Metabolic Effects of Liothyronine Therapy in Hypothyroidism: A Randomized, Double-Blind, Crossover Trial of Liothyronine *Versus* Levothyroxine

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Context: Levothyroxine (L-T_4) therapy is based on the assumption that the conversion of T_4 into T_3 provides adequate amounts of active hormone at target tissues. However, in rodents, L-T_4 alone does not restore a euthyroid state in all tissues. Previous combination L-T_4/liothyronine (L-T_3) therapy trials focused on quality-of-life endpoints, and limited information is available on the effects on other measures of thyroid hormone action.

Objective: Our objective was to evaluate the efficacy of thyroid hormone replacement with L-T_4 or L-T_3 at doses producing equivalent normalization of TSH.

Participants, Design, and Setting: Fourteen hypothyroid patients participated in this randomized, double-blind, crossover intervention at the National Institutes of Health Clinical Center.

Interventions: L-T_3 or L-T_4 were administered thrice daily to achieve a target TSH from 0.5–1.5 mU/liter. Volunteers were studied as inpatients after 6 wk on a stable dose and at the target TSH.

Main Outcome Measures: Serum thyroid hormones, lipid parameters, and indices of glucose metabolism were evaluated.

Results: No difference was observed in TSH between L-T_3 and L-T_4 treatments. L-T_3 resulted in significant weight loss [L-T_4, 70.6 ± 12.5, *vs.* L-T_3, 68.5 ± 11.9 kg ($P = 0.009$)] and in a 10.9 ± 10.0% decrease in total cholesterol ($P = 0.002$), 13.3 ± 12.1% decrease in low-density lipoprotein-cholesterol ($P = 0.002$), and an 18.3 ± 28.6% decrease in apolipoprotein B ($P = 0.018$). No significant differences were observed in high-density lipoprotein-cholesterol, heart rate, blood pressure, exercise tolerance, or insulin sensitivity.

Conclusions: The substitution of L-T_3 for L-T_4 at equivalent doses (relative to the pituitary) reduced body weight and resulted in greater thyroid hormone action on the lipid metabolism, without detected differences in cardiovascular function or insulin sensitivity. (*J Clin Endocrinol Metab* 96: 3466–3474, 2011)

Overt hypothyroidism has a prevalence of 0.3–2% in the general population (1, 2). Since 1891, hypothyroidism has been treated with replacement therapy, initially using desiccated thyroid gland (3), and since the 1930s with levothyroxine (L-T_4) (4), the sodium salt of the natural thyroid hormone (TH) tetraiodothyronine (T_4). Compared with desiccated gland, L-T_4 is devoid of antigenicity, is chemically stable, and has uniform potency and

Abbreviations: AUC, Area under the curve; DXA, dual-energy x-ray absorptiometry; HDL, high-density lipoprotein; LDL, low-density lipoprotein; L-T_3, liothyronine; L-T_4, levothyroxine; QOL, quality of life; REE, resting energy expenditure; TH, thyroid hormone.

prompt absorption allowing once-daily dosing. Therapy with L-T_4 is the current standard of care (5–8), based on the assumption that conversion of inactive T_4 into hormonally active T_3 provides an adequate amount of TH at the target end-organs (9). Adequate replacement therapy is defined by a serum TSH within the normal range, indicating a state of euthyroidism at the hypothalamus-pituitary axis level (7, 8).

However, rodent data indicate that replacement therapy with L-T_4 does not achieve adequate levels of T_3 in all tissues, whereas a combination of L-T_4 with liothyronine (L-T_3, synthetic drug formulation of T_3) does (10, 11). Furthermore, some patients treated with L-T_4 experience hypothyroid symptoms despite a serum TSH concentration within the normal laboratory reference limits (12). These findings inspired clinical trials of L-T_3/L-T_4 combination therapy. Although combination therapy improved quality of life (QOL) and depression scale outcomes either in the study populations (13–15) or in subgroups (16–18), other studies failed to replicate these findings (19–23). In addition, little is known about the effects of combination therapy on other measures of TH action.

In this study, we evaluated multiple measures of hormone action in response to doses of L-T_3 *vs.* L-T_4 that produce equivalent steady-state baseline and TRH-stimulated TSH levels.

Materials and Methods

Study design

The study was approved by the National Institute of Diabetes and Digestive and Kidney Diseases-National Institute of Arthritis and Musculoskeletal and Skin Diseases Institutional Review Board and conducted at the National Institutes of Health (NIH) Clinical Center. Informed consent was obtained from all study participants. Inclusion criteria were primary hypothyroidism on replacement therapy with a daily L-T_4 dose of at least 1.6 μg/kg, age at least 18 yr, and body mass index of at least 20 kg/m^2 and no higher than 30 kg/m^2. Exclusion criteria were clinical indication for TH suppressive therapy, thyroid residual function greater than 5% as measured by a 24-h ^{123}I thyroid uptake (in patients with thyroid gland or remnant greater than 1 cm^3 by ultrasound) while on replacement therapy; hypertension or cardiovascular disease; pregnancy or use of hormonal contraception; diabetes; serum total cholesterol 240 mg/dl or higher; serum triglycerides 220 mg/dl or higher; chronic liver disease or alanine aminotransferase level greater than 2-fold the upper laboratory reference limit; glomerular filtration rate (24) less than 50 ml/min · 1.73m^2; history or symptoms compatible with psychosis; major depression; use of antipsychotic medications; and use of drugs or dietary supplements known to influence thyroid status or TH pharmacokinetics. The details of this randomized, double blind, crossover intervention have been described elsewhere (25). After randomization, the study participants were assigned to a preprandial thrice-daily regimen of L-T_3 or L-T_4 with the therapeutic target of a serum TSH level within the range 0.5–1.5 mU/liter. Each capsule, with the exception of the placebo for sham adjustments, contained active drug (L-T_3 2.5, 10, or 16 μg, L-T_4 5, 10, or 33 μg). Adherence to the regimen was assessed by direct questioning during the follow-up visits and by pill counts. Patients returned to the clinic for biweekly follow-up visits and TSH sampling. Three unblinded investigators (M.Z., M.C.S., and F.P.) performed dosage adjustments. No dietary recommendations were given. Once the patients maintained a serum TSH level within the target range on a stable replacement dose for three consecutive follow-up visits, they were admitted for a 5-d hospital stay for metabolic testing. Upon discharge, the patients crossed over to the alternate treatment arm following the same scheme.

Study procedures

The technical references relative to the study procedures are reported in the Supplemental Material (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Blood collection

Fasting samples were collected on study d 1. TH samples were collected every 4 h for a 24-h period. All assays were performed by the Department of Laboratory Medicine, NIH Clinical Center.

Exercise test

A graded exercise test, with recording of anaerobic threshold, maximal oxygen consumption, maximal power (peak Watts), resting and maximal heart rate, and baseline and exercise-induced cardiac output was performed on study d 1. Gas exchange and electrocardiogram were measured by a metabolic cart (Ultima; Med Graphics Inc., Minneapolis, MN). Anaerobic threshold was determined using the V-slope method using an electromagnetically braked cycloergometer (Lode Corvival; Lode BV, Groningen, The Netherlands). Cardiac output was measured using impedance cardiography (Physioflow, Manatec Inc., Paris, France).

Array meal and equilibration diet

A standard buffet meal of 7566 kcal with 24 food items (46% carbohydrate, 14% protein, and 40% fat) was offered at lunchtime of study d 1. Study participants were instructed to eat to satiety and to indicate on a 10-cm visual-analog scale their level of hunger before and after the meal. The weight of each item was measured before and after the meal. After the array meal and throughout hospitalization, a 55% carbohydrate, 15% protein, and 30% fat caffeine-free, low-nitrate weight-maintenance diet was provided. Resting energy requirements were calculated using the Harris-Benedict equation with a standard activity factor of 1.5.

Physiology and body composition measurements

Body composition and bone mineral density were measured by dual-energy x-ray absorptiometry (DXA) with a QDR 4500A scanner (Hologic, Bedford, MA). Body weight was measured with a digital scale (Scale-Tronix 5702; Carol Stream, IL) the morning of study d 2. Resting energy expenditure (REE) and the respiratory quotient were measured the morning of study d 4 by indirect calorimetry after a 12-h fast using the ventilated hood technique (ParvoMedics, Sandy, UT).

Hyperinsulinemic-euglycemic clamp

The morning of study d 4, a hyperinsulinemic-euglycemic clamp was performed using an insulin infusion rate of 40 mU/$m^2 \cdot$ min. Serum glucose was measured every 5 min by glucose analyzer (YSI, Yellow Springs, OH). After equilibration, glucose disposal rate was calculated over a 20-min period while serum glucose was maintained within the range 95–105 mg/dl.

Quality of life assessment

A psychological well-being metric (SF-36) and the hypothyroid-specific health-related QOL questionnaire were administered on study d 2. Questionnaires were evaluated by a clinical psychologist, blinded to the treatment assignments. At the completion of the second hospitalization, the study patients were asked which of the two treatments they preferred.

Cardiovascular function

Echocardiograms were obtained according to established guidelines. Endothelial function was assessed on study d 5 after a 12-h fast using an Acuson Sequoia C512 (Siemens, Malvern, PA) with a 15L8 transducer. Brachial artery flow-mediated vasodilation was assessed using established methods. Studies were acquired at baseline and during reactive hyperemia for measurement of endothelium-dependent vasodilation. Sublingual nitroglycerin (0.4 mg) was also given for measurement of nitrate-induced, endothelium-independent vasodilation.

Statistical analysis

A two-tailed paired *t* test was used to compare data between treatment arms. Nonparametric data were analyzed using Wilcoxon signed rank sum test. Results are expressed as mean ± SD; $P < 0.05$ was considered the threshold for statistical significance. Analyses were performed using Prism version 5 (GraphPad, La Jolla, CA).

Results

Study population characteristics

During the period August 2005 to July 2010, approximately 110 prospective volunteers contacted the NIH recruitment office in reference to this study. Most of the patients declined to participate because of the time commitment and/or the thrice-daily drug administration regimen. Twenty-nine patients (25 females and four males) were screened in the outpatient clinic for the participation in the study, and 18 (16 females and two males) of 20 eligible subjects agreed to enroll and were randomized. Four patients (three females and one male) withdrew from the study, one because of relocation and three because of poor compliance with the medication regimen. All of the dropouts were recorded during the first phase of the study (two while on L-T_3 and two while on L-T_4). Fourteen patients (seven initially allocated to L-T_3 and seven to L-T_4 treatment arm) completed the study. Patients' baseline characteristics are reported in Table 1. Drug dosage, serum TH levels, and TRH-stimulation test of the first 10

TABLE 1. Baseline characteristics of the study population

	Mean ± SD (range)[a]
Female/male (n)	13/1
Age (yr)	49.3 ± 8.0 (41–72)
Ethnicity (n)	
Caucasian	10
Asian	2
Hispanic	1
African-American	1
Height (cm)	162.8 ± 6.1 (151.5–173.9)
Weight (kg)	69.7 ± 12.5 (51.3–89.9)
Body mass index (kg/m²)	26.3 ± 4.3 (20.3–30.0)
Etiology of hypothyroidism (n)	
Multinodular goiter	7
Follicular adenoma	1
Papillary thyroid cancer	2
Follicular thyroid cancer	1
Medullary thyroid cancer	1
Hashimoto thyroiditis	1
^{131}I treatment for Graves' disease	1
Duration of hypothyroidism (yr)	8.6 ± 13.1 (0.5–50)
TSH (mU/liter)	1.1 ± 1.4 (0.04–4.52)
T_3 (ng/dl)	99.0 ± 24.2 (65–141)
Free T_4 (ng/dl)	1.76 ± 0.46 (1.1–2.5)
Time to therapeutic target L-T_4 (d)	153 ± 70.5 (60–298)
Time to therapeutic target L-T_3 (d)	132 ± 71.1 (53–275)
L-T_4 dose [µg/d (µg/kg · d)]	120.3 ± 39.8 (1.68 ± 0.33)
L-T_3 dose [µg/d (µg/kg · d)]	39.7 ± 10.2 (0.57 ± 0.08)

Conversion factors for SI units are as follows: nanograms per deciliter × 0.0154 nanomoles per liter for T_3; nanograms per deciliter × 12.87 picomoles per liter for free T_4. The differences in time to therapeutic target were not significant ($P = 0.335$).

[a] Unless indicated otherwise.

patients who completed the study have been reported elsewhere (25). No treatment sequence effect was observed for any of the parameters analyzed. No serious adverse events were recorded. One patient experienced an episode of generalized anxiety disorder while receiving L-T_4 with a TSH of 0.27 mU/liter. The patient underwent cognitive behavior therapy with a clinical psychologist and completed the study without receiving anxiolytic therapy.

Replacement therapy and TH levels

The dose of L-T_3 was 0.57 ± 0.08 µg/kg · d, whereas the L-T_4 was 1.68 ± 0.33 µg/kg · d with a microgram/microgram L-T_3 to L-T_4 ratio of 0.34 ± 0.05 (Table 1). No difference was observed in the TSH values during the periods preceding the achievement of the therapeutic target (L-T_3 2.07 ± 1.28 *vs.* L-T_4 2.11 ± 1.56 mU/ml, $P = 0.945$). At the time of admission, there was no difference in the morning TSH values (L-T_3 1.44 ± 0.79 *vs.* L-T_4 1.30 ± 0.79 mU/ml, $P = 0.674$) or in the average TSH levels in the three follow-up visits preceding the admission (L-T_3
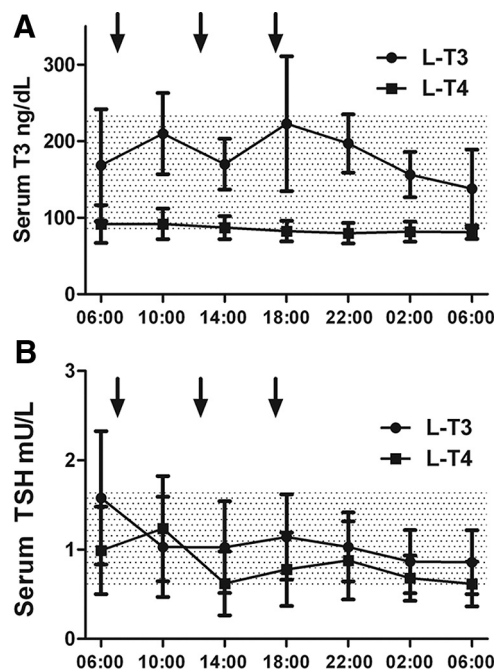
FIG. 1. The 24-h profile of serum T_3 (A), and TSH (B). The *arrows* indicate the administration of the study medication. For total T_3, to convert to SI units, multiply nanograms per deciliter × 0.0154 to get nanomoles per liter.

0.92 ± 0.18 *vs.* L-T_4 0.92 ± 0.25 mU/ml, $P = 0.999$). As expected, during L-T_3 therapy, the morning fT4 levels were below the detection limits (L-T_3 < 0.3 *vs.* L-T_4 1.57 ± 0.30 ng/dl). L-T_3 replacement resulted in higher serum morning T3 levels (L-T_3 172.0 ± 88.25 *vs.* L-T_4 92.86 ± 19.01 ng/dl, $P = 0.003$). The difference in serum T3 was evident throughout the 24-h [L-T_3 area under the curve (AUC) $261,214 \pm 85,309$ *vs.* L-T_4 AUC $126,531 \pm 33,934$ ng/dl · min, $P < 0.0001$]. No difference was observed in the 24-h profile of serum TSH (L-T_3 AUC 2332 ± 1312 *vs.* L-T_4 AUC 2495 ± 1786 mU/liter · min, $P = 0.789$) (Fig. 1). Although more variable than on L-T_4, the average serum T_3 levels remained within the normal range throughout the 24-h serial sampling period on L-T_3 (Fig. 1).

Body weight and body composition data (Table 2)

L-T_3 treatment resulted in a significant decrease in body weight (L-T_3 68.5 ± 11.9 *vs.* L-T_4 70.6 ± 12.5 kg, $P = 0.009$) with an average individual weight loss of -1.8 ± 1.9 kg and a nonsignificant decrease in total fat mass ($-5.3 \pm 8.5\%$, $P = 0.052$). The individual changes in body weight and fat mass are reported in Fig. 2. No significant difference in fat-free mass, bone mineral density, or REE was observed between the treatment arms.

Lipid and other laboratory data (Table 2)

Compared with the L-T_4 arm, L-T_3 resulted in a $10.9 \pm 10.0\%$ decrease in total cholesterol ($P = 0.002$), a $13.3 \pm 12.1\%$ reduction in low-density lipoprotein (LDL)-cho-

lesterol ($P = 0.002$), a $12.1 \pm 13.8\%$ reduction in non-high-density lipoprotein (non-HDL) cholesterol, ($P = 0.008$), and an $18.3 \pm 28.6\%$ decrease in apolipoprotein B ($P = 0.018$). A $7.0 \pm 14.6\%$ reduction in HDL-cholesterol with L-T_3 treatment did not reach statistical significance ($P = 0.067$). Similarly, there was no significant change in apolipoprotein A-I levels. Individual changes and average point estimates are shown in Fig. 2.

L-T_3 resulted in a $22.3 \pm 27.0\%$ increase in SHBG levels ($P = 0.038$). A significant correlation was observed among the changes in lipid parameters, whereas no significant correlation was observed among the changes in other parameters (Fig. 2). No significant differences were observed in fasting glucose, insulin, or insulin sensitivity as measured by the homeostatic model assessment index or hyperinsulinemic-euglycemic clamp.

QOL questionnaires and array meal

No difference between treatments was observed in the SF-36 (26), and no significant difference was observed in health-related QOL (19). The array meal showed no significant difference either in total calorie intake or in macronutrient preference. Similarly, no difference was observed in degree of hunger before and after the array meal test (data not shown). At the end of the study, four patients stated they had no preference in treatment, five preferred L-T_4, and five preferred L-T_3.

Cardiovascular function and exercise test (Table 2)

There were no statistically significant differences in resting heart rate or blood pressure between the two treatments, although a nonsignificant increase in diastolic blood pressure was observed during L-T_3 treatment (L-T_3 73.1 ± 10.0 *vs.* L-T_4 68.2 ± 7.3 mm Hg, $P = 0.078$). No significant differences between treatments were observed either in chamber size or systolic function parameters. Although there was a significantly shorter isovolumic relaxation time in the L-T_3 treatment arm, most diastolic function parameters showed no differences. No significant differences were observed in flow-mediated vasodilation or exercise tolerance test (Table 2). None of the subjects experienced anginal symptoms, and no ST-T wave changes were observed during exercise or during the recovery phase.

Discussion

L-T_4 is the standard medication for the treatment of hypothyroidism (6), and the single daily dosing makes it an ideal drug for life-long management of this disease. The sensitivity of the pituitary to TH and accurate assays of TSH allow precise therapeutic titration, based on the assumption that a state of pituitary euthyroidism equates to

TABLE 2. Physiology and body composition data

Parameter	L-T$_4$	L-T$_3$	*P* value (Δ %)
Physiology and body composition			
Weight (kg)	70.6 ± 12.5	68.5 ± 11.9	0.009 (−2.4 ± 2.6)
Fat mass (kg)[a]	25.5 ± 6.9	24.1 ± 6.9	0.052 (−5.3 ± 10.0)
Heart rate (bpm)	65.0 ± 8.1	68.2 ± 7.3	0.200
Systolic blood pressure (mm Hg)	113.4 ± 10.0	119.3 ± 10.6	0.122 (5.7 ± 11.6)
Diastolic blood pressure (mm Hg)	68.2 ± 7.3	73.1 ± 10.0	0.078 (7.7 ± 14.3)
Pulse pressure (mm Hg)	45.2 ± 9.0	46.2 ± 10.2	0.771
L1–L5 bone densitometry (g/cm^2)	1.1 ± 0.1	1.1 ± 0.1	0.707
Lumbar spine Z-score	0.55 ± 1.3	0.61 ± 1.3	0.312
Resting energy expenditure (kcal/24 h)	1201 ± 281.5	1177 ± 322.6	0.717
Respiratory quotient	0.86 ± 0.05	0.86 ± 0.05	0.841
Laboratory data			
Total cholesterol (mg/dl)	195.9 ± 25.9	173.9 ± 27.7	0.002 (−10.9 ± 10.0)
Non-HDL cholesterol (mg/dl)	132.9 ± 28.2	116.4 ± 31.6	0.008 (−12.1 ± 13.8)
LDL-cholesterol (mg/dl)	122.6 ± 25.2	106.2 ± 27.7	0.002 (−13.3 ± 12.1)
HDL-cholesterol (mg/dl)	63.0 ± 15.0	57.5 ± 11.7	0.067 (−7.0 ± 14.6)
Triglycerides (mg/dl)	78.2 ± 30.8	78.8 ± 28.6	0.937
Apolipoprotein A-I (mg/dl)	151.6 ± 30.9	144.2 ± 16.0	0.230 (−3.1 ± 16.1)
Apolipoprotein B (mg/dl)	86.6 ± 18.9	72.4 ± 14.8	0.018 (−18.3 ± 28.6)
Apolipoprotein B/apolipoprotein A-1 ratio	0.59 ± 0.17	0.51 ± 0.13	0.021 (−9.0 ± 13.8)
SHBG (nmol/liter)	49.9 ± 22.6	58.4 ± 26.8	0.038 (22.3 ± 27.0)
Fasting glucose (mg/dl)	90.3 ± 9.8	90.3 ± 5.8	0.325
Fasting insulin (μ U/ml)	6.3 ± 4.4	7.5 ± 3.9	0.889
HOMA-IR	1.31 ± 1.1	1.42 ± 0.8	0.604
Glucose disposal rate (mg/kg · min)	7.26 ± 2.7	7.37 ± 4.4	0.889
Echocardiogram			
Left ventricle mass index (g/m^2)	71.27 ± 16.1	71.15 ± 18.5	0.973
Left ventricle mass (g)	127.0 ± 37.66	126.9 ± 42.38	0.980
Left ventricle end-diastolic volume (ml)	70.53 ± 22.72	76.61 ± 22.22	0.07
Left ventricle end-diastolic volume/BSA (g/m^2)	39.4 ± 10.7	44.02 ± 10.0	0.07
Left ventricle end-systolic volume (ml)	22.46 ± 9.27	22.56 ± 7.17	0.672
Left ventricle end- systolic volume/BSA (g/m^2)	11.6 ± 5.5	12.8 ± 3.9	0.546
Isovolumetric relaxation time (msec)	97.83 ± 14.83	89.19 ± 11.52	0.04
E/A ratio	1.19 ± 0.53	1.22 ± 0.30	0.626
Deceleration time (msec)	227.0 ± 58.1	230.0 ± 35.2	0.766
Septal E/Ea ratio	7.83 ± 2.5	7.20 ± 3.6	0.893
Lateral E/Ea ratio	6.38 ± 2.1	5.91 ± 2.8	0.732
Flow-mediated dilation of the brachial artery			
Flow-mediated vasodilatation (%)	13.1 ± 7.1	12.7 ± 5.1	0.857
Nitroglycerin-mediated vasodilatation (%)	22.2 ± 8.7	21.7 ± 8.2	0.776
Exercise tolerance test			
Heart rate rest (bpm)	81.2 ± 12.3	79.9 ± 10.0	0.622
Heart rate maximal (bpm)	162.8 ± 12.3	163.1 ± 18.3	0.385
Stroke volume rest (ml)	77.2 ± 14.6	74.5 ± 14.5	0.796
Stroke volume maximal (ml)	112.9 ± 19.5	117.2 ± 19.1	0.372
VO$_2$Max (ml/min)	1669 ± 478.2	1660 ± 500.4	0.896
Anaerobic threshold (ml/min)	917.5 ± 311.9	925.0 ± 298.8	0.840
Power (Watts)	136.2 ± 49.3	137.6 ± 44.4	0.829

bpm, Beats per minute; BSA, body surface area; E/A ratio, early/late (atrial) ventricular filling velocity; E/Ea ratio, early diastolic transmitral velocity/early mitral annular diastolic velocity; HOMA-IR, homeostatic model assessment of insulin resistance; VO$_2$Max, maximal oxygen consumption. Conversion factors for SI units are as follows: milligrams per deciliter × 0.0259 millimoles per liter for total cholesterol and cholesterol fractions; milligrams per deciliter × 0.01 grams per liter for apolipoproteins; milligrams per deciliter × 0.0555 millimoles per liter for glucose; and microunits per milliliter × 6.945 picomoles per liter for insulin.

[a] Estimated by DXA scan.

euthyroidism throughout the target organs of the hormonal action. However, tissue euthyroidism is the net result of multiple steps including conversion of the prohormone T$_4$ into its active metabolite T$_3$, which is ultimately responsible for signaling at the end-organ target level. The circulating and intracellular pools of T$_3$ of treated hypothyroid patients (*i.e.* devoid of endogenous TH production) depend entirely on the conversion of exogenous L-T$_4$ into T$_3$. This is assured by the deiodinases that regulate the circulating levels of T$_3$ and its availability at the target tissue level, acting as a form of prereceptor, tissue-specific modulation of the TH action (27).
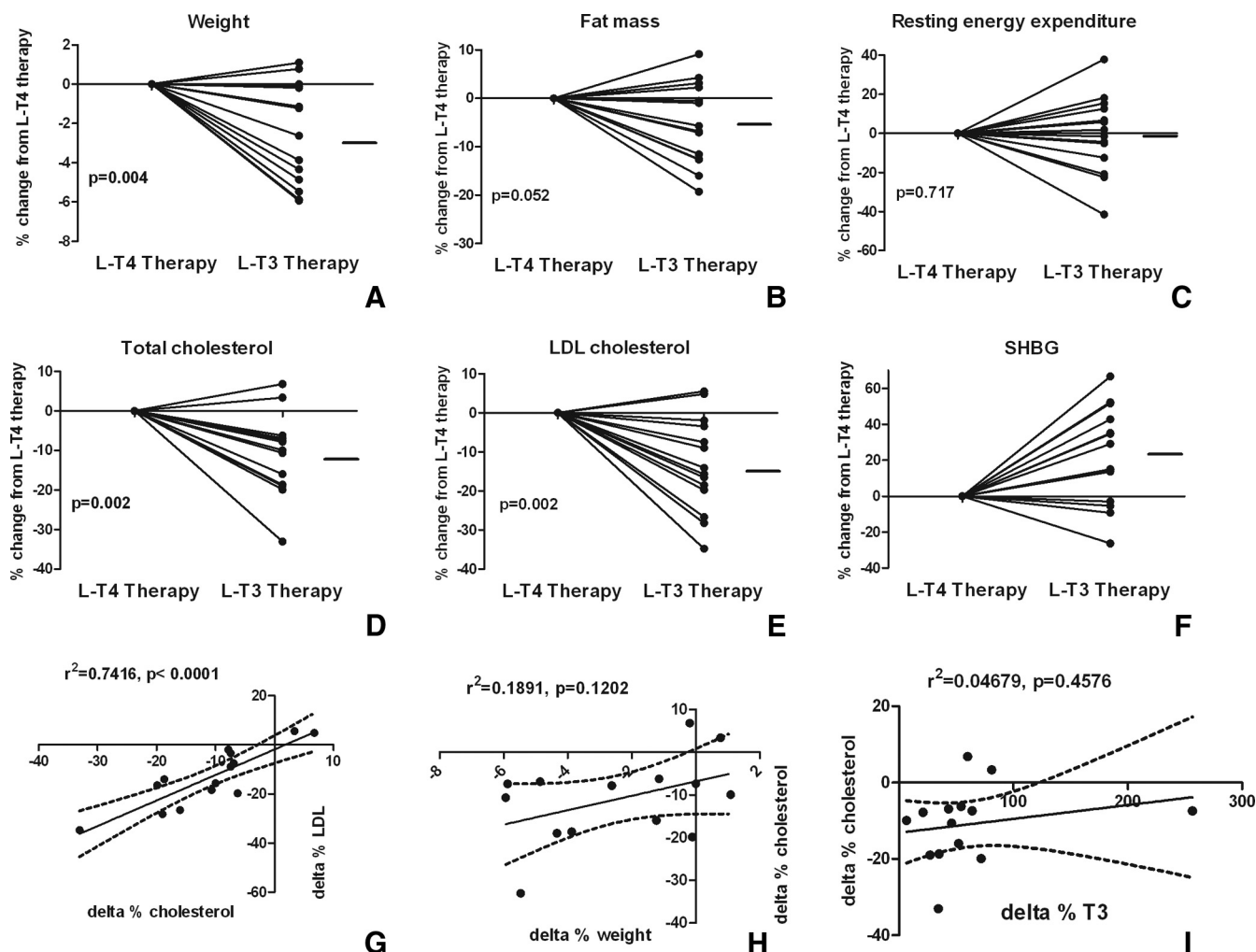
FIG. 2. A–F, Individual percent change after substitution of L-T_3 for L-T_4. The data are presented as changes from L-T_4 therapy: weight (A), fat mass (B), REE (C), total cholesterol (D), LDL-cholesterol (E), and SHBH (F). The *horizontal line* represents the average percent difference from L-T_4 therapy. G–I, Intra-individual correlations of changes in various parameters between treatments: total *vs.* LDL-cholesterol (G), weight *vs.* total cholesterol (H), and serum T_3 *vs.* total cholesterol (I). The *dashed lines* represent the 95% CI of the regression.

Replacement therapy with L-T_4/L-T_3 combinations has been suggested as a treatment for patients who do not achieve control of symptoms on L-T_4 monotherapy (28). Several studies have compared L-T_4/L-T_3 *vs.* L-T_4 monotherapy (13–23). However, these studies have focused on QOL and psychological endpoints, reporting only limited data on changes in other measures of TH action (16). Furthermore, therapy was administered as a once-daily regimen and/or using a fixed L-T_3 dose, and the interventions were not tailored to a target TSH level (13–18, 20, 22, 23). A single study allowed dose titration, but the data reported were limited to QOL and psychological endpoints (21).

Recently, we demonstrated the feasibility of sustained replacement therapy with L-T_3 alone on a thrice-daily regimen in a subgroup of patients presented in the current study (25). Aside from its potential use during TH withdrawal for management of thyroid cancer, the treatment with L-T_3, bypassing the need for deiodination, allows the

evaluation *in vivo* of the role of TH conversion. To this end, we systematically assessed the differences in the targets of the TH action, measured at a clamped, euthyroid state in the pituitary. The 0.34 ± 0.05 $\mu g/\mu g$ ratio of L-T_3/L-T_4 daily dose is in agreement with previous estimates obtained by compartmental analysis (29). Although the mean serum T_3 level remained within the normal range, L-T_3 treatment resulted in significantly higher T_3 serum concentrations. This is not surprising because type-2 deiodinase (30) is particularly active in the thyrotroph, and it is thought to play a major role in the hypothalamus-pituitary-thyroid axis (27, 31). Hence, this axis is probably more responsive to the inhibitory effects of T_4 than T_3.

The substitution of L-T_3 for L-T_4 caused a significant weight loss. TH is the major regulator of basal metabolic rate (32), so it is likely that this weight reduction is due to an increase in metabolic rate rather than a decrease in food intake. The lack of a detectable change in REE is likely due to the small magnitude of the effect, because a negative

energy balance of only about 50 kcal/d is thought to be required to maintain the reduced body weight observed in our study (33). Given the sensitivity of indirect calorimetry using the hood technique (34) and the variation observed in this study, a 50 kcal/d change in resting metabolic rate is not expected to be detected. We cannot rule out that the effect could be secondary to changes in other components of the energy balance, such as the thermic effect of food or physical activity not captured by the hood indirect calorimetry technique.

The substitution of L-T_3 for L-T_4 caused a significant reduction in lipid parameters. These data suggest that the TH action is increased in the liver, and the SHBG increase supports this hypothesis. The changes in serum lipid metabolism parameters are similar to the effects observed with drugs approved for the treatment of dyslipidemia (35) or TH analogs (36). The increase in liver TH tone could be due to a first-pass effect because orally delivered L-T_3 presumably produces higher concentrations in the liver than in other organs. This differential response appears to be limited to the lipid metabolism and SHBG, whereas no differences in indices of insulin resistance were detected. This is remarkable because hyperthyroid states are associated with an increase in hepatic gluconeogenesis (37), and overt thyrotoxicosis is a known cause of secondary diabetes.

Although the intra-individual changes in lipid parameters were strongly correlated, the changes among the other parameters were not. Due to the small number of observations, we cannot rule out the possibility of differential intra-individual tissue response to L-T_3 therapy.

Despite the increase in serum T_3, the L-T_3 treatment did not cause major changes in cardiovascular or musculoskeletal function, as indicated by the echocardiographic and maximal exercise tolerance tests and DXA studies. Similarly, no significant differences were observed in blood pressure, heart rate, or endothelial vascular function. The shorter isovolumic relaxation time observed in the L-T_3 treatment arm might suggest that the myocardium is more sensitive to this treatment, but the lack of significance in the other diastolic function indexes and the limited number of patients studied do not fully support this hypothesis. The presence of type-2 deiodinase in myocardium (38), skeletal muscle (38), and skeleton (39) could explain these findings. These tissues, like the pituitary, rely at least in part, on the intracellular conversion of T_4 to maintain the cellular levels of T_3 and thus are presumably less sensitive to the elevated serum T_3 levels resulting from L-T_3 therapy. This condition is opposite to the one observed in liver, whereby the tissue concentration of T_3 depends almost entirely on the circulation levels of T_3 (40).

Previous studies on L-T_3/L-T_4 combination therapy observed effects on QOL and/or behavior (13–23). Our lack of detected effects in these metrics could be due to the relatively small sample size. Furthermore, the duration of the intervention might have prevented the detection of significant changes in cardiovascular and musculoskeletal parameters.

The double-blind, crossover study design is robust and minimizes confounders due to inter-individual variability or comorbidity. Moreover, the sustained treatment on a stable dose of study drug with TSH levels within a narrow range minimizes carryover effects from previous treatment or unstable dosage. Finally, the stable serum T_3 levels over the 24-h period, and the inpatient stay under a controlled diet, further reduced the variability of the data and improved the likelihood of detecting differences in the parameters analyzed. Nonetheless, it is possible that the observed changes do not represent the full effects of the L-T_3 treatment because of the relatively short duration of the intervention while at a stable dose and target TSH. Alternatively, the nonsignificant differences in duration of the treatment might have affected the results of the study. This is unlikely because before reaching the therapeutic target, the patients spent similar time with TSH levels above or below the target range.

In conclusion, the results of this pharmacology, proof-of-concept study indicate that replacement therapy of hypothyroidism with L-T_3, compared with L-T_4 causes weight loss and favorable changes in the lipid profile without appreciable side effects. This intervention could be relevant for hypothyroid patients affected by comorbid conditions such as cardiovascular disease, diabetes, dyslipidemia, or obesity, where weight control and aggressive lowering of serum cholesterol are particularly important. However, presently, the prolonged use of L-T_3 alone for the treatment of hypothyroidism cannot be advocated in a clinical setting, because thrice-daily dosing is not practical and may affect patients' adherence with treatment. Further studies are needed to characterize the long-term effects and the subset of patients that might benefit from L-T_3 therapy, and the metabolic effects of L-T_3/L-T_4 combination therapy.

Acknowledgments

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