Higher thyroid function is associated with accelerated hippocampal volume loss in Alzheimer’s disease

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ABSTRACT

Background: In epidemiological studies, higher thyroid hormone (TH) levels have been associated with lower brain volume and increased risk of Alzheimer’s disease (AD) in elderly individuals. However, the relationships between serum THs and hippocampal atrophy rates have previously not been investigated.

Methods: A prospective study of patients with AD (n = 55), stable mild cognitive impairment (sMCI; n = 84) and healthy controls (n = 29) recruited at a single memory clinic. We investigated whether serum THs were associated with magnetic resonance imaging (MRI)-estimated hippocampal volumes at baseline and with longitudinal alterations, defined as annualized percent changes.

Results: Serum levels of free triiodothyronine (FT3) and FT3/free thyroxine (FT4) ratio were reduced in AD and sMCI patients compared with the controls (p < 0.05). Hierarchical linear regression analyses showed that higher serum FT3/FT4 ratio was associated with greater baseline hippocampal volume in all study groups. Only in AD patients, higher serum FT4 was associated with lower baseline volume of the left hippocampus. Finally, exclusively in the AD group, higher serum levels of FT3 and FT3/FT4 ratio, and lower serum TSH levels, were associated with greater annual hippocampal volume loss.

Conclusions: In all study groups, FT3/FT4 ratio was related to baseline hippocampal volume. However, only in AD patients, higher levels of THs were associated with greater annual loss of hippocampal volume, suggesting that excessive TH levels exert a deleterious effect on the hippocampus in the presence of existing AD neuropathology.

1. Introduction

Thyroid hormones (THs) are pivotal for the function of many organs including the brain. During aging, serum levels of thyroid-stimulating hormone (TSH) and the bioactive triiodothyronine (T3) tend to decline, while free levels of thyroxine (T4), often viewed as a pro-hormone to T3, are maintained (Boelaert, 2013). The importance of TSH and the bioactive triiodothyronine (T3) tend to decline, while free levels of thyroxine (T4) tend to increase, often viewed as a pro-hormone (TSH) and the bioactive triiodothyronine (T3) tend to increase, often viewed as a pro-
progression to manifest AD (Quinlan et al., 2019). Moreover, there is scarce data whether THs contribute to hippocampal atrophy in healthy elderly individuals or AD patients. Hippocampal atrophy is an early neuropathological hallmark of AD (Albert et al., 2018). Through a dense distribution of TH receptors, and high deiodinase activity, THs promote neurogenesis, myelination, plasticity and neuroprotection in the hippocampus (Dratman et al., 1983; Greenberg et al., 2006; Lin et al., 2011; Remaud et al., 2014). THs are crucial for hippocampal development, and deficiency of THs result in decreased hippocampal growth (Wheeler et al., 2011). However, in elderly individuals, one study showed that high-normal serum FT4 was associated with smaller hippocampal volume (de Jong et al., 2006). Another cross-sectional study did not find any relation between THs and hippocampal volume in AD patients (Quinlan et al., 2020).

In summary, the majority of epidemiological studies suggest that high-normal thyroid function is associated with increased risk of AD. To our knowledge, only one cross-sectional study has previously evaluated the relationship between THs and hippocampal volume specifically in AD patients. In the present study, we therefore investigated the associations between serum THs and baseline levels as well as longitudinal changes in magnetic resonance imaging (MRI)-estimated hippocampal volumes. We included AD patients, patients with stable MCI (sMCI), and healthy controls recruited at a single memory clinic.

2. Material and methods

2.1. Study participants

Patients and controls were recruited from the longitudinal Gothenburg MCI study performed at the memory clinic at Sahlgrenska University Hospital (Wallin et al., 2016). All subjects underwent a thorough baseline investigation including medical history and physical, radiological, neurological and psychiatric examinations, and were then followed biannually. Inclusion criteria comprised age > 40 and < 79 years, Mini Mental State Examination (MMSE) score > 19, and self- or informant-reported cognitive decline with a duration > 6 months. The exclusion criteria were designed to prevent the enrollment of patients with somatic and psychiatric disorders that could cause cognitive impairment (Wallin et al., 2016). Therefore, patients with subdural hemorrhage, malignant disease including brain tumor, encephalitis, and unstable heart disease were excluded as well as patients with major affective disorder, schizophrenia, substance abuse, and confusion. Additional exclusion criteria in our study were a non-AD dementia diagnosis, diabetes mellitus, and thyroid disease. We excluded participants receiving treatment at baseline or during the study period with levothyroxine or other medications altering TH levels such as amiodarone, lithium, and thyreostatics. Healthy controls without present, or past, history of, cognitive impairment were recruited through senior citizen diarone, lithium, and thyreostatics. Healthy controls without present, or past, history of, cognitive impairment were recruited through senior citizen habits and hypertension was evaluated at each visit by a specialist physician.

2.2. Ethical considerations

The study was approved by the ethical committee at University of Gothenburg. Oral and written informed consent was obtained from all participants. The study was conducted according to the Declaration of Helsinki.

2.3. Diagnostic procedures

For quantification of cognitive impairment, the global deterioration scale (GDS) was used (Reisberg et al., 1982). GDS stage 1 corresponds to no subjective or objective cognitive decline. GDS stage 3 is defined as MCI and GDS stage 4 equals mild dementia (Reisberg et al., 1982). The GDS classification was based on the medical history (self-reported and medical record review) and assessment of cognitive symptoms including the cognitive variables 13–20 of the Stepwise Comparative Status Analysis (STEP) (Wallin et al., 1996); I-FLEX, a short form of the Executive Interview (EXIT) (Royall et al., 1992); MMSE (Folstein et al., 1975); and the Clinical Dementia Rating Scale (CDR) (Morris, 1997). The CDR rating was based on information provided by the participant and an informant. Healthy controls had to meet the algorithm for GDS 1: STEP = 0, I-FLEX = 0, CDR ≤ 0.5, MMSE ≥ 27. The algorithm for GDS 3 was: STEP ≤ 1; I-FLEX ≤ 3; CDR > 0.5; MMSE ≥ 26. However, for the final GDS stage classification, a consensus decision was made by the specialized physicians at the memory clinic.

MCI patients (GDS = 3) who did not convert to dementia during the follow-up were classified as stable MCI (sMCI). For GDS stage 4, the dementia subtypes were diagnosed by specialized physicians who had access to clinical information and MRI data, but were blinded to the results of imaging volumetry/rating scales, cerebrospinal fluid (CSF) biomarkers, and neuropsychological tests. AD was diagnosed according to The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). Patients (n = 22) who fulfilled the AD criteria, but also had concomitant MRI findings of cerebral white matter hyperintensities (WMHs) were included in the AD group as vascular contribution is common in AD (Lo and Jagust, 2012). Totally, we included 84 sMCI patients, 55 AD patients (17 of these had MCI at baseline but converted to AD during the study period), and 29 healthy controls.

2.4. Assessment of covariates

Body weight was recorded to the nearest 0.5 kg, and body height was measured to the nearest 0.5 centimetres. Body mass index (BMI) was calculated as kilograms per meter squared (kg/m²). Medication, smoking habits and hypertension was evaluated at each visit by a specialist physician.

2.5. MRI-estimated hippocampal volumes

For the magnetic resonance data acquisition, a 1.5 tesla MRI scanner (Siemens Symphony, Erlangen, Germany) was used. The imaging protocol and sequence as well as volumetric procedures have been described previously (Eckerstrom et al., 2018). T1-weighted brain images were analyzed using the FreeSurfer automated segmentation software (version 5.3.0; https://surfer.nmr.mgh.harvard.edu/) to estimate intracranial volume (ICV) and left and right hippocampal volumes. To reduce head size variability, hippocampal volumes for each individual were corrected for ICV using the residual normalization method (Voedovskaya et al., 2014). First, a regression analysis was performed in the control group between the region of interest raw volume and the ICV to obtain the regression coefficient β. Then, the regression coefficient β was applied to the entire study sample, and adjusted hippocampal volumes (in cm³) were calculated according to the formula: Volume_adjusted i = Volume_raw i − β(ICV_raw i − ICV_mean).

Of the 139 patients (sMCI, n = 84; AD, n = 55) and 29 healthy controls, 73 patients (sMCI, n = 47; AD, n = 26) and 13 controls had at least one follow-up MRI. Baseline characteristics were similar in participants with or without follow-up MRI (not shown). The mean follow-up time was 3.5 (SD 1.9) years. The annualized percent change for hippocampal volumes was computed as: the volume at the last available follow-up time was 3.5 (SD 1.9) years. The annualized percent change for hippocampal volumes was computed as: the volume at the last available MRI scan minus the volume at the baseline MRI scan divided by the volume at the baseline MRI scan divided by the duration (years) between the measurements (x100).

2.6. Biochemical methods

Blood was drawn in the fasted state between 8AM and 10AM and then stored at – 80 °C pending biochemical analyses. Serum levels of TSH, FT4, and FT3 were determined at one occasion in 2015 using...
Elecys electrochemiluminescent immunoassays on a Cobas 8000 instrument (Roche Diagnostics Scandinavia AB, Stockholm, Sweden). All analyses were performed at the central laboratory of Sahlgrenska University Hospital. The reference ranges were: TSH: 0.30–4.2 mIU/L, FT4: 12–22 pmol/L, and FT3: 3.1 – 6.8 pmol/L. Low-density lipoprotein (LDL)-cholesterol was calculated according to Friedewald’s formula (Friedewald et al., 1972) based on routine clinical measurements of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides.

2.7. Statistical analyses

All statistical analyses were performed using SPSS for Windows version 25 (IBM Corp., Armonk, NY, USA). Means and standard deviations (SDs) are presented for continuous variables. Between-group differences were examined using Chi-square tests for categorical data, and for continuous variables, ANOVA was performed followed by post-hoc analyses using Tukey’s honestly significant difference.

We evaluated the associations between serum THs and hippocampal volumes using hierarchical linear regression analyses. All associations are presented after adjustment for age, sex and education. Additional adjustment for baseline hippocampal volume did not change the results (data not shown). Quadratic and cubic terms of serum TSH, FT4, FT3 and FT3/FT4 ratio did not contribute significantly to the model fit (p > 0.05) and were therefore excluded from further analyses, as a linear fit of the data was considered adequate.

In the hierarchical linear regression analyses, we built sequential models to evaluate the independent effect of each TH on hippocampal volume. In the total study population, the first model included a variable for study group membership and covariates. In the next step, a TH variable was added to the model. Finally, we also included a two-way interaction term (TH x study group) in the model. In terms of significant interaction effects, post-hoc analyses were conducted using univariate linear regression models to quantify the associations between THs and outcome variables within each study group. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographical and clinical characteristics

Characteristics of the study groups are shown in Table 1. Compared with sMCI patients and healthy controls, AD patients had higher age, reduced cognitive function (MMSE), lower BMI, and higher frequency of the APOE ε4 allele. sMCI patients had lower MMSE scores and a higher frequency of beta-blocker treatment than the control group. The study groups did not differ in terms of sex, education (years), LDL/HDL ratio, hypertension, or smoking.

3.2. Serum thyroid hormones at baseline

AD and sMCI patients had lower serum FT3 levels and reduced FT3/FT4 ratios compared with the healthy controls, whereas serum TSH and FT4 levels were unchanged (Table 1). None of the study participants had serum TSH or FT3 levels outside the reference range. However, nine participants (AD, n = 4; sMCI, n = 4; controls, n = 1) had serum FT4 levels of 11 pmol/L, just below the lower limit of the reference range. Two sMCI patients had mildly elevated serum FT4 (23 and 25 pmol/L, respectively). Furthermore, none of the participants showed clinical signs of thyroid disease. Therefore, all subjects were regarded as euthyroid.

In the total study population as well as in the individual study groups, carriers of the APOE ε4 allele had similar serum levels of TSH, FT4, FT3 and FT3/FT4 ratio compared with non-carriers (not shown).

Table 1

| Variable                  | Controls (n = 29) | Stable MCI (n = 84) | AD (n = 55) | p-value |
|---------------------------|------------------|---------------------|-------------|---------|
| Age (years)               | 61 (6.9)         | 64 (8.5)            | 68 (7.2)    | < 0.001 |
| Men/women, n (%)          | 13/16 (45/55)    | 32/52 (38/62)       | 26/29 (47/53)| 0.610   |
| Education (years)         | 13.1 (3.0)       | 13.4 (3.3)          | 13.0 (3.9)  | 0.739   |
| MMSE score                | 29.2 (1.0)       | 28.5 (1.5)          | 25.8 (2.9)  | < 0.001 |
| Clinical variables        |                  |                     |             |         |
| BMI (kg/m²)               | 25.3 (2.6)       | 25.3 (4.1)          | 23.7 (2.8)  | 0.023   |
| LDL/HDL ratio (mmol/L)    | 2.1 (0.8)        | 2.1 (0.9)           | 1.9 (0.6)   | 0.305   |
| Hypertension, n (%)       | 4 (18.2)         | 29 (23.5)           | 6 (11.3)    | 0.142   |
| Current smoking, n (%)    | 1 (4.5)          | 4 (4.8)             | 6 (11.1)    | 0.295   |
| Beta-blocker treatment, n (%) | 1 (3.4) | 19 (22.6)      | 7 (12.7)    | 0.047   |
| APOE ε4 allele (0/1/2; n, %) | 13/8/1 (59/32/5) | 43/28/5 (57/37/6) | 15/24/12 (29/47/24) | < 0.001 |
| Thyroid hormones          |                  |                     |             |         |
| TSH (mIU/L)               | 2.2 (0.7)        | 1.9 (0.8)           | 2.0 (0.8)   | 0.184   |
| FT4 (pmol/L)              | 15.9 (1.7)       | 15.8 (2.3)          | 16.0 (2.1)  | 0.878   |
| FT3 (pmol/L)              | 5.3 (0.6)        | 4.9 (0.6)           | 4.9 (0.5)   | 0.002   |
| FT3/FT4 ratio (%)         | 33.7 (4.1)       | 31.5 (4.1)          | 31.1 (4.9)  | 0.025   |

If not stated otherwise, values are given as means (SD). APOE genotyping was not performed in 7 controls, 8 stable MCI and 4 AD patients.

AD = Alzheimer’s disease, APOE ε4 = Apolipoprotein E ε4, BMI = Body mass index, FT3 = Free triiodothyronine, FT4 = Free thyroxine, LDL = Low density lipoprotein, HDL = High density lipoprotein, MCI = Mild cognitive impairment, MMSE = Mini Mental State Examination, TSH = Thyroid-stimulating hormone.

3.3. Baseline levels and annualized changes in hippocampal volumes

As shown in Table 2, AD patients had lower baseline hippocampal volumes than sMCI patients and healthy controls. Furthermore, in AD patients, the annual volume losses of 5.2 (2.9) % and 4.7 (2.3) % for the left and right hippocampus, respectively, were more marked than the corresponding losses in sMCI patients and healthy controls (Table 2). Baseline hippocampal volumes and the rates of annual volume loss were similar in sMCI patients and healthy controls.

Table 2

| Variable                  | Controls | Stable MCI | AD | p-value |
|---------------------------|----------|------------|----|---------|
| Adjusted brain volume (cm³) |          |            |    |         |
| Left hippocampus          |          |            |    |         |
| Baseline                  | 3.81 (0.4)| 3.66 (0.5) | 2.92 (0.6) | < 0.001 |
| Annualized change (%)     | -0.93 (1.1)| -1.54 (2.7)| -5.22 (2.9) | < 0.001 |
| Right hippocampus         |          |            |    |         |
| Baseline                  | 3.95 (0.4)| 3.76 (0.5) | 3.03 (0.6) | < 0.001 |
| Annualized change (%)     | -0.63 (1.0)| -1.63 (2.4)| -4.72 (2.3) | < 0.001 |

Values are presented as means (SD). Baseline magnetic resonance imaging (MRI) scans were available in 29 healthy controls and 139 patients (sMCI, n = 84; AD, n = 55). Of these, 13 controls and 73 patients (sMCI, n = 47; AD, n = 26) had follow-up MRI scans. Hippocampal volumes were adjusted for intracranial volume.

a p < 0.001 vs controls.

b p < 0.001 vs stable MCI.
were included in the analyses, the p-values for interaction were not significant (p > 0.05). There was no overall association between serum FT4 levels and hippocampal volumes in the entire study population. However, a significant interaction (serum FT4 x study group) was found (p = 0.04). We therefore performed post-hoc analyses, which demonstrated that higher serum FT4 was associated with lower baseline left hippocampal volume in AD patients (β = −0.311, 95% CI = −0.17 to −0.02, p = 0.02), but not in sMCI patients or healthy controls.

Serum FT3/FT4 ratios were associated with greater baseline volumes of the left (β = 0.17, 95% CI = 0.01 – 0.04, p = 0.004) and right hippocampus (β = 0.13, 95% CI = 0.00 – 0.04, p = 0.03). Both these associations were present in all study groups as the p-values for interaction were not significant (serum FT3/FT4 ratio x study group, p > 0.05).

3.5. Thyroid hormones and annualized changes in hippocampal volumes

In the total cohort, there was no overall association between serum TSH and the annualized percent changes in hippocampal volumes. However, in terms of left hippocampus annual decline, there was a significant interaction (serum TSH x study group, p = 0.04). Post-hoc analyses showed, in AD patients but not in sMCI patients or healthy controls, that serum TSH was associated with the annualized percent change in the left hippocampus (β = 0.45, 95% CI = 0.20 – 3.49, p = 0.03) (Table 3). Thus, only in the AD group, lower serum TSH was associated with greater annual loss of the left hippocampus (Table 3 and Fig. 1).

Higher serum FT3 levels were associated with larger annual volume loss of the left hippocampus (β = −0.22, 95% CI = −2.68 to −0.72, p = 0.01), but not of the right hippocampus in the total population. However, significant interactions (serum FT3 x study group) were found for the associations with the annualized percent change of the left (p = 0.03) and right (p = 0.04) hippocampus. Subsequent post-hoc analyses showed that only in AD patients, higher serum FT3 levels were associated with greater annual volume loss of the left and right hippocampus (Table 3). For each serum FT3 pmol/L increment, the annual volume loss was increased by 3.5% and 2.2%, respectively (Table 3 and Fig. 1). In contrast, serum FT4 levels were not associated with the annualized hippocampal volume decline and there were no significant interaction effects.

In the total population, serum FT3/FT4 ratios were not associated with the annualized decline in hippocampal volumes. However, there was a significant interaction effect (serum FT3/FT4 ratio x study group) on the annualized volume change of the left (p = 0.03) and right (p = 0.05) hippocampus. In the post-hoc analyses, in AD patients, higher serum FT3/FT4 ratio was related to greater annual volume loss of the left, but not of the right hippocampus (Table 3 and Fig. 1).

4. Discussion

The present study is the first one that has evaluated the association between serum TH levels and longitudinal changes in MRI-estimated hippocampal volumes. Only in the AD group, lower serum TSH and higher serum levels of FT3 and FT3/FT4 ratio were associated with greater annual loss of hippocampal volumes. In AD patients, the annual loss of left hippocampal volume was 3.5% greater per pmol/L increase in serum FT3. This corresponds to a ~9% annual loss of left hippocampal volume in AD patients having high-normal serum FT3 (6.1 pmol/L) vs. ~1.5% for AD patients with low-normal serum FT3 (3.7 pmol/L). Similar effects sizes were observed for the association between serum TSH and FT3/FT4 ratio and the annual change in left hippocampal volume (Fig. 1 and Table 3). Thus, specifically in AD patients, high-normal thyroid function was associated with accelerated annual loss of hippocampal volume.

We studied a well-defined euthyroid population of patients with AD or sMCI as well as healthy controls at a single memory clinic. In MCI, the risk of conversion to manifest dementia is increased, and MCI is often regarded as a transitory state between early disease stages and manifest dementia. However, MCI may be a heterogeneous condition and we included sMCI patients to investigate a study group with cognitive impairment that do not convert to manifest dementia. We found that the associations between serum THs and hippocampal volumes are, at least partly, different in manifest AD compared with both the sMCI patients with cognitive impairment and the cognitively healthy controls. Speculatively, this could support that the associations found only in AD patients are due to interactions between THs and AD neuropathology and not due to cognitive impairment in general.

The cross-sectional analyses in our study showed, also exclusively in AD patients, that serum FT4 levels were related with lower left hippocampal volume at baseline. In the only previous study in manifest AD, serum TH levels did not associate with hippocampal volumes in a relatively small group of AD patients (Quinlan et al., 2020). Two previous epidemiological studies found inverse associations between serum FT4 and total brain volumes (Chaker et al., 2018) or hippocampal volumes (de Jong et al., 2006) in elderly populations. Thus, high-normal serum FT4 may have adverse effects on brain morphology in the elderly, and the results of the present study additionally suggest that high serum FT4 is more detrimental in AD patients than in non-demented individuals.

In some contrast, a higher FT3/FT4 ratio, which reflects increased peripheral conversion of FT4 to the bioactive FT3, was associated with greater baseline left and right hippocampal volumes in the total cohort. In sMCI patients and healthy controls, this was the only measure of thyroid function that was associated with hippocampal volumes. In some agreement, higher serum TT3 was associated with higher hippocampal volumes in the only previous study in manifest AD (Quinlan et al., 2020). Two previous epidemiological studies found inverse associations between serum FT4 and total brain volumes (Chaker et al., 2018) or hippocampal volumes (de Jong et al., 2006) in elderly populations. Thus, high-normal serum FT4 may have adverse effects on brain morphology in the elderly, and the results of the present study additionally suggest that high serum FT4 is more detrimental in AD patients than in non-demented individuals.

In some contrast, a higher FT3/FT4 ratio, which reflects increased peripheral conversion of FT4 to the bioactive FT3, was associated with greater baseline left and right hippocampal volumes in the total cohort. In sMCI patients and healthy controls, this was the only measure of thyroid function that was associated with hippocampal volumes. In some agreement, higher serum TT3 was associated with higher hippocampal volumes in healthy controls in a cross-sectional study (Quinlan et al., 2020). Moreover, independent of serum T3 levels, higher serum FT3/FT4 ratio provided protection from physical and cognitive decline as well as mortality in patients admitted to a geriatric ward (Pasqualetti et al., 2018). The importance of the peripheral conversion of T4 to T3 may be further supported by the finding that carriers of a common polymorphism (D2-Thr92Ala) in the DIO2 gene, which encodes the T4 to...
T3 converting type 2 deiodinase (D2) enzyme, showed transcriptional alterations associated with neurodegeneration (McAninch et al., 2015). However, polymorphisms in the DIO1 (encoding type 1 deiodinase) and DIO2 genes did not associate with hippocampal volume in the Rotter-dam Scan Study (de Jong et al., 2007). Thus, the significance of the peripheral conversion of T4 to T3 is still unclear and needs to be clarified in further studies.

At baseline, we found reduced serum levels of FT3 and FT3/FT4 ratio in AD and sMCI, whereas serum TSH and FT4 were unchanged compared with healthy controls. In previous cross-sectional studies of manifest AD, serum levels of FT4 and TSH have been variable, whereas serum FT3 or TT3 levels have been unchanged or even decreased (Chang et al., 2018; Chiaravalloti et al., 2017; Johansson et al., 2013; Nomoto et al., 2019; Quinlan et al., 2020; Thomas et al., 1987). Thus, our finding of reduced serum FT3 at baseline with unchanged levels of other THs are in line with several, but not all, earlier studies of manifest AD. Furthermore, serum FT3 and FT3/FT4 ratio were decreased also in the sMCI patients who did not convert to dementia. Although the cause of these reductions in FT3 and FT3/FT4 ratio is unclear, it cannot be excluded that they are protective mechanisms in order to alleviate potentially harmful effects of THs on the brain.

In the present study, higher thyroid function was associated with greater annual loss of hippocampal volume only in the AD group. It could therefore be speculated that THs interact with AD neuropathological changes. In support of this, excessive TH levels increase the production of reactive oxygen species, which may contribute to neurodegeneration in AD (Aslan et al., 2011; Llanos-González et al., 2020). Moreover, greater exposure to THs could induce aberrant phosphorylation of tau (Lovell et al., 2004) and adversely affect neuroplasticity in the adult AD hippocampus (Taskin et al., 2011). THs may also affect the survival of hippocampal neurons by regulating glutamate release during Aβ induced excitotoxicity (Shuaib et al., 1994; Yeung et al., 2020). Thus, experimental and postmortem data provide further evidence that THs could aggravate hippocampal neurodegeneration by interactions with AD-associated neuropathological alterations.

Another mechanism underlying that higher thyroid function was associated with greater annual loss of hippocampal volume only in the AD group could be the co-existence of limbic-predominant age-related TDP-43 encephalopathy (LATE). In LATE, the SLC01A2/IAPP and ABCC9 risk associated genotypes have been related to TH dysregulation (Nelson et al., 2019b, 2016). LATE neuropathology is often present in the aged AD brain,
and has been associated with aggravation of AD-related hippocampal atrophy (Josephs et al., 2017). We cannot determine the degree of LATE comorbidity in our AD group due to the lack of autopsy confirmation. Furthermore, we did not determine genetic risk variants (e.g. polymorphisms in the TMEM106B, GRN, and ABCC9 genes) to account for concomitant LATE or hippocampal sclerosis. However, the maximum baseline age in our AD group was 78 years, whereas LATE is mainly seen above 80 years of age (Nelson et al., 2019a). Therefore, co-existing LATE likely has greater impact in AD patients that are older than those included in our study.

Strengths of the present study include the well-characterized euthyroid and non-diabetic study population, the mono-center design, and that longitudinal investigations of MRI-estimated hippocampal volumes were available in part of the study population. Furthermore, the annual loss of hippocampus volume of ~5% in the AD group was, as expected, greater than in the other study groups and approximately similar as that previously reported (Barnes et al., 2009). All blood samples were analyzed at one occasion (in 2015) and included measurements of TSH, FT4 as well as FT3. However, as thyroid function was assessed only at baseline, we could not evaluate if there were changes in TH levels across the study period. Also, as the relationship between THs in the circulation and the central nervous system is not well defined, further studies are needed to determine the impact of brain TH levels on hippocampal atrophy in AD. Moreover, our sample size was relatively small in the longitudinal analyses, which could have resulted in limited statistical power. Finally, we cannot evaluate whether the observed associations between serum THs and hippocampal volumes are causal.

5. Conclusion
In this mono-center-study, our main finding is that the associations between serum THs and hippocampal volumes are different in manifest AD compared with sMCI patients and cognitively healthy controls. Exclusively in AD patients, higher serum FT4 was associated with lower baseline volume of the left hippocampus. Furthermore, only in AD patients, lower serum TSH and higher serum levels of FT3 and FT3/FT4 ratio were associated with larger annual losses of hippocampal volumes. This suggests that higher thyroid function exerts detrimental effects on the hippocampus in manifest AD, and it could be speculated that THs interact with established disease mechanisms. In contrast, in sMCI patients and healthy controls, the role of THs is less clear and needs to be explored in more detail in further studies.

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All authors contributed to the design of the study and the collection of data. P.Q. and J.S. performed the statistical analyses and wrote a first draft manuscript. All authors read the manuscript and contributed to the finalizing of the manuscript. All authors have approved the final article.

Conflict of interest
There is nothing to disclose. None of the authors has any conflict of interest.

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References
Albert, M., Zhu, Y., Moghobak, A., Mori, S., Müller, M.I., Soldan, A., Pettigrew, C., Selnes, O., Li, S., Wang, M.-C., 2018. Predicting progression from normal cognition to mild cognitive impairment for individuals at 5 years. Brain 141, 877–887.
Aadan, M., Cosar, N., Celik, H., Aksog, N., Bulger, A.C., Begnink, H., Soyolar, Y.U., Kurucakoglu, M.E., Selik, S., 2011. Evaluation of oxidative status in patients with hyperthyroidism. Endocrine 40, 285–289.
Barnes, J., Bartlett, J.W., van de Pol, L.A., Ley, C.T., Scabill, R.L., Frost, C., Thompson, P., Fox, N.C., 2009. A meta-analysis of hippocampal atrophy rates in Alzheimer’s disease. Neurobiol. Aging 30, 1711–1723.
Boelart, K., 2013. Thyroid dysfunction in the elderly. Nat. Rev. Endocrinol. 9, 194–204.
Cappola, A.R., Arnold, A.M., Wulczyn, K., Carlson, M., Robbins, J., Peit, B.M., 2015. Thyroid function in the euthyroid range and adverse outcomes in older adults. J. Clin. Endocrinol. Metab. 100, 1088–1096.
Chaker, L., Cremer, L.G.M., Korevaar, T.L.M., de Groot, M., Dehghan, A., Franco, O.H., Nielsen, W.J., Ibram, M.A., Peeters, R.P., Vernooij, M.W., 2018. Age-dependent association of thyroid function with brain morphology and microstructural organization: evidence from brain imaging. Neurobiol. Aging 61, 44–51.
Chaker, L., Wolters, F.J., Bos, D., Korevaar, T.L., Hofman, A., van der Lugt, A., Koudstaal, P.J., Franco, O.H., Dehghan, A., Vernooij, M.W., Peeters, R.P., Ibram, M.A., 2016. Thyroid function and the risk of dementia: The Rotterdam Study. Neurology 87, 1688–1695.
Chang, Y.S., Wu, Y.H., Wang, C.J., Tang, S.H., Chen, H.L., 2018. Higher levels of thyroxine may predict a favorable response to donepezil treatment in patients with Alzheimer disease: a prospective, case-control study. BMC Neurosci. 19, 36.
Chiaravalloti, A., Ursini, F., Fiorentini, A., Barbagallo, G., Mortarana, A., Koch, G., Tavolozza, M., Schillaci, O., 2017. Functional correlates of TSH, FT3 and FT4 in Alzheimer disease: A F-18 FDG PET/CT study. Sci. Rep. 7, 6220.
de Jong, F.J., den Heijer, T., Visser, T.J., de Rijke, Y.B., Deexhage, H.A., Hofman, A., Breteler, M.M., 2006. Thyroid hormones, dementia, and atrophy of the medial temporal lobe. J. Clin. Endocrinol. Metab. 91, 2569–2573.
de Jong, F.J., Masaki, K., Chen, H., Remaley, A.T., Breteler, M.M., Petrovitch, H., Whit, L.R., Launder, L.J., 2009. Thyroid function, the risk of dementia and neurodegenerative changes: the Honolulu-Asia aging study. Neurobiol. Aging. 30, 600–606.
de Jong, F.J., Peeters, R.P., den Heijer, T., van der Deure, W.M., Hofman, A., Uitterlinden, A.G., Visser, T.J., Breteler, M.M., 2007. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. J. Clin. Endocrinol. Metab. 92, 636–640.
Draught, M.B., Crutchfield, F.L., Gordon, J.T., Jennings, A.S., 1983. Iodothyronine homeostasis in rat brain during hypo- and hyperthyroidism. Am. J. Physiol. 245, E185–E193.
Eckerstrom, C., Klasson, N., Olsson, E., Selnes, P., Rostad, S., Wallin, A., 2018. Similar pattern of atrophy in early- and late-onset Alzheimer’s disease. Alzheimers Dement (Amst.) 10, 253–259.
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. ‘Mini-mental state’. A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.
Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.
Greenberg, J.H., Revich, M., Gordon, J.T., Schenoff, M.B., Patlak, C.S., Draught, M.B., 2006. Imaging triiodothyronine binding kinetics in rat brain: a model for studies in human subjects. Synapse 60, 212–222.
Gusseklo, J., van Exel, E., de Craen, A.J., Meinders, A.E., Frolich, M., Westendorp, R.G., 2004. Thyroid status, disability and cognitive function, and survival in old age. Jama 292, 2591–2599.
Johansson, P., Almqvist, E.G., Johansson, J.O., Mattsson, N., Hansson, O., Wallin, A., Blennow, K., Zetterberg, H., Svensson, J., 2013. Reduced cerebrospinal fluid level of thyroxine in patients with Alzheimer’s disease. Psychoneuroendocrinology 38, 1058–1066.
Josephs, K.A., Dickson, D.W., Tosakulwong, N., Tamas, J., Murray, M.E., Petrucelli, L., Liesting, A.M., Senjou, M.L., Spychalla, A.J., Knopman, D.S., Parisi, J.E., Petersen, R.C., Jack, Jr., C.R., Whitwell, J.L., 2017. Rates of hippocampal atrophy and presence of post-mortem TDP-43 in patients with Alzheimer’s disease: a longitudinal retrospective study. Lancet Neurol. 16, 917–924.
Lin, H.Y., Davis, F.B., Luidens, M.K., Mousa, S.A., Cao, J.H., Zhou, M., Davis, P.J., 2011. Molecular basis for certain neuroprotective effects of thyroid hormones. Front Mol. Neurosci. 4, 29.
Llanos-González, E., Henares-Chavarino, Á.A., Pedrero-Prieto, C.M., García-Carpintero, S., Frondoso-Rubio, J., Sancho-Bielso, F.J., Alcain, F.J., Sanz-Taberner, J.R., Rabanal-Ruiz, Y., Durán-Prado, M., 2020. Interplay between mitochondrial oxidative disorders and proteinopathy in Alzheimer’s Disease. Front. Neurosci. 13.
Lo, R.Y., Jagust, W.J., 2012. Vascular burden and Alzheimer disease pathologic progression. Neurology 79, 1349–1355.
Lovell, M.A., Xiong, S., Xie, C., Davies, P., Markenberg, W.R., 2004. Induction of hyperphenylhydrated tau in primary rat cortical neuron cultures mediated by oxidative stress and glycogen synthase kinase-3. J. Alzheimer’s Dis. 6, 659–681.

McAninch, E.A., Jo, S., Preite, N.Z., Farkas, E., Mohasck, P., Fekete, C., Egri, P., Gereben, B., Li, Y., Deng, Y., Patil, M.E., Zevenbergen, C., Peeters, R.P., Mash, D.C., Bianco, A.C., 2015. Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. J. Clin. Endocrinol. Metab. 100, 920–933.

McKinnon, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stellar, E.M., 1984. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 34, 939–944.

Morris, J.C., 1997. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. Int. Psychogeriatr. 9 (Suppl 1), 173–176 discussion 177–178.

Nelson, P.T., Dickson, D.W., Trojanowski, J.Q., Jack, C.R., Boyle, P.A., Arfanakis, K., Redammers, R., Alafuzoff, I., Attwell, R., Brayne, C., Gilchrist, L.T.S., Chui, H., Fardo, D.W., Flanagan, M.E., Halliday, G., Hokken-Koelega, S.R.K., Hunter, S., Jicha, G.A., Katsurada, Y., Kwas, T.H., Keene, C.D., Kovacs, G.G., Kukull, W.A., Levey, A.E., Makinejad, N., Montine, T.J., Murayama, S., Murray, M.E., Nag, S., Risman, R.A., Seeley, W.W., Sperling, R.B., White, C.L., Yu, Y., Schneider, J.A., 2019a. Limbic–predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain 142, 1503–1527.

Nelson, P.T., Gal, Z., Wang, W.X., Niedowicz, D.M., Artiushin, S.C., Wycoff, S., Wei, A., Jicha, G.A., Fardo, D.W., 2019b. TDP-43 proteinopathy in aging: Associations with risk-associated gene variants and with brain parenchymal thyroid hormone levels. Neurobiol. Dis. 125, 67–76.

Nelson, P.T., Katsurada, T., Nito, K., Artiushin, S.C., Jicha, G.A., Wang, W.X., Aker, E.L., Saykin, A.J., Kukull, W.A., Fardo, D.W., 2016. Genomics and CSF analyses implicate thyroid hormone in hippocampal sclerosis of aging. Acta Neuropathol. 132, 841–858.

Nomoto, S., Kinno, R., Uchida, K., Kubota, S., Moriyama, T., Futamura, A., Sugimoto, A., Kuroda, T., Yano, S., Murakami, H., Shirasawa, T., Yoshimoto, T., Mineura, A., Okazaki, A., Ono, K., 2019. The relationship between thyroid function and cerebral survival in hospitalized older patients. J. Clin. Endocrinol. Metab. 103, 1867–1876.

Quinlan, P., Horvath, A., Eckerstrom, C., Wallin, A., Svensson, J., 2020. Altered thyroid hormone profile in patients with Alzheimer’s disease. Psychoneuroendocrinology 121, 104844.

Quinlan, P., Horvath, A., Wallin, A., Svensson, J., 2019. Low serum concentration of free triiodothyronine (FT3) is associated with increased risk of Alzheimer’s disease. Psychoneuroendocrinology 99, 112–119.

Reisberg, B., Ferris, S.H., de Leon, M.J., Crook, T., 1982. The Global Deterioration Scale for the Assessment of Primary Degenerative Dementia. Am. J. Psychiatry 139, 1139–1143.

Rieben, C., Segna, D., da Costa, B.R., Collet, T.H., Chaker, L., Aubert, C.E., Baumgartner, C., Almeida, O.P., Hogervorst, E., Trompet, S., Masaki, K., Mookerjea, S.P., Gunekkoo, J., Peeters, R.P., Bauer, D.C., Aujeszky, D., Rodondi, N., 2016. Subclinical thyroid dysfunction and the risk of cognitive decline: a meta-analysis of prospective cohort studies. J. Clin. Endocrinol. Metab. 101, 4945–4956.

Royall, D.R., Maharin, R.K., Gray, K.F., 1992. bedside assessment of executive cognitive impairment: the executive interview. J. Am. Geriatr. Soc. 40, 1221–1226.

Shabbi, A., Izaj, S., Hennings, S., Galazka, P., Ishaqay, R., Liu, L., Ruvindran, J., Miyashita, H., 1994. Decreased glutamate release during hypothyroidism may contribute to protection in cerebral ischemia. Exp. Neurol. 128, 260–265.

Taskin, E., Arts, A.S., Bitikas, S., Dolu, N., Liman, N., Suer, C., 2011. Experimentally induced hyperthyroidism disrupts hippocampal long-term potentiation in adult rats. Neuroendocrinology 94, 218–227.

Thomas, D.R., Hallwood, R., Harris, B., Williams, P.A., Scanlon, M.F., John, R., 1987. Thyroid status in senile dementia of the Alzheimer type (SDAT). Acta Psychiatr. Scand. 76, 158–162.

van den Belt, A.W., Kaufman, J.-M., Zilkens, M.C., Lamberts, S.W.J., Egan, J.M., van der Lely, A.J., 2018. The physiology of endocrine systems with ageing. Lancet Diabetes Endocrinol. 6, 647–658.

van Vliet, N.A., van Heemst, D., Almeida, O.P., Årvid, B.O., Aubert, C.E., Bae, J.B., Barnes, L.E., Bauer, D.C., Blauw, G.J., Brayne, C., Cappola, A.R., Ceresini, G., Comijs, H.C., Dartiges, J.F., Degryse, J.M., Dullaart, R.P.F., van Eersel, M.E.A., den Elzen, W.P.J., Ferrucci, L., Fink, H.A., Flicker, L., Grabe, H.J., Han, J.W., Helmer, C., Huisman, M., Ikram, M.A., Imamura, M., de Jongh, R.T., Jukema, J.W., Kim, K.W., Kohlmann, L.H., Lopez, O.L., Miojantaa, S.P., Moon, J.H., Moutzouri, E., Nauck, M., Parle, J., Peeters, R.P., Samuels, M.H., Schmidt, C.O., Schminke, U., Slagboom, P.E., Stordal, E., Vaes, B., Völzke, H., Westendorp, R.G.J., Yamada, M., Yeap, B.B., Rodondi, N., Gunekkoo, J., Trompet, S., 2021. Association of thyroid dysfunction with cognitive function: an individual participant data analysis. JAMA Intern. Med. 181, 1440–1450.

Voevodskaya, O., Simoons, A., Nordenkajold, R., Kullberg, J., Ahlstrom, H., Lind, L., Wahlund, L.O., Larsson, E.M., Westman, E., 2014. The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer’s disease (Alzheimer’s Disease Neuroimaging, I). Front. Aging Neurosci. 6, 264.

Wallin, A., Edman, Å., Blennow, K., Gottfries, C.G., Karlsson, I., Regland, B., Sjögren, M., 1996. Stepwise Comparative Status Analysis (STEP): a tool for identification and classification of regional brain syndromes in dementia. J. Geriatr. Psychiatr. Neurol. 9, 185–199.

Wallin, A., Nordlund, A., Jonsson, M., Lind, K., Edman, A., Gothlin, M., Stallhammar, J., Eckerstrom, M., Kern, S., Bojesson-Hanson, A., Carlsson, M., Olsson, E., Zetterberg, H., Blennow, K., Svensson, J., Ohrfelt, A., Bjerke, M., Rolstad, S., Eckerstrom, C., 2016. The Gothenburg MCI study: design and distribution. Scand. J. Clin. Lab. Invest. 76, 158–163.

Wallin, A., Edman, Å., Blennow, K., Gottfries, C.G., Karlsson, I., Regland, B., Sjögren, M., 1996. Stepwise Comparative Status Analysis (STEP): a tool for identification and classification of regional brain syndromes in dementia. J. Geriatr. Psychiatr. Neurol. 9, 185–199.

Wallin, A., Nordlund, A., Jonsson, M., Lind, K., Edman, A., Gothlin, M., Stallhammar, J., Eckerstrom, M., Kern, S., Bojesson-Hanson, A., Carlsson, M., Olsson, E., Zetterberg, H., Blennow, K., Svensson, J., Ohrfelt, A., Bjerke, M., Rolstad, S., Eckerstrom, C., 2016. The Gothenburg MCI study: design and distribution. Scand. J. Clin. Lab. Invest. 76, 158–163.