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FEATURED ARTICLE

The serum metabolome mediates the concert of diet, exercise, and neurogenesis, determining the risk for cognitive decline and dementia

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Abstract

Introduction: Diet and exercise influence the risk of cognitive decline (CD) and dementia through the food metabolome and exercise-triggered endogenous factors, which use the blood as a vehicle to communicate with the brain. These factors might act in concert with hippocampal neurogenesis (HN) to shape CD and dementia.

Methods: Using an *in vitro* neurogenesis assay, we examined the effects of serum samples from a longitudinal cohort (n = 418) on proxy HN readouts and their association with future CD and dementia across a 12-year period.

Results: Altered apoptosis and reduced hippocampal progenitor cell integrity were associated with exercise and diet and predicted subsequent CD and dementia. The effects of exercise and diet on CD specifically were mediated by apoptosis.

Discussion: Diet and exercise might influence neurogenesis long before the onset of CD and dementia. Alterations in HN could signify the start of the pathological process and potentially represent biomarkers for CD and dementia.

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KEYWORDS
adult hippocampal neurogenesis, cognitive decline, dementia, diet, exercise

1 | INTRODUCTION

Cognitive decline (CD) is an increasing concern, not only due to the resulting deterioration in overall quality of life and independence worldwide,[1] but also because of the well-known elevated risk to develop dementia.[2] Given that both CD and dementia are aging-related conditions[3] and life expectancy continues to increase, CD and dementia are expected to triple in prevalence by 2040.[4] While our understanding of some of the potential mechanisms of cognitive aging has improved,[5] effective strategies for preventing/slowing CD have so far remained limited. However, several lifestyle factors have emerged as potentially modifiable risk factors for CD and dementia,[6] which may offer promising targets for early preventive strategies.

Two key lifestyle modifications that offer considerable promise pertain to exercise and diet. Indeed, several clinical studies have documented the protective effects of increased exercise against CD/dementia,[7] and revealed how higher adherence to a Mediterranean diet slowed CD and reduced dementia risk.[8,9] There is also evidence of a protective association between certain candidate nutrients and foods (e.g., folate and flavonoids in dark leafy vegetables, omega-3 fatty acids, and vitamin D in seafood, caffeine) and cognitive outcomes in aging human populations.[10] In this respect, we recently identified a signature of metabolites predictive of CD and found a protective role of coffee, cocoa, fish, and red wine.[11] However, before we can effectively exploit the effects of exercise and incorporate protective bioactive compounds into targeted dietary interventions to attenuate/prevent CD and potentially dementia, the role of exercise, and of these dietary modulators, needs to be better understood at the neurobiological level.

Importantly, animal research has shown how diet and exercise can directly influence hippocampal neurogenesis (HN, i.e., the birth of new neurons in the adult brain[12]), which specifically increases in response to exercise[13] and to diets rich in omega-3 fatty acids, polyphenols, and vitamins D and E.[14] Moreover, HN plays a vital role in memory and cognition[15] and, like diet and exercise, has been associated with CD/dementia in human postmortem research,[16] suggesting that some of the protective effects of lifestyle factors on cognitive health could be mediated by a modulation of HN. However, currently it is impossible to directly test the effects of diet and/or exercise on human adult HN, which is why we turned to a recently developed, well-established *in vitro* proxy readout of human neurogenesis (**Box 1**).[17,18]

Therefore, using serum samples collected at inclusion from a longitudinal dementia cohort, we investigated the effect of the systemic environment (i.e., serum) of participants with, and without, subsequent CD and dementia on the neurogenic process using the *in vitro* assay. More specifically, we: (1) determined whether markers of HN were associated with CD and/or dementia 12 years after the samples were taken, and (2) whether exercise and key nutritional factors, including our previously identified metabolites[11] and lipids,[19] could modulate the neurogenic process.

2 | METHODS AND MATERIALS

2.1 | Population and study design

Serum samples were from participants of the Three-City (3C) cohort,[20] specifically, from a case-control study on CD (n = 418) nested within the 3C-Bordeaux centre study as described before.[11] Briefly, as highlighted in Figure 1A, the 3C cohort (n = 9,294) is a French population-based cohort on dementia, started in 1999-2000 and consisting of community dwellers aged >65 years. Participants were recruited from three French cities: Bordeaux (n = 2,104), Dijon (n = 4,931), and Montpellier (n = 2,259), and specifically a case-control study nested within the 3C-Bordeaux cohort (n = 418) was constructed for the purposes of this research as previously described[11] and detailed in the **Supplementary materials**.

As depicted in Figure 1A, at baseline, fasting blood samples, and sociodemographic, lifestyle, and clinical measures were collected from all participants. Follow-up visits were performed every 2 to 3 years over a 12-year period during which cognition status and dementia incidence were assessed. The 3C study protocol was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital (Paris, France). For further details on all baseline and outcome measures collected on the 3C study, see Table 1 and the **Supplementary materials**.

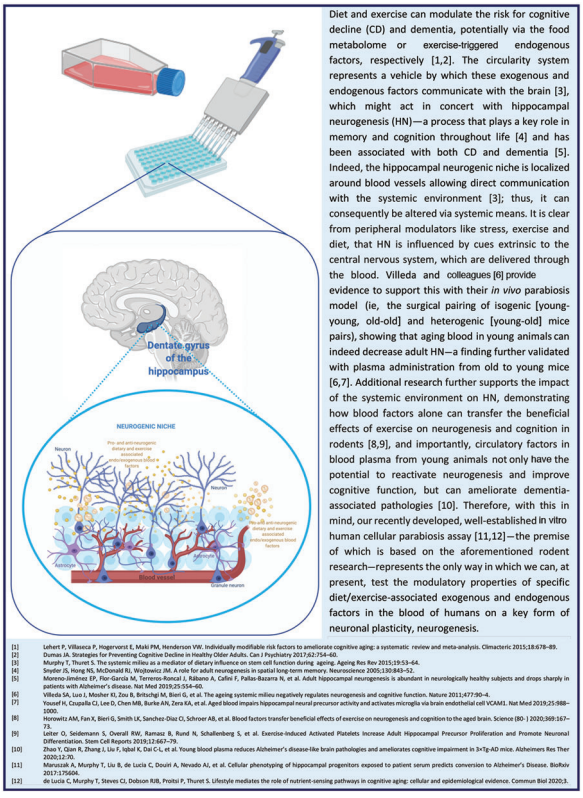
2.2 | Cognitive outcomes

Participants were retrospectively classified as either cognitively stable (control) or with accelerated CD (case) based on their trajectory of cognitive evolution over 12 years as previously detailed.[11] Cases were defined as participants with the worst slopes of CD across the 12-year follow-up and controls as those with CD below median value (i.e., >median slope; Figure 1A). For further details, see the **Supplementary materials**.

2.3 | Dementia outcomes

Participants were dementia-free (and cognitively stable) at baseline and were followed-up for incident dementia across years. Clinical diagnosis was established and validated by an independent committee of neurologists, using the Diagnostic and Statistical Manual of Mental Disorders IV as described elsewhere.[21] Dementia subtypes included Alzheimer's disease (AD, including cases of probable or possible AD and mixed dementia) or vascular dementia and other (including cases of vascular dementia, Parkinson dementia, Lewy body dementia, and frontotemporal dementia). Due to small numbers the seven subtypes were retrospectively pooled into two main categories for analyses. For further details, see the **Supplementary materials**.

BOX 1: The concept behind the *in vitro* human neurogenesis assay



Diet and exercise can modulate the risk for cognitive decline (CD) and dementia, potentially via the food metabolome or exercise-triggered endogenous factors, respectively [1,2]. The circularity system represents a vehicle by which these exogenous and endogenous factors communicate with the brain [3], which might act in concert with hippocampal neurogenesis (HN)—a process that plays a key role in memory and cognition throughout life [4] and has been associated with both CD and dementia [5]. Indeed, the hippocampal neurogenic niche is localized around blood vessels allowing direct communication with the systemic environment [3]; thus, it can consequently be altered via systemic means. It is clear from peripheral modulators like stress, exercise and diet, that HN is influenced by cues extrinsic to the central nervous system, which are delivered through the blood. Villeda and colleagues [6] provide evidence to support this with their *in vivo* parabiosis model (ie, the surgical pairing of isogenic [young-young, old-old] and heterogenic [young-old] mice pairs), showing that aging blood in young animals can indeed decrease adult HN—a finding further validated with plasma administration from old to young mice [6,7]. Additional research further supports the impact of the systemic environment on HN, demonstrating how blood factors alone can transfer the beneficial effects of exercise on neurogenesis and cognition in rodents [8,9], and importantly, circulatory factors in blood plasma from young animals not only have the potential to reactivate neurogenesis and improve cognitive function, but can ameliorate dementia-associated pathologies [10]. Therefore, with this in mind, our recently developed, well-established in vitro human cellular parabiosis assay [11,12]—the premise of which is based on the aforementioned rodent research—represents the only way in which we can, at present, test the modulatory properties of specific diet/exercise-associated exogenous and endogenous factors in the blood of humans on a key form of neuronal plasticity, neurogenesis.

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RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using PubMed, Embase, and PsycINFO. While the impact of diet and exercise on cognitive decline (CD) and dementia has been consistently demonstrated (cited herein), research on the association between hippocampal neurogenesis (HN) and these aging-associated outcomes is in its infancy. Moreover, the relationship between diet, exercise, neurogenesis, CD, and dementia remains elusive.
2. **Interpretation**: We demonstrate that HN is both modulated by diet and exercise and predicts future CD and dementia rates across a 12-year period. These data support the literature revealing that neurogenesis may be a key pathway in the relationship between diet, exercise, and cognitive aging.
3. **Future directions**: Future efforts are needed to mechanistically understand how diet could modulate HN and subsequent CD/dementia, given that it is modifiable and may represent an effective early preventative strategy against future CD and dementia.

2.4 | Dietary variables

The concentrations of 23 nutritional biomarkers were determined in total plasma as previously described.[21–23] The metabolite and lipid data were extracted from serum using untargeted liquid-chromatography (LC) mass spectrometry (MS) metabolomics and shotgun MS lipidomics, respectively, as described previously.[11,19] For a full list of all dietary variables, see Table 2.

2.5 | Cell line and culture conditions

We used the immortalized human fetal hippocampal multipotent progenitor cell line *HPC0A07/03* (HPC; ReNeuron Ltd, UK) as described before.[17,18] HPCs were cultured in medium (constitution as previously described[18]) and grown on tissue culture flasks (Nunclon, Denmark), incubated at 37°C, with 5% CO2 and saturated humidity. Cells were routinely passaged at 80% confluency before being plated for experiments. For details on the cell line and culture conditions, see the **Supplementary materials** and Figure S1.

2.6 | *In vitro* neurogenesis assay

HPC0A07/03 cells (of passage number 15 to 21) were treated with participant serum as described before.[17,18] Briefly, as detailed in Figure 1B, 1% serum was added to the cell culture during both proliferation (48 hours) and differentiation (7 days) before being fixed in 4% paraformaldehyde and stained for proliferation- and differentiation-specific markers, respectively. All experiments were carried out in technical triplicates by an experimenter blinded to caseness. For further detail on the *in vitro* assays, see the **Supplementary materials**.

2.7 | Immunocytochemistry

Cell count, progenitor cell integrity, proliferation, cell death, and differentiation were visualized using 4′,6-diamidino-2-phenylindole (DAPI), Nestin and SRY-Box Transcription Factor 2 (SOX2), Ki67, cleaved caspase-3 (CC3), doublecortin (DCX), and microtubule-associated protein 2 (MAP2), using immunocytochemistry as previously described.[18] All immunocytochemistry was performed by an experimenter blinded to caseness. For protocol details, antibodies used, and representative images, see the **Supplementary materials** and Figure S2.

2.8 | Image analysis

All immunostainings were quantified using the semi-automated CellInsight NXT High Content Screening (HCS) platform (ThermoScientific) and Studio Cell Analysis Software (ThermoScientific)

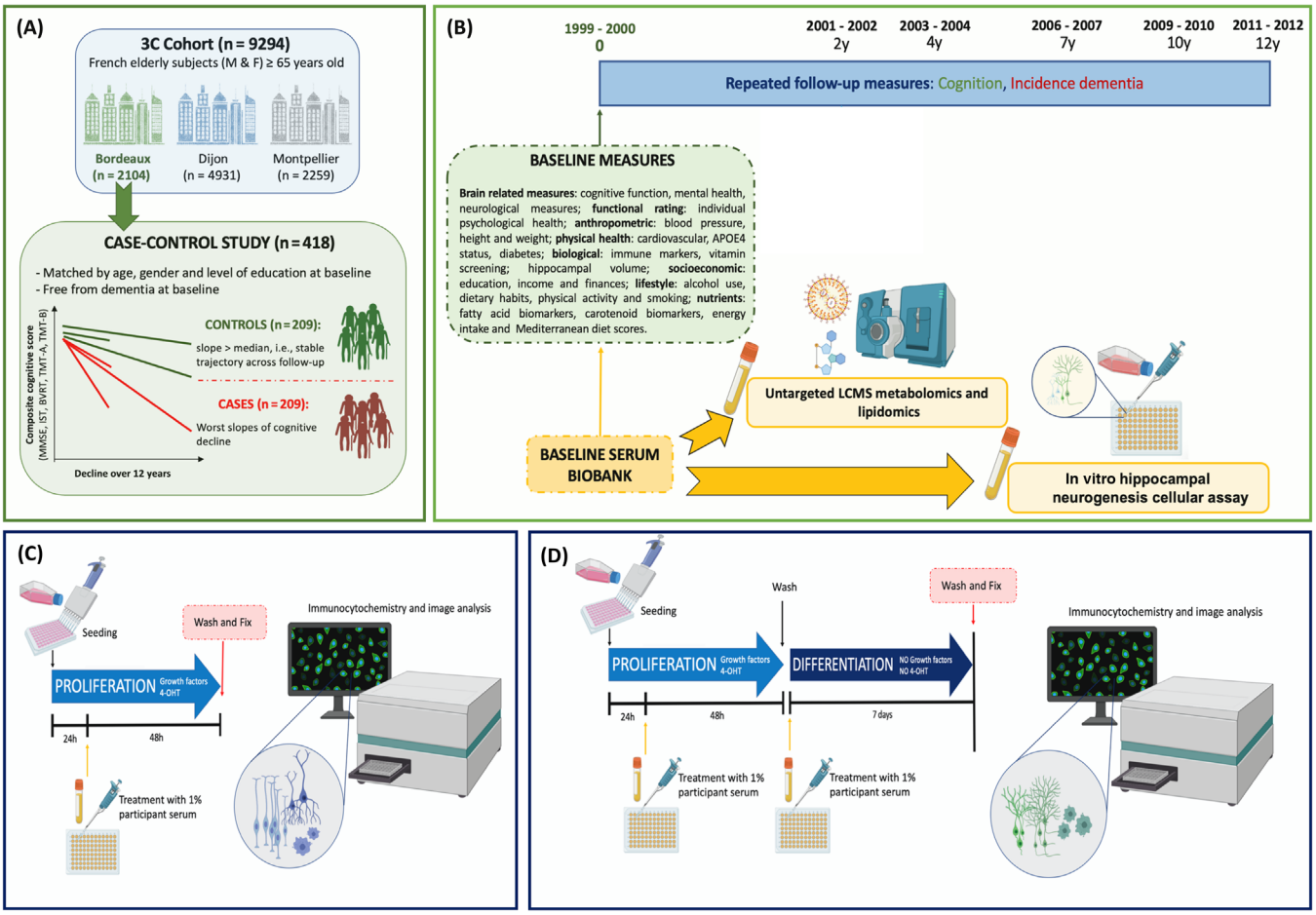

FIGURE 1 **Population, study design, and cellular assays. (A) Three-City (3C) cohort and sample**: The 3C cohort is a French population-based cohort on dementia that started in 1999-2000 and consists of male and female community dwellers aged >65 years (n = 9,294). Participants from the 3C study were recruited from three French cities, Bordeaux (n = 2,104), Dijon (n = 4,931), and Montpellier (n = 2,259), and a case-control study on cognitive decline (CD) nested within the 3C-Bordeaux cohort (n = 418) was constructed for the purposes of this study. Participants in our sample were all free from dementia at baseline, and cases and controls (for CD) were matched at baseline for age, gender, and education; therefore, our sample consisted of 209 cases and 209 controls. Using a linear mixed model, individual slopes of cognitive change for each participant over the 12-year follow-up period were estimated. The primary outcome represents the change in a composite score of global cognition, defined as the average of Z-scores of five neuropsychological tests (Mini-Mental State Examination [MMSE], Benton Visual Retention Test [BVRT], Isaac's Set Test [IST], Trail-Making Test part A [TMT-A], Trail-Making Test part B [TMT-B]) across the five follow-up visits. *Cases* were defined as participants with the worst slopes of cognitive decline and *controls* as those with a more stable trajectory (i.e., >median slope).
(B) Longitudinal study design and measures: At baseline, face-to-face interviews were conducted to collect sociodemographic, lifestyle, and medical data, plus cognitive testing, blood pressure, and anthropometric measurements from all participants. Additionally, fasting blood samples were collected for constituting a plasma and serum biobank, from which serum samples were used for the metabolomics, lipidomics, and *in vitro* cellular assays. Follow-up visits were performed every 2 to 3 years over a 12-year period and included in-person neuropsychological assessments carried out by a trained psychologist. **(C) *In vitro* cellular assay:** Proliferation timeline: Twenty-four hours after seeding, cell medium was replaced with fresh medium containing 1% serum and 1:100 penicillin streptomycin (PenStrep; 10 000 U/mL) and subsequently left to incubate for 72 hours before being fixed in 4% paraformaldehyde (PFA) and stained for proliferation specific markers. Specifically, to assess proliferation, sex determining region Y (SRY)-box 2 (SOX2) and Nestin were used to measure hippocampal progenitor cell integrity and maintenance, while Ki67 was used to measure proliferation; cleaved caspase-3 (CC3) was also used to assess cell death during these early stages of the neurogenic process.
(D) *In vitro* cellular assay: Differentiation timeline: After 48 hours of proliferation in the presence of 1% serum and 1:100 PenStrep, cells were washed and treated with another serum supplementation, this time in medium lacking 4-hydroxytamoxifen (4-OHT) and growth factors (epidermal growth factor and basic fibroblast growth factor), to allow cells to differentiate spontaneously. Serum-treated cells were subsequently left to differentiate for a further 7 days before being fixed in 4% PFA and stained for differentiation specific markers. To assess differentiation, cells were stained with doublecortin and microtubule-associated protein 2 to look at early differentiation overall, and CC3 to assess cell death.
Abbreviations: M, male; F, female; LCMS, liquid chromatography mass spectroscopy. Image created using BioRender software.

TABLE 1 Baseline participant characteristics and *in vitro* neurogenesis readouts according to caseness

	Cognitive decline			Incident dementia: Alzheimer's disease[a]			Incident dementia: Vascular and other[b]		
Measures	Cases (n = 209)	Controls (n = 209)	*Adjusted p*[c]	Cases (n = 87)	Controls (n = 305)	*Adjusted p*[d]	Cases (n = 26)	Controls (n = 305)	*Adjusted p*[d]
Sociodemographic characteristics									
Age (years)	76 (1.6)	76 (1.6)	–	**77 (4.2)**	**75 (4.3)**	**0.02***	77 (4.8)	75 (4.3)	0.14
Gender; female	138 (66)	138 (66)	–	**67 (77)**	**214 (64)**	**0.02***	19 (73)	214 (64)	0.21
Education ≥ secondary school	60 (29)	60 (29)	–	25 (29)	99 (30)	0.65	7 (27)	99 (30)	0.91
Revenue (monthly income):			0.16			**0.04***			0.94
Minimum	34 (16)	22 (11)	–	10 (12)	40 (13)	–	5 (19)	40 (13)	–
Low	78 (37)	81 (39)	–	33 (38)	115 (38)	–	9 (35)	115 (38)	–
Middle	44 (21)	49 (23)	–	16 (18)	71 (23)	–	5 (19)	71 (23)	–
High	53 (26)	57 (27)	–	28 (32)	79 (26)	–	7 (27)	79 (26)	–
Health indicators									
BMI (kg m^{-2})	26.8 (4.4)	26.1 (3.6)	0.30	26.1 (4.6)	26.5 (3.9)	0.13	27.0 (2.4)	26.5 (3.9)	0.61
Plasma total cholesterol (mmol L^{-1})	5.8 (1.0)	5.8 (0.9)	0.73	5.9 (1.0)	5.8 (0.9)	0.10	**5.8 (0.8)**	**5.8 (0.9)**	**0.001****
Plasma LDL cholesterol (mmol L^{-1})	3.7 (0.8)	3.6 (0.8)	0.52	3.7 (0.9)	3.6 (0.8)	0.29	**3.7 (0.6)**	**3.6 (0.8)**	**0.001****
Plasma HDL cholesterol (mmol L^{-1})	1.6 (0.4)	1.6 (0.4)	0.50	**1.5 (0.4)**	**1.6 (0.3)**	**0.04***	**1.7 (0.4)**	**1.6 (0.3)**	**0.001****
Plasma triglycerides (mmol L^{-1})	1.4 (0.8)	1.3 (0.6)	0.22	1.3 (0.7)	1.3 (0.7)	0.64	1.4 (0.6)	1.3 (0.7)	0.13
Plasma glucose (mmol L^{-1})	5.4 (1.4)	5.2 (1.2)	0.22	5.3 (1.6)	5.3 (1.2)	0.62	**5.5 (1.2)**	**5.3 (1.2)**	**0.01***
ApoE-ε4 carrier[e]	**54 (26)**	**25 (12)**	**<0.001*****	**25 (29)**	**16 (5.4)**	**0.003****	4 (15)	16 (5.4)	0.46
Medical factors									
Hypertension[f]	164 (79)	159 (76)	0.98	65 (75)	261 (78)	0.61	25 (96)	261 (78)	0.08#
Diabetes[g]	**27 (13)**	**12 (6)**	**0.01***	11 (13)	28 (8)	0.06#	**7 (27)**	**28 (8)**	**0.004****
Hypercholesterolemia[h]	127 (61)	128 (61)	0.47	58 (67)	199 (59)	0.69	**22 (85)**	**199 (59)**	**0.01***
Antecedents of CVD[i]	70 (34)	58 (28)	0.36	29 (33)	102 (30)	0.78	11 (42)	102 (30)	0.22
Neuro/psychological factors									
Depression[j]	70 (34)	52 (25)	0.053#	30 (35)	93 (28)	0.71	8 (31)	93 (28)	0.74
Dementia	**106 (51)**	**7 (3)**	**<0.001*****	–	–	–	–	–	–
Cognitive decline	–	–	–	**82 (94)**	**131 (39)**	**<0.001*****	**24 (92)**	**131 (39)**	**<0.001*****
Biological factors									
Inflammation: plasma IL6 (pg/mL)	11.7 (38.3)	7.5 (13)	0.30	11.2 (41.4)	8.5 (13.8)	0.62	10.6 (13.7)	8.5 (13.8)	0.69
Inflammation: plasma LBP (pg/mL)	33.9 (13.1)	31.5 (14.4)	0.24	33.4 (12.7)	32.3 (14.3)	0.11	35.1 (14.4)	32.3 (14.3)	0.24
Inflammation: plasma sCD14 (pg/mL)	3.6 (2.3)	3.7 (1.9)	0.24	3.7 (2.5)	3.6 (1.8)	0.89	3.4 (1.6)	3.6 (1.8)	0.96
Total hippocampal volume (mm^3)	**6828.1 (862)**	**7222.7 (653.1)**	**0.004****	6809.3 (602.7)	7087.1 (803.2)	0.06#	7046.0 (1057.8)	7087.1 (803.2)	0.90

(Continues)

TABLE 1 (Continued)

Measures	Cognitive decline			Incident dementia: Alzheimer's disease[a]			Incident dementia: Vascular and other[b]		
	Cases (n = 209)	Controls (n = 209)	*Adjusted p*[c]	Cases (n = 87)	Controls (n = 305)	*Adjusted p*[d]	Cases (n = 26)	Controls (n = 305)	*Adjusted p*[d]
Medication									
Antihypertensive use[k]	**132 (61)**	**112 (54)**	**0.047***	49 (56)	197 (59)	0.51	20 (77)	197 (59)	0.08#
Diabetic medication use[l]	14 (7)	17 (8)	0.57	3 (3)	28 (8)	0.70	**5 (19)**	**28 (8)**	**0.04***
Lipid lowering medication use[m]	79 (38)	69 (33)	0.32	34 (39)	114 (34)	0.91	10 (39)	114 (34)	0.85
Psychotropics and antidepressants use[n]	70 (34)	57 (27)	0.17	**34 (39)**	**97 (29)**	**0.04***	9 (35)	97 (29)	0.64
Vitamin D supplements	10 (5)	8 (4)	0.63	1 (1)	18 (5)	0.09#	2 (8)	18 (5)	0.84
Lifestyle characteristics									
Regular physical exercise[o]	**45 (22)**	**70 (34)**	**0.04***	16 (18)	98 (29)	0.42	4 (15)	98 (29)	0.28
Alcohol use (per week)	13 (14.6)	14.6 (17.4)	0.43	10.5 (11.4)	14.7 (16.9)	0.29	14.6 (15.5)	14.7 (16.9)	0.83
Smoking status			0.55			0.47			0.59
Never	141 (68)	136 (65)	–	64 (74)	195 (64)	–	16 (62)	195 (64)	–
Former	58 (28)	64 (31)	–	19 (22)	95 (31)	–	8 (30)	95 (31)	–
Current	10 (5)	9 (4)	–	4 (5)	15 (5)	–	2 (8)	15 (5)	–
Technical factors									
Passage number	18 (1.9)	18 (1.8)	0.99	18 (1.9)	18 (1.9)	0.99	18 (1.8)	18 (1.9)	0.93
Serum batch	8.2 (3.9)	8.5 (4.2)	0.87	8.1 (4.2)	8.4 (6.7)	0.93	8.5 (4.3)	8.4 (6.7)	0.72
Batch number	2 (1.3)	2 (1.1)	0.99	2.4 (1.2)	2.1 (1.1)	0.92	2.3 (1.1)	2.1 (1.1)	0.81
In vitro readouts of the hippocampal neurogenic process									
Proliferation readouts									
DAPI-positive cells (cell number; n)	348.0 (84.8)	339.6 (81.7)	0.30	333.7 (82.9)	344.1 (83.4)	0.38	372.8 (80.6)	344.1 (83.4)	0.11
Nestin-positive cell density (%)	92.7 (3.5)	93.0 (3.2)	0.49	93.1 (3.2)	92.8 (3.4)	0.60	92.1 (3.4)	92.8 (3.4)	0.24
SOX2-positive cell density (%)	**92.7 (3.8)**	**93.6 (3.6)**	**0.03***	93.0 (4.1)	93.3 (3.8)	0.70	**90.8 (2.8)**	**93.3 (3.8)**	**0.01***
Nestin/SOX2-positive cell density (%)	87.2 (5.3)	88.0 (4.8)	0.13	87.6 (5.4)	87.8 (5.0)	0.80	**85.3 (4.8)**	**87.8 (5.0)**	**0.01***
Ki67-positive cell density (%)	81.1 (7.5)	81.3 (7.6)	0.71	81.4 (7.5)	81.3 (7.5)	0.98	79.8 (7.4)	81.3 (7.5)	0.30
CC3-positive cell density (%)	1.3 (1.0)	1.3 (1.0)	0.67	**1.1 (1.0)**	**1.3 (1.0)**	**0.03***	1.3 (0.87)	1.3 (1.0)	0.93
Ki67/CC3-positive cell density (%)	0.64 (0.6)	0.68 (0.6)	0.56	0.60 (0.6)	0.67 (0.6)	0.20	0.73 (0.9)	0.67 (0.6)	0.49
Differentiation readouts									
DAPI-positive cells (cell number; n)	375.4 (93.4)	382.5 (91.8)	0.43	368.0 (98.2)	382.2 (95.4)	0.53	354.6 (71.7)	382.2 (95.4)	0.71
Ki67-positive cell density (%)	38.0 (9.7)	37.7 (9.6)	0.74	37.3 (9.1)	38.0 (10.0)	0.45	39.0 (8.0)	38.0 (10.0)	0.79
DCX-positive cell density (%)	43.7 (8.2)	42.9 (8.4)	0.33	43.7 (7.8)	43.3 (8.4)	0.89	42.5 (8.3)	43.3 (8.4)	0.92
Ki67/DCX-positive cell density (%)	18.9 (6.6)	18.4 (6.2)	0.43	19.0 (6.6)	18.7 (6.4)	0.53	18.1 (5.7)	18.7 (6.4)	0.68

(Continues)

TABLE 1 (Continued)

Measures	Cognitive decline			Incident dementia: Alzheimer's disease[(a)]			Incident dementia: Vascular and other[(b)]		
	Cases (n = 209)	Controls (n = 209)	*Adjusted p*[(c)]	Cases (n = 87)	Controls (n = 305)	*Adjusted p*[(d)]	Cases (n = 26)	Controls (n = 305)	*Adjusted p*[(d)]
MAP2-positive cell density (%)	46.1 (12.0)	45.2 (12.1)	0.47	45.1 (11.9)	45.7 (12.0)	0.80	47.3 (13.2)	45.7 (12.0)	0.26
CC3-positive cell density (%)	**7.5 (3.0)**	**6.7 (3.0)**	**0.004****	7.7 (4.1)	7.0 (3.9)	0.70	6.8 (4.8)	7.0 (3.9)	0.51
MAP2/CC3-positive cell density (%)	4.5 (3.2)	4.0 (2.9)	0.10	4.7 (3.3)	4.2 (2.9)	0.90	4.1 (3.1)	4.2 (2.9)	0.4

Abbreviations: ApoE-ε4, allele ε4 for the apolipoprotein E gene; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; CVD, cardiovascular disease; DAPI, 4′,6-diamidino-2-phenylindole; SOX2, sex determining region Y (SRY)-box 2; CC3, cleaved caspase 3; DCX, doublecortin; MAP2, microtubule-associated protein 2; SD, standard deviation.

Values represent mean (SD) or N (%) of non-missing values. Characteristics (and associated values) in bold are covariates, all of which are controlled for in relevant models. # Also adjusted for in further analyses where relevant. .

(a) Alzheimer's Disease (AD) cases included all diagnoses of probable AD, possible AD, and mixed dementia.

(b) Vascular and other dementia included all diagnoses of vascular dementia, Parkinson dementia, Lewy body dementia, and frontotemporal dementia.

(c) Estimated using conditional logistic regression, conditioned on age, gender, and education to account for matched cases and controls.

(d) Estimated using logistic regressions controlling for age, gender, and education. False discovery rate correction was applied to control for multiple testing; *p* values represent adjusted *p* values.

(e) ApoE genotype was considered dichotomously: presence of at least one ε4 allele. (f) Blood pressure ≥ 140/90 mmHg or antihypertensive medication use.

(g) Glucose ≥ 7.2 mmol/L or antidiabetic medication use.

(h) Fasting plasma total cholesterol ≥ 6.2 mmol/L or lipid-lowering medication use.

(i) History of cardiovascular or cerebrovascular disease.

(j) Assessed using the Center for Epidemiological Studies-Depression scale (CES-D).[1,2] High depressive symptomology score ≥ 17 men, ≥23 for women or too depressed to answer.

(k) Includes all antihypertensive drugs, calcium channel blockers, diuretics, beta-blockers, and drugs acting on the renin-angiotensin system.

(l) Includes all antidiabetic drugs except insulin.

(m) Includes all statins, fibrates, or bile acid sequestrants.

(n) Includes all psycholeptics and psychoanaleptics—antidepressants, psychostimulants, and nootropics.

(o) Practice and intensity of physical exercise was assessed using a physical activity questionnaire for the elderly.[3] Regular exercise was classified as doing sport regularly or having at least one hour of leisure or household activity per day. Described in detail in.[4]

Dementia incidence: n = 113 participants developed dementia over the 12-year follow-up period (Median of follow-up = 9.1 years, range = 0.6–13.5) with an incidence of 22.5 cases per 1,000 person-years. Cellular readouts expressed as a percentage relative to neural (DAPI) cell number. Cell line: HPC0A07/03; Passage number: P15-21; Technical replicates: n = 3.

*$p < .05$; ** $p < .01$; *** $p < .001$.

References:

[1] Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas* 1977;1:385–401. https://doi.org/10.1177/014662167700100306.

[2] Lyness JM, Noel TK, Cox C, King DA, Conwell Y, Caine ED. Screening for Depression in Elderly Primary Care Patients: A Comparison of the Center for Epidemiologic Studies—Depression Scale and the Geriatric Depression Scale. *Arch Intern Med* 1997;157:449–54. https://doi.org/10.1001/archinte.1997.00440250107012.

[3] Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. *Med Sci Sports Exerc* 1991;23:974–9.

[4] Dupré C, Bongue B, Helmer C, Dartigues JF, Hupin D, Roche F, et al. Physical activity types and risk of dementia in community-dwelling older people: the Three-City cohort. *BMC Geriatr* 2020;20:132. https://doi.org/10.1186/s12877-020-01538-3.

as previously described.[18] Protocols were set by an experimenter blinded to caseness and parameters were kept constant across all experiments. For details on the protocols and parameters used, see the **Supplementary materials**.

2.9 | Statistical analysis

Data analyses were conducted using SPSS Statistics 26 and R software (version 3.6.3). We first studied the association between each of the proliferation and differentiation markers and caseness for CD and dementia using conditional logistic regressions and logistic regression models, respectively. Models were primarily adjusted for age, sex, and education; in case of association, further adjustment was performed, by including potential confounders that were associated with CD and/or dementia in the present population (those in bold font in Table 1). Additionally, to further explore the relationship between the neurogenic process and exercise, interactions were tested between each HN readout and physical exercise in our models, adjusting for age, sex, and education, and potential confounders as aforementioned.

TABLE 2 Associations between participant characteristics, exercise, nutritional data and altered *in vitro* neurogenesis readouts

Measures	SOX2-positive cells		CC3-positive cells during proliferation		CC3-positive cells during differentiation	
	Beta estimate (95% Confidence intervals)	***Adjusted p*[(a)]**	**Beta estimate (95% Confidence intervals)**	***Adjusted p*[(a)]**	**Beta estimate (95% Confidence intervals)**	***Adjusted p*[(a)]**
Sociodemographic characteristics						
Revenue	0.10 (-0.2; 0.7)	0.06[#]	-0.05 (-0.1; 0.05)	0.37	0.08 (-0.2; 0.7)	0.75
Health indicators						
BMI (kg m-^{2})	**-0.13 (-1.2; -0.1)**	**0.01***	-0.08 (-0.3; 0.06)	0.20	-0.02 (-0.8; 0.5)	0.71
Plasma total cholesterol (mmol L^{-1})	0.35 (-0.2; 3.1)	0.08[#]	-0.06 (-0.5; 0.4)	0.75	0.11 (-1.3; 2.3)	0.59
Plasma LDL cholesterol (mmol L^{-1})	**-0.38 (-3.5; -0.04)**	**0.04***	-0.01 (-0.5; 0.5)	0.94	-0.13 (-2.6; 1.3)	0.53
Plasma HDL cholesterol (mmol L^{-1})	0.02 (-1.2; 1.7)	0.75	-0.01 (-0.4; 0.4)	0.87	0.03 (-1.4; 1.9)	0.75
Plasma triglycerides (mmol L^{-1})	-0.02 (-0.7; 0.4)	0.64	0.04 (-0.1; 0.2)	0.42	**0.12 (0.1; 1.3)**	**0.02***
Plasma glucose (mmol L^{-1})	-0.03 (-0.4; 0.3)	0.59	0.01 (-0.1; 0.1)	0.80	-0.03 (-0.5; 0.2)	0.53
ApoE-ε4 carrier [(b)]	-0.05 (-1.4; 0.4)	0.28	0.02 (-0.2; 0.4)	0.67	-0.02 (-1.4; 1.1)	0.77
Medical factors						
Hypertension [(c)]	-0.04 (-1.2; 0.6)	0.46	0.001 (-0.3; 0.3)	0.99	0.02 (-1.0; 0.8)	0.79
Diabetes [(d)]	0.03 (-0.9; 1.7)	0.53	-0.06 (-0.6; 0.2)	0.26	0.06 (-0.9; 2.6)	0.34
Hypercholesterolemia [(e)]	-0.05 (-1.2; 0.4)	0.30	-0.05 (-0.3; 0.1)	0.38	0.05 (-0.6; 1.3)	0.44
Antecedents of CVD [(f)]	0.39 (-0.5; 1.1)	0.44	0.03 (-0.2; 0.3)	0.54	0.09 (-0.2; 1.8)	0.12
Neuro/psychological factors						
Depression [(g)]	0.03 (-0.6; 1.0)	0.58	-0.08 (-0.4; 0.05)	0.13	0.04 (-0.5; 1.3)	0.37
Biological factors						
Inflammation: plasma IL6 (pg/mL)	0.04 (-0.1; 0.1)	0.79	-0.13 (-0.03; 0.01)	0.36	-0.23 (-0.1; 0.1)	0.11
Inflammation: plasma LBP (pg/mL)	0.03 (-0.1; 0.1)	0.86	-0.20 (-0.05; 0.01)	0.20	0.09 (-0.1; 0.1)	0.56
Inflammation: plasma sCD14 (pg/mL)	0.17 (-0.2; 0.6)	0.29	-0.20 (-0.2; 0.05)	0.23	-0.02 (-0.4; 0.4)	0.89
Total hippocampal volume (mm^3)	-0.03 (-0.01; 0.02)	0.83	0.25 (0.001; 0.002)	0.10	-0.16 (-0.003; 0.001)	0.29
Medication						
Antihypertensive use[(h)]	0.004 (-0.7; 0.8)	0.94	0.02 (-0.2; 0.2)	0.76	**0.12 (0.1; 1.8)**	**0.02***
Diabetic medication use [(i)]	-0.03 (-1.9; 0.9)	0.49	0.04 (-0.2; 0.5)	0.38	-0.02 (-1.9; 1.1)	0.64
Lipid lowering medication use [(j)]	-0.002 (-1.1; 1.1)	0.97	-0.04 (-0.3; 0.1)	0.43	0.06 (-0.3; 1.4)	0.20
Psychotropics and antidepressants use [(k)]	-0.03 (-1.1; 0.5)	0.50	0.01 (-0.2; 0.2)	0.81	0.04 (-0.6; 1.2)	0.48
Vitamin D supplements	-0.03 (-2.4; 1.2)	0.51	-0.07 (-0.8; 0.1)	0.17	-0.02 (-2.5; 1.5)	0.63
Lifestyle characteristics						
Regular physical exercise [(l)]	0.04 (-0.5; 1.2)	0.46	-0.02 (-0.3; 0.2)	0.78	**-0.16 (-2.4; -0.3)**	**0.009****
Alcohol use (per week)	-0.02 (-0.03; 0.02)	0.70	-0.003 (-0.01; 0.01)	0.95	-0.10 (-0.05; 0.007)	0.13
Smoking status	-0.05 (-1.1; 0.4)	0.34	-0.04 (-0.3; 0.1)	0.45	-0.01 (-0.9; 0.8)	0.88
Energy intake (Kcal/day)	0.06 (0.001; 0.01)	0.25	-0.05 (0.001; 0.002)	0.51	-0.05 (-0.002; 0.001)	0.46

(Continues)

TABLE 2 (Continued)

Measures	SOX2-positive cells		CC3-positive cells during proliferation		CC3-positive cells during differentiation	
	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]
Mediterranean diet (score) [(m)]	-0.8 (-0.9; 0.1)	0.15	0.14 (-0.1; 0.3)	0.40	0.23 (-0.5; 2.9)	0.17
Technical factors						
Passage number	-0.09 (-0.4; 0.04)	0.11	-0.04 (-0.1; 0.04)	0.55	-0.04 (-0.3; 0.1)	0.48
Serum batch	0.02 (0.03; 0.2)	0.11	-0.08 (-0.06; 0.01)	0.19	0.05 (-0.1; 0.1)	0.55
Batch number	0.02 (-0.3; 0.1)	0.21	-0.02 (-0.1; 0.06)	0.64	-0.06 (-0.2; 0.1)	0.39
Nutritional data						
***Fatty acid biomarkers (% of total fats in plasma)*:**						
Myristic acid	-0.06 (81; 107.6)	0.50	-0.06 (-0.6; 0.3)	0.49	0.11 (-0.4; 2.6)	0.14
Palmitic acid	-0.02 (-0.3; 0.2)	0.90	-0.17 (-0.1; 0.03)	0.28	-0.27 (-0.5; 0.03)	0.09[#]
Stearic acid	0.04 (-0.03; 0.3)	0.79	0.11 (-0.04; 0.1)	0.41	0.20 (-0.02; 0.5)	0.07[#]
Total saturated fats (myristic + palmitic + stearic acid)	0.14 (-0.3; 0.2)	0.15	-0.18 (-0.05; 0.003)	0.08[#]	-0.04 (-0.1; 0.1)	0.67
Palmitoleic acid	-0.8 (-1.2; 0.49)	0.42	0.09 (-0.1; 0.3)	0.31	0.03 (-0.5; 0.7)	0.70
Oleic acid	0.03 (-0.2; 0.3)	0.78	0.13 (-0.02; 0.1)	0.22	0.14 (-0.02; 0.3)	0.09[#]
Total monosaturated fats (palmitoleic + oleic acid)	-0.1 (0.3; 0.1)	0.30	0.03 (-0.03; 0.05)	0.70	-0.09 (-0.3; 0.1)	0.80
Alpha linoleic acid (ALA)	-0.02 (-2.7; 2.0)	0.76	0.06 (-0.3; 0.8)	0.42	0.01 (-2.1; 2.4)	0.90
Eicosapentaenoic acid (EPA)	-0.1 (-1.8;0.4)	0.24	0.01 (-0.2; 0.3)	0.93	-0.03 (-1.1; 0.7)	0.62
Docosapentaenoic acid (DPA)	-0.03 (-2.5; 1.7)	0.70	-0.04 (-0.6; 0.4)	0.61	-0.03 (-2.6; 1.5)	0.60
Docosahexaenoic acid (DHA)	0.04 (-0.7; 1.1)	0.68	-0.06 (-0.3; 0.1)	0.48	0.12 (-0.1; 1.4)	0.10
Total long-chain omega-3 fatty acids (ALA+EPA+DPA+DHA)	0.17 (-2.0; 2.3)	0.87	-0.003 (-0.5; 0.5)	0.99	0.09 (-1.9; 2.5)	0.80
Linoleic acid (LA)	-0.03 (-0.2; 0.2)	0.98	0.04 (-0.04; 0.05)	0.76	**0.32 (0.1; 0.4)**	**0.001****
γ-linolenic acid (GLA)	0.09 (-1.7; 5.9)	0.27	0.07 (-0.3; 1.1)	0.25	0.03 (-2.5; 4.0)	0.64
Arachidonic acid (AA)	-0.03 (-0.5; 0.3)	0.80	-0.09 (-0.1; 0.02)	0.14	0.17 (-0.3; 0.4)	0.83
Total omega-6 fatty acids (LA+GLA+AA)	0.16 (-0.02; 0.2)	0.10	-0.14 (-0.6; 0.02)	0.29	0.07 (-0.04; 0.2)	0.16
Total polyunsaturated fats	0.21 (-0.2; 0.2)	0.84	**-0.23 (-0.06; -0.003)**	**0.03***	-0.04 (-0.2; 0.2)	0.78
Vitamin D and Malnutrition biomarkers:						
Plasma 25 (OH) Vitamin D (ng/ml)	-0.02 (-13.2; 10.6)	0.83	**0.14 (0.001; 0.03)**	**0.03***	0.03 (-0.05; 0.1)	0.87
Plasma prealbumin (g/L)	-0.03 (-0.1; 0.04)	0.68	**0.17 (0.8; 5.4)**	**0.01***	**-0.16 (-23.2; -2.8)**	**0.01***
Carotenoid biomarkers:						
α-carotene (μg/L)	-0.12 (-0.2; 0.004)	0.25	-0.01 (-0.003; 0.003)	0.93	0.07 (-0.01; 0.02)	0.55
β -carotene (μg/L)	0.12 (-0.2; 0.005)	0.30	0.11 (-0.001; 0.001)	0.38	-0.12 (-0.01; 0.002)	0.31
lutein (μg/L)	-0.04 (-0.01; 0.006)	0.69	**-0.22 (-0.005; -0.3)**	**0.04***	0.03 (-0.01; 0.001)	0.78

(Continues)

TABLE 2 (Continued)

Measures	SOX2-positive cells		CC3-positive cells during proliferation		CC3-positive cells during differentiation	
	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]
lycopene (μg/L)	-0.05 (-0.01; 0.002)	0.43	-0.03 (-0.001; 0.001)	0.67	0.06 (-0.002; 0.01)	0.40
zeaxanthin (μg/L)	0.05 (-0.02; 0.04)	0.61	0.08 (-0.005; 0.01)	0.46	-0.09 (-0.05; 0.02)	0.38
β-cryptoxanthin (μg/L)	**0.13 (< 0.001; 0.008)**	**0.01***	-0.03 (-0.002; 0.001)	0.72	-0.10 (-0.01; 0.001)	0.17
α-tocopherol (mg/L)	0.03 (-0.1; 0.2)	0.67	0.08 (-0.02; 0.07)	0.33	0.12 (-0.03; 0.3)	0.11
γ-tocopherol (mg/L)	-0.12 (-2.2; 0.3)	0.15	-0.08 (-0.6; 0.2)	0.26	0.04 (-1.0; 1.7)	0.58
Retinol (μg/L)	0.06 (-0.002; 0.005)	0.40	-0.04 (-0.001; 0.001)	0.64	-0.11 (-0.01; 0.001)	0.11
Metabolites(n):						
M497_23830689T10_1167019511755 identified as Atractyligenin glucuronide	-0.10 (-0.001; 0.001)	0.62	0.18 (0.001; 0.002)	0.27	-0.07 (-0.001; 0.001)	0.69
M144_10186339T0_96749736196272 identified as Proline betaine	0.03 (0.001; 0.002)	0.86	0.04 (0.001; 0.002)	0.77	0.21 (0.001; 0.002)	0.17
M195_08767297T8_09546229270978 identified as Caffeine	0.07 (0.001; 0.002)	0.70	0.17 (0.001; 0.002)	0.35	-0.01 (0.001; 0.002)	0.95
M251_12784054T13_2571318403737 identified as CMPFP	-0.08 (0.001; 0.002)	0.64	-0.21 (0.001; 0.002)	0.21	-0.09 (0.001; 0.002)	0.60
M129_06580978T0_904033275275335	0.23 (0.001; 0.002)	0.23	**-0.37 (0.001; 0.002)**	**0.04***	**0.12 (0.001; 0.002)**	**0.03***
M160_13318885T0_97439997580839	-0.14 (0.001; 0.002)	0.50	-0.17 (0.001; 0.002)	0.39	0.05 (0.001; 0.002)	0.80
M271_20560881T12_9976137225527	-0.15 (-0.002; 0.001)	0.33	-0.23 (0.001; 0.002)	0.15	-0.20 (-0.002; 0.001)	0.22
M197_12846996T7_95463273162836 identified as Cylco(pro-val)	0.15 (-0.002; 0.001)	0.43	0.22 (0.0001; 0.002)	0.24	-0.11 (-0.002; 0.001)	0.56
M372_31086618T13_1253091214364 identified as Myristoylcarnitine	-0.11 (-0.002; 0.001)	0.66	0.20 (0.001; 0.002)	0.41	0.10 (-0.001; 0.002)	0.69
M626_35364866T11_6944007396207 identified as Glycodeoxycholic acid-3-glucuronide	-0.03 (-0.001; 0.001)	0.86	-0.03 (0.001; 0.002)	0.86	**-0.31 (-0.001; 0.001)**	**0.04***
M383_11602175T0_87755517430863 identified as Glucose	0.09 (0.001; 0.002)	0.50	0.06 (0.001; 0.002)	0.63	0.10 (0.001; 0.002)	0.45
M114_06608452T0_88400356559869 identified as Creatinine	0.04 (0.001; 0.002)	0.86	-0.06 (0.001; 0.002)	0.78	0.22 (0.001; 0.002)	0.29

(Continues)

TABLE 2 (Continued)

Measures	SOX2-positive cells		CC3-positive cells during proliferation		CC3-positive cells during differentiation	
	Beta estimate (95% Confidence intervals)	***Adjusted p*$^{(a)}$**	**Beta estimate (95% Confidence intervals)**	***Adjusted p*$^{(a)}$**	**Beta estimate (95% Confidence intervals)**	***Adjusted p*$^{(a)}$**
M189_15976101T0_79895784276683 identified as N-trimethyl-Lysine	0.08 (-0.001; 0.001)	0.64	0.13 (0.001; 0.002)	0.49	**0.26 (0.001; 0.002)**	**0.04***
M159_02763805T1_15990298079185	0.25 (0.001; 0.002)	0.10	0.07 (0.001; 0.002)	0.64	-0.11 (-0.001; 0.001)	0.46
M211_14411442T8_78455609427413 identified as Cyclo(leucyl-prolyl)	-0.13 (-0.002; 0.001)	0.55	-0.08 (0.001; 0.002)	0.69	-0.04 (-0.002; 0.001)	0.84
M287_625696T14_0906860659992 identified as lysoPC (18:3)	0.02 (-0.002; 0.002)	0.89	-0.06 (-0.001; 0.001)	0.70	**-0.23 (-0.002; -0.001)**	**< 0.001*****
M363_21662646T10_8052397329281 identified as Cortisol	-0.06 (-0.001; 0.001)	0.68	-0.16 (0.001; 0.002)	0.27	-0.12 (-0.001; 0.001)	0.42
M330_26397929T11_5819601029136 identified as Undecanoylcarnitine /4,8 dimethylnonanoylcarnitine	0.15 (-0.001; 0.001)	0.48	-0.22 (0.001; 0.002)	0.30	-0.14 (-0.001; 0.001)	0.51
M245_07686764T1_15630605014072	-0.01 (-0.003; 0.003)	0.95	-0.06 (-0.001; 0.001)	0.76	-0.04 (-0.003; 0.003)	0.82
M256_6796231T14_2723319681386	-0.11 (-0.003; 0.002)	0.54	-0.21 (-0.001; 0.001)	0.23	-0.15 (-0.004; 0.002)	0.04
M175_11895877T0_81149358234335 identified as Arginine	-0.12 (0.001; 0.002)	0.42	0.03 (0.001; 0.002)	0.85	0.05 (0.001; 0.002)	0.75
M344_27959058T12_2384972676736 identified as Lauroylcarnitine	0.07 (0.001; 0.002)	0.78	0.02 (0.001; 0.002)	0.95	-0.09 (-0.001; 0.001)	0.72
***Lipids (%pmol per total lipid)(o)*:**						
PCO32:0(16:0/16:0)	**-0.13 (-2.5; -0.1)**	**0.03***	-0.004 (-0.5; 0.4)	0.96	-0.04 (-2.3; 1.4)	0.62
PCO34:1(16:1/18:0)	**0.11 (0.4; 11.1)**	**0.045***	-0.05 (-2.5; 1.1)	0.45	-0.03 (-9.1; 5.6)	0.64
PE38:5(18:1/20:4)	0.01 (-0.7; 0.8)	0.89	0.08 (-0.1; 0.3)	0.17	**-0.13 (-1.7; -0.05)**	**0.03***
PEO34:3(16:1/18:2)	0.11 (-0.1; 1.6)	0.11	-0.12 (-0.4; 0.1)	0.06#	0.01 (-0.8; 1.0)	0.83

(Continues)

Next, for each marker of the hippocampal neurogenic process that we found to have a significant linear association with CD and/or dementia, we explored associations with nutrient biomarkers and previously identified blood metabolites and lipids using linear regression models initially adjusted for age, sex, and education. Further adjustment was performed including potential confounders associated with the HN and nutrient-related data (those in bold font in Table 2).

To determine the potential mediating role of HN outcomes in the relationship between diet/exercise and CD/dementia, mediation analyses were conducted using the causal step method[24] and based on recommendations by MacKinnon et al.[25] Models were adjusted for age, sex, and education, and for relevant potential confounders (as above).

Finally, we explored whether neurite morphology was associated with CD/dementia and used principal component analysis to combine

TABLE 2 (Continued)

Measures	SOX2-positive cells		CC3-positive cells during proliferation		CC3-positive cells during differentiation	
	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]	Beta estimate (95% Confidence intervals)	*Adjusted p*[(a)]
SM40:2,2	-0.02 (-0.2; 0.1)	0.77	-0.03 (-0.05; 0.03)	0.66	-0.01 (-0.2; 0.1)	0.91
TAG50:5	-0.06 (-0.4; 0.2)	0.35	-0.04 (-0.1; 0.1)	0.55	0.10 (-0.05; 0.6)	0.10

Abbreviations: ApoE-ε4, allele ε4 for the apolipoprotein E gene; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; CVD, cardiovascular disease; SOX2, sex determining region Y (SRY)-box 2; CC3, cleaved caspase 3.
Participant characteristics and nutritional related data in bold are covariates, all of which are controlled for in relevant models. # Also adjusted for in further analyses where relevant.
(a) Estimated using linear regressions controlling for age, gender, and education. False discovery rate correction was applied to account for multiple testing; *p* values represent adjusted *p* values.
(b) ApoE genotype was considered dichotomously: presence of at least one ε4 allele.
(c) Blood pressure ≥ 140/90 mmHg or antihypertensive medication use.
(d) Glucose ≥ 7.2 mmol/L or antidiabetic medication use.
(e) Fasting plasma total cholesterol ≥ 6.2 mmol/L or lipid-lowering medication use.
(f) History of cardiovascular or cerebrovascular disease.
(g) Assessed using the Center for Epidemiological Studies-Depression scale (CES-D).[1,2] High depressive symptomology score ≥ 17 men, ≥23 for women or too depressed to answer.
(h) Includes all antihypertensive drugs, calcium channel blockers, diuretics, beta-blockers, and drugs acting on the renin-angiotensin system.
(i) Includes all antidiabetic drugs except insulin.
(j) Includes all statins, fibrates, or bile acid sequestrants.
(k) Includes all psycholeptics and psychoanaleptics—antidepressants, psychostimulants, and nootropics.
(l) Practice and intensity of physical exercise was assessed using a physical activity questionnaire for the elderly.[3] Regular exercise was classified as doing sport regularly or having at least one hour of leisure or household activity per day. Described in detail in.[4]
(m) Derived from a food frequency questionnaire (FFQ) and a 24-hour dietary recall. A Mediterranean diet score was generated by adding the scores for each food group considered to be part of the Mediterranean diet.[5]
(n) Metabolites we previously identified as being associated with cognitive decline in our sample.[6]
(o) Lipids we previously identified (and validated in an independent cohort) as being associated with cognitive decline in our sample.[7]
Cell line: HPC0A07/03; Passage number: P15-21; Technical replicates: n = 3 .
* $p < .05$; ** $p < .01$; *** $p < .001$.

References:
[1] Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Appl Psychol Meas 1977;1:385–401. https://doi.org/10.1177/014662167700100306.
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[3] Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. Med Sci Sports Exerc 1991;23:974–9.
[4] Dupré C, Bongue B, Helmer C, Dartigues JF, Hupin D, Roche F, et al. Physical activity types and risk of dementia in community-dwelling older people: the Three-City cohort. BMC Geriatr 2020;20:132. https://doi.org/10.1186/s12877-020-01538-3.
[5] Féart C, Samieri C, Barberger-Gateau P. Mediterranean diet and cognitive function in older adults. Curr Opin Clin Nutr Metab Care 2010;13:14–8. https://doi.org/10.1097/MCO.0b013e3283331fe4.
[6] Low DY, Lefèvre-Arbogast S, González-Domínguez R, Urpi-Sarda M, Micheau P, Petera M, et al. Diet-Related Metabolites Associated with Cognitive Decline Revealed by Untargeted Metabolomics in a Prospective Cohort. Mol Nutr Food Res 2019;63:1–10. https://doi.org/10.1002/mnfr.201900177.
[7] Lefèvre-Arbogast S, Hejblum BP, Helmer C, Klose C, Manach C, Low DY, et al. Early signature in the blood lipidome associated with subsequent cognitive decline in the elderly: A case-control analysis nested within the Three-City cohort study. EBioMedicine 2021;64:103216. https://doi.org/10.1016/j.ebiom.2021.103216.

HN readouts into a neurogenic profile that we investigated in relation to CD/dementia (**Supplementary materials**).

False discovery rate correction[26] was applied to account for multiple testing throughout and all models were bootstrap enhanced to obtain robust estimates of standard errors. For mediation models, bootstrapping was used to estimate the percentage of the association between diet/exercise and CD/dementia mediated by HN outcomes.

3 | RESULTS

Participants were, on average, 76 years-old at baseline and 66% were female (Table 1). The characteristics of our sample by CD and dementia status are detailed in Table 1. Participant cognition was assessed for an average of 8.5 years and the average age of dementia onset was 85 years.

We first determined the association between individual markers of the neurogenic process and subsequent CD and incident dementia. As indicated in Table 1, levels of %CC3-positive cell density (during proliferation and differentiation) and %SOX2 were all significantly associated with CD and/or dementia.

3.1 | Cell death during differentiation is associated with future CD while the association of hippocampal stem cell integrity with CD is modified by regular exercise

As shown in Figure 2A-B, increased baseline levels of %CC3-positive cells during differentiation were significantly associated with CD across a 12-year period in a fully adjusted model ($p = .006$; Model 1A). Moreover, we found a significant interaction between baseline levels of %SOX2-positive cells and physical exercise on CD ($p = .049$), such that reduced baseline levels of %SOX2 were significantly associated with CD but only in participants who did not regularly exercise ($p = .04$; Figure 2A: Model 1B; Figure 2C).

3.2 | The association between HN and incident dementia depends on specific aspects of the neurogenic process and the type of dementia

Interestingly, no significant difference in any baseline neurogenesis readout was found between cases of dementia and controls. However, when we conducted separate models for each dementia subtype (i.e., AD and vascular dementia/other), we found that particular neurogenic markers were associated with specific dementia subtypes (Figure 3). Specifically, we found that decreased levels of baseline %CC3-positive cells during proliferation were associated with an increased risk of AD ($p = .04$; Figure 3A: Model 4; Figure 3B), whereas reduced baseline %SOX2-positive cell levels were significantly associated with an increased risk of vascular dementia/other ($p = .02$; Figure 3A: Model 5; Figure 3C).

Thus far we have shown that only specific HN readouts are associated with CD and incident dementia up to 12 years prior to caseness, and that physical exercise can modify the association of %SOX2 with CD. No differences in any other individual neurogenesis readout, that is, densities of %Nestin-, %Ki67-, %DCX- or %MAP2-positive cells, was observed between groups (Table 1). Moreover, we found no difference in the neurite morphology of %DCX- and %MAP2-positive cells between cases and controls (**Supplementary materials** and Table S1). Additionally, principal component analyses revealed no differences in the overall neurogenic profiles between groups at baseline (**Supplementary materials**).

Next, starting with our HN-associated readouts relevant to CD (i.e., %CC3 during differentiation and %SOX2), we subsequently assessed their relationship with exercise and our baseline dietary variables (Table 2).

3.3 | Global (mal)nutrition status and exercise are associated with cell death during differentiation whereas cholesterol and carotenoid levels are associated with hippocampal progenitor cell integrity among non-exercisers

Both exercise and diet were found to be associated with levels of %CC3 (during differentiation). Specifically, we found that reduced levels of plasma prealbumin ($p = .01$) and increased levels of an unidentified metabolite (i.e., M129_06580978T0_904033275275335; $p = .04$) were both associated with increased levels of %CC3 (during differentiation) in a fully adjusted model (Figure 2D-E). Furthermore, we found that a lack of regular physical exercise was also independently associated with increased levels of %CC3 (during differentiation) ($p = .001$;Figure 2D).

Since %SOX2 was modified by exercise in the context of CD in our sample, we subsequently investigated the association between this particular HN marker and the nutritional factors, as stratified by exercise. Specifically, we found that increased plasma levels of low density lipoprotein (LDL) cholesterol ($p = .004$) and reduced plasma levels of ß-cryptoxanthin ($p = .002$) were both associated with reduced levels of baseline %SOX2 among non-regular exercisers only in a fully adjusted model (Figure 2F-G).

Finally, we focused on our two HN markers associated with AD and vascular dementia/other (i.e., %CC3 during proliferation and %SOX2 [in the sample as a whole]). Here, we found that diet, but not exercise, was associated with these two neurogenesis readouts (Table 2).

3.4 | Vitamin D levels and global (mal)nutrition, but not exercise, are associated with cell death during proliferation, whereas carotenoid levels and altered lipid metabolism are associated with hippocampal progenitor cell integrity

As shown in Figure 3D-E, reduced plasma levels of vitamin D ($p = .04$) and prealbumin ($p = .009$) were both independently associated with reduced baseline levels of %CC3 (during proliferation). Moreover, increased levels of the same unidentified metabolite found to be associated with %CC3 (during differentiation) was also associated with cell death during proliferation ($p = .03$).

By contrast, as shown in Figure 3F-G, reduced levels of phosphatidylcholine ether (PCO)34:1(16:1/18:0) ($p = .04$) and plasma ß-cryptoxanthin ($p = .001$) were both independently associated with reduced levels of %SOX2—the latter of which was replicated among non-exercisers with CD in our sample. Furthermore, we found that increased levels of PCO32:0(16:0/16:0) were also associated with reduced %SOX2 ($p = .002$).

For results on the associations between these nutritional factors and CD/dementia, see the **Supplementary materials**.

Association between the hippocampal neurogenic process and cognitive decline across the 12-year follow-up

(A)

Model 1A: Association between baseline levels of cell death during differentiation (ie, %CC3-positive cells) and hippocampal progenitor cell integrity (ie, %SOX2-positive cells) and odds of future cognitive decline over the 12-year follow-up

Variable	OR	Standard Error	95% Confidence levels	*P*
%CC3-positive cells	1.1	0.03	1.01; 1.11	0.006**
%SOX2-positive cells	0.96	0.03	0.91; 1.02	0.20
%SOX2-positive cells*regular exercise	0.94	0.02	0.87; 0.98	0.049*
APOE4 carrier	2.7	0.31	1.61; 4.61	0.001**

Model 1B: Association between baseline levels of hippocampal progenitor cell integrity (i.e., %SOX2-positive cells) and odds of future cognitive decline over the 12-year follow-up stratified by regular exercise

Regular exercisers

Variable	OR	Standard Error	95% Confidence levels	*P*
%SOX2-positive cells	1.07	0.14	0.81; 1.42	0.62
APOE4 carrier	0.30	1.22	0.03; 3.22	0.32
Total hippocampal volume (mm^3)	0.99	0.001	0.995; 0.999	0.01*

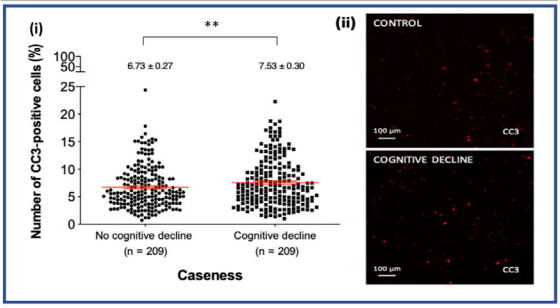
Non-regular exercisers

Variable	OR	Standard Error	95% Confidence levels	*P*
%SOX2-positive cells	0.93	0.04	0.87; 0.99	0.04*
APOE4 carrier	1.40	0.44	4.07; 1.73	0.01*

Conditional logistic regression models

Models conditioned for age, gender, education (ie, matching variables) and adjusted for diabetes, incident depression, incident dementia, total hippocampal volume, antihypertensive medication use and regular physical exercise (Model 1A only). * $P < .05$; ** $P < .01$

(B) Increased baseline levels of cell death during differentiation are associated with future cognitive decline



(C) Reduced baseline levels of hippocampal progenitor cell integrity are associated with future cognitive decline in non-regular exercisers

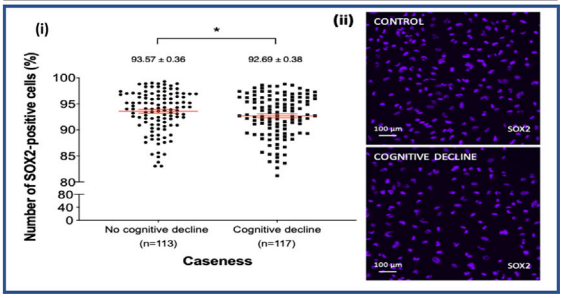


FIGURE 2 **Relationship between the hippocampal neurogenic process, future cognitive decline, exercise, and nutritional measures. (A)** Associations between baseline cell death during differentiation, hippocampal progenitor cell integrity, and future cognitive decline. Model 1A: Association between the hippocampal neurogenic process on cognitive decline using conditional logistic regression. Baseline levels of cleaved caspase-3 (CC3)-positive cell density during differentiation (odds ratio [OR] 1.1 [95% confidence interval (CI) 1.01; 1.11]; $p = .006$) and the apolipoprotein E4 gene (APOE4) status (OR 2.7 [95% CI 1.61; 4.61]; $p = .001$) were both independently associated with future cognitive decline across the 12-year follow-up period in a fully adjusted model. However, although baseline levels of sex determining region Y (SRY)-box 2 (SOX2)-positive cell density were no longer significantly associated with future cognitive decline in a fully adjusted model (OR 0.96 [95% CI 0.91; 1.02]; $p = .20$), there was a significant interaction between regular exercise and %SOX2 (OR 0.94 [95% CI 0.87; 0.98]; $p = .049$). Model 1B: Further analyses, stratifying the sample by exercise, revealed that %SOX2 levels were in fact modified by exercise such that reduced %SOX2-positive cells were associated with future cognitive decline in cases that did not regularly exercise (OR 0.93 [95% CI 0.87; 0.99]; $p = .04$) but not for those that did exercise regularly (OR 1.07 [95% CI 0.81; 1.42]; $p = .62$). **(B) (i)** Baseline levels of %CC3-positive cells during differentiation stratified by caseness for future cognitive decline. Cases, that is, those with an accelerated rate of cognitive decline across the 12-year follow-up, had significantly higher levels of %CC3-positive cells during differentiation (M = 7.53 [0.30] vs M = 6.73 [0.27]). Cellular readout expressed as a percentage relative to neural cell number. Participants matched for age, gender, and education. Cell line: *HPC0A07/03*; Passage number: P15-21; Technical replicates: n = 3; Data represents mean ± SEM. ** $p < .01$. **(ii)** Representative immunostaining demonstrating %CC3-positive cell density during differentiation for representative case and control. Images taken at x10 objective; scale bar represents 100 μm. **(C) (i)** Baseline levels of %SOX2-positive cells stratified by caseness for cognitive decline in non-regular exercisers only. Cases had significantly lower levels of baseline %SOX2-positive cells (M = 92.69 [0.38] vs M = 93.57 [0.36]). Cellular readout expressed as a percentage relative to neural cell number. Sample = non-regular exercisers only. Participants matched for age, gender, and education. Cell line: *HPC0A07/03*; Passage number: P15-21; Technical replicates: n = 3; Data represents mean ± SEM. * $p < .05$. **(ii)** Representative immunostaining demonstrating %SOX2-positive cell density for representative case and control. Images taken at x10 objective; scale bar represents 100 μm. **(D)** Associations between diet, exercise, and the altered hippocampal neurogenesis readouts. Model 2: Association between baseline prealbumin, an unidentified metabolite, and regular exercise on cell death during differentiation using linear regression. Baseline levels of prealbumin (ß = −0.25 [95% CI −37.6; −4.4] 8.6; $p = .01$), an unidentified metabolite, M129, (ß = 0.20 [95% CI 0.001; 0.0015] 0.06; $p = .04$), and regular exercise (ß = −0.30 [95% CI −4.1; −1.1] 0.75; $p = .001$) were all significantly associated with baseline %CC3-positive cell levels during differentiation in a fully adjusted model. **(E) (i)** Scatterplot showing negative relationship between prealbumin levels and %CC3 levels at baseline (blue). **(ii)** Scatterplot showing positive relationship between the metabolite, M129, and %CC3 levels at baseline (green). **(F)** Model 3: Association between baseline nutritional levels and baseline levels of hippocampal progenitor cell integrity using linear regression in non-regular exercisers. Plasma levels of low density lipoprotein (LDL) cholesterol (ß = −1.5 [95% CI −2.5; −0.48] 0.007; $p = .004$) and ß-cryptoxanthin (ß = 0.03 [95% CI 0.004; 0.01] 0.0001; $p = .002$) were both associated with baseline %SOX2-positive cell levels in a fully adjusted model in those that did not regularly exercise. **(G) (i)** Scatterplot showing negative relationship between baseline plasma levels of LDL cholesterol and %SOX2 levels in non-exercisers (blue). **(ii)** Scatterplot showing positive relationship between baseline plasma levels of ß-cryptoxanthin and %SOX2 levels in non-exercisers (green). **(H)** There was a significant indirect effect of baseline plasma prealbumin levels on future cognitive decline through baseline %CC3-positive cell levels during differentiation (*ab* = −0.86 [−2.21; −0.04]). The mediator (i.e., %CC3 levels) accounted for 25% of the total effect ($P_M = .25$). **(I)** There was a significant indirect effect of baseline regular exercise status on future cognitive decline through baseline %CC3 levels during differentiation (*ab* = −0.08 [−0.21; −0.02]). The mediator (i.e., %CC3 levels) accounted for 18% of the total effect ($P_M = .18$).

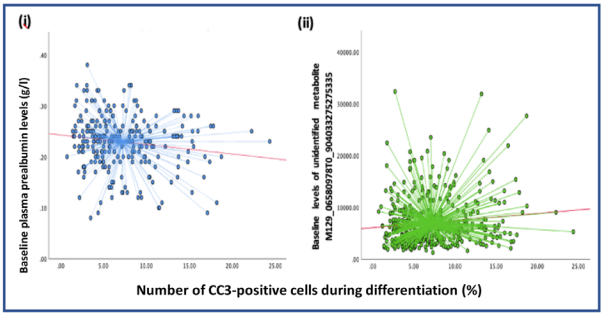
Association between diet, exercise, cell death during differentiation and hippocampal progenitor cell integrity (in non-regular exercisers)

(D)
Model 2: Association between baseline prealbumin levels, an unidentified metabolite and regular exercise, and baseline levels of cell death during differentiation (ie, %CC3-positive cells)

Variable	Standardised estimate (a)	Standard Error	95% Confidence levels	P
Plasma prealbumin (g/l)	−0.25	8.60	−37.64; −4.48	0.01*
Unidentified metabolite M129_06580978T0_904033275275335	0.20	0.06	0.001; 0.0015	0.04*
Regular physical exercise	−0.30	0.75	−4.11; −1.14	0.001**

Linear regression model
Model adjusted for age, gender, education, plasma triglyceride levels, antihypertensive medication use, plasma linoleic acid (% total fats), stearic acid (% total fats), palmitic acid (% total fats), oleic acid (% total fats), metabolite glycodeoxycholic acid-3-glucuronide, metabolite N-trimethyl-Lysine, metabolite lysophosphatidylcholine (18:3), lipid phosphatidylethanolamine (PE)38:5(18:1/20:4.
(a) Increments are the estimates expressed as a 1 standard deviation-increase. * $P < .05$; ** $P < .01$.

(E) Reduced baseline prealbumin levels and increased levels of an unidentified metabolite are associated with increased baseline levels of cell death during differentiation

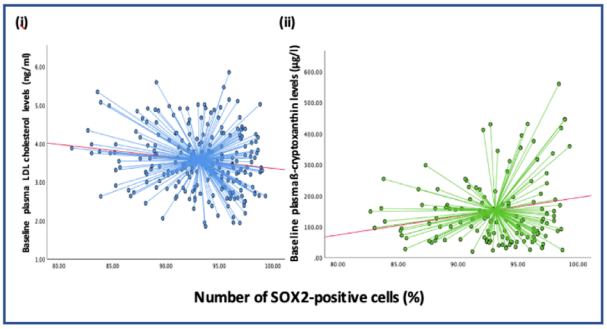


(F)
Model 3: Association between baseline plasma LDL cholesterol levels, plasma ß-cryptoxanthin levels and baseline levels of hippocampal progenitor cell integrity (ie, %SOX2-positive cells) in participants that do not regularly exercise

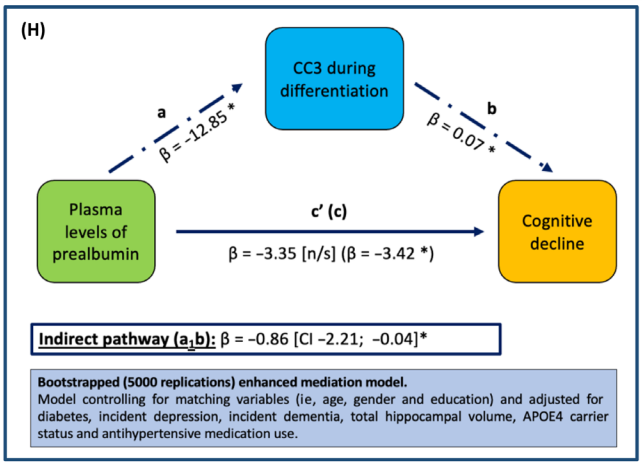
Variable	Standardised estimate (a)	Standard Error	95% Confidence levels	p
Baseline plasma LDL cholesterol levels (mmol L^{-1})	−0.51	0.007	−2.5; −0.48	0.004**
Baseline plasma ß-cryptoxanthin levels (µg/l)	0.03	0.0001	0.004; 0.01	0.002**

Linear regression model
Model adjusted for age, gender, education, socioeconomic status, and body mass index.
(a) Increments are the estimates expressed as a 1 standard deviation-increase. ** $P < .01$

(G) Reduced baseline plasma levels of LDL cholesterol and increased baseline plasma levels of ß-cryptoxanthin are associated with hippocampal progenitor cell integrity in non-regular exercisers



Association between diet, exercise, cell death during differentiation and future cognitive decline: Cell death mediates the relationship between diet/exercise and cognitive decline



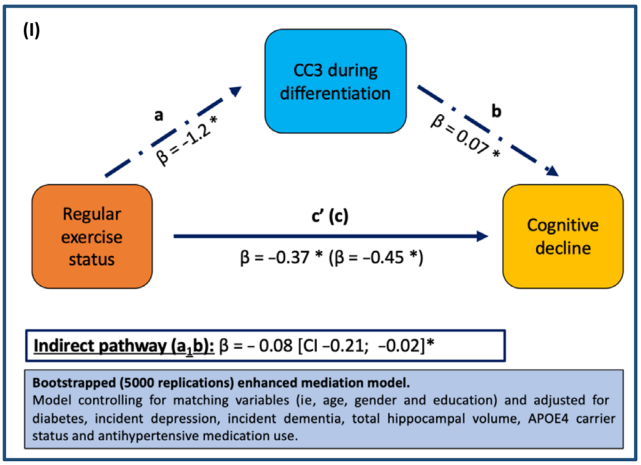


FIGURE 2 Continued

3.5 | The effect of regular exercise and prealbumin levels on future CD is partially mediated by cell death during differentiation

In order to more fully understand the relationship between our observed associations with exercise, diet, HN, and CD/dementia, mediation analyses were performed. As depicted in Figure 2H-I, we found a significant indirect effect of plasma prealbumin levels (*ab* = -0.86 [−2.21; −0.04]) and regular exercise status (*ab* = -0.08 [−0.21; −0.02]) on future CD as mediated through %CC3 levels during differentiation. No significant indirect effects of %CC3 during proliferation or %SOX2 and our associated dietary variables (i.e., vitamin D, prealbumin,

Association between the hippocampal neurogenic process and incident dementia across the 12-year follow-up

(A)

Model 4: Association between baseline levels of cell death during proliferation (ie, %CC3-positive cells) and odds of future Alzheimer's disease over the 12-year follow-up

Variable	OR	Standard Error	95% Confidence levels	*P*
%CC3 positive cells	0.25	0.17	0.56; 0.97	0.04*
Cognitive decline status	3.56	0.49	13.59; 68.72	0.001**

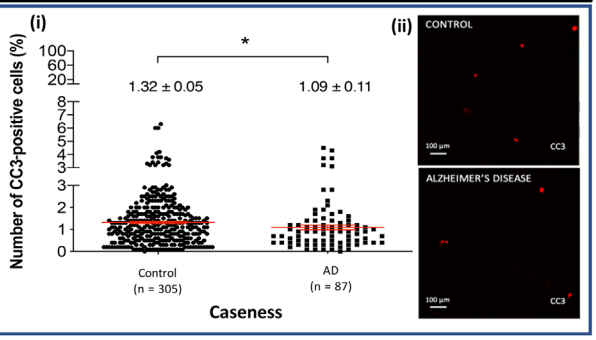
Logistic regression model
Model adjusted for age, gender, education, age of dementia onset, revenue, plasma levels of HDL cholesterol, ApoE-ε4 carrier status, diabetes, total hippocampal volume, psychotropics and antidepressants use and vitamin D supplement use. * *P* < .05; ** *P* < .01

Model 5: Association between baseline levels of hippocampal progenitor cell integrity (ie, %SOX2-positive cells) and odds of vascular dementia/ other over the 12-year follow-up

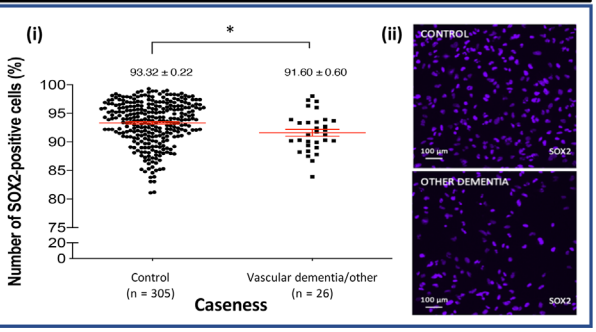
Variable	OR	Standard Error	95% Confidence levels	*P*
%SOX2 positive cells	0.88	0.06	0.78; 0.98	0.02*
Diabetes	5.32	0.55	1.80; 15.53	0.002**
Hypercholesterolemia	4.10	0.59	1.29; 12.91	0.02*
Cognitive decline status	8.42	0.77	5.38; 18.0	<0.001***

Logistic regression model
Model adjusted for age, gender, education, age of dementia onset, plasma glucose levels, plasma cholesterol levels (ie, total, LDL and HDL), hypertension, anti-hypertensive medication use and diabetic medication use. * *P* < .05; ** *P* < .01; *** *P* < .001

(B) Reduced baseline levels of cell death during proliferation are associated with an increased odds of future Alzheimer's disease



(C) Reduced baseline levels of hippocampal progenitor cell integrity are associated with an increased odds of future vascular dementia/other



Association between diet, exercise, cell death during proliferation and hippocampal progenitor cell integrity

(D)

Model 6: Association between plasma vitamin D levels, plasma prealbumin and an unidentified metabolite and baseline levels of cell death during proliferation (ie, %CC3-positive cells)

Variable	Standardised estimate [a]	Standard Error	95% Confidence levels	*P*
Baseline plasma Vitamin D levels (ng/ml)	0.11	0.006	0.002; 0.02	0.04*
Baseline plasma prealbumin levels (g/l)	0.21	1.27	1.36; 6.37	0.009**
Unidentified metabolite M129_065809781O_90403327527335	−0.15	0.0001	0.0001; 0.00015	0.03*

Linear regression model
Model adjusted for age, gender, education, total saturated fats, total polyunsaturated fats, lutein levels and lipid PEO34:3(16:1/18:2).
(a) Increments are the estimates expressed as a 1 standard deviation-increase. * *P* < .05; ** *P* < .01.

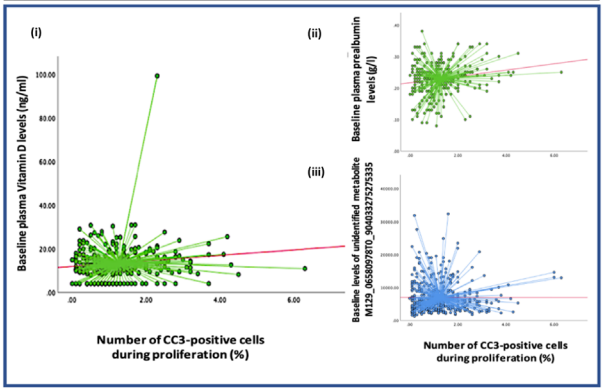
(F)

Model 7: Association between plasma ß-cryptoxanthin levels, two phosphatidylcholine ethers and baseline levels of hippocampal progenitor cell integrity (ie, %SOX2-positive cells)

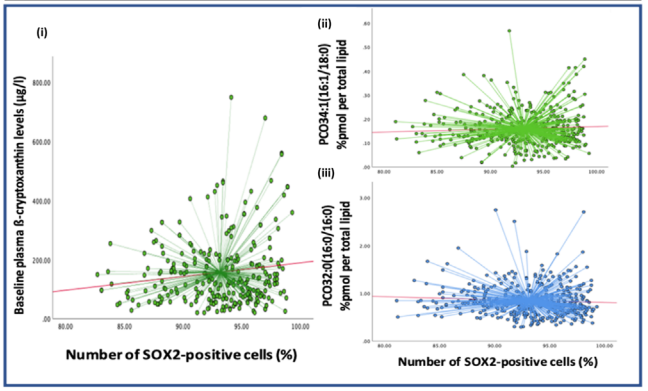
Variable	Standardised estimate [a]	Standard Error	95% Confidence levels	*P*
Baseline plasma ß-cryptoxanthin levels (µg/l)	0.17	0.002	0.002; 0.009	0.001**
PCO32:0(16:0/16:0) % pmol per total lipid	−0.20	0.72	−3.77; -0.93	0.002**
PCO34:1(16:1/18:0) % pmol per total lipid	0.10	2.96	0.56; 11.01	0.04*
BMI	−0.15	0.30	−1.35; -0.18	0.03*

Linear regression model
Model adjusted for age, gender, education, socioeconomic status, and baseline plasma LDL cholesterol levels.
(a) Increments are the estimates expressed as a 1 standard deviation-increase. * *P* < .05; ** *P* < .01.

(E) Reduced baseline plasma levels of vitamin D and plasma prealbumin, and increased levels of an unidentified metabolite were associated with reduced cell death during proliferation (associated with AD)



(G) Reduced baseline plasma levels of ß-cryptoxanthin and lipid PCO34:1(16:1/18:0), and increased levels of lipid PCO32:0(16:0/16:0) were associated with hippocampal progenitor cell integrity (associated with vascular dementia/other)



unidentified metabolite M129, ß-cryptoxanthin, PCO32:0[16:0/16:0], or PCO34:1[16:1/18:0]) on dementia outcomes were observed.

4 | DISCUSSION

Previous *in vivo* and *in vitro* parabiosis research supports that the systemic environment plays a significant role in brain aging and may determine the fate of hippocampal progenitor cells (**Box 1**). Here, we further demonstrate the ability of the human systemic environment, in the context of CD and dementia, to differentially regulate HN in an *in vitro* model. Using baseline serum samples from a prospective dementia cohort, we show that not only are individual markers of HN associated with CD and dementia 12 years later, but that there is also some degree of specificity with respect to dementia subtype diagnosis. Specifically, we find that increased baseline levels of cell death during differentiation (i.e., %CC3) significantly predicts CD over the following 12 years, while *decreased* baseline levels of cell death during *proliferation* and reduced hippocampal progenitor cell integrity (i.e., %SOX2) predict AD and vascular dementia/other, respectively. Moreover, we find that exercise, malnutrition, vitamin D, carotenoid, and lipid levels are associated with these changes in cell death and hippocampal progenitor cell integrity, and that specifically reduced physical activity and increased levels of malnutrition increase cell death during differentiation, which in turn increases the risk for future CD.

One of the brain structures that shows the earliest changes in relation to CD is the hippocampus.[27] It is also one of the niches within the human adult brain where neurogenesis occurs,[12] and has consistently been implicated in learning and memory.[15] Therefore, it is highly encouraging that we observe such early changes in HN in participants with future CD and dementia. Indeed, while our HN measures are only proxy measures of *in vivo* neurogenesis, our finding that those with future CD have increased apoptosis during differentiation is in line with human postmortem[28] and human *in vitro* work.[17] Importantly, animal research supports a role for apoptosis in both CD and dementia through promoting neuronal loss,[29] and it has become commonly acknowledged that increased neuronal death due to apoptosis is a key characteristic of CD and AD[30]—outcomes of which could be driven by inflammatory mediators released by microglia and astrocytes, which subsequently compromise the function and structure of neurons.[31]

Our most interesting finding is that in participants who subsequently develop AD, we observe a decrease in apoptosis during proliferation, which was not observed in those with future CD. The likely impact of this change will be a disruption in HN (i.e., an increase), given that less apoptosis occurs in the early trajectory of the neurogenic process.[32] Indeed, previous clinical work supports an increase in proliferation and HN in early AD,[33] so our finding is somewhat in line

FIGURE 3 **Relationship between the hippocampal neurogenic process, future dementia, exercise, and nutritional measures. (A)** Associations between baseline cell death during proliferation and hippocampal progenitor cell integrity and dementia. Model 4: Association between baseline levels of cleaved caspase-3 (CC3)-positive cell density during proliferation and future Alzheimer's disease (AD) over the 12-year follow-up using a logistic regression model. Reduced baseline levels of %CC3-positive cells during proliferation (odds ratio [OR] 0.25 [95% confidence interval (CI) 0.56; 0.97]; $p = .04$) and cognitive decline status (OR 3.56 [95% CI 13.59; 68.72]; $p = .001$) were both independently associated with increased odds of future AD in a fully adjusted model. Model 5: Association between baseline hippocampal progenitor cell integrity and vascular dementia/other using logistic regression. Baseline levels of sex determining region Y (SRY)-box 2 (SOX2)-positive cell density (OR 0.88 [95% CI 0.78; 0.98]; $p = .02$), diabetes (OR 5.32 [95% CI 1.80; 15.53]; $p = .002$), hypercholesterolemia (OR 4.10 [95% CI 1.29; 12.91]; $p = .02$), and cognitive decline status (OR 8.42 [95% CI 5.38; 18.0]; $p < .001$) were all associated with increased odds of developing future vascular dementia/other in a fully adjusted model. **(B) (i)** Baseline levels of %CC3-positive cells during proliferation stratified by caseness for AD. Cases of AD had significantly lower levels of baseline levels of %CC3 during proliferation (M = 1.32 [0.05] vs M = 1.11 [0.10]). Cellular readout expressed as a percentage relative to neural cell number. Cell line: *HPC0A07/03*; Passage number: P15-21; Technical replicates: n = 3; Data represents mean ± SEM. * $p < .05$. **(ii)** Representative immunostaining demonstrating %CC3-positive cell density during proliferation for representative case and control. Images taken at x10 objective; scale bar represents 100 μm. **(C) (i)** Baseline levels of %SOX2-positive cells stratified by caseness for vascular dementia/other. Cases of vascular dementia/other had significantly lower levels of baseline levels of %SOX2 (M = 93.32 [3.75] vs M = 90.81 [2.81]). Cellular readout expressed as a percentage relative to neural cell number. Cell line: *HPC0A07/03*; Passage number: P15-21; Technical replicates: n = 3; Data represents mean ± SEM. * $p < .05$. **(ii)** Representative immunostaining demonstrating %SOX2-positive cell density for representative case and control. Images taken at x10 objective; scale bar represents 100 μm. **(D)** Associations between diet, exercise, and the altered hippocampal neurogenesis readouts. Model 6: Association between baseline levels of Vitamin D, prealbumin, and an unidentified metabolite on baseline levels of cell death during proliferation using linear regression. Plasma levels of Vitamin D (ß = 0.11 [95% CI 0.002; 0.02] 0.006; $p = .04$), plasma prealbumin levels (ß = 0.21 [95% CI 1.36; 6.37] 1.27; $p = .009$) and unidentified metabolite, M129, levels (ß = -0.15 [95% CI 0.0001; 0.00015] 0.0001; $p = .03$) were all associated with baseline %CC3-positive cell density during proliferation in a fully adjusted model. **(E)** Scatterplot showing **(i)** positive relationship between plasma levels of Vitamin D and %CC3 levels during proliferation at baseline (green), **(ii)** positive relationship between plasma levels of prealbumin and %CC3 levels during proliferation at baseline (green), **(iii)** negative relationship between unidentified metabolite M129 and %CC3 levels during proliferation at baseline (blue). **(F)** Model 7: Association between baseline levels of ß-cryptoxanthin and two lipids on baseline levels of hippocampal progenitor cell integrity using linear regression. Plasma levels of ß-cryptoxanthin (ß = 0.17 [95% CI 0.003; 0.009] 0.002; $p = .001$), lipid phosphatidylcholine ether (PCO)34:1 (16:1/18:0) (ß = 0.10 [95% CI 0.56; 11.01] 2.96; $p = .04$), lipid PCO32:0(16:0/16:0) (ß = −0.20 [95% CI −3.77; −0.93] 0.72; $p = .002$), and body mass index (BMI; ß = −.15 [95% CI −1.35; −0.18] 0.30; $p = .03$) were all associated with baseline %SOX2 levels in a fully adjusted model. **(G)** Scatterplot showing **(i)** positive relationship between baseline plasma levels of ß-cryptoxanthin and %SOX2 levels at baseline (green), **(ii)** positive relationship between baseline plasma levels of lipid PCO34:1 (16:1/18:0) and %SOX2 levels at baseline (green), **(iii)** negative relationship between baseline plasma levels of lipid PCO32:0(16:0/16:0) and %SOX2 levels at baseline (blue). Abbreviations: ApoE-ε4, allele ε4 for the apolipoprotein E gene; HDL, high density lipoprotein; LDL, low density lipoprotein.

with previous research. Interestingly, increased platelet levels of anti-apoptosis protein, Bcl2, in amnestic mild cognitive impairment (MCI) has recently been reported, implying that abnormal apoptosis may appear in the early stage of AD and increase with the development of the disease.[34] Moreover, previous work using the *in vitro* HN assay found that increased neurogenesis characterized progression of MCI one year before conversion to AD,[17] so it remains possible that these even earlier changes in apoptosis could precede the later neurogenesis outcomes. It is noteworthy that the majority of HN and AD human research evaluates neurogenesis during the later stages of AD, making it difficult to extrapolate these findings to prodromal stages of the disease. In the context of our work this is even more challenging when our measures represent the state of HN up to 12 years prior to AD diagnosis.

It is also exciting that we can distinguish between dementia subtypes (i.e., AD vs vascular dementia/other). Unlike AD, we find that a reduction in baseline levels of %SOX2, a marker of hippocampal progenitor integrity (i.e., the maintenance of the stem cell pool and its proliferative/differentiative capacity), is predictive of vascular dementia/other—an outcome we also find in CD in individuals not regularly exercising. Given the strong implication of SOX2 in regulating the fate of stems cells, it is unsurprising that this transcription factor could play an important role in the development of neurodegenerative disease.[35] Indeed, previous animal work demonstrates how SOX2 deficiency not only impairs HN, but can also promote neuronal degeneration.[36] Importantly, decreased levels of SOX2 have been found in dementia postmortem, the decrease of which also correlates with disease severity, albeit in the context of AD.[37] Moreover, SOX2-positive neural stems cells may play a key role in cognitive-reserve capacity, shown previously to correlate with human cognition,[38] and increase upon the restoration of cognitive impairment in an AD mouse model.[39] To our knowledge, our finding that SOX2 is specifically associated with vascular dementia/other is novel. Therefore, it is difficult to extrapolate our findings to the wider literature on other forms of dementia specifically, which have largely been understudied in the context of HN research.[40]

Interestingly, SOX2 also plays a key role in vascularization,[41] and it is noteworthy that vascular dementia, which is associated with reduced cerebral blood flow (CBF),[42] was most prevalent within our vascular dementia/other category. Animal research shows how SOX2 deficiency can lead to abnormal vasculature development,[43] and has been specifically linked to vascular disease brought on by abnormal endothelial-mesenchymal transitions, for which SOX2 is an essential mediator.[44] Furthermore, given that exercise promotes CBF,[45] it might explain why SOX2 can predict CD in participants that do not regularly exercise. Indeed, previous clinical research reports memory and executive function improvement in individuals with exercise-associated increases in CBF.[46]

Based on these findings, we sought to determine whether exercise could be modulating these HN outcomes and find that reduced exercise specifically increases cell death during differentiation which then subsequently increases the risk for future CD within our sample. This is supported by both animal and human research showing how exercise inhibits apoptosis,[47] via suppression of the immune system,[48] and importantly, can attenuate CD in a clinical sample[49]—findings all observed in the context of our work. However, while we show that exercise plays an important role in CD-associated apoptosis, we find no evidence to support an association between exercise and AD or vascular dementia/other in our sample.

With respect to diet, we find that overall nutritional status may be a key factor in determining apoptosis at any point during the neurogenic process. Specifically, we find a positive association between prealbumin—a biomarker for malnutrition[50]—and apoptosis during proliferation but a negative association with apoptosis during differentiation—suggesting a biphasic relationship between these two factors. Importantly, malnutrition has been frequently associated with increased apoptosis and immune system activation both clinically and in animal models.[51,52] Moreover, poor nutritional status is known to accelerate CD/dementia in aging human populations,[53] and altered apoptosis could represent one of the mechanisms of action contributing to CD/dementia risk. Indeed, in our sample, we find a significant indirect effect of reduced prealbumin levels on future CD mediated by increased cell death during differentiation, emphasizing that the observed association between apoptosis and CD is influenced by the negative effect of poor overall nutrition. This supports the idea that global nutritional status may be more effective in slowing CD/dementia in clinical populations[54] by promoting brain health. However, it is noteworthy that while we find significant independent associations between prealbumin levels, apoptosis, and AD (see the **Supplementary materials**), we find no evidence to support that apoptosis during proliferation mediates the observed relationship between AD and poor nutrition, pointing to other key mediators not identified in the context of our work.

In addition to the observed positive association with prealbumin, we find that vitamin D levels are also positively associated with cell death during proliferation, which is in line with human *in vitro* cellular work showing that increased vitamin D promotes apoptosis in a dose-dependent manner.[55] Furthermore, we also find that AD cases within our sample have significantly lower levels of vitamin D at baseline (see the **Supplementary materials**), which is also consistent with previous clinical research that supports vitamin D deficiency as a risk factor for AD.[56] However, similar to prealbumin, while we observe independent associations between apoptosis during proliferation, vitamin D levels, and future AD, we find no statistical evidence to support that the relationship between vitamin D and AD is mediated by apoptosis. Therefore, other (as yet unidentified) mediators are likely playing a more substantial role in the trajectory of AD. However, we are mindful that insufficient power may have limited mediation analyses in the context of AD, increasing the likelihood of type II error.[57] Future work should aim to replicate these findings in a larger sample while using a longitudinal design to maximize the identification of other key mediators of HN in the trajectory of dementia.

Although we show that global (mal)nutrition may be important with respect to apoptosis, we find that single dietary nutrients may be more relevant to %SOX2. Specifically, we see a positive association between

%SOX2 and ß-cryptoxanthin—a xanthophyll and biomarker for fruit and vegetable intake.[58] This is particularly interesting, given that both have been associated with cancers, and cardiovascular and neurodegenerative disease, with increased levels of ß-cryptoxanthin known to have a protective effect against these conditions.[35,59] Furthermore, reduced carotenoid levels have been associated with dementia,[22] suggesting that this particular dietary measure could play a key role in modulating SOX2 levels that subsequently promote or aggravate disease pathology. However, similar to AD, we could only demonstrate associations between these factors and did not find a mediatory effect of %SOX2. Moreover, we did not find a difference in ß-cryptoxanthin levels at baseline between controls and individuals diagnosed with vascular dementia/other, although it remains possible that the effect of ß-cryptoxanthin on hippocampal stem cell integrity could influence other downstream processes that subsequently promote dementia pathology years later, an effect not captured due to our study design.

Interestingly, carotenoids are also key modulators of lipid membranes and play a vital role in their protection and in the maintenance of the lipid bilayer,[60] which is consistent with our finding that ß-cryptoxanthin levels are negatively correlated with PCO32:0(16:0/16:0) ($p = .008$, **Supplementary materials**)—an ether phospholipid that forms a key component of the lipid membrane and lipid rafts.[61] Importantly however, we find that %SOX2 is positively associated with unsaturated PCO34:1(16:1/18:0) and negatively associated with saturated PCO32:0(16:0/16:0)—all of which play a critical role in neurotransmission and synaptic plasticity.[62] Moreover, we find that the positive association between ß-cryptoxanthin and %SOX2 is mediated through levels of PCO32:0(16:0/16:0), such that reduced ß-cryptoxanthin is associated with increased PCO32:0(16:0/16:0), which in turn is associated with decreased %SOX2 (**Supplementary materials**). This partial mediation suggests that disruption in lipid homeostasis could be one of the mechanisms contributing to the observed changes in %SOX2. Although the dysregulation of the aforementioned PCO species has been consistently observed in CD/dementia,[63] due to limited sample size we were unable to further explore the complex interactions that exist between %SOX2, ß-cryptoxanthin, the two PCO species, and this dementia category.

The strength of our work lies in the use of a well-characterized prospective cohort to evaluate the impact of exercise and nutrition (including metabolomics and lipidomics) on *in vitro* neurogenesis measures early (i.e., up to 12 years) in the trajectory of CD and dementia. However, our study also has limitations. First, our HN measures are only proxy measures of *in vitro* neurogenesis; therefore, they might not mirror HN *in vivo*. Moreover, we recognize that our assay does not reconstitute the neurogenic niche in its entirety, and future work should expand the model to include other important players (e.g., microglia) and extend its duration to also monitor synaptic formation and plasticity. Finally, we only assessed a single baseline measure of the neurogenic process, deemed necessary to determine temporality. Due to time and logistical constraints, serum samples at later times points were unavailable for analysis. To more fully understand the impact of the neurogenesis-associated outcomes across the trajectory of CD and dementia, it would be highly profitable for future research to adopt a longitudinal approach and compare HN markers and overall neurogenic profiles at multiple timepoints.

In summary, we show that HN, altered by exercise and diet, can predict future CD and dementia, and can potentially distinguish between dementia diagnoses. The observed changes in apoptosis and hippocampal progenitor cell integrity, up to 12 years prior to the development of CD and/or dementia, could signify the start of the pathological process and potentially represent early functional biomarkers for these conditions. However, more work is needed to more fully understand how exercise and diet might be modulating HN and subsequent CD/dementia, given that it is modifiable and may represent an effective early preventative strategy.

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CONFLICTS OF INTEREST

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- MP: Identificación de moduladores dietéticos del envejecimiento cognitivo y plasticidad cerebral, y evidencia del concepto de eficacia en la prevención/reversión del declive cognitivo. Ministerio de

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None of the authors declare to have received royalties or licenses in the past 36 months.

None of the authors declare to have received consulting fees in the past 36 months.

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- CD declares having received personal reimbursement of travel fees from Laboratoires Théa.
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All Patents:

- ST declares: Patent application PCT/GB2017/052904 Assay for predicting conversion to Alzheimer's Disease and Monitoring disease progression.
- CD declares: Patent for a biomarker of retinal status in omega 3 polyunsaturated fatty acids (pending).
- MP declares: Inventor/s (signature): María Carmen Escolano Mirón; Mercè Pallàs Lliberia; Christian Gaspar Griñán Ferre; Sònia Abás Prades; Luis-Felipe Callado Hernando; Jesús A. García Sevilla Title: Synthetic I2 imidazoline receptor ligands for prevention or treatment of human brain disorders Application number: EP17382879.9 First priority country: European Patent Convention Countries Date of priority: 21/12/2017 Universitat de Barcelona / Universidad del País Vasco / Euskal Herriko Unibertsitatea / Universitat de les Illes Balears. Model: Request priority Inventor/s (signature): Tamara Maes, David Rotllant Pozo, Christian Griñán-Ferré, Mercè Pallàs Lliberia, Roser Nadal Alemany, Antonio Armario García. Title: Methods of treating behavior alterations. Application number: PCT/EP2019/071120. Pub. NO.: WO/2019/025588 Inventor/s (signature): Mónica Olivares Martín. Luis Pérez Martínez. Óscar Bañuelos Hortigüela. José Luís López Larramendi. Mercè Pallàs Lliberia. Cristian Gaspar Griñan Ferre. Title: Composiciones para uso en el tratamiento de trastornos cognitivos Application number:PCT/ES2020/070005.
- LA declares: Patent applications on the use of a buccal film of montelukast in diseases associated with neuroinflammation. This is not related to the present work.

None of the authors declare to have participated on a Data Safety Monitoring Board or Advisory Board in the past 36 months.

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- ST declares being the Chair of the Research Degree Examination Board at King's College London (KCL) and the Deputy Head of the Basic and Clinical Neuroscience Department (KCL) For both, payment is part of her salary.
- DYL declares being Vice President of LEARN, LKCMedicine early career researcher network (established Oct 2020).

- CF declares being Member of the board of directors of the French Nutrition Society without payment.
- MP declares being the secretary of the Spanish Pharmacology Society from September 2019
- AK declares chairing the Dutch neuroscience meeting and Treasurer of the European Brain and Behavior Society.

None of the authors declare to have held stock or stock options in entities related to the current manuscript and/or area of research included in this manuscript or related area of research in the past 36 months.

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CS declares to be co-chair of Executive Committee of Alzheimer's Association ISTAART Nutrition Metabolism and Dementia Professional Interest Area.

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SUPPORTING INFORMATION

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

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