.04; 0.19)	0.10 (-0.00; 0.21)	-0.02 (-0.14; 0.09)	-0.06 (-0.19; 0.06)	0.13 (0.02; 0.24)*	0.03 (-0.09; 0.15)

Midx models: adjusted for age, sex, intracranial volume. Reference categories for all categorical risk factors is absence of that risk factor. ¹Adjusted for sex and intracranial volume. ²Adjusted for age and intracranial volume. ³Current and former smoking were compared to never smoking. ⁴Alcohol: data of 241 participants used due to missing data ($n = 42$). ⁵Body mass index, obesity: data of 282 participants on body mass index and obesity ($n = 1$ missing). ⁶Diabetes mellitus: data of 281 participants on diabetes mellitus ($n = 2$ missing). ⁷For easier interpretation, we used the continuous systolic and diastolic blood pressure divided by a factor ten. *significant p -value < 0.05. **significant p -value < 0.01.

difference between the effect estimates for CA4/DG and CA1 volume.

DISCUSSION

In this study among middle-aged and older adults without clinical dementia, we observed that increasing age was associated with smaller volumes of all subfields, except CA4/DG. Current smoking was associated with smaller volumes of the entorhinal cortex and subiculum. Moderate alcohol use was associated with smaller volumes of CA1 and CA4/DG. Obesity was associated with smaller volumes of the majority of subfields. Diabetes mellitus was associated with smaller subiculum volume. Other risk factors studied were not associated with hippocampal subfield volumes.

Although we observed that risk factors were associated with specific subfield volumes, and that some effect estimates were relatively large compared to others, none of the subfields was differentially affected by any of the risk factors when formally tested. Of note, this study included the largest number of participants with 7T MRI segmentations to date; nevertheless, it is possible that we lacked sufficient power to detect small differences between subfields. Also, we tried to avoid multiple comparisons by using one subfield (CA1) as a reference subfield to test if the subfields were differentially affected by a risk factor. CA1 is the largest subfield and thus least prone to measurement error. This already resulted in 30 comparisons. It is thus possible that we missed differences between other subfields. However, comparing all risk factors with all subfields would have largely increased the number of comparisons and thus the likelihood of chance findings.

Our finding that increasing age was associated with smaller subfield volumes is consistent with previous findings from other *in vivo* studies in humans that found increasing age to be associated with smaller volumes in one or more subfields [10, 14–26] (Table 1). Although these studies did not examine any potential differential effects on individual subfields, the variety in subfields affected between studies suggests that all subfields are to a lesser or greater extent affected. This is consistent with our observation that subfields were not differentially affected when formally tested, and is in line with *in vivo* literature reporting that aging is associated with hippocampal shrinkage [27]. An histological study, showed most neuron loss in the subiculum and CA4 (or hilus)

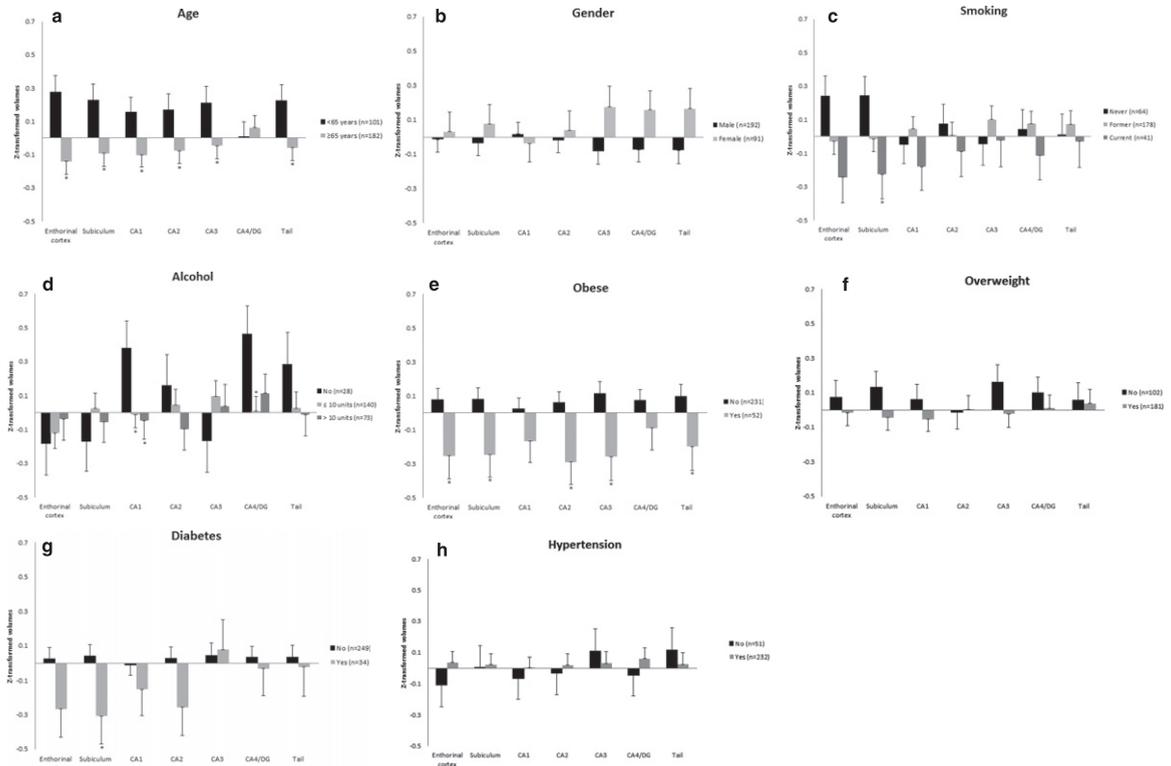


Fig. 1. Non-adjusted estimated means and standard errors of age, sex, and vascular risk factors with z-transformed volumes of each hippocampal subfield. CA, Cornu Ammonis; DG, dentate gyrus. * significant difference between the groups compared in this subfield, p -value<0.05. In the cases that there are 3 groups visualized per subfield, the significant difference is between the vascular risk factor and the group where the risk factor is absent (reference category). Note: Plots of 'age' and 'overweight' include groups generated for visualization purpose only, these dichotomous groups were not used in an analysis.

with ageing [28], although the effect on the volume of the subfields with this neuron loss was not specified.

In this study, sex was not associated with any subfield volume reduction, which is consistent with most literature [14, 21, 25]. To our knowledge only one study reported that men had a larger CA2 volume than women (Table 1) [23]. Overall, sex does not seem to influence a decrease in hippocampal (subfield) volume. Current smoking, but not former smoking, was associated with smaller entorhinal cortex and subiculum volumes. Another study found a trend for a decrease in subiculum volume and smaller volumes of CA1, CA2-3, and CA4/DG (Table 1) [29]. Smoking is an important risk factor for AD [30] and previous studies, *in vivo* and histological, report that the entorhinal cortex and subiculum are among the first structures affected in AD [8, 28]. It is interesting to note that in our study also the entorhinal cortex and subiculum show the strongest associations with smoking status. Although we did not find that the

entorhinal cortex and subiculum were significantly more affected than CA1 as reference subfield when formally tested, our findings support the notion that smoking contributes to AD development through hippocampal volume loss. We also found an association between alcohol use up to 10 units per week and more than 10 units per week and a smaller CA1 volume, and also between alcohol use up to 10 units per week and a smaller CA4/DG. One other study also found a smaller CA1 volume, CA4, and DG in patients with alcohol use disorder compared to control participants, which may be consistent with our findings. However, that study also found a smaller subiculum volume in alcohol use disorder compared to control participants, which we did not observe (Table 1) [15]. Another study in patients with alcohol use disorder found a smaller subiculum volume, but no associations with CA2-3 or CA4/DG (Table 1) [31]. It should be noted that we did not examine alcohol use disorder, but units per week in participants not selected on alcohol dependence. In alcohol use disorder it

Table 4
Age, vascular risk factors and volumes in standardized z-scores of hippocampal subfields compared to the volume of Cornu Ammonis 1

	Cornu Ammonis 1	Entorhinal cortex	Subiculum	Cornu Ammonis 2	Cornu Ammonis 3	Cornu Ammonis 4 / Dentate Gyrus	Tail
Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Age	reference	0.01 (-0.00; 0.03)	0.01 (-0.01; 0.03)	0.00 (-0.01; 0.02)	-0.00 (-0.02; 0.01)	-0.00 (-0.02; 0.01)	-0.00 (-0.02; 0.02)
Vascular risk factors							
Current smoking	reference	0.10 (-0.33; 0.53)	0.09 (-0.34; 0.52)				
Alcohol ≤10 units/week ¹	reference	-0.04 (-0.37; 0.29)	-0.19 (-0.51; 0.13)	-0.15 (-0.48; 0.17)	-0.19 (-0.53; 0.14)	0.12 (-0.20; 0.43)	-0.04 (-0.37; 0.29)
Alcohol >10 units/week ¹	reference	-0.18 (-0.54; 0.18)	-0.04 (-0.39; 0.31)	0.07 (-0.29; 0.42)	-0.07 (-0.43; 0.29)	-0.13 (-0.48; 0.21)	-0.02 (-0.38; 0.35)
Body mass index ²	reference	0.01 (-0.03; 0.05)	0.01 (-0.03; 0.05)	0.00 (-0.04; 0.04)	0.02 (-0.02; 0.06)		
Obesity ²	reference	0.14 (-0.25; 0.53)	0.14 (-0.25; 0.52)	0.16 (-0.23; 0.55)	0.18 (-0.22; 0.58)		0.11 (-0.29; 0.50)
Diabetes Mellitus ³	reference						
Diastolic blood pressure ⁴	reference		0.21 (-0.25; 0.67)				

Mixed models: adjusted for age, sex and intracranial volume. Volumes of hippocampal subfields with Cornu Ammonis 1 as reference volume (all z-transformed), for vascular risk factors that showed a significant association with volumes in Table 2. ¹Alcohol: data of 241 participants used due to missing data (n = 42). ²Body Mass Index, obesity: data of 282 participants on body mass index and obesity (n = 1 missing). ³Diabetes mellitus: data of 281 participants on diabetes mellitus (n = 2 missing). ⁴For easier interpretation we used the continuous diastolic blood pressure divided by a factor ten.

is reported that individuals on average have an 8% smaller hippocampus [5].

Higher BMI and obesity were associated with volume reductions in almost all hippocampal subfields. One other study that investigated ERC found no association with obesity (Table 1) [20], and another investigated SUB, CA2-3, and CA4/DG and also found no associations (Table 1) [31]. Reviews report that obesity is associated with a smaller total hippocampal volume [5, 27], possibly due to several different effects leading to a decrease in insulin sensitivity [27]. Insulin resistance in the hippocampus is thought to be an important factor in cognitive dysfunction and AD [32].

DM was associated with significant volume reduction in the subiculum, which is consistent with one other study, although that study also observed an association with volume reduction in CA1 (Table 1) [33]. It has been reported that patients with DM have increased atrophy of the total hippocampus [5], and DM is one of the more consistently reported risk factors for AD and vascular dementia [3] and is thought to affect cognition by the mechanism of insulin resistance as mentioned above.

We found no association between hypertension and hippocampal subfield volumes, which is consistent with two other studies (Table 1) [20, 25]. However, one other study found a smaller CA1-2 volume in older adults with hypertension compared to normotensive older adults (Table 1) [26]. To our knowledge, no studies investigated the associations between continuous systolic or diastolic blood pressure and subfield volumes in humans *in vivo*. We did not find evidence for an association between systolic blood pressure and smaller hippocampal subfield volumes. In our study, higher diastolic blood pressure was associated with larger CA4/DG volume, but not with other subfield volumes. CA4/DG was not differentially affected when compared to CA1 volume. To explore if pulse pressure could explain this unexpected finding, we performed a *post-hoc* mixed effect regression model, and found that pulse pressure was not associated with any of the subfields volumes. Overall, the associations between blood pressure and hippocampal subfield volumes are inconsistent, and there does not seem to be a differential effect on subfield volumes. Hypertension, systolic and diastolic blood pressure levels and their association with dementia is complex. Both hypertension and low diastolic blood pressure are associated with a decrease in hippocampal volume [5].

Strengths of this study include the large number of participants with 7T brain imaging. Further, we used 7T MRI to visualize the hippocampal subfields and used a freely available fully automated segmentation program (ASHS) that has been validated and showed high accuracy compared to the labor-intensive manual segmentation [13]. Previous studies were conducted on lower resolution MRI scanners which make it more difficult to delineate the subfields and studies therefore combined different subfields in their analyses. Furthermore, the method of delineation of the subfields differs across studies. Some studies used manual protocols, while others used automatic methods of delineation. These automatic programs included Freesurfer version 5.1 up to version 5.3, which have been criticized before as these subfields did not agree well with histological boundaries and other studies using manual protocols [34]. The subfield boundaries have been improved in version 6.0, although caution with interpretation of the volumetric data on the subfields is still advised as some boundaries are not visual in training data [35]. In Table 1, we included those studies that used a manual or automated delineation method of hippocampal subfield volumes, to increase comparability with our study, although it must be noted that there are many interesting other studies using different techniques (e.g., [36–44]) on the association between age or other risk factors and the hippocampus). Another strength of this study is that we examined differential effects of the vascular risk factors on subfield volumes. As can be seen from Table 1, few studies investigated the associations between vascular risk factors and hippocampal subfield volumes *in vivo*, and none examined any potential differential effects of the risk factors on subfield volumes. Further, we included participants from various settings, which increased variability in risk factor distribution. At the same time, it should be noted that the majority of participants in the study came from the SMART-MR study that had a history of vascular disease and were survivors of the cohort still willing and able to undergo a 7T MRI. The results may therefore not be generalizable to the general population without dementia.

A limitation of this study is the cross-sectional design, and we therefore do not know to what extent the smaller subfield volumes observed represent volume loss in the context of normal aging or neurodegenerative diseases, such as AD. The participants were not selected on having an increased risk for AD, although a portion of the sample may have a

family history of AD or experience subjective cognitive decline. Also, we did not have *APOE* genotype for all participants available nor did we have amyloid- β or tau. Also, we were not able to determine to what extent hippocampal subfields show differential volume loss over time. Prospective studies are needed to determine to what extent the observed hippocampal subfield volume reductions increase the risk of AD or are part of normal aging.

In conclusion, in older adults without dementia, age, smoking, alcohol use, obesity and diabetes mellitus were associated with smaller volumes of specific hippocampal subfields on 7T MRI; however, no statistical evidence was found that these subfields were differentially affected by these risk factors. More studies are needed to confirm our findings and investigate to what extent hippocampal subfields are differentially affected by risk factors and contribute to AD development.

ACKNOWLEDGMENTS

We gratefully acknowledge the time and effort of all participants. We would like to thank all general practitioners from the general practice “Huisartsenpraktijk Bosboomstraat” for their help in inclusion of the participants. Furthermore, we would like to thank all the people involved in data acquisition in the memory clinics, and people involved in the data acquisition of the PREDICT-MR study.

Financial support was received by the Alzheimer Nederland - Internationale Stichting Alzheimer Onderzoek (AN-ISA0) (Grant number 12504). The funding source had no involvement in writing of this article or the decision to submit it for publication.

We gratefully acknowledge the contribution of the SMART research nurses; R. van Petersen (data-manager); B.G.F. Dinther (vascular manager) and the members of the Utrecht Cardiovascular Cohort-Second Manifestations of ARterial disease-Study Group (UCC-SMART-Study Group): F.W. Asselbergs and H.M. Nathoe, Department of Cardiology; G.J. de Borst, Department of Vascular Surgery; M.L. Bots and M.I. Geerlings, Julius Center for health Sciences and Primary Care; M.H. Emmelot, Department of Geriatrics; P.A. de Jong and T. Leiner, Department of Radiology; A.T. Lely, Department of Obstetrics & Gynecology; N.P. van der Kaaij, Department of Cardiothoracic Surgery; L.J. Kappelle and Y.M. Ruigrok, Department of Neurology; M.C. Verhaar, Department of Nephrology,

F.L.J. Visseren (chair) and J. Westerink, Department of Vascular Medicine, University Medical Center Utrecht and Utrecht University. Special thanks go to N.P.A. Zuithoff, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht and Utrecht University, for his assistance with SAS.

Authors' disclosures available online (<https://www.j-alz.com/manuscript-disclosures/20-0159r1>).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/JAD-200159>.

REFERENCES

- [1] World Health Organization, Fact Sheet Dementia, <https://www.who.int/news-room/fact-sheets/detail/dementia>, Last updated September 19, 2019, Accessed on October 8, 2019.
- [2] World Health Organization, Alzheimer's Disease International (2012) Dementia, A public health priority.
- [3] Bellou V, Belbasis L, Tzoulaki I, Middleton LT, Ioannidis JPA, Evangelou E (2017) Systematic evaluation of the associations between environmental risk factors and dementia: An umbrella review of systematic reviews and meta-analyses. *Alzheimers Dement* **13**, 406-418.
- [4] Kockelkoren R, De Vis JB, Stavenga M, Mali WPTM, Hendrikse J, Rozemuller AM, Koek HL, van der Schaaf IC, Velthuis BK, de Jong PA (2018) Hippocampal calcification on brain CT: prevalence and risk factors in a cerebrovascular cohort. *Eur Radiol* **28**, 3811-3818.
- [5] Fotuhi M, Do D, Jack C (2012) Modifiable factors that alter the size of the hippocampus with ageing. *Nat Rev Neurol* **8**, 189-202.
- [6] Pini L, Pievani M, Bocchetta M, Altomare D, Bosco P, Cavado E, Galluzzi S, Marizzoni M, Frisoni GB (2016) Brain atrophy in Alzheimer's disease and aging. *Ageing Res Rev* **30**, 25-48.
- [7] Yushkevich PA, Amaral RSC, Augustinack JC, Bender AR, Bernstein JD, Boccardi M, Bocchetta M, Burggren AC, Carr VA, Chakravarty MM, Chételat G, Daugherty AM, Davachi L, Ding SL, Ekstrom A, Geerlings MI, Hassan A, Huang Y, Iglesias JE, La Joie R, Kerchner GA, LaRocque KF, Libby LA, Malykhin N, Mueller SG, Olsen RK, Palombo DJ, Parekh MB, Pluta JB, Preston AR, Pruessner JC, Ranganath C, Raz N, Schlichting ML, Schoemaker D, Singh S, Stark CEL, Suthana N, Tomparay A, Turowski MM, Van Leemput K, Wagner AD, Wang L, Winterburn JL, Wisse LEM, Yassa MA, Zeineh MM (2015) Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in *in vivo* MRI: Towards a harmonized segmentation protocol. *Neuroimage* **111**, 526-541.
- [8] Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011) A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci* **12**, 585-601.
- [9] Wu W, Brickman AM, Luchsinger J, Ferrazzano P, Pichiule P, Yoshita M, Brown T, Decarli C, Barnes CA, Mayeux R, Vannucci SJ, Small SA (2008) The brain in the age of old: The hippocampal formation is targeted differentially by diseases of late life. *Ann Neurol* **64**, 698-706.
- [10] Wisse LEM, Biessels GJ, Heringa SM, Kuijff HJ, Koek DL, Luijten PR, Geerlings MI (2014) Hippocampal subfield volumes at 7T in early Alzheimer's disease and normal aging. *Neurobiol Aging* **35**, 2039-2045.
- [11] Jellinek, Hoeveel alcohol bevat een standaardglas bier, wijn of sterke drank? <https://www.jellinek.nl/vraag-antwoord/hoeveel-alcohol-bevat-een-glas-bier-wijn-of-sterke-drank/>, Last updated January, 2016, Accessed on May 26, 2020.
- [12] Yushkevich PA, Pluta JB, Wang H, Xie L, Ding SL, Gertje EC, Mancuso L, Kliot D, Das SR, Wolk DA (2015) Automated volumetry and regional thickness analysis of hippocampal subfields and medial temporal cortical structures in mild cognitive impairment. *Hum Brain Mapp* **36**, 258-287.
- [13] Wisse LEM, Kuijff HJ, Honingh AM, Wang H, Pluta JB, Das SR, Wolk DA, Zwaneburg JJM, Yushkevich PA, Geerlings MI (2016) Automated hippocampal subfield segmentation at 7T MRI. *Am J Neuroradiol* **37**, 1050-1057.
- [14] Daugherty AM, Bender AR, Raz N, Ofen N (2016) Age differences in hippocampal subfield volumes from childhood to late adulthood. *Hippocampus* **26**, 220-228.
- [15] Zahr NM, Pohl KM, Saranathan M, Sullivan E V., Pfefferbaum A (2019) Hippocampal subfield CA2+3 exhibits accelerated aging in Alcohol Use Disorder: A preliminary study. *NeuroImage Clin* **22**, 101764.
- [16] Malykhin NV, Huang Y, Hrybouski S, Olsen F (2017) Differential vulnerability of hippocampal subfields and anteroposterior hippocampal subregions in healthy cognitive aging. *Neurobiol Aging* **59**, 121-134.
- [17] de Flores R, La Joie R, Landeau B, Perrotin A, Mézenge F, de La Sayette V, Eustache F, Desgranges B, Chételat G (2015) Effects of age and Alzheimer's disease on hippocampal subfields: Comparison between manual and freesurfer volumetry. *Hum Brain Mapp* **36**, 463-474.
- [18] Nadal L, Coupé P, Helmer C, Manjon JV, Amieva H, Tison F, Dartigues JF, Catheline G, Planche V (2020) Differential annualized rates of hippocampal subfields atrophy in aging and future Alzheimer's clinical syndrome. *Neurobiol Aging* **90**, 75-83.
- [19] Stoub TR, Barnes CA, Shah RC, Stebbins GT, Ferrari C, deToledo-Morrell L (2012) Age-related changes in the mesial temporal lobe: The parahippocampal white matter region. *Neurobiol Aging* **33**, 1168-1176.
- [20] Armstrong NM, An Y, Beason-Held L, Doshi J, Erus G, Ferrucci L, Davatzikos C, Resnick SM (2019) Sex differences in brain aging and predictors of neurodegeneration in cognitively healthy older adults. *Neurobiol Aging* **81**, 146-156.
- [21] La Joie R, Fouquet M, Mézenge F, Landeau B, Villain N, Mevel K, Pélerin A, Eustache F, Desgranges B, Chételat G (2010) Differential effect of age on hippocampal subfields assessed using a new high-resolution 3T MR sequence. *Neuroimage* **53**, 506-514.
- [22] Lee J, Im SJ, Lee SG, Stadlin A, Son JW, Shin CJ, Ju G, Lee SI, Kim S (2016) Volume of hippocampal subfields in patients with alcohol dependence. *Psychiatry Res Neuroimaging* **258**, 16-22.
- [23] Mueller SG, Stables L, Du AT, Schuff N, Truran D, Cashdollar N, Weiner MW (2007) Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4 T. *Neurobiol Aging* **28**, 719-726.

- [24] Mueller SG, Weiner MW (2009) Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus* **19**, 558–564.
- [25] Raz N, Daugherty AM, Bender AR, Dahle CL, Land S (2015) Volume of the hippocampal subfields in healthy adults: differential associations with age and a pro-inflammatory genetic variant. *Brain Struct Funct* **220**, 2663–2674.
- [26] Shing YL, Rodrigue KM, Kennedy KM, Fandakova Y, Bodammer N, Werkle-Bergner M, Lindenberger U, Raz N (2011) Hippocampal subfield volumes: Age, vascular risk, and correlation with associative memory. *Front Aging Neurosci* **3**, 2.
- [27] Bettio LEB, Rajendran L, Gil-Mohapel J (2017) The effects of aging in the hippocampus and cognitive decline. *Neurosci Biobehav Rev* **79**, 66–86.
- [28] West MJ, Coleman PD, Flood DG, Troncoso JC (1994) Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.
- [29] Durazzo TC, Meyerhoff DJ, Nixon SJ (2013) Interactive effects of chronic cigarette smoking and age on hippocampal volumes. *Drug Alcohol Depend* **133**, 704–711.
- [30] Durazzo TC, Mattsson N, Weiner MW (2014) Smoking and increased Alzheimer's disease risk: A review of potential mechanisms. *Alzheimers Dement* **10**, S122–S145.
- [31] Mole TB, Mak E, Chien Y, Voon V (2016) Dissociated accumbens and hippocampal structural abnormalities across obesity and alcohol dependence. *Int J Neuropsychopharmacol* **19**, pyw039.
- [32] Biessels GJ, Reagan LP (2015) Hippocampal insulin resistance and cognitive dysfunction. *Nat Rev Neurosci* **16**, 660–671.
- [33] Zhang YW, Zhang JQ, Liu C, Wei P, Zhang X, Yuan QY, Yin XT, Wei LQ, Cui JG, Wang J (2015) Memory dysfunction in type 2 diabetes mellitus correlates with reduced hippocampal CA1 and subiculum volumes. *Chin Med J (Engl)* **128**, 465–471.
- [34] Wisse LEM, Biessels GJ, Geerlings MI (2014) A critical appraisal of the hippocampal subfield segmentation package in FreeSurfer. *Front Aging Neurosci* **6**, 261.
- [35] Giuliano A, Donatelli G, Cosottini M, Tosetti M, Retico A, Fantacci ME (2017) Hippocampal subfields at ultra high field MRI: An overview of segmentation and measurement methods. *Hippocampus* **27**, 481–494.
- [36] Frisoni GB, Ganzola R, Canu E, Rüb U, Pizzini FB, Alessandrini F, Zoccatelli G, Beltramello A, Caltagirone C, Thompson PM (2008) Mapping local hippocampal changes in Alzheimer's disease and normal ageing with MRI at 3 Tesla. *Brain* **131**, 3266–3276.
- [37] Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, Jones M, Martin-Harris L, Ercoli LM, Miller KJ, Small GW, Bookheimer SY (2010) Family history of Alzheimer's disease and hippocampal structure in healthy people. *Am J Psychiatry* **167**, 1399–1406.
- [38] Ziegler G, Dahnke R, Jäncke L, Yotter RA, May A, Gaser C (2012) Brain structural trajectories over the adult lifespan. *Hum Brain Mapp* **33**, 2377–2389.
- [39] Apostolova LG, Green AE, Babakchanian S, Hwang KS, Chou YY, Toga AW, Thompson PM (2012) Hippocampal atrophy and ventricular enlargement in normal aging, mild cognitive impairment (MCI), and Alzheimer disease. *Alzheimer Dis Assoc Disord* **26**, 17–27.
- [40] Yang X, Goh A, Chen SHA, Qiu A (2013) Evolution of hippocampal shapes across the human lifespan. *Hum Brain Mapp* **34**, 3075–3085.
- [41] Kerchner GA, Berdnik D, Shen JC, Bernstein JD, Fenesy MC, Deutsch GK, Wyss-Coray T, Rutt BK (2014) APOE e4 worsens hippocampal CA1 apical neuropil atrophy and episodic memory. *Neurology* **82**, 691–697.
- [42] Shi J, Leporé N, Gutman BA, Thompson PM, Baxter LC, Caselli RJ, Wang Y (2014) Genetic influence of apolipoprotein E4 genotype on hippocampal morphometry: An N=725 surface-based Alzheimer's disease neuroimaging initiative study. *Hum Brain Mapp* **35**, 3903–3918.
- [43] Kurth F, Cherbuin N, Luders E (2017) The impact of aging on subregions of the hippocampal complex in healthy adults. *Neuroimage* **163**, 296–300.
- [44] Dong S, Dongwei L, Zhang J, Liang J, Sun Z, Fang J (2019) Individuals in the prediabetes stage exhibit reduced hippocampal tail volume and executive dysfunction. *Brain Behav* **9**, e01351.
- [45] Doshi J, Erus G, Ou Y, Resnick SM, Gur RC, Gur RE, Satterthwaite TD, Furth S, Davatzikos C (2016) MUSE: MULTI-atlas region Segmentation utilizing Ensembles of registration algorithms and parameters, and locally optimal atlas selection. *Neuroimage* **127**, 186–195.
- [46] Erus G, Doshi J, An Y, Verganelakis D, Resnick SM, Davatzikos C (2018) Longitudinally and inter-site consistent multi-atlas based parcellation of brain anatomy using harmonized atlases. *Neuroimage* **166**, 71–78.
- [47] Bender AR, Daugherty AM, Raz N (2013) Vascular risk moderates associations between hippocampal subfield volumes and memory. *J Cogn Neurosci* **25**, 1851–1862.
- [48] Harding AJ, Halliday GM, Kril JJ (1998) Variation in hippocampal neuron number with age and brain volume. *Cereb Cortex* **8**, 710–718.
- [49] Malykhin N V., Bouchard TP, Ogilvie CJ, Coupland NJ, Seres P, Camicioli R (2007) Three-dimensional volumetric analysis and reconstruction of amygdala and hippocampal head, body and tail. *Psychiatry Res* **155**, 155–165.
- [50] Malykhin N V., Lebel RM, Coupland NJ, Wilman AH, Carter R (2010) In vivo quantification of hippocampal subfields using 4.7 T fast spin echo imaging. *Neuroimage* **49**, 1224–1230.
- [51] Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW (2008) Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage* **42**, 42–48.
- [52] Goncharova II, Dickerson BC, Stoub TR, DeToledo-Morrell L (2001) MRI of human entorhinal cortex: A reliable protocol for volumetric measurement. *Neurobiol Aging* **22**, 737–745.
- [53] Wisse LEM, Gerritsen L, Zwanenburg JJM, Kuijff HJ, Luijten PR, Biessels GJ, Geerlings MI (2012) Subfields of the hippocampal formation at 7T MRI: In vivo volumetric assessment. *Neuroimage* **61**, 1043–1049.