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Levo-thyroxine Replacement in Obese Adults: the Role of Metabolic Variables and Aging on Thyroid Testing Abnormalities

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Context. General rates of over- and under-replacement in levo-thyroxine (L-T4) users with primary hypothyroidism are variably high. No information on L-T-4 adequacy exists in obesity.
Objective: We explored rates and factors relating to L-T4 adequacy in obese patients with primary hypothyroidism.
Setting. Tertiary care center.
Design. Among 4954 consecutive obese patients admitted between 2011-2014, 691 hypothyroid patients on L-T4 therapy and 691 BMI-, age- and gender-matched euthyroid controls underwent analysis of thyroid function, gluco-lipid profile, body composition and indirect calorimetry. L-T4 users were classified into low TSH (<0.27 mU/liter), euthyroid (0.27-4.2 mU/liter) and high TSH (>4.2 mU/liter).
Results. L-T4 users constituted 13.9% of the incident population. TSH was low in 7.5%, high in 17.2% and normal in 75.2% of L-T4 users. Over-treatment decreased with aging and more L-T4 users ≥65y had normal TSH than those <65y ($p<0.05$). Compared to the euthyroid obese group, L-T4 users showed higher adiposity, similar insulin resistance but an healthier lipid profile. In multivariable analyses, L-T4 dose was predicted by fat-free mass, hypothyroidism cause, and gender ($p<0.0001$ to <0.05). Risk of L-T4 over-replacement increased with younger age (OR 0.96; 95% CI 0.94-0.99), higher L-T4 dose (OR 2.98; 95% CI 1.44-6.14) and lower BMI (OR 0.93; 95% CI 0.88-0.99). Male gender increased the likelihood of L-T4 under-replacement (OR 2.37; 95% CI 1.10-5.11).
Conclusions. Obesity is associated with milder rates of inadequate L-T4 treatment compared to non-obese populations. L-T4 adequacy increases with aging. Age, body composition and gender are main the determinants of L-T4 requirements in obesity.

Rates of over- and under-treatment are milder in obese L-T4 users than reference non-obese populations and improve with aging. Age, body composition and gender influence L-T4 requirements in obesity.

Introduction

Obesity and thyroid abnormalities are common conditions closely linked together from clinical and molecular viewpoints (1). This relationship has become clinically relevant in the context of an unprecedented rise in the prevalence of obesity worldwide (2). Because thyroid hormone plays important roles in energy homeostasis and gluco-lipid metabolism (1), thyroid dysfunctions are often associated with perceived or factual changes in body composition and resting energy expenditure (REE) independent of physical activity (3).

Levo-thyroxine (L-T4) is the treatment of choice when the diagnosis of persistent thyroid hormone deficiency is confirmed (4-6). With the exception of central hypothyroidism or peripheral thyroid hormone resistance, individualized L-T4 dose up-titration is conventionally determined by circulating TSH, the established biomarker of L-T4 replacement (7-11). Reaching a TSH value within the age-adjusted euthyroid range is the accepted therapeutic target (4,5,12-14), as it promotes improvements in symptoms, quality of life and cardiovascular risk (15-22). The therapeutic management of primary hypothyroidism with L-T4 is considered relatively uncomplicated, and aided by reliably accurate TSH and thyroid hormone immunoassays. Despite reaching TSH within the euthyroid range, however, some patients face modest improvements in bodyweight and lipid abnormalities during long-term L-T4 replacement (5,23,24). Moreover, epidemiology studies found that as many as 57% of patients undergoing L-T4 replacement can experience therapy over- or under-replacement, even when frequent biochemical monitoring is accomplished (13,25-27). Aspects of previously unrecognized complexity in the therapeutic management of hypothyroidism have also been underscored in special populations, in particular older patients, children, pregnant women and obese individuals (4-6,13,14,28). Common reasons for incorrect L-T4 replacement include non-adherence, medication administration errors, dietary factors, medication interference, as well as impaired L-T4 absorption (4-6,14,29-31).

To date, the rate of abnormal thyroid function testing and its metabolic associates in obese subjects undergoing L-T4 treatment for primary hypothyroidism remains unknown. Given the detrimental consequences of inadequate L-T4 replacement especially in the aging population, we conducted a cross-sectional investigation to define the prevalence and the factors associated with inadequate L-T4 replacement, and to evaluate the metabolic phenotype associated with L-T4 treated hypothyroidism in obese patients.

Patients and Methods

Of 4954 consecutive de novo patients admitted between 7th January 2011 and 28th December 2014 to our Institution for work-up and rehabilitation of morbid obesity, 691 obese patients suffering from primary hypothyroidism on stable treatment with tablet L-T4 for at least 6 months were included in the study [605 F/86 M; age, 59 yrs (IQR, 50-69 yrs); BMI, 43.9 kg/m^2 (IQR 40.1-48.4 kg/m^2)]. All patients' charts were individually reviewed where available to exclude inappropriate L-T4 prescriptions. The cause of primary hypothyroidism was autoimmune thyroid disease (AITD) in 609 patients (88.1%) and total thyroidectomy in 82 cases (11.9%). None had suffered from thyroid cancer. A group of 691 BMI-, age- and gender-matched biochemically euthyroid obese patients referred to our unit for obesity in the same period, were enrolled according to the exclusion criteria and constituted the control group. Exclusion criteria were comorbidities affecting the evaluation of thyroid function, acute illness and/or acute inflammation, use of T3 or medications potentially interfering with thyroid function (such as amiodarone, steroids or lithium carbonate therapy) or L-T4 absorption (such as cholestyramine, antacids, ferrous sulfate, sucralfate, laxatives, calcium carbonate, proton pump inhibitors, lovastatin, bile acid sequestrants) and pregnancy. In all patients, body weight was stable for ≥6 months prior to study enrolment. The investigation was approved by the local Ethical Committee, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research On admission, and

written consent was obtained from all patients. Main study outcomes included rates of over- and under-treatment in L-T4 users and factors associated with L-T4 adequacy. Secondary outcome measures included assessment of differences in metabolic profile between L-T4 users and euthyroid controls.

In all participants, body measurements were conducted on fasting patients wearing light underwear. Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, and BMI was expressed as body mass (kg)/height $(m)^2$. The criterion of obesity was BMI ≥30 kg/m^2. Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest after gentle aged expiration; hip was measured as the greatest circumference around the nates. Bio-impedance analysis (BIA, 101/S Akern; Florence, Italy) allowed measurement of percent fat mass (%FM) expressed and free fat mass (FFM, kg). Methodology, variation coefficients and exclusion criteria have been previously detailed (32). Resting energy expenditure (REE) was expressed in kilocalories per 24h and determined in a thermo-regulated room (22-24 °C) by computed open-circuit indirect calorimetry, measuring resting oxygen uptake and resting carbon dioxide production by a ventilated canopy (Sensormedics, Milan, Italy) at 1-min intervals for 30 min and expressed as 24 h value. Methodology, equation formulae and performance of different equations to predict measured REE have been previously described (33,34).

Undiluted serum samples were assayed in duplicate for fT4 and TSH using an automated chemiluminescence assay system (Immulite 2000; DPC, Los Angeles, CA). The principle of the method is a two-site, solid-phase chemiluminescent immunometric assay or competitive immunoassay. L-T4 users were classified according to TSH levels, into one of the following three groups: patients with low serum TSH levels (TSH <0.27 mU/liter), euthyroid patients (TSH 0.27-4.20 mU/liter), and patients with high serum TSH (TSH >4.20 mU/liter). Within these groups, patients were then stratified according to their fT4 levels: high (fT4 >17.0 ng/liter), normal (fT4 ≥9.0-17.0 ng/liter) and low fT4 (fT4 <9 ng/liter).

Obese euthyroid controls underwent measurement of anti-thyroperoxidase (TPOAb) and anti-thyroglobulin (TgAb) antibodies by automated chemiluminescence assay system (Anti-Tg, Anti-TPO Ready Pack, Siemens Healthcare Diagnostics, Milan). Thyroid ultrasound examination was performed in a subgroup of 47 controls having their TSH levels above the reference TSH range, to exclude antibody-negative AITD. Routine laboratory data included glucose, total cholesterol (CHO), high-density (HDL) and low-density lipoprotein (LDL) cholesterol and triglycerides (TG) measured by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Ultrasensitive C-reactive protein (CRP) was measured by CRP (latex) HS Roche kit. Insulin levels were measured using a Cobas Integra 800 Autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA), and insulin resistance was expressed as HOMA-IR [insulin (mIU/L) × [glucose (mmol/L)/22.5]]. Type 2 diabetes mellitus (T2DM) was ascertained by patients' history and/or biochemistry analyses on admission according to current guidelines (35).

Statistical analyses

Statistical analyses were performed using SPSS version 21 (Somers, NY, USA) on log transformed data to correct for the non-Gaussian distribution obtained by the Shapiro-Wilk test. In the text and tables, values are expressed as median and interquartile ranges. Univariate ANOVA was used for comparison between groups. Spearman's correlation analysis was used to identify significant associations between the variables of interest.

Stepwise multivariable regression analysis was conducted to identify predictors of L-T4 dose and the multilinear model included age, gender (female=0, male=1), BMI, FFM and %FM, REE, aetiology of hypothyroidism (AITD = 0, total thyroidectomy = 1), daily L-T4 dose µg/kg of body weight (BW), duration of L-T4 treatment, and T2DM. β coefficients and significance values obtained from the models were reported. Multinomial logistic regression

analysis was performed to test the effect of variables of interest on the risk of under- and over-treatment with L-T4 therapy. Statistical significance was set at 5%.

Results

Obese L-T4 users

L-T4 users accounted for 13.9% of all patients referred to our unit in the study period for morbid obesity. Most patients of the study population were severely obese: BMI was >40 kg/m^2 in 75.8%, >35.0-39.9 kg/m^2 in 18.4%, and >30.0-34.9 kg/m^2 in 5.8% of L-T4 users. Females accounted for 87.6%. Previously diagnosed or de novo T2DM affected 47.7% of cases. Anthropometric and metabolic profiles are summarized in Table 1.

In the population as a whole, the median daily dose of L-T4 was 100 µg (IQR, 75-125), equivalent to 0.91 µg/kg/day (IQR, 0.61-1.17) when expressed relative to BW. As expected, the daily L-T4 µg/kg dose was lower in AITD than athyreotic patients [0.85 (IQR, 0.55-1.10) vs. 1.20 µg/kg/day (IQR, 0.91-1.40), p<0.01]. The difference in L-T4 µg/kg dose between females and males was not significant [(0.84 (IQR, 0.45-1.16) vs. 0.91 µg/kg/day (IQR, 0.61-1.17), respectively].

The overall rate of patients with abnormal TSH was 24.7%. Individually, TSH levels were low in 7.5% (52 cases: TSH <0.27-0.1 mU/liter in 27 and <0.1 mU/liter in 25), high in 17.2% (119 cases; TSH >4.20-10 mU/liter in 101 and >10 mU/liter in 18) and normal in the remaining 75.3% of cases. Moreover, high fT4 levels were observed in 13.4% of patients with low TSH, while 12.6% of those with high TSH had low fT4 levels. According to serum TSH, the rate of under-treated patients was higher than that of over-treated ones (χ^2=17.80, p<0.0001). Compared to L-T4 users whose TSH was in the euthyroid range (Table 2), over-replaced patients were younger, less obese, and took a higher daily dose of L-T4 µg/kg, while under-replaced patients were younger, predominantly males, showed higher abdominal obesity, FFM, insulin and insulin resistance, and took a lower daily dose of L-T4 µg/kg. A declining trend in FFM-adjusted REE was observed with decreasing L-T4 replacement. In analysis restricted to diabetic L-T4 users on chronic metformin treatment, this subgroup exhibited non-significantly lower TSH levels compared to non-diabetic L-T4 users [TSH, 1.83 (IQR, 0.34-3.46) vs. 2.6 (IQR, 0.01-3.25) mU/liter; p=0.7], while proportions of over-treated (5.2% vs. 7.3%) and under-treated (15.7% vs. 13.0%) patients were comparable between subgroups.

TSH levels did not appear to change with increasing age (Figure 1). In the 238 L-T4 users aged 65y or older, however, the frequency of euthyroid TSH was marginally higher than in the younger counterpart (84% vs. 70.6%, p<0.05), owing to a progressive reduction in over-treatment rates across increasing age decades (Figure 2). Cumulatively, low TSH levels suggestive of over-treatment were observed in <5% of patients aged >65 yr, while the rate of those with high TSH remained relatively constant across age decades. Gender distribution was similar between elderly and non-elderly subgroups. The anthropometric and metabolic study showed that elderly L-T4 users had a lower body weight, were more sarcopenic and, expectedly, took a lower L-T4 dose than their younger counterpart (Table 3).

Correlation and regression analyses in obese L-T4 users

Bivariate correlation analysis showed that the daily L-T4 dose was associated with many bodily variables, although FFM was the strongest correlate (Table 4). In a model including age, gender, cause of hypothyroidism, diabetes mellitus, %FM and FFM, as well as duration of L-T4 treatment, a stepwise multivariable regression analysis documented that daily L-T4 µg/kg dose was best predicted by FFM (β=0.262, p<0.0001), total thyroidectomy (β=0.225, p<0.0001), and gender (β=-0.122, p<0.05). In multinomial logistic regression analysis, the likelihood of L-T4 over-replacement was associated with younger age, higher daily L-T4

µg/kg dose and lower BMI (Table 5). In contrast, the risk of under-replacement was only associated with male gender ($p<0.05$).

Comparison with an euthyroid obese population

To test for potential anthropometric and metabolic effects relating to L-T4 treatment in the obese setting, a comparably obese group of age-, sex- and BMI-matched euthyroid subjects was enrolled among inpatients regularly admitted to our unit for obesity workup during the study period. In this group, BMI was >40 kg/m^2 in 77.6%, >35.0-39.9 kg/m^2 in 16%, and >30.0-34.9 kg/m^2 in 6.4% of cases. None suffered from inflammatory conditions and CRP levels were similar between populations. Despite having comparable BMIs, groups differed in terms of %FM and, non-significantly, FFM. Nevertheless, FFM-adjusted REE was higher in L-T4 users than euthyroid controls. There was no between-group difference in glucose and insulin levels, hence insulin resistance. Oppositely, L-T4 users exhibited a healthier lipid profile owing to lower total and LDL-cholesterol levels than euthyroid controls. These differences remained unchanged after excluding the subgroup of over- and under-replaced patients from the L-T4 group.

Discussion

L-T4 represents the first-line replacement treatment in primary hypothyroid patients with the aim to restore normal TSH secretion, promote patients' well-being and reduce the systemic consequences of hypothyroidism (14). Studies in the general population reported a variably high proportion of patients with abnormal TSH during replacement therapy with L-T4, even in the case of frequent monitoring for dosing adjustments. In the Colorado Thyroid Study, abnormal TSH levels were documented in 40% of 1525 patients taking thyroid medications, with over a fifth of cases showing low TSH values (25). A UK survey on 1037 patients noted abnormal thyroid function testing in 37.2% of cases, with 17.4% being over-treated and 19.8% being under-treated (26). Further, in 29.5% of 266 L-T4 users from the Pomerania study TSH levels were outside the reference range, and again more patients were over-replaced than under-replaced, namely 19.5% vs. 10% of cases (27). Our analysis on obese individuals with primary hypothyroidism evaluated the prevalence of adequate L-T4 treatment and attempted to gain insights on factors relating to abnormal thyroid testing in this cohort. Results highlight a peculiar role for obesity on L-T4 requirements, with nearly 75% of participants showing TSH levels within the euthyroid range. Among those with abnormal TSH, the rate of L-T4 under-replacement predominated as compared to that of over-replacement, namely 17.2% vs. 7.5% of cases. The reason for this outcome remains unclear. Current general recommendations toward specialised management of obesity increase the chance of stricter follow-ups (36), thus that our finding could simply be the result of improved obesity management. Alternatively, several studies have shown that body weight is maintained at a stable range, known as the set-point, despite the variability in energy intake and expenditure (37). This regulatory physiology is largely governed by leptin, a known hypothalamic modulator of TRH secretion, which could act to cushion TSH variations in obese patients until a new metabolic set-point develops, such as that generated by weight loss obtained during an inpatient regime (33) or after bariatric surgery (38). Because of the cross-sectionality of this study, the aptness of our interpretation remains to be verified in prospective studies achieving adequate variations in metabolic set-point.

TSH was unrelated to age but aging was associated with an improving rate of euthyroid TSH levels. We observed that median dose of L-T4 µg/kg BW declined with age, a finding that corroborates previous studies and, in this cohort, is potentially linked to underlying sarcopenic obesity. In older people, inflammatory conditions or non-thyroidal illness could per se alter TSH and fT4 adequacy to assess genuine euthyroidism, while fT3 measurement

can improve results accuracy (39). As we did not measure fT3 levels, the potential influence of extra-thyroidal conditions on our results cannot be discriminated. However, acute illness and/or inflammation was verified upon clinical assessment and by measurement of CRP levels at study entry to avoid including potential cases. Of note, the prevalence of over-treatment, a matter of concern for cardiac and skeletal health in the elderly, decreased with age and totalled 5% in patients aged 65y and over. This figure differs from data reported in the Cardiovascular Heart Study (13) and the Framingham Heart Study (40), where overtreatment rates in the elderly were 41% and 43%, respectively, while it overlaps with that obtained in an endocrine clinic population (41). The discrepancy could be explained by the circumstance that our referral center may admit patients undergoing endocrine surveillance elsewhere. Thus, we cannot dismiss the possibility of over-appraisal of L-T4 treatment adequacy in this subgroup. While endorsing current recommendations on adequate thyroid function monitoring in older L-T4 users (12,14), the apparent favourable outcome observed in our elderly cohort should be interpreted with caution, based on the evidence that target TSH levels during L-T4 treatment should be age-adjusted to avoid the risk of overtreatment in the elderly (12,14).

In terms of L-T4 requirement, the median L-T4 dose was 0.91 µg/kg/day. Body weight is a good indicator for calculating an appropriate starting dose of L-T4, with other determinants including age, gender, body composition, cause and validation of hypothyroidism, as well as comorbidities, drugs, and adherence to therapy (4-11,14,42,43). Notwithstanding the suggestion that severe obesity may require higher than normal L-T4 doses due to impaired L-T4 pharmacokinetics and T4 to T3 conversion (31), we failed to observe apparent defects in L-T4 absorption in this large obese group and confirmed earlier evidence showing that the weight-adjusted L-T4 dose decreases with increasing BMI (44,45). Moreover, our findings on fat-free mass confirm that lean mass exerts a predictive role on L-T4 dose (38,44), which has been linked to cellular processes of deiodination and thyroid hormone metabolism taking place in muscle cells (46).

In the search for factors potentially associated with abnormal thyroid function testing, the role of anthropometric and metabolic variables of L-T4 users was explored according to patients' TSH. Patients with low TSH were younger, took a higher L-T4 dose and exhibited a poorer lean mass relatively to body mass when compared to those with euthyroid TSH values. On the other hand, patients with high TSH levels also were younger and were more frequently males, more abdominally obese, and their L-T4 dose appeared disproportionately low relative to their lean mass. Resting energy expenditure normalized by fat-free mass appeared to decline across categories of L-T4 users with low to high TSH. This result substantiate the notion that thyroid hormone and thyrotoxicosis exerts stimulatory effects on protein metabolism, heat production and metabolic efficiency (1). Likewise, a recent 6-month interventional study by Samuels and coworkers (47), while failing to document a direct effect of L-T4 dose modifications on resting energy expenditure normalized for lean body mass, noticed that its increases correlated directly with increases in fT4 and fT3 levels and inversely with increases in TSH levels across the achieved range of TSH levels. At odds with findings obtained in the general population on the favourable effect of L-T4 replacement on the lipid profile (18-22), we could document only modest decrements of total and LDL-cholesterol along with declining levels of serum TSH in L-T4 users. In terms of glucose homeostasis, the high TSH subgroup harboured significantly higher insulin resistance and non-significantly higher glucose levels compared to the other subgroups. Previous studies informed on associations between insulin resistance and thyroid function summarized as follows: 1) a cross-sectional association exists between insulin resistance and TSH levels (48); 2) the hypothyroid state is linked to a cellular condition of insulin resistance in adipocytes and muscle cells (49,50); 3) treatment of hypothyroidism improves insulin resistance (51). While

these and our findings pinpoint the potential link between L-T4 adequacy and insulin resistance, we found that neither diabetes mellitus nor metformin treatment altered the risk of thyroid testing abnormalities. This lack of result adds argument to the ongoing debate on the relationship between diabetes mellitus and the risk of L-T4 over-treatment in the general population, where both positive (13) and negative (26) associations have been identified. With reference to the risk of abnormal thyroid testing, logistic regression analysis showed that the likelihood of overtreatment was associated with younger age, higher L-T4 dose and lower BMI, while only male gender increased the risk of L-T4 under-treatment. These results corroborate those of studies in non-obese populations, reporting a relationship between L-T4 dose and overtreatment risk on one side, and between male gender and under-treatment risk on the other (13,52). Together, our results suggest that the adequacy of treatment in obesity is potentially associated with anthropometric and metabolic outcomes, and underscore the role of lean mass and age in regulating L-T4 requirements. Whether this implies that assessment of body composition in this setting may help titrating individual L-T requirements remains to be demonstrated.

To implement our understanding of residual metabolic risks in obese subjects on L-T4, their metabolic profile was examined in comparison to an equally obese group of euthyroid controls. Despite the similarities in BMI distribution, adiposity was higher in L-T4 users than in euthyroid obese controls, which agrees with previous evidence that hypothyroid patients only experience modest reductions in fat mass (1). Moreover, about 15% of L-T4 treated subjects do not reach clinical euthyroidism (14) as a result of impaired intracellular T3 production determined by the down-regulation of the deiodinase pathway (53). Hypothetically, the existence of underlying peripheral hypothyroidism may ultimately influence long term regulation of body composition and contribute to explain current observations. Nonetheless, the levels of total cholesterol and LDL-cholesterol were significantly lower in L-T4 treated than in euthyroid controls, without differences in HDL-cholesterol and triglyceride levels. These findings fall in line with those of previous randomized crossover (19,23), double-blind placebo-controlled (20,21) and longitudinal studies (22) investigating on L-T4 effects on lipids in non-obese populations (19-23). Current results also agree with those of a meta-analysis showing a decreasing effect of L-T4 replacement on total and LDL-cholesterol in L-T4 users, while HDL-cholesterol and triglyceride levels remained unaltered (54). Oppositely, a meta-analysis of 99 studies documented persistently higher total and LDL cholesterol levels were observed in L-T4 users compared to controls (24). Because of the lack of studies on lipids in L-T4 treated obese populations (55), we cannot draw conclusive clinical implications from our results.

Our study depicts a real-world snapshot of L-T4 therapy in hypothyroid obesity. As such, it conveys a number of limitations, such as the cross-sectional design, the lack of fT3 measurement and information on therapeutic adherence (56-59). As individual adequacy of L-T4 therapy was based on the incidental finding of normal TSH, this measure may inadequately reflect the long-term thyroid hormone replenishment status of these patients. Furthermore, this study did not include a group of lean hypothyroid L-T4 users to compare rates of inadequate L-T4 replacement in the general population. Rather, our study aim was to discriminate the effect of L-T4 on the metabolic phenotype of obesity. The strength of the clinical information originating from our investigation includes a lower than expected rate of inadequate L-T4 replacement, an improving L-T4 adequacy with aging, and a relationship between L-T4 requirement and gender, age and lean body mass in this obese cohort. While our results imply that a limited proportion of L-T4 users is over-treated among obese patients referring to an obesity clinic, current data also support the recommendation that TSH target during L-T4 replacement should be age-adjusted, and L-T4 adequacy should be especially monitored in elderly obese individuals to avoid the detrimental effect of over-treatment.

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No competing financial interests exist.

Data Availability
All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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Figure 1: Correlation between serum TSH levels and age in obese L-T4 users.

Figure 2: Prevalence of obese L-T4 users with low TSH (over-treated), euthyroid (adequately-treated) and high TSH (under-treated) subdivided according to the age decades. For each decade, proportions are expressed as percent values and the number of subjects is displayed in parentheses on the horizontal axis.

Table 1. Summary of anthropometric and biochemical data obtained in obese L-T4 users and obese euthyroid controls (Eu-Thyr group). Data are expressed as median, with interquartile range in parentheses, or percentage. Comparison between populations was performed by ANOVA and χ^2 test.

Variables	L-T4 group (n = 691)	Eu-Thyr group (n = 691)	p
Males/females (N, %)	86/605 (12.4/87.6)	86/605 (12.4/87.6)	1
Age (years)	59 (50-69)	58.0 (50.9-66)	0.9
BMI (kg/m^2)	43.9 (40.1-48.4)	44.3 (39.8-48.2)	0.5
BMI >40 kg/m^2	75.8%	77.6%	0.2
Waist (cm)	123 (114-132)	122 (111-131)	0.6
FM (%)	50.9 (46.6-54.2)	48.9 (44.4-53.0)	<0.0001
FFM (kg)	54.0 (48.3-59.8)	54.4 (49.1-61.6)	0.2
REE (kcal/day)	1731 (1557-1940)	1711 (1491-1949)	0.2
REE$_{FFM}$	31.5 (2.6-34.4)	30.7 (27.1-33.8)	<0.001
TSH (mU/liter)	2.08 (0.96-3.69)	1.78 (1.20-2.70)	<0.0001
fT4 (ng/liter)	12.1 (10.8-13.6)	11.2 (10.2-12.5)	<0.0001
Glucose (mg/dL)	97.0 (88.0-114.8)	97.0 (86.0-111.0)	0.1
Insulin (mU/liter)	13.1 (8.9-18.4)	13.4 (9.6-18.9)	0.2
HOMA-IR	3.2 (2.1-4.8)	3.3 (2.2-5.2)	0.1
CHO (mg/dL)	191 (166-219)	208 (182-233)	<0.0001
LDL-CHO (mg/dL)	120 (97-144)	129 (106-153.9)	<0.0001
HDL-CHO (mg/dL)	47 (39-56)	47 (39-54)	0.7
TG (mg/dL)	130 (100-175)	130 (103-169)	0.5
CRP (mg/dL)	0.7 (0.4-1.2)	0.7 (0.4-1.2)	0.7

For abbreviations: BMI, body mass index; WHR, Waist-to-hip ratio, FM, fat mass; FFM, fat-free mass; REE, resting energy expenditure; TSH, thyroid-stimulating hormone; fT4, free thyroxine; HOMA-IR, homeostatic model of insulin resistance; CHO, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; CRP, C-reactive protein.

Table 2. Summary of data obtained in obese L-T4 users, subdivided as adequately, under-treated and over-treated patients. Data are expressed as median, with interquartile range in parentheses. Comparison between populations was performed by ANOVA test and χ^2 test. Significant differences between adequately treated and under-treated or over-treated group are expressed as: *, p<0.05; *1, p<0.01; *2, p<0.001; *3, p<0.0001. Significant differences between under-treated and over-treated group are expressed as: §, p<0.05; §, 1p<0.01; §2, p<0.001; §3, p<0.0001.

Variables	Adequately treated (n = 519)	Under-treated (n = 119)	Over-treated (n = 52)
Males/females (N, %)	50/469 (9.6/90.4)	30/89 (25.2/74.8)^{*3}	6/46 (11.5./98.5)§
Age (years)	60 (51-70)	57 (49-64)*	54 (48-63)^{*2}
BMI (kg/m^2)	44.1 (40.2-48.5)	44.5 (41.0-49.6)	40.6 (37.1-43.7)$^{*2, §3}$
Waist (cm)	123 (113-132)	127 (116-139)*	120 (110-125)$^{*, §2}$
FM (%)	51.0 (47.2-54.5)	49.5 (43.6-53.9)	50.0 (44.7-53.0)
FFM (kg)	53.7 (47.9-58.8)	58.6 (50.9-68.4)^{*3}	51.1 (47.7-56.8)^{§2}

REE (kcal/day)	1720 (1549-1920)	1809 (1631-2057)*	1711 (1570-1904)
REE_{FFM}	31.5 (28.6-34.4)	30.5 (27.7-33.3)*	32 (30.2-35.5)§
L-T4 dose (µg/day)	100 (75-125)	100 (50-136)	125 (92-150) *1, §
L-T4 dose BW (µg/kg/day)	0.90 (0.62-1.14)	0.84 (0.49-1.14)*	1.16 (0.94-1.37)*2, §3
TSH (mU/liter)	1.80 (1.02-2.80)	5.90 (4.92-8.12)*3	0.07 (0.03-0.15)*3, §3
fT4 (ng/liter)	12.1 (11.0-13.6)	11.1 (9.9-12.4)*3	14.3 (12.7-16.0)*3, §3
Glucose (mg/dL)	96 (88-113)	101 (90-119)	95 (88-107)
Insulin (mU/liter)	12.7 (8.9-17.7)	14.3 (8.8-21.8)*	13.8 (9.6-18.3)
HOMA-IR	3.0 (2.1-4.6)	3.6 (2.1-6.0)*	3.2 (2.2-5.0)
CHO (mg/dL)	192 (168-218)	189 (166-226)	181 (155-213)
LDL-CHO (mg/dL)	120 (99-143)	121 (95-147)	104 (89-140)
HDL-CHO (mg/dL)	47 (38-57)	46 (40-55)	47 (41-53)
TG (mg/dL)	127 (102-174)	141 (100-183)	126 (85-153)
CRP (mg/dL)	0.65 (0.38-1.17)	0.64 (0.39-1.23)	0.82 (0.50-1.05)

For abbreviations: BMI, body mass index; WHR, Waist-to-hip ratio, FM, fat mass; FFM, fat-free mass; REE, resting energy expenditure; TSH, thyroid-stimulating hormone; fT4, free thyroxine; HOMA-IR, homeostatic model of insulin resistance; CHO, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides.

Table 3. Summary of anthropometric and thyroid function data obtained in obese L-T4 users divided according to age <65 and ≥65 years. Data are expressed as median, with interquartile range in parentheses, or percentage. Comparison between populations was performed by ANOVA and χ^2 test.

Variables	**Patients aged <65 yr (n = 453)**	**Patients aged ≥65 yr (n = 238)**	**p**
Males/Females (N, %)	55/397 (12.1/87.9)	31/207 (13/87)	0.7
Age (years)	54 (45-58)	71 (69-74)	<0.0001
BMI (kg/m^2)	44.3 (40.3-49.4)	43.2 (39.7-47.2)	<0.01
FM (%)	50.1 (45.9-53.5)	52.4 (48.0-55.4)	<0.0001
FFM (kg)	55.3 (50.7-61.6)	49.5 (45.5-56.5)	<0.0001
L-T4 dose (µg/day)	100 (75-132)	100 (50-125)	<0.0001
L-T4 dose (µg/kg/day)	0.93 (0.66-1.18)	0.88 (0.55-1.14)	<0.05
TSH (mU/liter)	1.99 (0.88-3.83)	2.14 (1.10-3.26)	0.3
fT4 (ng/liter)	11.9 (10.8-13.6)	12.3 (10.9-13.7)	0.5
CRP (mg/dL)	0.7 (0.4-1.3)	0.5 (0.3-1)	<0.001
Adequately treated (%)	319 (70.6%)	200 (84.0%)	<0.05
Under-treated (%)	92 (20.4%)	38 (16.0%)	0.2
Over-treated (%)	41 (9.1%)	11 (4.6%)	<0.05

For abbreviations: BMI, body mass index; TSH, thyroid-stimulating hormone; fT4, free thyroxine; BW, body weight.

Table 4. Bivariate correlation analysis between the daily L-T4 and L-T4 µg/kg dose and anthropometric or metabolic variables in L-T4 users. Significant correlations are shown in bold character and expressed as ρ values. For significance: *, *p <0.05*, *1, *p<0.01*, *2, *p<0.001*, *3, *p<0.0001*.

Variables	**L-T4/day**	**L-T4 µg/kg/day**
Gender	0.04	-0.05
Age (years)	**-0.15*3**	-0.05
BMI (kg/m^2)	**0.09***	**-0.19*3**
WC (cm)	**0.11***	**0.16*3**
FM (%)	-0.003	**-0.12*1**
FFM (kg)	**0.19*3**	**-0.11*1**
REE (kcal/day)	**0.14*1**	**-0.11***
REE_{FFM}	0.02	0.04
TSH (mU/liter)	**-0.13*3**	**-0.20*3**
fT4 (ng/liter)	**0.24*3**	**0.28*3**
Glucose (mg/dL)	0.02	0.01
Insulin (mU/liter)	-0.02	-0.07
HOMA-IR	-0.01	-0.05
CHO (mg/dL)	-0.04	-0.03
LDL-CHO (mg/dL)	-0.01	-0.01
HDL-CHO (mg/dL)	-0.06	-0.02
TG (mg/dL)	-0.01	-0.01

For abbreviations: BMI, body mass index; WC, Waist Circumference, FM, fat mass; FFM, fat-free mass; REE, resting energy expenditure; TSH, thyroid-stimulating hormone; fT4, free thyroxine; HOMA-IR, homeostatic model of insulin resistance; CHO, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides.

Table 5. Odds ratios (ORs) for L-T4 over-treatment and under-treatment vs. adequate treatment. Significant correlations are shown in bold character.

Covariates	**L-T4 over-treatment**		**L-T4 under-treatment**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	**0.96 (0.94-0.99)**	**0.001**	0.99 (0.97-1.01)	0.2
Gender				
Female	1	Ref.	1	Ref.
Male	1.75 (0.55-5.59)	0.3	**2.37 (1.10-5.11)**	**0.03**
BMI	**0.93 (0.88-0.99)**	**0.01**	1.01 (0.98-1.05)	0.5
L-T4 dose (µg/kg/day)	**2.98 (1.44-6.14)**	**0.003**	1.02 (0.59-1.78)	0.9
Duration of hypothyroidism	0.99 (0.95-1.04)	0.8	0.99 (0.96-1.02)	0.5
FFM (kg)	0.98 (0.95-1.02)	0.3	1.01 (0.99-1.04)	0.3
Cause of hypothyroidism				
AITD (0)	1	Ref.	1	Ref.
Thyroidectomy (1)	1.38 (0.58-3.25)	0.5	0.56 (0.29-1.27)	0.2
Presence of diabetes mellitus				
No (0)	1	Ref.	1	Ref.
Yes (1)	1.30 (0.45-3.22)	0.7	0.47 (0.40-1.55)	0.5

For abbreviations: BMI, body mass index; FFM, free fat mass; AITD, autoimmune thyroid disease

Figure 1

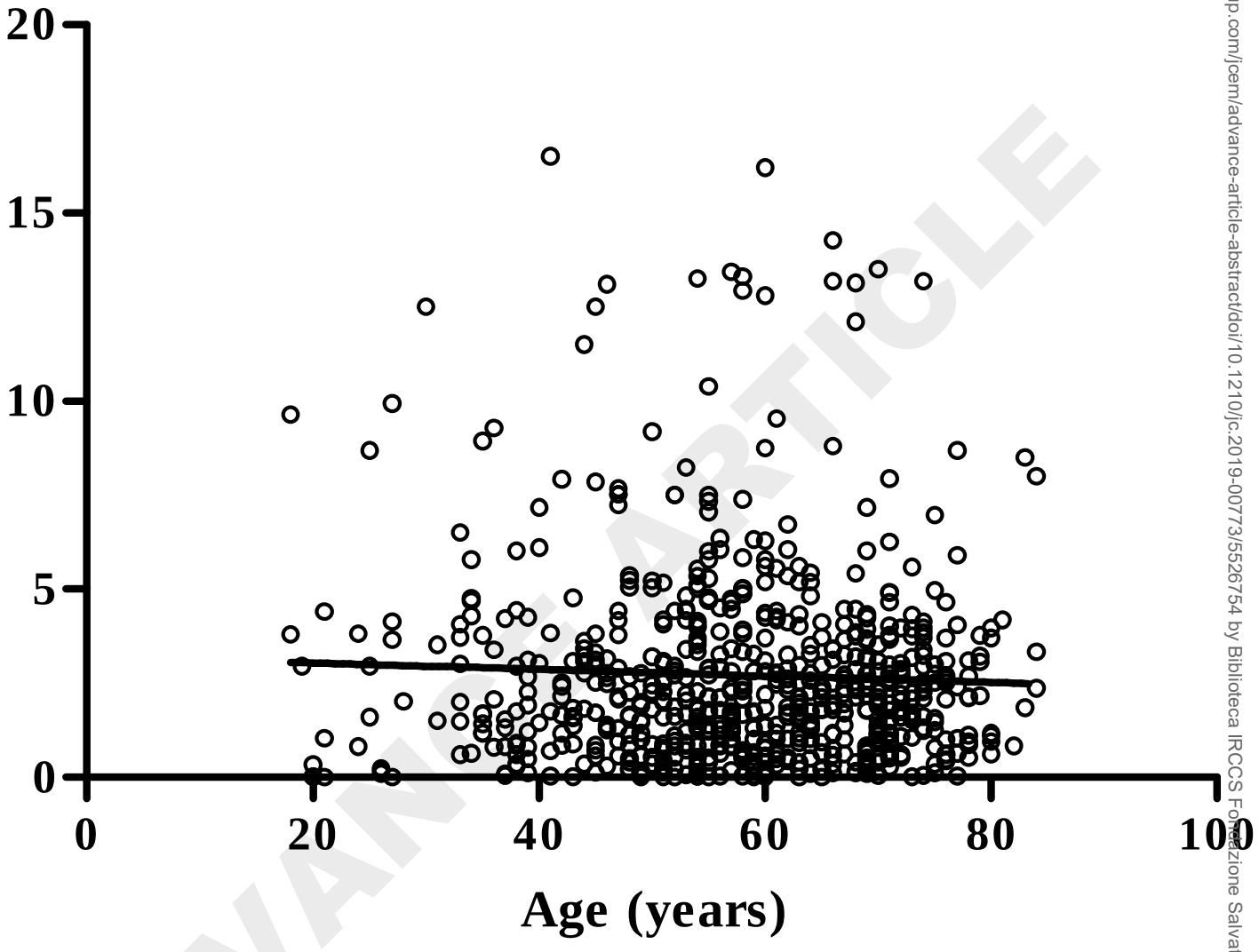

Figure 2