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Heterogeneity of thyroid function and impact of peripheral thyroxine deiodination in centenarians and semi-supercentenarians: association with functional status and mortality

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Abstract

Thyroid hormones (FT3, FT4) and TSH were evaluated in a population of 672 well-characterized Italian subjects (age range: 52-113 years), including an unprecedented number of centenarians, semi-supercentenarians, as well as centenarian's offspring and age-matched elderly (CENT, 105+, CENTOFF and CTRL, respectively). The results show that FT3 level and FT3/FT4 ratio decrease while FT4 and TSH increase in an age-dependent manner. In CENT/105+, higher FT4 level and lower FT3/FT4 ratio are associated with an impaired functional status and an increased mortality. A cluster analysis identified three clusters of CENT/105+ based on their FT3, FT4 and TSH levels. Cluster3, characterized by lower FT3 and TSH and higher FT4, shows the worst health status and the shortest survival. Thus, the age-related changes of thyroid hormones extend to the most advanced age and CENT/105+ are highly heterogeneous regarding thyroid function. This heterogeneity is related to different health, functional and cognitive status, as well as with survival/mortality in CENT/105+. Finally, we investigated a remarkable number of CENT/105+ showing a thyroid profile suggestive of non-thyroidal illness syndrome (NTIS) (excluded from the previous analysis). NTIS CENT/105+ are characterized by a worse functional and cognitive status and an increased mortality with respect to CENT/105+ without NTIS.

Keywords: Human aging, longevity, mortality, health, thyroid hormones

Introduction

Human aging is currently defined as a historical, dynamic and context-dependent process involving the continuous adaptation of the organism to life-long exposure to internal and external insults, that we conceptualized in the ‘‘remodeling theory of aging’’(1). Consequently, the aging phenotype in humans is very heterogeneous and can be described as a complex counterintuitive mosaic of increasing frailty and robustness (2,3). Such heterogeneity is particularly evident in the oldest old who can be characterized by a health status ranging from remarkably good to severely bad, regarding the major functions (physical, cognitive and psychological) fundamental for the quality of life and health span, and deeply associated with survival.

A remodeling of the endocrine system also occurs during aging (4,5). Thyroid gland produces 3,3’,5-triiodothyronine (T3) and 3,3’,5,5’-tetraiodothyroxine (T4). These hormones play a crucial role in the homeostasis of the organism at multiple levels. A large amount of serum T3 is generated by the deiodination of the outer ring of T4 in extra-thyroidal tissues. While the removal of an atom of iodine in the inner ring of T4 produces the inactive thyroid metabolite reverse T3 (3,3’,5’-triiodothyronine or rT3) (6). More than 99% of the circulating T3 and T4 is bound to carrier proteins. However, only free and unbound thyroid hormones (FT3 and FT4) are capable of entering tissue cells. Significant changes in thyroid parameters are observed throughout life. However, it is still unclear if this is a normal adaptive response associated with senescence or a progressive thyroid dysfunction (7,8). The clinical features of healthy elderly subjects are often similar to the signs and symptoms of hypothyroidism (fatigue, cold intolerance, constipation, depression). In addition, cardiovascular, musculoskeletal, cognitive and metabolic functions are commonly impaired in both aging and hypothyroidism. On the other hand, experimental evidence suggests that the hypothyroid state may favour longevity by reducing metabolic rate, oxidative stress and cell senescence (9,10). Several clinical studies have been performed to investigate the role of thyroid function in the aging process obtaining contrasting results (11–14). Indeed, nutritional status,

concomitant illness and drug therapy can affect thyroid function in the elderly, making difficult the interpretation of the results.

Centenarians are considered the best example of successful aging, and a precious model to study the complex traits of human longevity. They reach the very extremes of the human lifespan and have escaped neonatal mortality, pre-antibiotic era illnesses and fatal outcomes of age-related diseases (15). Centenarian's offspring represent another informative model to study determinants involved in the modulation of longevity. Relatives of long-lived individuals show a lower morbidity and higher survival compared to a demographically-matched control group (subjects matched for age, sex, ethnicity, parent year of birth, but born from non-long-lived parents) (16–18).

To investigate the impact of the thyroid hormones in aging and longevity, we evaluated the thyroid function profile in a well-characterized population of 672 adult and old Italian subjects, ranging from 52 to 113 years old. This population consisted of centenarians, semi-supercentenarians (*i.e.* persons who reach the age of 105 years), centenarian's offspring and elderly subjects age-matched with centenarian's offspring, hereafter indicated as CENT, 105+, CENTOFF and CTRL, respectively. In order to understand the impact of thyroid function on the health status of the oldest old (CENT and 105+), the association between free thyroid hormones and thyrotropin (TSH) and functional, cognitive and depression status as well as mortality, was evaluated. In addition, a considerable number of CENT and 105+ (excluded from the previous analysis) showed a thyroid profile suggestive of non-thyroidal illness syndrome (NTIS), a condition characterized by a reduced peripheral conversion of FT4 to FT3 and normal or low TSH probably as consequence of different acute and chronic systemic disorders (19). Thus, it seemed worthwhile to describe their health status and mortality.

Methods

Study design and participants. A total of 672 subjects were enrolled by three Italian study centers (Bologna, Milan, and Cosenza) and surrounding areas. The group of centenarians (CENT)

consisted of 144 subjects (32 men, mean age 101.2 ± 1.9 years and 112 women, mean age 100.8 ± 1.7 years). The group of semi-supercentenarians (*i.e.* persons who reach the age of 105 years, 105+) consisted of 70 subjects (16 men, mean age 105.6 ± 0.9 years and 54 women, mean age 106.3 ± 1.6 years). The group of centenarian offspring (CENTOFF) consisted of 308 subjects (125 men, mean age 70.2 ± 6.5 years and 183 women, mean age 70.8 ± 7.1 years), having a centenarian or semi-supercentenarian parent born between 1899 and 1909. The group of controls (CTRL) consisted of 150 age-matched subjects (80 men, mean age 70.5 ± 6.7 years and 70 women, mean age 70.3 ± 6.5 years) with no long-lived parents. The lists of centenarians, semi-supercentenarians and their offspring were obtained by the Register Office, while the controls were identified by checking the birth and death dates of their parents in paper population records from Registers Office. All participants signed the informed consent before undergoing the questionnaires, measurements and blood sampling. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy).

Measurement tools. Functional status was assessed by ADL-Activities of Daily Living scale (scores ranging from 0 [all functions lost] to 5 [all functions preserved]) (20).

Physical performance was assessed by Handgrip strength test measuring the maximum isometric strength of the hand and forearm muscles. Handgrip strength was measured using a hand-held dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark) for two performances with each hand. The best performance was selected for the analysis. Cognitive status was assessed by SMMSE-Standardized Mini-Mental State Examination test (21). Depression status was investigated by Geriatric Depression Scale short form (GDS, 15 items) (22).

History of past and current diseases was accurately collected by checking the participants' medical documentation and addressing the major age-related pathologies. Current use of medication (including inspection of the drugs by the interviewer) was recorded. Thyroid dysfunctions suggestive of clinical hypothyroidism and hyperthyroidism, subclinical hypothyroidism and

hyperthyroidism, central hypothyroidism and NTIS were identified based on serum FT4, FT3 and TSH concentrations, as indicated in Supplemental Table 1. The criteria for the classification of the above-mentioned conditions were defined with respect to population-based reference ranges of different study centers.

Laboratory measurements. Overnight fasting blood samples were obtained in the morning. Serum was obtained after clotting and centrifugation at 760 g for 20 min at 4°C, rapidly frozen and stored at -80°C. Plasma was obtained within 2 hours from venipuncture by centrifugation at 2000 g for 20 min at 4°C, rapidly frozen and stored at -80°C. Serum total and HDL cholesterol, triglycerides, C-Reactive Protein (CRP) and glycaemia were measured by standard biochemical assays. Thyroid hormones (FT3, FT4) and TSH were measured according to standard procedures in three study centers (Bologna: ElectroChemiLuminescence ImmunoAssay (ECLIA), Elecsys Roche Diagnostics S.p.A.; Milan: ElectroChemiLuminescence ImmunoAssay (ECLIA), Cobas Roche Diagnostics GmbH; Cosenza: ChemiLuminescence ImmunoAssay, ADVIA Centaur®, Siemens Healthcare Diagnostics Inc.). Each study center used its appropriate population-based reference ranges to define euthyroidism and thyroid dysfunctions/pathologies as shown in Supplemental Table 1. Serum insulin was measured by a chemiluminescent immunoassay (LIAISON® Insulin assay, DiaSorin, Saluggia, Italy) and analyzed by the LIAISON® Analyzer. Insulin resistance status was assessed as homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula [67]: insulin (IU/mL) x glucose (mmol/L) /22.5 (23).

Statistical analysis**.** According to the parametric test Shapiro–Wilk, all the continuous variables are not normally distributed. Therefore, non-parametric tests were applied. The correlations between thyroid hormones and age were calculated by a Spearman's rank correlation. Differences among groups (CENT, 105+, CENTOFF and CTRL; NTIS CENT/105+ *vs* CENT/105+ without NTIS) were assessed by Kruskal–Wallis *H* test or Mann–Whitney *U* test for continuous variables as appropriate. The association between thyroid hormones and ADL score, Handgrip

strength, SMMSE score and GDS score in CENT/105+ was evaluated by a General Linear Model adjusted for age, gender and study center. A Two-Step Cluster Analysis was applied to determine three clusters within CENT/105+ group according to their levels of thyroid hormones (FT3, FT4) and TSH. Differences among clusters (Cluster1, Cluster2, and Cluster3) were assessed by Kruskal–Wallis *H* test or Mann–Whitney *U* test for continuous variables as appropriate. Logistic regression analysis was used to determine if categorical variable, such as gender, the presence of a thyroid dysfunction, the inability to perform Handgrip strength test or MMSE and the incapacity or the refusal to answer to GDS, is more associated with a group or a cluster. Kaplan–Meier survival curves were used to display five-year all-cause mortality in CENT/105+ clusters and in NTIS CENT/105+ and CENT/105+ without NTIS. Univariate Cox regression model adjusted for age, gender and study center was used to evaluate the association between FT3, FT4 and TSH and the five-year survival in CENT/105+. All analyses were executed by means of SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Thyroid hormones according to age and gender

A total of 672 subjects were enrolled for this study: the group of CENT consisted of 144 subjects, the group of 105+ consisted of 70 subjects, the group of CENTOFF consisted of 308 subjects and the group of CTRL consisted of 150 subjects and the baseline characteristics of each group are shown in Supplemental Table 2. The number and prevalence of euthyroid subjects, subjects taking medications for thyroid dysfunction and newly diagnosed thyroid disease in each group of the study population are shown in Table 1. No significant differences were found between CENTOFF and CTRL, while the prevalence of NTIS was higher in CENT compared to 105+ (p=0.046) (Table 1). Subjects with primary clinical hypothyroidism (n=11), central hypothyroidism (n=24), NTIS (n=58), or taking medications known to affect thyroid function (thyroid hormone preparations, methimazole, propylthiouracil, amiodarone, lithium carbonate, carbamazepine, or

phenytoin, etc.) (n=33), were excluded from the analysis related to correlation with age, functional and cognitive status, and mortality.

The correlations between age and thyroid hormones are shown in Figure 1. FT3 level and FT3/FT4 ratio were inversely related (Figure 1A, ρ= -0.573, $p<0.001$ and Figure 1C, ρ= -0.517, $p<0.001$, respectively), while FT4 and TSH levels were directly related (Figure 1B, ρ=0.132, p=0.002 and Figure 1D, ρ=0.216, $p<0.001$, respectively) to age. FT3 level and FT3/FT4 ratio were lower in 105+ compared to CENT (p=0.012 and p=0.003 respectively, Supplemental Figure 1A, 1C and 1E), while no differences in the levels of FT4 and TSH were found between CENT and 105+ (p=0.381 and p=0.902 respectively, Supplemental Figure 1B, 1D and 1E).

No significant differences in the levels of FT3, FT4, TSH and FT3/FT4 ratio were found between CENTOFF and age-matched CTRL (p=0.361, p=0.750, p=0.346 and p=0.403, respectively, (Supplemental Figure 1A, 1B, 1C, 1D and 1E). Therefore, CENTOFF and CTRL were put together in the group CENTOFF/CTRL for the analysis of gender differences. FT3, FT4, TSH levels and FT3/FT4 ratio were compared between men and women in CENTOFF/CTRL, CENT and 105+. FT3 level and FT3/FT4 ratio are higher in men compared to women in the group of CENTOFF/CTRL (p=0.007 and p=0.035, respectively) and CENT (p=0.005 and p=0.023, respectively) (Supplemental Figure 2A and 2C). TSH level was higher in men compared to women in the group of CENT (p=0.023) (Supplemental Figure 2D). No difference in FT4 level was observed between genders in each group (Supplemental Figure 2B).

Association among thyroid hormones and functional, cognitive, depression status and mortality in CENT/105+

The association among the levels of thyroid hormones and functional, cognitive, depression status and mortality in CENT and 105+ was evaluated (Supplemental Table 3A, 3B, 3C, 3D and3E) by a General Linear Model and by Univariate Cox regression model adjusted for age, gender and study center. The trends of the association analysis were the same in CENT and 105+, therefore, to

increase the statistical power of the regression analysis, CENT and 105+ were put together in the group CENT/105+.

The results indicated that FT4 level was negatively associated, while FT3/FT4 ratio was positively associated with ADL score ($p<0.001$ for both regressions, Table 2A). FT3 level and FT3/FT4 ratio were positively associated with handgrip strength ($p=0.026$ and $p<0.001$, respectively, Table 2A), while FT4 level was negatively associated with handgrip strength ($p<0.001$, Table 2A). FT4 level was negatively associated, while TSH level was positively associated with SMMSE score ($p=0.005$ and $p=0.006$, respectively, Table 2B). No significant association was observed between thyroid hormones and GDS score (Table 2B).

FT4 level was associated with an increased mortality hazard ($p=0.001$) while FT3/FT4 ratio is associated with a decreased mortality hazard ($p=0.002$) in CENT/105+ (Table 2C). No association was observed between FT3 and TSH level and mortality in CENT/105+ (Table 2C).

Clustering of CENT/105+ according to thyroid hormones levels

A two-step cluster analysis based on the levels of thyroid hormones (FT3, FT4) and TSH identified three clusters of CENT/105+ (Figure 2). Cluster1 consisted of 78 CENT/105+ (21 men and 57 women) and was considered as reference being the most numerous cluster. Cluster2 consisted of 24 CENT/105+ (7 men and 17 women) with a lower level of FT3 and a higher level of TSH compared with Cluster1 ($p<0.001$ for both comparisons, Figure 2A and 2B). Cluster3 consisted of 43 CENT/105+ (7 men and 36 women) with a lower level of FT3 and TSH ($p=0.028$ and $p=0.041$ respectively) and a higher level of FT4 ($p<0.001$) compared to Cluster1 (Figure 2A and 2B). Median age is higher in Cluster2 compared to Cluster1 ($p=0.039$) while no difference in age was found between Cluster3 and Cluster1 ($p=0.451$) (Figure 2A).

No significant difference was found between CENT/105+ belonging to Cluster1 and Cluster2 regarding functional, cognitive and depression status, BMI and biochemical parameters as well as survival (Figure 2A). CENT/105+ belonging to Cluster3 had a worse functional, cognitive

and depression status compared to Cluster1: Cluster3 showed lower ADL score and handgrip strength (p=0.002 and p=0.015, respectively), SMMSE score (p=0.041) and higher GDS score (p=0.046) (Figure 2A). Moreover, the percentages of subjects not performing Handgrip strength test, unable to perform SMMSE and not answering to GDS were higher in Cluster3 compared to Cluster1 (p<0.001, p=0.033 and p=0.048, respectively, Figure 2A). Cluster3 showed lower insulin (p=0.014) and HOMA-IR (p=0.023) compared to Cluster1 (Figure 2A). Survival differed significantly between Cluster3 and Cluster1. Cluster3 showed lower estimated survival time compared to Cluster1 (p=0.001) (last row of Figure 2A). The association between clusters and mortality (estimated by Univariate Cox regression model, adjusted for age, gender and study center) and the cumulative survival curves for each cluster are reported in Figure 2C and 2D, respectively. Cluster3 was associated with an increased mortality hazard, compared to Cluster1 (p=0.001, Figure 2C).

Functional, cognitive and depression status and mortality in CENT/105+ with NTIS

A considerable percentage of CENT and 105+ (excluded from the previous analysis, 28.5% and 15.7%, respectively) showed a thyroid profile suggestive of NTIS (Table 1). NTIS typically manifests as reduced peripheral conversion of FT4 to FT3 and normal or low TSH as a consequence of different acute and chronic systemic conditions (19). NTIS is still a debated topic because it is not yet clear whether it represents an adaptive response or a real hypothyroidism at the tissue level (19). Accordingly, we considered interesting to analyse the phenotypical characteristics of CENT/105+ with NTIS (hereafter indicated as NTIS CENT/105+, n=52) in comparison to the previously considered group of CENT/105+ (n=145) without NTIS. NTIS CENT/105+ are younger (p=0.029) and, as expected, have lower levels of FT3 and TSH (p<0.001 and p=0.001, respectively) in comparison to CENT/105+ (Figure 3A).

Functional, cognitive and depression status, BMI, biochemical parameters and survival were compared between NTIS CENT/105+ and CENT/105+ without NTIS. NTIS CENT/105+ showed a

worse functional (lower ADL score, $p<0.001$; a higher percentage of subjects not performing Handgrip strength test, $p=0.029$) and cognitive (a

higher percentage of subjects unable to perform SMMSE, $p=0.037$) status. Moreover, they were characterized by lower levels of insulin, HOMA-IR index, HDL cholesterol and total protein ($p=0.022$, $p=0.012$, $p=0.038$ and $p=0.016$, respectively), as well as a reduced estimated survival time ($p<0.001$) (Figure 3A). The association between NTIS and mortality hazard (estimated by Univariate Cox regression model, adjusted for age, gender and study center) and the cumulative survival curves for NTIS CENT/105+ and CENT/105+ are shown in Figure 3C and 3D, respectively. NTIS CENT/105+ display an increased mortality hazard ($p<0.001$) and decreased survival in comparison to CENT/105+ without NTIS.

Discussion

As pointed out by the "remodeling theory of aging" (1), a variety of parameters and functions changes during aging, and the major problem is to understand whether these modifications represent a positive adaptation or a progressive, negative deterioration. The main result of this adaptive process is an increase of the inter-individual variability. Indeed, an important characteristic of old and very old people is their large heterogeneity, regarding health status, biomedical, functional and cognitive parameters, as well as the response to pharmacological treatment (2). These considerations apply also to the changes described in the last 30 years regarding thyroid aging (10). With age, volume and function of thyroid gland decrease while the prevalence of thyroid diseases increases (4). However, the studies about the relationship between serum thyroid parameters and longevity provided conflicting results in humans (24). Centenarians represent an informative model to disentangle the relationship between thyroid and aging, as they are exceptional individuals who reached the extreme limits of human lifespan postponing or escaping the main age-related diseases. Moreover, the levels of thyroid hormones (FT3, FT4) and TSH have a pervasive effect on many crucial functions, being associated with functional, cognitive

and depression status, as well as survival/mortality in long-lived subjects (8,13,25,26). The present paper is aimed to clarify these aspects studying the thyroid function in a sizeable Italian population, covering a wide age range (from 50 to 113 years) including an unprecedented number of CENT (n=144), 105+ (n=70), CENTOFF (n=308) and age-matched CTRL (n=150). All the subjects were in-depth characterized, paying particular attention to CENT and 105+.

The major findings of the present investigation can be summarized as follow:

i) FT3 level and FT3/FT4 ratio decrease while FT4 and TSH increase in an age-dependent manner.

ii) Higher FT4 level and lower FT3/FT4 ratio in CENT/105+ are associated with an impaired functional status and an increased mortality. In particular, a cluster analysis identified three clusters of CENT/105+ based on their FT3, FT4 and TSH levels. The Cluster3, characterized by lower FT3 and TSH and higher FT4, shows the worst health status and the shortest survival.

iii) A conspicuous group of CENT/105+ showing a thyroid profile suggestive of NTIS (excluded from the previous analysis) was characterized by a worse functional and cognitive status and by an increased mortality.

Regarding the first point, our data are partially discordant with previous studies. Mariotti et al. (27) as well as Magri et al. (28), found an age-related decline of TSH and FT3 levels and a significant increase of rT3 in centenarians compared with old controls, while the level of FT4 was found similar (27) or increased (28,29) or decreased (30) in comparison with younger subjects. Baranowska et al. (31) found that serum TSH and T4 concentrations in centenarian women were comparable with that observed in elderly and young women, while serum T3 levels in centenarians were lower compared with younger groups. These contrasting results could be related to the presence of several confounding factors, such as ethnicity, environment, age-related chronic diseases, pharmacological treatments and iodine intake able to influence the results of thyroid

function tests in long-lived individuals (10). It is worth to consider that the above-mentioned studies (published at least 10 years ago) were conducted on centenarians born 10 or even 20 years before than centenarians and 105+ enrolled in the present study. Birth year cohort has a strong impact on the health status and phenotype of oldest old (32,33) and it is conceivable that current centenarians, who have lived in an improved environment (health progress, disease prevention, increased educational, healthier lifestyle), have been differently selected respect to centenarians studied in the past.

Other data of the literature agree with our observations, showing a mild decline of thyroid function in centenarians (34,35)

The results of the present paper demonstrated that the progressive age-related reduction in serum FT3 level and FT3/FT4 ratio as well as the mild increase in serum FT4 and TSH levels were characteristics of centenarians and extended to individuals attaining extreme longevity, *i.e.* 105+. The age-related increase of serum TSH concentration may suggest either a decline in thyroid response or an altered negative-feedback between FT4 and TSH occurring with aging (34). These modifications may represent a pathological deficit of the thyroid system or an adaptive response of centenarians and semi-supercentenarians towards minor energy expenditure and/or requirements. In fact, the Basal Metabolic Rate (BMR), calculated by the Harris-Benedict formula (36) showed a significant decrease with age (Spearman Rank correlation, ρ=-0.626, $p<0.001$, data not shown). Moreover, a significant correlation between BMR and FT3 (Spearman Rank correlation, ρ=0.446, $p<0.001$, data not shown) was found supporting the hypothesis that the age-related decline in thyroid function corresponds to lower energy expenditure and/or requirements in CENT/105+.

A longitudinal study enrolling a cohort of elderly individuals (mean age 85 years) over a period of 13 years reported a decrease in total T3 levels and an increase in TSH and FT4 levels, probably due to an age-related alteration in TSH set point (26). Although our study was not longitudinal, it seemed that also exceptional long-lived individuals do not escape this phenomenon.

Our results indicated also that FT3/FT4 ratio decreased dramatically with age, suggesting a decline of 5'-deionidase activity as previously described by Magri et al. (28). It can be speculated that the increase of pro-inflammatory cytokines, *i.e.* TNF-α, IL-1 and IL-6, typical of aging and longevity (inflammaging) could inhibit the 5' deiodinase activity (37,38).

Regarding the second point, our results are in line with previous observations. In particular, healthy adults with higher serum FT4 (within the normal range) had worse physical performance scores and lower grip strength (39,40). Few recent studies reported an increased risk of dementia at lower concentrations of TSH (11,41) suggesting that an excessive production of FT4 may result detrimental for central nervous system (41).

Moreover, our data regarding the increase of FT4 and the decrease of FT3/FT4 ratio associated with lower survival in CENT/105+, were similar to what observed in Leiden 85-Plus Study (25) and CHS All-Stars study (26) reporting an association between higher FT4 level and mortality in 85+ subjects.

Our results showed that CENT/105+ with high FT3/FT4 ratio had a better functional capability and survival suggesting that they maintain a good hormonal negative feedback probably preserving an efficient 5'-deiodinase activity. Bearing in mind that FT3 is the bioactive form of thyroid hormone, during aging a decline in serum FT3 levels could be balanced by a compensatory increase in 5'-deiodinase activity, finalized to maintain an adequate local production of FT3 (6). It may be also interpreted in a more general strategy to increase lifespan through a slower loss of physiologic reserve capacity and a better integrity of biological systems. Although we have excluded subjects with thyroid diseases, the cross-sectional design of this study does not allow us to determine causality among the evaluated variables, therefore we cannot exclude that these hormonal changes are representative of a progressive deterioration of thyroid gland. However, our results are consistent with a recent prospective study showing that FT3/FT4 ratio represents an independent marker of frailty and survival in a population of euthyroid older patients (mean age 84 years),

hospitalized for acute disease. In this study FT3/FT4 ratio was inversely related to multi prognostic index (MPI), a reliable marker of frailty, even in subjects with normal FT3. In addition, Cox regression analysis documented a significantly, independent increase of long-term mortality moving from the highest through the lowest quartile of FT3/FT4 ratio (42).

Regarding the third finding, a remarkable percentage of CENT/105+ (24.3%) showed a thyroid profile suggestive of NTIS. NTIS typically manifests as reduced peripheral conversion of FT4 to FT3 and normal or low TSH probably a consequence of different acute and chronic systemic diseases (19,29). NTIS is still a debated topic because it is not yet clear whether it represents a consequence of these pathologic conditions or a real hypothyroidism at the tissue level (19). NTIS CENT/105+ characterized by high FT4 levels and very low FT3 levels, probably due to a peripheral reduced conversion of FT3 by the deiodination of FT4, had a worse functional and cognitive status and a reduced survival despite being younger than CENT/105+ without NTIS. These results could be interpreted as a maladaptive remodeling of NTIS CENT/105+. Indeed, a continuum age remodeling is needed to reach the extreme limits of life in good health conditions and when this adaptive capacity declines, deteriorative changes occur.

In conclusion, the change in thyroid hormones with age in humans is a phenomenon which extends to the most advanced age. Centenarians and semi-supercentenarians are highly heterogeneous regarding thyroid function and this heterogeneity has a counterpart regarding health, functional and cognitive status, and, eventually, mortality rate as shown by the presence within the centenarians and semi-supercentenarians of three clusters characterized by different levels of FT3, FT4 and TSH. Accordingly, we hypothesize that there is a limited number of adaptive strategies that thyroid can use to cope with the metabolic/energetic age-related requirements.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Figure and table legends

Tables

Table 1. Number and prevalence of euthyroid subjects, subjects taking medications for thyroid dysfunction and newly diagnosed thyroid disease in each group of the study population.

	CENT	**105+**	**p**	**CENTOFF**	**CTRL**	**p**
Euthyroidism, n (%)	83 (57.6)	48 (68.6)	-	255 (82.8)	126 (84.0)	-
Primary hypothyroidism on L-thyroxine therapy, n (%)	1 (0.7)	1 (1.4)	0.701	22 (7.1)	8 (5.3)	0.473
Hyperthyroidism on methimazole therapy, n (%)	0	0	-	0	1 (0.7)	-
Primary clinical hypothyroidism, n (%)	4 (2.8)	2 (2.9%)	0.869	4 (1.3)	1 (0.7)	0.544
Primary subclinical hypothyroidism, n (%)	8 (5.6)	3 (4.3%)	0.537	10 (3.2)	5 (3.3)	0.983
Subclinical hyperthyroidism, n (%)	1 (0.7)	2 (2.9%)	0.316	3 (1.0)	2 (1.3)	0.745
Central hypothyroidism, n (%)	6 (4.2)	3 (4.3%)	0.842	12 (3.9)	3 (2.0)	0.298
NTIS, n (%)	41 (28.5)	11 (15.7%)	**0.046**	2 (0.6)	4 (2.7)	0.109

Note: CENT: centenarians; 105+: semi-supercentenarians; CENTOFF: centenarian's offspring; CTRL: elderly subjects age-matched with centenarian's offspring; NTIS: non-thyroidal illness syndrome. Statistical analysis by a logistic regression was performed. Euthyroidism has been considered the reference condition.

Table 2. Association of FT3, FT4 and TSH levels with A) functional status (ADL score and handgrip strength), B) cognitive and depression status (SMMSE and GDS score) and C) mortality in CENT/105+.

A	**ADL score**		**Handgrip strength**[a]	
	B coefficient (95% C.I.)	p	B coefficient (95% C.I.)	p
FT3	0.6 (-0.2-1.6)	0.160	3.6 (0.4-6.8)	**0.026**
FT4	-0.3 (-0.5- -0.1)	**0.000**	-1.3 (-1.8 - -0.7)	**0.000**
FT3/FT4	14.5 (7.2-21.9)	**0.000**	64.1 (38.9-89.2)	**0.000**
TSH	0.1 (0.0-0.3)	0.088	0.3 (-0.2-0.9)	0.208

B	**SMMSE score**[b]		**GDS score**[c]	
	B coefficient (95% C.I.)	p	B coefficient (95% C.I.)	p
FT3	2.1 (-2.5-6.8)	0.368	-0.5 (-2.9-1.9)	0.696
FT4	-1.2 (-2.0- -0.4)	**0.005**	0.2 (-0.2-0.6)	0.332
FT3/FT4	26.2 (-1.9-54.4)	0.067	-9.1 (-27.6-9.4)	0.331
TSH	1.0 (0.3-1.8)	**0.006**	0.7 (-0.3-0.4)	0.489

C	**Hazard ratio**		
	B coefficient	Exp(B) (95% C.I.)	p
FT3	-0.3	0.8 (0.4-1.3)	0.325
FT4	0.2	1.2 (1.1-1.3)	**0.001**
FT3/FT4	-8.8	0.0 (0.0-0.1)	**0.002**
TSH	0.0	1.0 (0.9-1.1)	0.429

Note: ADL: Activities of Daily Living scale; SMMSE: Standardized Mini-Mental State Examination test; GDS: Geriatric Depression Scale. Statistical analysis was performed by a General Linear Model adjusted for age, gender and study center for ADL, SMMSE and GDS score and for handgrip strength and by a Cox regression model adjusted for age, gender and study center for mortality. [a]Handgrip strength has been evaluated on 85 subjects; [b]SMMSE score has been assessed on 104 subjects; [c]GDS has been performed on 97 subjects.

FIGURES CAPTIONS

Figure 1. Age-dependent variations of the levels of FT3 (A), FT4 (B), FT3/FT4 ratio (C) and TSH (D).

Figure 2. CENT/105+ have been clustered according to their levels of FT3, FT4 and TSH by a Two Step Cluster analysis. A) Comparison of thyroid hormones and TSH levels, depression, cognitive and functional status, BMI, biochemical parameters among clusters of CENT/105+ evaluated by Mann-Whitney *U* test. Data are expressed as mean (S.D.) for age, ADL, SMMSE and GDS scores and handgrip strength and median (min-max) for thyroid hormones, BMI and biochemical parameters. Comparison of survival between clusters is evaluated by Kaplan-Meier analysis and estimated survival time is expressed as median (95% C.I.). B) scatter-plot matrix of FT3, FT4 and TSH levels in Cluster1, Cluster2 and Cluster3; C) association of clusters with mortality performed by Cox regression model adjusted for age, gender and study center. Cluster1 is considered the reference for the regression analysis. D) cumulative survival curves of CENT/105+ according to clusters. [a]SMMSE has been assessed on 60 subjects belonging to Cluster1, 19 subjects belonging to Cluster 2 and 23 subjects belonging to Cluster3; [b]GDS has been performed on 59 subjects belonging to Cluster1, 13 subjects belonging to Cluster 2 and 25 subjects belonging to Cluster3; [c]Handgrip strength has been evaluated on 66 subjects belonging to Cluster1, 16 subjects belonging to Cluster 2 and 23 subjects belonging to Cluster3; [d]Total protein have been measured on 68 subjects belonging to Cluster1, 16 subjects belonging to Cluster 2 and 37 subjects belonging to Cluster3.

Figure 3. Analysis of NTIS CENT/105+ compared to CENT/105+ without NTIS. A) Comparison of thyroid hormones and TSH levels, depression, cognitive and functional status, BMI, biochemical parameters between CENT/105+ and NTIS CENT/105+ evaluated by Mann-Whitney U test. Data are expressed as mean (S.D.) for age, ADL, SMMSE, GDS scores and handgrip strength and

median (min-max) for thyroid hormones, BMI and biochemical parameters. Comparison of survival between CENT/105+ and NTIS CENT/105+ is evaluated by Kaplan-Meier analysis and estimated survival time is expressed as median (95% C.I.). B) association of NTIS with mortality. Statistical analysis was performed by Cox regression model adjusted for age, gender and study center. CENT/105+ without NTIS were considered the reference for the regression analysis. C) cumulative survival curves of CENT/105+ *vs* NTIS CENT/105+. [a]SMMSE has been assessed on 104 CENT/105+ and 29 NTIS CENT/105+; [b]GDS has been performed on 97 CENT/105+ and 29 NTIS CENT/105+; [c]Handgrip strength has been evaluated on 105 CENT/105+ and 29 NTIS CENT/105+; [d]Total protein have been measured on 121 CENT/105+ and 51 NTIS CENT/105+.

Figure 1

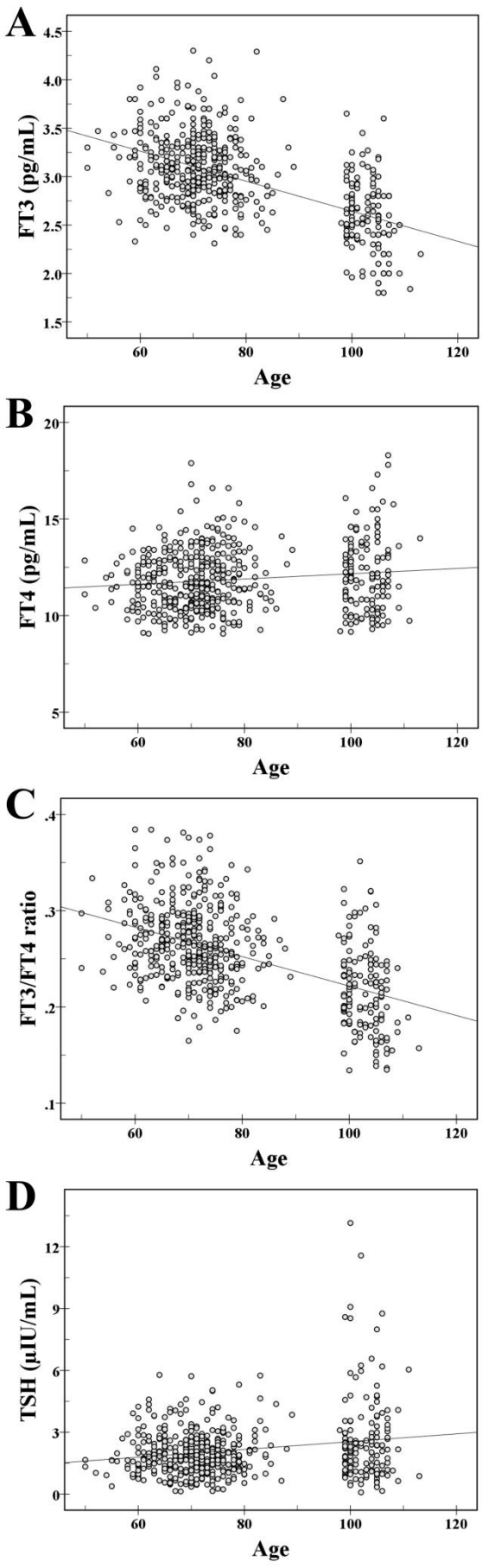
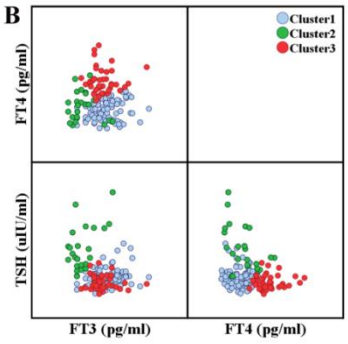

A
FT3 (pg/mL)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
60
80
100
120
Age
B
FT4 (pg/mL)
20
15
10
5
60
80
100
120
Age
C
FT3/FT4 ratio
.4
.3
.2
.1
60
80
100
120
Age
D
TSH (μIU/mL)
12
9
6
3
0
60
80
100
120
Age

Figure 2

A

	Cluster1	Cluster2	Cluster3	*p*		
	Reference	Lower FT3 and higher TSH	Lower FT3, higher FT4, lower TSH	1*vs*2	1*vs*3	2*vs*3
N	78	24	43			
Men:Women	21:57	7:17	7:36			
Age (yrs)	102.5 (2.9)	103.9 (3.2)	103.0 (3.2)	.039	.451	.194
FT3 (pg/mL)	2.7 (2.0-3.6)	2.0 (1.8-2.8)	2.5 (2.1-3.6)	<.001	.028	<.001
FT4 (pg/mL)	11.2 (9.2-13.4)	11.7 (9.5-15.2)	14.0 (12.2-18.3)	.352	<.001	<.001
TSH (µIU/mL)	2.2 (0.1-6.6)	5.0 (2.4-13.1)	1.4 (0.1-4.1)	<.001	.002	<.001
ADL score	3.5 (1.8)	2.9 (1.7)	2.6 (1.9)	.105	.002	.249
Handgrip strength (kg)	14.5 (5.8)	12.5 (6.4)	10.8 (5.1)	.178	.015	.544
Not performing Handgrip strength test, n (%)	12 (15.4)	8 (33.3)	20 (46.5)	.059	<.001	.296
SMMSE score[a]	19.6 (5.3)	20.9 (6.2)	18.4 (6.5)	.766	.041	.200
Unable to perform SMMSE, n (%)	18 (23.1)	5 (20.8)	18 (41.9)	.877	.033	.088
GDS score[b]	4.8 (3.4)	7.1 (4.9)	6.7 (4.0)	.402	.046	.927
Not answering to GDS, n (%)	19 (24.4)	11 (45.8)	18 (41.9)	.057	.048	.753
BMI (kg/m^2)	24.4 (17.7-34.7)	21.9 (20.1-26.6)	23.0 (13.3-30.4)	.134	.062	.131
C-Reactive Protein (mg/L)	3.4 (0.3-27.0)	5.0 (0.3-18.4)	3.9 (0.3-33.8)	.510	.059	.472
Glycaemia (mg/dL)	83 (62-269)	89 (64-221)	79 (41-142)	.580	.062	.131
Insulin (µIU/mL)	5.9 (0.2-72.9)	4.0 (1.4-32.6)	4.2 (1.3-14.8)	.067	.014	.951
HOMA-IR index	1.2 (0.1-2.3)	0.9 (0.2-8)	1.0 (0.2-3.3)	.200	.023	.690
Total cholesterol (mg/dL)	184 (117-301)	190 (122-284)	175 (103-322)	.941	.247	.590
HDL cholesterol (mg/dL)	47 (21-82)	48 (20-99)	47 (25-81)	.679	.490	.431
Triglycerides (mg/dL)	114 (55-535)	112 (62-209)	98 (56-220)	.974	.281	.484
Total protein[d] (g/dL)	6.8 (5.6-9.1)	6.7 (5.6-8.2)	6.7 (4.9-7.8)	.459	.082	.642
Estimated survival time (days)	932 (796-1068)	715 (525-905)	552 (409-694)	.098	.001	.256

B



C

	Hazard ratio		
	B coefficient	Exp(B) (95% C.I.)	*p*
Cluster1	-	-	-
Cluster2	0.353	1.4 (0.8-2.4)	.204
Cluster3	0.709	2.0 (1.3-3.1)	.001

D

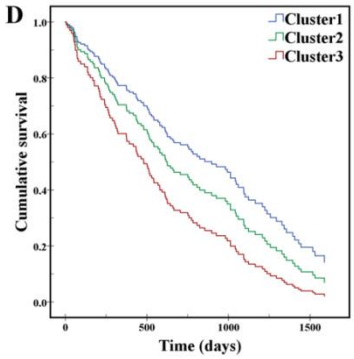


Figure 3

A

	CENT/105+	NTI CENT/105+	*p*
N	145	52	
ADL score	3.2 (1.8)	2.0 (1.5)	<.001
Handgrip strength[c] (kg)	13.3 (5.8)	10.2 (4.1)	.060
Not performing Handgrip strength test, n (%)	40 (27.6)	23 (44.2)	.029
SMMSE score[a]	19.5 (5.7)	17.6 (6.1)	.089
Unable to perform SMMSE, n (%)	41 (28.3)	23 (44.2)	.037
GDS score[b]	5.6 (3.8)	5.7 (3.5)	.684
Not answering to GDS, n (%)	48 (33.1)	23 (44.2)	.153
BMI (kg/m^2)	23.5 (13.3-34.7)	22.2 (15.2-34.1)	.230
C-Reactive Protein (mg/L)	3.8 (0.3-33.8)	4.4 (0.4-89.3)	.553
Glycaemia (mg/dL)	83 (41-269)	79 (48-172)	.196
Insulin (µIU/mL)	4.9 (0.2-72.9)	4.2 (0.2-81.7)	.022
HOMA-IR index	1.0 (0.1-23.0)	0.8 (0.1-23.0)	.012
Total cholesterol (mg/dL)	183 (103-322)	178.5 (71-318)	.726
HDL cholesterol (mg/dL)	47 (20-99)	43 (25-75)	.038
Triglycerides (mg/dL)	111 (55-535)	100 (37-342)	.173
Total protein[d] (g/dL)	6.8 (4.9-9.1)	6.6 (4.4-7.9)	.016
Estimated survival time (days)	791 (695-888)	419 (303-535)	<.001

B

	Hazard ratio		
	B coefficient	Exp(B) (95% C.I.)	*p*
CENT/105+	-	-	-
NTIS CENT/105+	0.956	2.6 (1.8-3.7)	<.001

C