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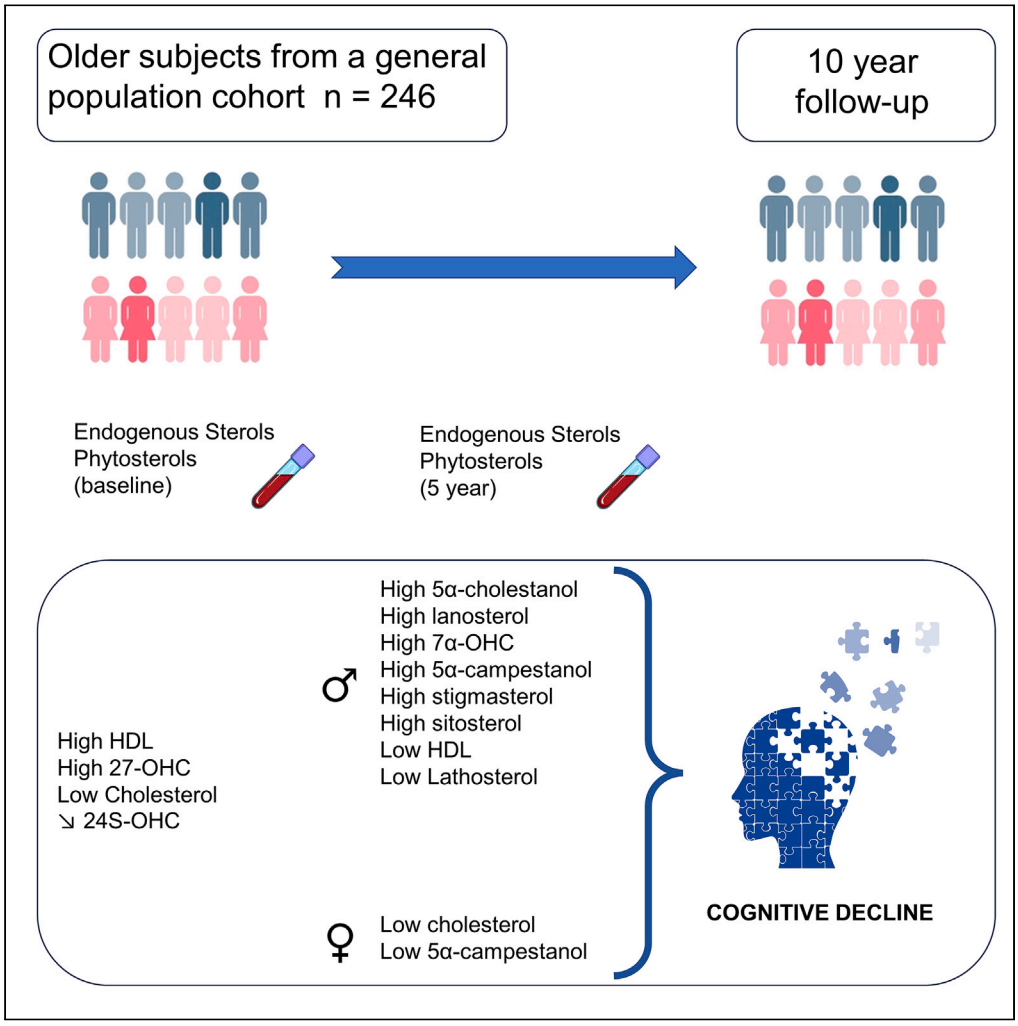

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Highlights

Endogenous cholesterol metabolism is associated with long-term cognitive decline

Changes over time in hydroxysterols are associated with long-term cognitive decline

Sex-specific cholesterol metabolites are associated with cognitive impairment

Phytosterols are associated with long-term cognitive decline

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Cholesterol-metabolism, plant sterols, and long-term cognitive decline in older people – Effects of sex and APOEe4

Matteo Spinedi,[1] Christopher Clark,[1] Leonardo Zullo,[2] Anja Kerksiek,[3] Giorgio Pistis,[4] Enrique Castelao,[4] Armin von Gunten,[2] Martin Preisig,[4] Dieter Lütjohann,[3] and Julius Popp[1,2,5,*]

SUMMARY

Neurodegenerative, vascular, and dementia diseases are linked to dysregulations in cholesterol metabolism. Dietary plant sterols, or phytosterols, may interfere to neurodegeneration and cognitive decline, and have cholesterol-lowering, anti-inflammatory, and antioxidant qualities. Here, we investigated the potential associations between circulating cholesterol precursors and metabolites, triglycerides, and phytosterols with cognitive decline in older people by performing multivariate analysis on 246 participants engaged in a population-based prospective study. In our analysis we considered the potential effect of sex and APOEe4. We reveal particular dysregulations of diet-derived phytosterols and endogenous cholesterol synthesis and metabolism, and their variations over time linked to cognitive decline in the general population. These results are significant to the development of interventions to avoid cognitive decline in older adults and suggest that levels of circulating sterols should be taken into account when evaluating risk.

INTRODUCTION

The prevalence of cognitive disorders increases exponentially with age.[1] In addition to age, genetic aspects such as the presence of the Apolipoprotein E (APOE) e4 allele, cardiovascular risk factors, and depression represent important risk factors for cognitive decline.[2]

There is evidence suggesting that cholesterol, cholesterol metabolites and precursors, and plant-derived phytosterols are associated with cognitive decline and dementia.[3] Circulating cholesterol is synthesized mainly in the liver. This process involves many precursors, such as lanosterol, dihydrolanosterol, lathosterol, and desmosterol.[4] Cerebral cholesterol is synthesized completely *in situ* by neurons and astrocytes and is unable to pass the blood-brain barrier (BBB).[5] In the event of excessive cerebral cholesterol accumulation, cerebral cholesterol is enzymatically converted into 24S-hydroxycholesterol (24S-OHC).[6,7] 24S-OHC serves as a vehicle for cholesterol removal from the brain[3] since it is able to pass the BBB[8] into circulating blood, where it can be considered a marker of cerebral cholesterol turnover. In the periphery, cholesterol is converted into 7α-hydroxycholesterol (7α-OHC) and 27-hydroxycholesterol (27-OHC) prior to the conversion of these oxysterols into bile acids. 27-OHC can pass the BBB from the periphery into the central nervous system (CNS).[9–11] Cholesterol can also be converted into the 5α-saturated metabolite 5α-cholestanol, which is a marker for cholesterol absorption in the periphery via the intestines.[12] Aside from cholesterol metabolites, circulating blood also contains plant sterols, which are entirely diet-derived and can cross the BBB, including sitosterol, campesterol, stigmasterol, and brassicasterol.[10,13,14]

Cholesterol, 27-OHC, and 24S-OHC in the cerebrospinal fluid (CSF) have been associated with cognitive impairment in memory clinic patients.[3,15] Cerebral pathology and neurodegeneration can affect cholesterol metabolism. For example, overproduction of cholesterol in the brain likely occurs by myelin breakdown, a by-product of neurodegeneration.[16] Cholesterol synthesis is also lowered in older people with impaired cognition.[17] Cholesterol metabolism dysregulations have also been associated with pathological manifestations of Alzheimer's disease (AD). Individual cholesterol metabolites in the CNS have also been associated with concentration changes in the CSF biomarkers of AD, including β-amyloid (Aβ) 1–42 and tau phosphorylated at threonine 181.[3,18–21] Some phytosterols have also been linked to neurodegeneration[3] and changes in Aβ 1–42 levels.[22]

Both APOE and sex are associated with cognitive decline and cholesterol metabolism.[23–27] APOE removes Aβ from the CNS across the BBB,[28] and carrying the APOEe4 allele is the most important genetic risk factor for non-familial AD.[29,30] APOE also transports cholesterol

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Table 1. Characteristics of the study population

	Total n = 246	CDR = 0 n = 137	CDR = 0 · 5 n = 106	p-value
Female % (n) female	63 (153)	72 · 99 (100)	50 (53)	**<0 · 001**
Age, mean (S·E·)	69 · 99 (0 · 24)	69 · 94 (0 · 32)	69 · 99 (0 · 37)	0 · 914
BMI, mean (S·E·)	26 · 27 (0 · 28)	26 · 11 (0 · 40)	26 · 56 (0 · 39)	0 · 427
MMSE, mean (S·E·)	29 · 38 (0 · 08)	29 · 72 (0 · 06)	28 · 94 (0 · 162)	**<0 · 001**
CDR-SoB, mean (S·E·)	0 · 79 (0 · 04)	0 · 46 (0 · 04)	1 · 23 (0 · 06)	**<0 · 001**
MDD, % (n)	3 · 0 (8)	2 · 9 (4)	3 · 8 (4)	0 · 711
Hypertension, % (n)	59 · 0 (146)	57 · 7 (79)	61 · 3 (65)	0 · 565
Diabetes, % (n)	13 · 0 (32)	8 · 0 (11)	19 · 8 (21)	**0 · 007**
Dyslipidemia, % (n)	51 · 0 (125)	46 · 7 (64)	56 · 6 (60)	0 · 126
APOEe4 carrier, % (n)	17 · 0 (36)	14 · 6 (20)	15 · 1 (16)	0 · 772
Statin treatment, % (n)	10 · 0 (24)	8 · 8 (12)	11 · 3 (12)	0 · 507
Education				
Basic, % (n)	15 · 9 (39)	15 · 3 (21)	17 (18)	0 · 208
Apprenticeship, % (n)	40 · 0 (98)	37 · 2 (51)	44 · 3 (47)	0 · 208
High school, % (n)	25 · 2 (62)	27 · 0 (37)	23 · 6 (25)	0 · 208
University, % (n)	17 · 9 (44)	20 · 4 (28)	15 · 1 (16)	0 · 208

Group comparisons between CDR = 0 and CDR = 0 · 5 for demographic and clinical features. Significant p values of T-tests and Mann-Whitney U-Tests are shown in bold. BMI: Body mass index, CDR: Clinical Dementia Rating, CDR-SoB: Clinical Dementia Rating Sum of Boxes, MMSE: Mini Mental State Examination, MDD: Major depressive disorder, S·E: Standard error; n: number of participants.

between glia and neurons, through specific receptors[31,32] and is associated with hypercholesterolemia and hyperlipidemia, leading to cerebral infarctions, atherosclerosis, and coronary artery disease, all risk factors for dementia.[33]

Regarding sex, the plasma lipid profile of males and pre- and postmenopausal females differs significantly. In comparison to females, males typically have higher levels of triglycerides, low-density lipoprotein (LDL) cholesterol, and total cholesterol as well as 27-OHC. Compared to males, females often have higher high-density lipoprotein (HDL) cholesterol levels.[34–36] Males also have less peripheral insulin resistance, which is linked to dyslipidemia and is a known risk factor for developing AD. Males are also more prone to type 2 diabetes, which is known to have an impact on blood lipid levels.[37–39]

While there is evidence that alterations in cholesterol synthesis and metabolism contribute to brain pathologies, their relationships with cognitive decline in the general older population are unknown. In this study, we investigate in a population-based cohort of older individuals whether cholesterol, its precursors and metabolites, triglycerides, and phytosterols circulating in the blood predict long-term cognitive decline. Furthermore, we address the role of sex and the APOEe4 status on the relationships between sterols and cognitive decline. The results may be important for assessing sex-specific risk and prevention strategies in older people in the general population.

RESULTS

Study population

Tables 1 and 2 show the clinical and demographic features of the study group. The 246 participants considered at TP0 differ across groups (CDR = 0 and CDR = 0 · 5) for sex, CDR-SoB score, MMSE score, and the presence of diabetes. 245 participants had longitudinal cognitive data (TP2), including MMSE data, and 237 participants had sterol concentrations at TP0 and TP1. At TP2, there were differences in all cognitive assessment scores between both groups (Table S1). We also compared the study sample with participants from the CoLaus/PsyCoLaus cohort with cognitive assessments and sterol plasma levels measured at TP0, but not at TP2 (n = 683), whom we considered as drop-outs from the present study. This revealed that the participants in this study were younger at inclusion and presented less cognitive impairment as well as lower rates of diabetes and hypertension (Table S2). In addition, there were differences between circulating levels of cholesterol, lanosterol, lathosterol, campesterol, and stigmasterol at TP0 (Table S3).

Associations with long-term cognitive decline

We first assessed the relationship between plasma levels of cholesterol, its precursors, and metabolites, and phytosterols at TP0 and decline in global cognition or in cognitive and functional decline over eight years (Table 3). Higher levels of HDL-cholesterol and lower levels of cholesterol (identified by backward regression) measured in peripheral blood at TP0 were associated with a worsening of cognitive and functional abilities. When specifically considering the APOEe4 carrier status, we observed the same results (data not shown). When sex was entered into the regression models before considering other confounders and sterols concentrations, we observed the same results, with

Table 2. Sterol plasma levels at TP0 in the study group

	Total n = 246	CDR = 0 n = 137	CDR = 0 · 5 n = 106	p-value
ENDOGENOUS STEROLS				
Cholesterol mg/dL, mean (S·E·)	214 · 79 (2 · 64)	214 · 01 (3 · 60)	215 · 40 (3 · 93)	0 · 795
Triglycerides mmol/L, mean (S·E·)	1 · 35 (0 · 06)	1 · 28 (0 · 06)	1 · 45 (0 · 12)	0 · 166
HDL-cholesterol mmol/L, mean (S·E·)	1 · 73 (0 · 03)	1 · 78 (0 · 04)	1 · 67 (0 · 04)	0 · 087
24S-OHC ng/mL, mean (S·E·)	56 · 41 (1 · 06)	57 · 38 (1 · 41)	54 · 52 (1 · 51)	0 · 170
27-OHC ng/mL, mean (S·E·)	166 · 32 (3 · 42)	162 · 55 (4 · 71)	169 · 81 (4 · 85)	0 · 291
Lanosterol µg/dL, mean (S·E·)	26 · 94 (1 · 08)	28 · 19 (1 · 43)	25 · 45 (1 · 69)	0 · 215
Desmosterol mg/dL, mean (S·E·)	0 · 15 (0 · 01)	0 · 15 (0 · 004)	0 · 15 (0 · 01)	0 · 365
Dihydro-Lanosterol µg/dL, mean (S·E·)	4 · 12 (0 · 21)	4 · 26 (0 · 29)	3 · 97 (0 · 29)	0 · 207
7α-OHC ng/mL, mean (S·E·)	139 · 70 (11 · 18)	120 · 43 (6 · 33)	136 · 07 (10 · 77)	0 · 285
5α-cholestanol mg/dL, mean (S·E·)	0 · 31 (0 · 01)	0 · 31 (0 · 01)	0 · 31 (0 · 01)	0 · 968
Lathosterol mg/dL, mean (S·E·)	0 · 25 (0 · 01)	0 · 26 (0 · 01)	0 · 23 (0 · 01)	0 · 186
PHYTOSTEROLS				
Brassicasterol µg/dL, mean (S·E·)	20 · 29 (0 · 78)	20 · 45 (1 · 04)	20 · 18 (1 · 19)	0 · 865
Campesterol mg/dL, mean (S·E·)	0 · 34 (0 · 01)	0 · 34 (0 · 02)	0 · 34 (0 · 02)	0 · 911
5α-campestanol µg/dL, mean (S·E·)	6 · 66 (0 · 31)	6 · 74 (0 · 38)	6 · 41 (0 · 47)	0 · 588
Stigmasterol µg/dL, mean (S·E·)	6 · 92 (0 · 27)	6 · 92 (0 · 36)	6 · 90 (0 · 43)	0 · 962
Sitosterol mg/dL, mean (S·E·)	0 · 27 (0 · 01)	0 · 27 (0 · 01)	0 · 25 (0 · 01)	0 · 271
5α-sitostanol µg/dL, mean (S·E·)	7 · 68 (0 · 27)	7 · 77 (0 · 33)	7 · 43 (0 · 41)	0 · 510

Group comparisons between CDR = 0 and CDR = 0 · 5 groups. Significant p values of T-tests are shown in bold; CDR: Clinical Dementia Rating, HDL high-density lipoprotein,24S-OHC: 24S-hydroxycholesterol, 27-OHC: 27-hydroxycholesterol, 7α-OHC: 7α-hydroxycholesterol, S·E: Standard error; n: number of participants.

the addition of higher plasma levels 27-OHC associated with a worsening in cognitive and functional abilities (**Δ**CDR; B 0 · 011, p value 0 · 044). When performing the analysis in males and females independently (Table 4), we found that in males, lower levels of HDL-cholesterol and lathosterol were associated with a decrease in global cognition and a decline in cognitive and functional performance respectively. Higher blood values of 5α-cholestanol, lanosterol, and 7α -OHC were associated with a decline in cognitive and functional performance in males. Backward stepwise regression models further identified associations between worsening cognitive and functional performance in males and higher blood values of 5α-campestanol, stigmasterol, and sitosterol. In females, backward stepwise regression analysis showed that lower levels of cholesterol were associated with decreased cognitive and functional performance. When specifically addressing the interactions between sex and circulating sterol levels in the whole cohort these associations were confirmed except for the following: the association of

Table 3. Results of logistic regression separately considering TP0 endogenous sterol levels and TP0 phytosterol levels and their associations with a decline in global cognition (ΔMMSE) or in cognitive and functional performance (ΔCDR and ΔCDR-SoB) at TP2 in the whole cohort

	B	p value
ENDOGENOUS STEROLS		
ΔCDR		
HDL-cholesterol	1 · 605	0 · 018
Δ24S-OHC	−0·054	0 · 033
5α-cholestanol	14 · 45[a]	0 · 006
ΔCDR-SoB		
HDL-cholesterol	1 · 00	0 · 050
Cholesterol	−0·566[a]	0 · 004
Δ24S-OHC	−0·042	0 · 026

B: beta coefficient.; CDR: Clinical Dementia Rating, CDR-SoB: Clinical Dementia Rating Sum of Boxes, MMSE: Mini Mental State Examination, HDL high-density lipoprotein, 24S-OHC: 24S-hydroxycholesterol; **Δ**: concentration changes over 5 years from baseline (TP1-TP0) for sterols or change in score TP2-TP0 for cognitive assessments.

[a]Results obtained from backward regression models.

Table 4. Results of logistic regression separately considering TP0 endogenous sterol levels and TP0 phytosterol levels and their associations with a decline in global cognition (ΔMMSE) or in cognitive and functional performance (ΔCDR and ΔCDR-SoB) at TP2 considering males and females independently and the interactions of sex with TP0 endogenous sterol levels and TP0 phytosterol in all participants

	Males only		Females only		Whole cohort	
	B	p value	B	p value	B	p value
ENDOGENOUS STEROLS						
ΔMMSE						
HDL-cholesterol	−3 · 308	0 · 042				
ΔCDR						
5α-cholestanol						
5α-cholestanol x Female sex					−15 · 66[a]	0 · 002
ΔCDR-SoB						
Cholesterol			−0·543[a]	0 · 018		
Lathosterol	−23 · 97	0 · 020				
Lathosterol x Female sex					−12.95	0 · 011
Lanosterol	0 · 178	0 · 005				
Lanosterol x Female sex					−0 · 084	0 · 005
7α -OHC	0 · 017	0 · 015				
7α -OHC x Female sex					−0 · 007	0 · 050
5α-cholestanol	10 · 748	0 · 015				
PHYTOSTEROLS						
ΔMMSE						
Stigmasterol	0 · 29[a]	0 · 031				
ΔCDR						
Sitosterol	10 · 99[a]	0 · 004				
ΔCDR-SoB						
5α-campestanol	0 · 20[a]	0 · 004				
5α-campestanol x Female sex					−0 · 49[a]	<0 · 001
Sitosterol x Female sex					12 · 80[a]	0 · 001

B: beta coefficient.; CDR: Clinical Dementia Rating, CDR-SoB: Clinical Dementia Rating Sum of Boxes, MMSE: Mini Mental State Examination, HDL high-density lipoprotein, 7α-OHC: 7α-hydroxycholesterol; x: interaction variable; Δ: concentration changes over 5 years from baseline (TP1-TP0) or change in score TP2-TP0 for cognitive assessments.

[a]Results obtained from backward regression models.

HDL-cholesterol and stigmasterol with a decline in global cognition in males, and the association of cholesterol with cognitive and functional decline in females which were no longer significant (Table 4). We found no interactions between the APOEe4 carrier status and circulating sterol levels with global cognition or in cognitive and functional decline in the whole cohort. In models considering cholesterol, its precursors, and metabolites, the occurrence of treatment with statins showed a significant positive association with a decline in cognitive and functional abilities (data not shown). The diagnosis of diabetes was associated with a decline in global cognition for all considered sterols when considering the whole cohort. When considering females only, diabetes was not associated with a decline in cognition (data not shown). When we considered the concentrations of non-cholesterol sterols corrected for cholesterol, all previously observed associations remained significant, except for the associations of (1) lower levels of cholesterol with worsening of cognitive and functional abilities in the whole cohort and (2) lower levels of HDL-cholesterol with a decrease in global cognition in males. These analyses also revealed the additional association of higher levels of TP0 lanosterol with cognitive and functional decline in the whole cohort. In females, we also observed lower levels of triglycerides were associated with cognitive and functional decline when correcting for overall cholesterol levels.

Association between changes in sterols levels at TP1 and cognitive decline at TP2

We next investigated how incident changes in plasma sterol levels between TP0 and TP1 were associated with changes in cognition at TP2. The decline in cognitive and functional performance was associated with a decrease in 24S-OHC levels in the blood over 5 years (Table 3). In this model, the diagnosis of diabetes was associated with a decline in global cognition. Statins treatment was not associated with changes in cognition in these models (data not shown). After correcting non-cholesterol sterol levels for overall cholesterol, we observed that a decrease

in 24S-OHC was associated with cognitive and functional performance decline in the whole cohort. An increase in stigmasterol levels was associated with cognitive and functional performance decline in females only (Table S4).

DISCUSSION

We found that higher levels of HDL-cholesterol and 27-OHC and lower levels of cholesterol were associated with cognitive and functional decline up to ten years later. These associations were not affected by the APOEe4 carrier status. Decreasing 24S-OHC plasma levels over five years were associated with a decline in cognitive and functional performance up to ten years later. When considering males only, lower levels of HDL-cholesterol and lathosterol were associated with a decrease in global cognition and a decline in cognitive and functional performance. Moreover, elevated blood levels of 5α-cholestanol, lanosterol, 7α-OHC, 5α-campestanol, stigmasterol, and sitosterol were associated with a decline in cognitive and functional performance in males. In females, lower levels of cholesterol and 5α-campestanol were associated with cognitive and functional decline.

Previous studies showed that high levels of serum cholesterol in mid-life are associated with a higher risk of developing AD in later life.[40,41] Furthermore, in patients with Alzheimer's disease higher levels of total cholesterol were related to cortical amyloid-beta deposition and accelerated cognitive deterioration.[42] We observed that lower levels of cholesterol at TP0 were associated with cognitive and functional decline up to ten years later in the whole group. This is in line with previous evidence from a population-based study suggesting that in older people hypercholesterolemia may be associated with better cognitive performance.[43] This suggests a changing relationship between circulating blood cholesterol and cognitive decline where in mid-life high cholesterol levels are a risk factor for dementia while in later life lower cholesterol levels may reflect ongoing disease processes.[40] Low circulating cholesterol levels have been associated with loss of gray matter in medial temporal brain regions.[44] These regions are also associated with prodromal AD[45] suggesting low circulating levels of cholesterol are associated with a higher risk of cognitive decline and/or dementia due to AD by affecting brain structure. Alternatively, alterations in the synthesis and metabolism of cholesterol could be explained by neurodegeneration in these regions.[46,47] Our results suggest that lower or decreasing cholesterol circulating levels in older people may indicate a higher risk of cognitive decline, possibly reflecting ongoing cerebral pathological changes.

While previous evidence suggests a protective effect of HDL-cholesterol against cognitive decline and dementia,[43,48–51] we observed an association between higher levels of HDL-cholesterol at TP0 and cognitive and functional decline. One explanation for this association could be the complex interplay between different lipid fractions and their effects on brain health. While HDL-cholesterol can remove excess cholesterol from the bloodstream, it is important to note that HDL particles are not a homogeneous entity. HDL particles can vary in size, composition, and functionality, which can lead to different physiological effects and effects on cognition via anti-inflammatory, antithrombic and cholesterol efflux regulation properties.[52] When performing the analysis in males and females independently, we found that in males, lower levels of HDL-cholesterol were associated with a decline in global cognition. Previous studies have shown that low serum levels of HDL-cholesterol are associated with risk factors of cognitive decline,[53] both in mid- and in late-life,[54,55] and that males have lower circulating levels of HDL-cholesterol. These associations suggest that cholesterol and HDL-cholesterol could be differentially associated with cognitive decline in males and females, and that males with lower circulating HDL-cholesterol are more at risk of future cognitive decline.

Previous studies suggest elevated plasma 24-OHC levels are associated with cognitive decline in older adults and could even occur before the development of cognitive impairment.[16] Increased levels of 24S-OHC have also been observed in AD,[3] which suggests its involvement in the disease process and/or that 24S-OHC is a by-product of myelin degradation triggered by neurodegeneration. However, there is also evidence pointing toward the potential neuroprotective effects of 24S-OHC through supporting myelination and/or synaptogenesis.[56] In addition, 24S-OHC has been suggested to suppress brain cholesterol biosynthesis in animal studies.[57] We observed an association between decreasing serum levels of 24S-OHC and cognitive decline. Considering that 24S-OHC is the primary metabolite of brain cholesterol,[58] its decreasing levels may indicate decreasing cerebral cholesterol levels.

Higher baseline levels of 27-OHC were associated with a decline in cognitive and functional abilities in females while the cholesterol-corrected 27-OHC levels were associated with decline in the whole cohort. Previous studies suggest that increased levels of 27-OHC in CSF are associated with enhanced amyloidogenesis, a key pathological hallmark of AD.[15,21] Since 27-OHC can cross the blood-brain barrier, its plasma levels may affect its concentration in the brain. The association observed here between circulating 27-OHC levels and decline in cognitive and functional abilities could be specific to participants with AD pathology. In our study, we cannot differentiate between AD and other causes of cognitive decline, however.

Higher levels of 7α-OHC, a metabolite of cholesterol produced by the CYP7A1 enzyme, were associated with a decline in cognition in males. This enzyme is regulated by cholesterol, with higher cholesterol levels leading to increased activity of CYP7A1.[59] Accordingly, higher levels of 7α-OHC may reflect an altered cholesterol metabolism in males associated with a higher risk of cognitive decline. Increased circulating levels of 7α-OHC may also result from the effect of sex hormones on the regulation and expression of CYP7A1 in aging males, in line with animal studies.[60]

Higher 5α-cholestanol levels were associated with a decline in cognitive and functional abilities in males. Elevated 5α-cholestanol levels have been linked to an increased risk of coronary events,[61] themselves risk factors of cerebral pathologies. Therefore, the association between high 5α-cholestanol levels and cognitive decline in males may represent, at least in part, the detrimental effects of vascular events on brain health. It is also possible that individuals with a high 5α-cholestanol-to-cholesterol ratio have higher levels of low-density lipoprotein (LDL) cholesterol.[62] This will result in lower HDL-cholesterol levels which are associated with an increased risk of cognitive decline in our study. In line with the present results, this mechanism could be specific to males considering serum testosterone levels are inversely correlated

with total cholesterol and LDL-cholesterol levels.[63,64] In addition, lower testosterone levels in aging males[65] reduce the liver's uptake of cholesterol.[66] Both mechanisms might contribute to decreasing circulating HDL-cholesterol.

In males, lower levels of lathosterol and higher levels of lanosterol were associated with cognitive decline. When normalized for cholesterol levels, higher levels of lanosterol were associated with a decline in cognitive and functional abilities in the whole cohort. These findings contrast with previous research reporting an association between higher circulating levels of lathosterol and decreased cognitive performance in the general population.[67] In AD, studies have consistently reported lower plasma and CSF levels of lathosterol and lanosterol.[68–70] Therefore, the role of both of these cholesterol precursors may differ in AD compared to other causes of cognitive impairment. Lathosterol is converted into cholesterol, via 7-dehydrocholesterol (provitamin D3) thanks to the activity of lathosterol oxidase. It is also considered a surrogate marker for cholesterol synthesis in the periphery.[71] Animal studies have shown that this enzyme is regulated by testosterone levels,[72] possibly explaining the sex-specific results. Taken together, these observations suggest that reduced levels of the cholesterol precursor lathosterol and the possible resulting decrease in cholesterol synthesis are particularly relevant for all-cause cognitive decline in males.

We observed higher levels of sitosterol and 5α-campestanol associated with cognitive decline in males. In females, higher levels of stigmasterol normalized for total cholesterol were also associated with cognitive decline. Both of these results are in line with previous work showing an increase in these phytosterols is associated with cognitive decline.[64,73] This could be explained by their potential to lower cholesterol levels by competing with cholesterol for reabsorption in the intestinal system.[74–80] These phytosterols have also been associated with decreased amyloidogenic processes which could be beneficial in AD. Indeed, treatment with sitosterol attenuates cognitive deficits and prevents amyloid plaque deposition in mouse models of AD.[81] Additionally, sitosterol has been found to increase the production of Aβ by upregulating the expression of the β-secretase gene.[22] In humans, stigmasterol was associated with lower Aβ[22] while campesterol and sitosterol have been associated with tau and p-tau.[3] Taken together, the mechanisms linking phytosterols to cognitive decline and whether they may represent targets for prevention interventions need further investigation.

Previous studies showed an association between the APOEe4 carrier status and dyslipidemia in late-onset AD and in the female general population.[82,83] Our results did not show any significant impact of the APOEe4 status on the associations between sterols and cognitive decline. One possible explanation is that the effects of sterols on cognitive decline may be mediated by mechanisms independent of APOEe4 carrier status. While APOE is known to play a crucial role in lipid metabolism and the accumulation of Aβ,[84] there could be additional pathways through which cholesterol and its derivatives may be related to cognitive decline. Another possibility is that our results are affected by the low number APOEe4 carriers amongst the study participants.

Statins are commonly prescribed to lower LDL-cholesterol levels and reduce the risk of cardiovascular disease. However, the effects of statins on cognitive function and the development of dementia remain controversial. Randomized controlled trials have failed to consistently demonstrate beneficial effects on cognitive decline. Some studies have even suggested that highly lipophilic statins such as simvastatin and atorvastatin can cross the blood brain barrier and may contribute to reversible cognitive impairment or increase the risk of cognitive decline by affecting CNS cholesterol physiology.[85–87] These effects can also be also influenced by the APOE haplotype.[88,89] In our study, statin treatment was associated with more marked cognitive and functional decline when considering cholesterol, its precursors, and metabolites. Together, no clear conclusion can be drawn on the association of statin use with cognitive decline, especially in people with specific risk profiles.

Conclusions

Plasma markers of cholesterol synthesis and metabolism, as well as phytosterols, are associated with cognitive decline in older non-demented people in the general population. The observed sex-specific associations highlight the necessity of considering sex when evaluating individual risk profiles and developing focused prevention and early treatment strategies.

Limitations of the study

The present study has some limitations. First, the absence of information on the precise pathologies that underlie cognitive impairment does not allow for distinguishing between cognitive decline brought on by vascular, mixed, or AD pathologies, or other brain-related disorders. This does, however, suggest that the associations observed here are readily generalizable to different possible causes of cognitive decline in the general population. Future studies should consider combining circulating sterol levels with biomarkers of brain pathologies, including AD, or with postmortem analysis, to better understand the association between circulating sterols and cognitive decline. In our study, unfortunately, a more precise distinction was not made on the type of statin used by the participants. In the future, it would be important to distinguish in the analysis between hydrophilic and lipophilic statins because fat soluble statins are able to cross the BBB and can cause cognitive impairment.[87] Furthermore, nutrition directly affects phytosterol levels and cholesterol metabolism, and it is probable that different diets in different individuals influence the relationships we observed. Collecting detailed dietary data from individuals would help better control dietary-related confounding variables. However, the group considered is large enough that the probable diet heterogeneity in our study group should alleviate this issue. The study is also limited by considering only participants with TP2 visits available. This survivor bias excludes participants that might experience more severe and rapid cognitive decline or even death in favor of participants with milder and/or slower cognitive deterioration. Indeed, comparing the included participants with those from the CoLaus/PsyCoLaus cohort without TP2 visits revealed that the participants in this study were generally healthier. This suggests our findings may be especially relevant for healthier older people. On the other hand, considering older individuals from the general population increases the generalizability of the results and represents a strength of this study. By studying a representative sample of older adults, the results hold implications for risk assessment and prevention strategies concerning cognitive decline in the aging population, especially considering that the associations observed in this study

span a longer period than is generally considered. We also considered a large array of confounders, including statin treatment, and specifically addressed the roles of sex and APOEe4 carrier status on the associations between sterols and cognitive decline. This approach provides valuable insights into sex-specific patterns independent of APOEe4 carrier status. Finally, this study considers multiple cholesterol-related markers providing a more comprehensive analysis of the relationship between cholesterol metabolism, plant sterols, and cognitive decline.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - CoLaus/PsyCoLaus study design
  - Study sample
- METHOD DETAILS
  - Cognitive measurements
  - Biological data
  - Ethics
  - Role of funders
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109013.

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AUTHOR CONTRIBUTIONS

J.P. and D.L. designed the study. A.v.G. and M.P. contributed to the CoLaus/PsyCoLaus study design. E.C. contributed to the data management. M.S. and C.C. performed the data analysis. M.S., C.C., D.L., and J.P. wrote the manuscript. L.Z., A.K., G.P., E.C., A.v.G., M.P., D.L., and J.P. critically revised the manuscript. All authors reviewed and approved the final manuscript. J.P. is the guarantor responsible for the contents of the manuscript. The authors read and approved the final manuscript.

DECLARATION OF INTERESTS

J.P. received consultation and speaker honoraria from Nestle Institute of Health Sciences, Innovation Campus, EPFL, Lausanne, Switzerland, Ono Pharma, Schwabe Pharma Switzerland, OM Pharma Switzerland, Roche Pharma, and Fujirebio Europe, all not related to the present work. C.C. received consultation and speaker honoraria from OM Pharma Suisse. The other authors declare no conflicts of interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
SPSS software (version 29.0.0.0)	IBM	RRID: SCR_016479

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Pr Julius Popp, e-mail: julius.popp@uzh.ch.

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request. Requests must be justified and will be subject to data access agreement between the parties.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

CoLaus/PsyCoLaus study design

We used data and biological samples from CoLaus/PsyCoLaus, an ongoing prospective cohort study designed to study mental disorders and cardiovascular risk factors as well as their associations in the general population. The study procedures were previously described in detail.[90,91] Briefly, between 2003 and 2006, 6734 people aged 35 to 75 years were randomly selected from residents of the city of Lausanne, Switzerland, according to the civil register and underwent physical and psychiatric evaluations. Follow-up (FU) evaluations of the cohort were completed between 2009-2014 (FU1), between 2014-2018 (FU2), and between 2018-2021 (FU3). At each psychiatric evaluation,[90,91] which took part approximately one year after the physical evaluation, diagnostic information was elicited using the semi-structured Diagnostic Interview for Genetic Studies (DIGS;[92]) In addition, participants aged 65 years and older, completed a comprehensive cognitive assessment at FU1, FU2, and FU3. For the present study, FU1 was used as the first assessment (Time-point 0 (TP0)), the subsequent evaluation as the 5-year follow-up (Time-point 1 (TP1), $4{\cdot}66 \pm 0{\cdot}70$ years), and FU3 as the 8-year follow-up (Time-point 2 (TP2), $8{\cdot}36 \pm 0{\cdot}69$ years).

Study sample

For the present analysis, we selected the participants aged 65 years and older at TP0 who performed the cognitive test at TP0 and TP2 and for which plasma samples (see below) at TP0 were available. Participants with a Clinical Dementia Rating (CDR,[93]) score ≥ 1 were excluded. This resulted in a total of 246 participants (153 females and 93 males). Of them, ninety-two percent were Caucasians. Individuals with a CDR score equal to 0 at TP0 (n=137) were considered cognitively healthy, whereas those with CDR = 0·5 at TP0 (n=106) were considered mildly impaired. At TP2, Mini Mental State Examination (MMSE,[94]) data of 245 participants and CDR and Clinical Dementia Rating Sum of Boxes (CDR-SoB) of 246 participants were available.

METHOD DETAILS

Cognitive measurements

A detailed neuropsychological assessment was performed at TP0 and TP2.[95] One year prior to TP0, the global cognitive performance score (MMSE) was derived from the original CoLaus survey. The cognitive test battery included assessments of verbal fluency with the DO40 picture-naming test, letter (phonemic) and category (semantic) fluency tasks, executive function with the Stroop test,[96] memory performance with the Grober and Buschke Double Memory Test,[97] and visuospatial construction with figures from the Consortium to Establish a Registry for Alzheimer's Disease neuropsychological test battery.[98] Overall cognitive and functional status was assessed using CDR and CDR-SoB scores.

Biological data

As described previously,[67,70,99,100] at TP0 and at TP1 plasma concentrations of cholesterol, cholesterol precursors (desmosterol, lanosterol, dihydro-lanosterol, lathosterol), the enzymatic metabolites of cholesterol (oxysterols: 24S-OHC, 7α-OHC, and 27-OHC), 5α-cholestanol, and phytosterols (campesterol, 5α-campestanol, stigmasterol, sitosterol, 5α-sitostanol, brassicasterol) were measured by combined gas chromatography-flame ionization mass spectrometry. At TP0, plasma concentrations of triglycerides and HDL-cholesterol were measured on fresh blood samples in the University Hospital Clinical Laboratory (CHUV, Lausanne, Switzerland) using standard analyses.[90] Fasting venous blood samples were centrifuged at 4°C aliquoted and frozen at -80°C before analysis.

The APOE haplotypes were determined using nuclear DNA extracted from whole blood obtained from all participants using the Affymetrix Axiom SNP array. Haplotypes were called using BRLMM "http://www.affymetrix.com/support/technical/whitepapers/brlmm_whitepap". We analyzed the alleles of two single nucleotide polymorphisms of the APOE gene, rs429358 and rs7412 and defined six haplotypes (e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4. Individuals with at least one e4 allele were considered carriers.

Ethics

The CoLaus/PsyColaus study was approved by the Ethics Committee of the Vaud Canton. All participants signed a written informed consent after having received a detailed description of the goal and funding of the study.

Role of funders

Funding sources had no role in the conduct or reporting of the research.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS software (Version 29.0.0.0). T-tests and Mann-Whitney U-Tests for continuous variables and Chi-Square tests for categorical variables were used to perform group comparisons between the CDR = 0 and CDR = 0·5 groups in the study sample. Normality was assessed using the Shapiro-Wilk test. The same group comparisons between participants in the present study and those in the CoLaus/PsyCoLaus without 10-year follow-up visits (n=683; considered as drop-outs) were performed to determine if a survivor bias was present in the study cohort. Concentrations of phytosterols and endogenous sterols (see below) at TP0 or their changes over four years (TP1-TP0) were associated with cognitive decline over time (TP2-TP0). We considered lipids that can derive from endogenous metabolic processes (endogenous sterols) which include cholesterol, triglycerides, HDL-cholesterol, lathosterol, desmosterol, lanosterol, 5α-cholestanol, dihydrolanosterol, 7α-OHC, 24S-OHC, 27-OHC and plant-derived sterols (phytosterols) including campesterol, stigmasterol, 5α-campestanol, 5α-sitostanol, sitosterol and brassicasterol separately. In these logistic regression analyses, performed in all participants, we considered the following confounders: APOEe4 carrier status, age, sex, statin treatment, diabetes, hypertension, body mass index (BMI), major depressive disorder (MDD) and years of education. To specifically address the effects of sex and of the APOEe4 carrier status, these confounders were entered into the models before considering other confounders and sterols concentrations in separate regression models. The analyses were also performed in males and females independently; in this case, we considered the same confounders without sex. Considering the limited statistical power, we opted not to conduct separate analyses in the APOEe4 carriers and non-carriers. In addition, we performed logistic regression with a backward selection method based on the significance of the score statistic to select the sterols most significantly associated with cognitive decline over time. This stepwise approach was applied in all the above models. A decline in global cognition was defined as $\Delta MMSE \geq 2$ between T0 and T2; decline in cognitive and functional performance was defined as $\Delta CDR\text{-}SoB \geq 0{\cdot}5$ or $\Delta CDR > 0$. The interactions between sex, circulating sterols at TP0, and cognitive decline at TP2 were further tested by creating interaction variables for all considered sterols as follows: sex (1= Male, 2=Female) × circulating sterol measurement. These interaction variables were then added to the regression models described above for the whole cohort. This approach was repeated for interactions between the APOEe4 carrier status and circulating sterols at TP0 and cognitive decline at TP2. To account for lipoprotein dependency for transport of non-cholesterol sterols in circulating plasma, we further normalized all non-cholesterol sterol levels according to total cholesterol levels at both TP0 and TP1, as measured using gas chromatography-mass spectrometry-selected ion monitoring and–flame ionization detection as described above. The regression models were repeated with these normalized values and the original cholesterol, HDL-cholesterol, and triglyceride levels.