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ORIGINAL ARTICLE

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Biological variation in thyroid function tests in older adults and clinical implications

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Thyroidea landsforeningen

Abstract

Objective: Interpreting thyroid function tests can be challenging due to inherent variation, and the need for tests rises with age. While age-related changes in thyrotropin (TSH) levels are known, the biological variation in older adults remains unclear.

Design: We recruited nineteen 65–99-year-old (older adults) without thyroid disease for monthly blood sampling for 1 year.

Patients and Measurements: Serum was stored at −20C°, and TSH, total thyroxine (TT4) and total triiodothyronine (TT3) were analysed in random order in a single batch for each participant. Results were compared to test results from 15 euthyroid men aged 24–53 years (younger adults) collected previously using a similar methodology.

Results: Interindividual coefficients of variation in older/younger adults were 46.7%/44.0% for TSH, 12.7%/19.5% for TT4 and 14.6%/22.4% for TT3. Intraindividual coefficients of variation (CV_I) were 19.0%/25.4% for TSH, 5.5%/10.8% for TT4 and 6.9%/13.2% for TT3. The index of individuality was below 0.6 for all hormones in all age groups. The number of samples required to determine the homoeostatic set-point at 10% precision in older adults was 14–21 for TSH and 2 for TT4 and TT3. TT4 in older adults was the only parameter in any group with comparable CV_I between individuals ($p = .22$).

Conclusions: CV_I for TT4 and TT3 was halved in older compared to younger adults with two tests of TT4 needed to describe the individual set-point. Similar CV_I between older adults caused TT4 to provide a reliable estimate of thyroid function, and the added value of measuring thyroxine could improve clinical practice.

KEYWORDS

biological variation, coefficient of variation, intraindividual variation, older adults, thyroid function

1 | INTRODUCTION

The occurrence of thyroid dysfunction rises with age, and hypothyroidism is seen in up to one in five older adults.[1–3] Diagnosis and monitoring of thyroid dysfunction depend on population-based reference intervals, and slight deviations are classified as subclinical thyroid dysfunction with serum thyrotropin (TSH) outside the reference range and serum thyroxine (T4) and triiodothyronine (T3) within the reference ranges. The prevalence of thyroid dysfunctions varies in older adults across populations depending on iodine intake levels.[4,5] The high prevalence in some populations has led to a debate on whether the upper limit of the reference range for TSH should be raised in old age.[2,6] This stance implies that subclinical hypothyroidism is an age-related physiological condition, which may be supported by the low specificity of symptoms and the lack of treatment effect in this group.[7–9] Still, some progress to overt hypothyroidism, and careful monitoring with repeated thyroid function tests is recommended.[10,11]

The reliability of population-based reference ranges depends on biological variation.[12] Previous studies have consistently shown large interindividual coefficients of variation compared to intraindividual coefficients of variation (CV_I) variation in thyroid hormone tests in young to younger adults,[13–15] suggesting low reliability of population-based reference ranges for the interpretation of test results for an individual. However, the reliability of population-based reference ranges for older adults remain unknown and biological variation has not been reported for this age group.

Previous studies have reported less stable pituitary function and dampened secretion of TSH from the pituitary gland in older adults.[16–18] Thus, it may be hypothesised that variation changes with advancing age. Moreover, an increased CV_I for TSH would cause a more frequent detection of TSH above the upper reference limit in older adults, raising the risk of overdiagnosis and treatment.[19,20]

We generated data on biological variation in thyroid function tests in older adults. These data were used to evaluate the significance of changes in serial testing, the utility of reference ranges and the number of samples needed to settle the individual set-point in older adults. Finally, results were compared with previously collected data in younger adults.

2 | MATERIALS AND METHODS

2.1 | Participants, setting and sampling

We included a population of older adults from volunteers at Aalborg University Hospital, local senior sports associations and local nursing homes in the spring 2019. Participants had to be ≥65 years of age, had no history of past or present thyroid disease, had no intake of thyroid-interfering drugs (including steroid hormones, lithium and amiodarone), systemic oestrogen, had not received contrast containing iodine 6 months prior and were able to give informed consent for participation.

At inclusion, participants were asked to fill in a self-report questionnaire regarding height, weight, smoking status, alcohol consumption, family history of thyroid disease, medical conditions, medication and use of vitamin supplements containing iodine. Frailty was assessed using the Clinical Frailty Scale (CFS).[21]

Participants had blood samples drawn at 4-week intervals for 12 months. Samples were collected between 800 and 1500 h, with sampling around the same time of day for each participant. Community dwellers visited the hospital, while nursing home residents were offered home visits. Urine samples were delivered at the first visit to determine urinary iodine concentration (UIC). Participants were asked to inform study staff when receiving new medication, in case of acute illness, and if receiving iodine-containing contrasts.

2.2 | Laboratory methods

Blood samples were centrifuged at 2200*g* for 10 min, and serum was separated and stored below −20C° until analysis. Samples were subsequently analysed in random order in the same batch for each participant. Serum TSH, total T4 and total T3 were analysed using the Cobas 8000, module e602 (Roche Diagnostics). According to the manufacturer, the intraassay CV was 1.5% for TSH, 1.3% for TT4 and 3.3% for TT3. We analysed the sixth sample for each participant in triplicate to determine analytical variation. The first run was used for determining intraindividual variation. After analyses of TSH, T4 and T3, sample number one and 12 for each participant were transferred to a Kryptor Compact PLUS (BRAHMS) and analysed for thyroid peroxidase antibodies (TPOAb) and TSH-receptor antibodies (TRAb) with cut-offs of 30 (TPOAb) and 1.0 IU/L (TRAb)[22,23]

UIC was determined using the ceri/arsen method after alkaline ashing, as is standard in our laboratory.[24]

2.3 | Comparison with younger adults

We compared the findings among old adults to a previously collected sample from a parallel study of younger adults, previously described in detail.[13] In brief, 15 euthyroid men aged 24–53 years (younger adults) with no known thyroid disease had 12 blood samples collected a month apart. Samples were collected between 0900 and 1200 h. After collecting all samples, they were thawed and analysed for TSH, TT4 and TT3 in random order in the same batch for each participant.

2.4 | Statistical methods

Demographic characteristics were presented with categorical variables as numbers and percentages and continuous variables as means with standard deviation or medians with interquartile range (IQR) as appropriate.

We assumed that samples were independent and compared variances between individuals using Bartlett's test of homogeneity of variance. Individual set points (means) were compared by the Kruskal–Wallis test. Coefficients of variance, index of individuality and number of samples to determine an individual homoeostatic set point were determined using nested analysis of variance (ANOVA).[25] Confidence intervals for coefficients of variance were estimated using the McKay approximation, and reference change values were determined using the asymmetric method.[26] We had no missing data.

The normality of distribution was assessed by the Shapiro–Wilk test at the group and individual levels before conducting nested ANOVA. We examined for trend by individual linear regression, and in the case of trend, participants were excluded from the primary analysis of the measurand. We included all participants regardless of the trend for subgroup analyses due to limited sample sizes in subgroups. Using the Cochran test, we tested for outliers in the replicate analysis of the analytical coefficient of variance. The Cochran test was also used to identify between-individual outliers, while we used the Dixons Q test to identify within-subject outlying values. Outlying samples within individuals were excluded from the analysis, and outlying individuals were excluded when calculating the intraindividual coefficient of variance. A sensitivity analysis was performed including all samples. All tests of assumptions in nested ANOVA used a significance level of 1% due to the number of tests.

In our analysis, we included participants with potential thyroid function-affecting events during the study to mimic daily clinical practice. We presented the developments in individual TSH, TT4 and TT3 indexed to the individual mean graphically to assess any potential impact of potential thyroid function-affecting events.

All analyses were performed using R version 4.0.3 (R Core Team [2020]). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/).

3 | RESULTS

3.1 | Participant and sample characteristics

Participants were 19 adults aged 66 through 99 years at inclusion. We recruited volunteers at Aalborg University Hospital ($n = 11$), at local senior sports associations ($n = 4$) and from local nursing homes ($n = 4$). All Nursing home residents were frail (all, CFS > 5), while none of the volunteers or those from senior sports associations were frail (all, CFS < 3). None of the participants had elevated TRAb at the baseline or conclusion of the study, while two hosted TPOAb throughout the study (Table 1).

All 19 participants completed the study, and all had 12 monthly blood samples drawn, adding up to 228 samples. The median number of days between sampling was 29 (IQR: 28–31). Four participants did not complete the study before the national lockdown due to the COVID-19 pandemic and, therefore, had a 2-month intermission before having their final samples collected.

TABLE 1 Characteristics of the participants.

	N = 19
Age, years—median (IQR)	76 (70, 83)
Sex, women—*n* (%)	13 (68.4%)
UIC, µg/L—median (IQR)	90 (60, 121)
BMI, kg/m^2—mean (SD)	25.0 (3.5)
Smoking—*n* (%)	
Prior	7 (36.8%)
Current	3 (15.8%)
Alcohol, above 7 units/week—*n* (%)	3 (15.8%)
Family history of thyroid disease—*n* (%)	2 (10.5%)
Hypertension—*n* (%)	12 (63.2%)
Diabetes—*n* (%)	2 (10.5%)
Heart disease—*n* (%)	5 (26.3%)
Neurological conditions—*n* (%)	3 (15.8%)
History of cancer—*n* (%)	2 (10.5%)
No. of drugs—median (IQR)	3 (2, 6)
Vitamins containing iodine—*n* (%)	6 (31.6%)
TRAb ≥ 1 IU/L[a]—*n* (%)	0 (0.0%)
TPOAb ≥ 30 IU/L[a]—*n* (%)	7 (36.8%)
TPOAb ≥ 60 IU/L[a]—*n* (%)	2 (10.5%)

Abbreviations: anti-TPO, thyroid peroxidase antibodies; BMI, body mass index; IQR, interquartile range; SD, standard deviation; TRAb, thyrotropin receptor antibodies; UIC, urinary iodine concentration.

[a]TRAb and anti-TPO were measured at the first and last data collection. The number of positives in the table indicates the total number of participants positive at any measurement.

Samples were collected around the same time of the day for all participants, with 12 (5.3%) of 228 samples collected more than 60 min from the individual mean sampling time. The greatest time difference between any two samples for any individual was 180 min, while most participants had a narrower maximum time difference between sampling (median 70 min, IQR 40–97 min).

3.2 | Variation in thyroid function tests in old adults

Figure 1 illustrates unique set-points for each individual for TSH, TT4 and TT3 ($p < .001$ for all variables). The figure also illustrates different magnitudes of individual variation between participants' TSH and TT3, which was confirmed by Bartlett's test for homogeneity of variances (TSH, $p < .001$; TT3, $p = .003$). In contrast, the intraindividual variation around the individual set-points was similar for TT4 ($p = .22$).

Table 2 lists intra- and interindividual variation for TSH, TT3 and TT4, and one individual with subclinical hypothyroidism was excluded

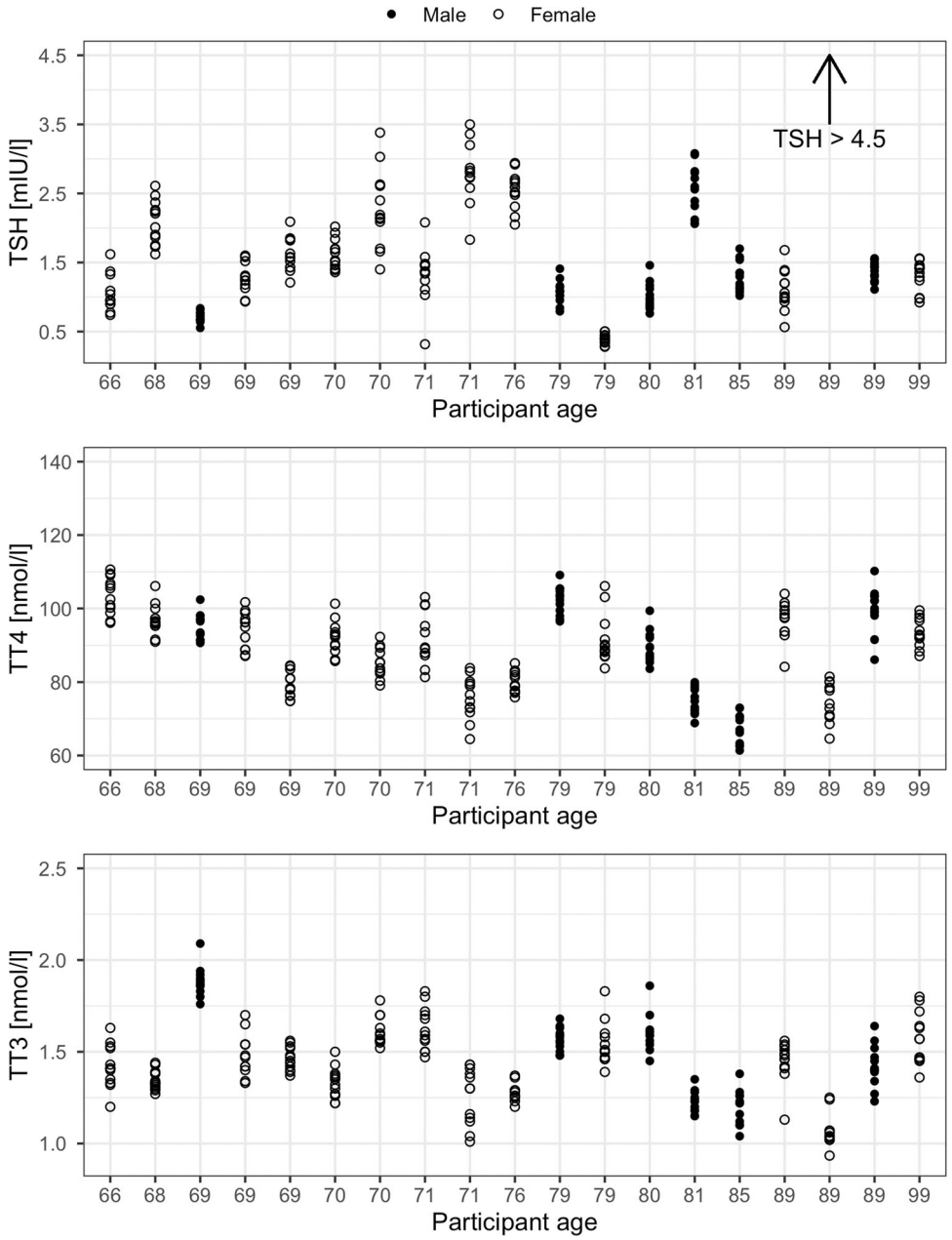

FIGURE 1 Results of 12 monthly thyroid function tests for each participant sorted by age. Values on the *y*-axis were cropped to references ranges to support the presentation of test results and, therefore, TSH measurements for one participant with subclinical hypothyroidism are not displayed (indicated by arrow). TSH, thyrotropin; TT3, total triiodothyronine; TT4, total thyroxine.

from further TSH analyses. TSH results were normally distributed, no individuals had a trend in TSH, and no samples within individuals were outlying. However, three participants had outlying values compared to the entire sample and were excluded from the analysis of interindividual variation of TSH, while included for calculation of intraindividual variation.

TT4 measurements were normally distributed for all individuals, and three were excluded due to a trend in TT4. There were no outliers within or between individuals.

TT3 measurements were normally distributed, and four samples were excluded as outliers. Two individuals were excluded from the analysis due to a trend in TT3. No between-individual outlier was identified.

Intraindividual variation was unaltered for TT4 and 0.8% higher for TT3 in the sensitivity analysis including all data (Supporting Information: Table 1).

3.3 | Results in subgroups

We classified participants into three subgroups: TPOAb positive (above 30 IU/L) at baseline, participants with potential thyroid

TABLE 2 Variation in thyroid function tests in older adults.

	TSH *N* = 18	TT4 *N* = 16	TT3 *N* = 17
Analytical CV[a] (95% CI)	0.8% (0.7%–0.9%)	1.4% (1.2%–1.7%)	1.5% (1.3%–1.8%)
Intraindividual CV (95% CI)	18.6% (17.2%–20.2%)	5.4% (4.9%–5.9%)	6.2% (5.8%–6.8%)
Interindividual CV (95% CI)	44.3% (33.3%–72.4%)	13.4% (10.3%–19.4%)	14.8% (11.5%–21.5%)
Index of individuality	0.43	0.35	0.42
Number of tests for[b]			
5% precision	54	5	7
10% precision	14	2	2
25% precision	3	1	1
RCV[c]			
Increase	66.3%	16.6%	19.5%
Decrease	39.9%	14.2%	16.3%

Abbreviations: CI, confidence interval; CV, coefficient of variation; RCV, reference change value; SD, standard deviation; TSH, thyrotropin; TT3, total triiodothyronine; TT4, total thyroxine.

[a]CV calculated from our data.

[b]Number of tests required to determine an individual's homoeostatic set point at a given precision.

[c]Reference change value, the percentage change that is statistically significant at a 95% confidence level.

function-affecting events during the study, and frail older adults (nursing home participants).

Four participants experienced potential thyroid function-affecting events during the study period, none of whom were frail. These events did not affect intraindividual variation (Supporting Information: Figure 1).

The four nursing home residents neither experienced potential thyroid function-affecting events nor hosted TPOAb at baseline. All four were included in the primary analysis of TSH, two were excluded in the primary analysis of TT3, and three were excluded from the primary analysis of TT4 due to a trend.

The subgroup analyses showed that participants with TPOAb had a lower variation for all thyroid parameters, while frail older adults had the largest variation. This difference was most pronounced for TSH, and there was no overlap in the confidence intervals in TPOAb positive participants and results from primary analysis (Supporting Information: Figure 2).

The were no marked sex differences in intraindividual variation in older participants, but a trend toward higher variation in women (Supporting Information: Table 2).

3.4 | Comparison with younger adults

Intraindividual variation in the previously collected sample of younger adults using similar methods and sampling interval was higher for all hormone tests. The higher variance was most pronounced for peripheral hormones, where the variance was doubled in younger compared to older adults (Figure 2).

4 | DISCUSSION

The frequency of thyroid function testing has increased in recent years, and screening for thyroid dysfunction is included in most routine health checks, often measuring just TSH.[27] This is particularly important among older adults, many of whom undergo frequent health care checks, as the symptoms of thyroid dysfunction are particularly insensitive in this group.[7] Moreover, thyroid dysfunction is frequent among older adults, with one in five women aged 68 having thyroid dysfunction.[3] Therefore, knowing how to interpret thyroid function test results in older adults is paramount. Our findings of biological variation in thyroid function tests with monthly samplings for 1 year in older adults aged 66 through 100 years facilitate clinical decision-marking in older adults by identifying true changes in thyroid function tests at the individual patient level.

Importantly, we found that the variation was similar between older adults for TT4 while not for TSH and TT3. In addition, the smallest intraindividual variation was observed for TT4, while TSH exhibited the most considerable variation. We also found that the index of individuality was <0.6, corroborating a low sensitivity of reference ranges to detect slight abnormalities in thyroid hormones in the individual—also in old age.

It may improve clinical practice that only two samples are required to describe the homoeostatic set point for the individual patient at 10% precision for TT4 and TT3. Consequently, a change in TT4 from 100 to 85 nmol/L an older patient would be a true decrease in T4 even when it occurs within the laboratory reference range. A similar difference in T4 in younger adults may not be a true change as illustrated by our comparison to younger adults.

The intraindividual variation of TT4 and TT3 found in our sample of older adults was about half that of the variation in younger adults in our previous study.[13] The two samplings were conducted in the same geographical setting using similar methodologies. The only differences were the inclusion of individuals with potential thyroid function-altering events and a wider time for blood sampling, adding to circadian variation. These differences could have led to higher variation in older adults, yet we found the opposite.

Data on the intraindividual variation in thyroid function tests in older adults is scarce. We found studies of younger adults, with one study of TT4 and two studies of TT3 using monthly samplings. These studies showed similar or larger intraindividual variation in younger adults than in our sample.[14,28] Browning et al. found an

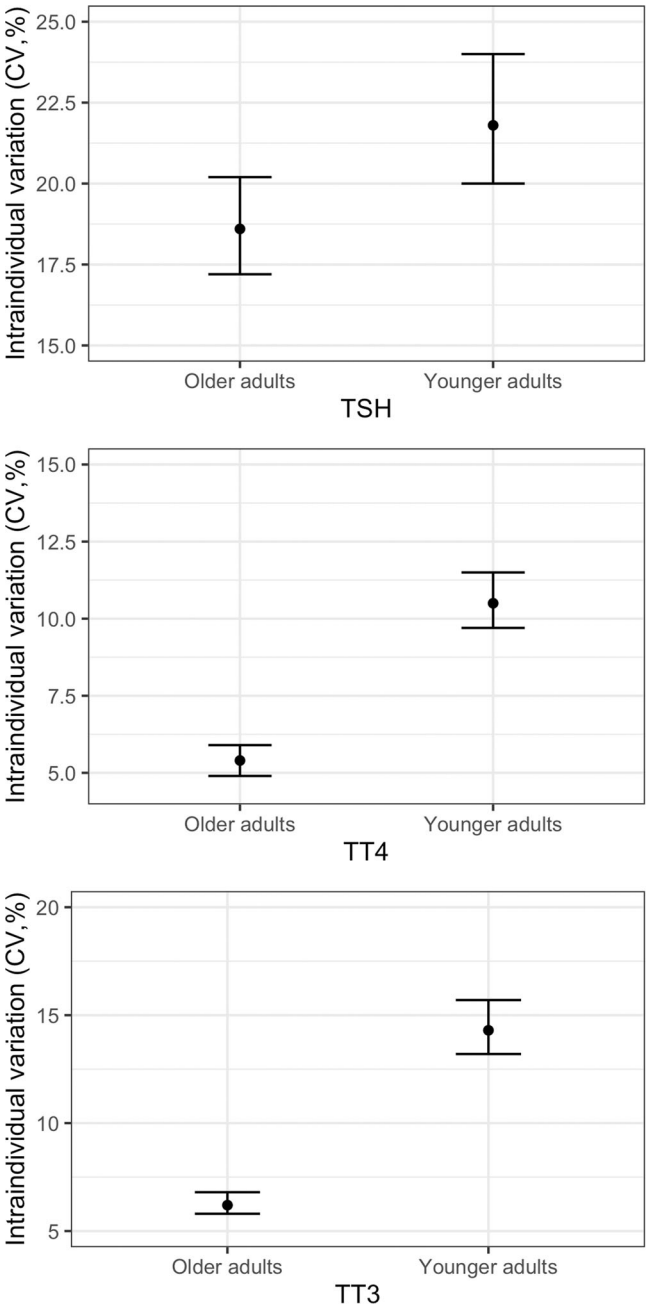

FIGURE 2 Intraindividual variation in younger compared to older adults. Results of intraindividual variation in younger compared to older adults. CV, coefficient of variation; TSH, thyrotropin; TT3, total triiodothyronine; TT4, total thyroxine.

intraindividual variation in TT3 close to the level we found in younger adults (CV 10.4%), while it was closer to our finding in older adults for TT4 (CV 5.1%).[15] However, Browning et al. had a sampling interval of only 2 to 5-day, suggesting serial correlations.[29] Serial correlations reduce measured variation, suggesting a higher true variation of thyroid function tests.[30,31]

As for TSH, both within- and between individual variation in old adults was similar to that of younger adults. Thus, our initial hypothesis of an age-related decrease in stability of the pituitary gland leading to a larger intraindividual variation in TSH in older adults was not supported by over findings. Moreover, we found a similar distribution of concordant changes in TSH and peripheral thyroid hormones in older and younger adults, which contrasts with previous findings that pituitary function may be dampened and less stable in older adults and, instead, relate to unaltered variation.[16]

Population-based reference ranges are just as insensitive for older as for younger adults for detecting true changes in thyroid function in the individual.[13,32] Our data also illustrate that aligning the upper limit of the reference range with the population's 97.5th upper percentile would increase the insensitivity to true changes in TSH in the individual. Thus, including our participant with subclinical hypothyroidism further decreased the individuality index.

Overall, our findings for TSH indicate "business as usual" for interpretation based on reference ranges with a rule of thumb that true change in serial testing occurs when TSH changes 1.0 IU/L or more.[13] Interestingly, we found both (1) a similar variation between older adults for TT4 and (2) a narrow variation in TT4 compared to the population reference range. Consequently, variation in TT4 was not unique to the individual as is seen in younger adults. Hence, the number of tests needed to settle an individual set-point is similar between individuals in old age, and, importantly, just two samples are required for a precision of 10% in old age. It may thus be recommended to add TT4 to TSH as part of the clinical routine when measuring and reporting thyroid function test results in subjects aged 65 years or above.

5 | STRENGTHS AND LIMITATIONS

Strengths of our study include careful planning to reduce circadian and preanalytical variation and allowing sufficient time between samplings to meet the requirements for independent samples.[29] Further, we included older adults from both nursing homes and community dwellers, and it should be noted that data collection was complete in all participants despite facing COVID-19. Finally, the sample size was three times the recommended lower number of participants for the study of biological variation.[25]

Some of our participants experienced potential thyroid function-altering events during the year of data collection. It has been recommended to exclude such participants as such events are assumed to confound results.[33,34] We included these participants and, interestingly, the events influenced neither variation nor mean values. These results suggest that a more liberal approach can be taken, allowing for less strict inclusion criteria when surveying old individuals to enhance external validity.

It was a limitation that just one participant had TT4 levels close to the upper limit of the population-based reference range, which could affect generalisability to this group. Total thyroid hormones were measured to support the comparison with our previous study of biological variation in younger men, and to reduce analytical imprecision.[35] This may influence the external validity when applying data to estimates of free thyroid

hormones. We did not include urinary iodine measurement at every visit, and we thus lack information on variation in iodine intake in old age and an assessment of the possible influence on the thyroid.

The present study of old subjects including both women and men was compared to only younger men. Our data do not support that the lower intraindividual variation in old compared to younger subjects was related to differences in sex distribution, which is in keeping with previous findings.[26] Finally, our results would have been strengthened by parallel sampling and analysis in younger and older adults. Therefore, our results should be confirmed in future research, including data on the sensitivity of the pituitary gland.

6 | CONCLUSIONS

Our initial hypothesis of increasing variation in thyroid function with old age was not supported as we found similar intraindividual variation in TSH in older and younger adults. Importantly, the intraindividual variation in TT4 was similar between individuals in older adults only, while variation in TT4 and TT3 was about half of that of younger adults. This lower and homogeneous variation in T4 supports adding T4 in clinical practice when performing thyroid screening in older adults to inform diagnosis and monitoring of thyroid dysfunction in older people.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Some or all data sets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

ETHICS STATETMENT

The study was approved by the Scientific Ethics Committee of Northern Jutland (reference number N-20170043) and registered with the Danish Data Protection Agency (reference number 2017-54). All participants gave written informed consent.

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SUPPORTING INFORMATION

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

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