RESEARCH

Effects of levothyroxine substitution therapy on hunger and food intake in individuals with hypothyroidism

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Abstract

Context: In individuals with hypothyroidism and overweight, levothyroxine substitution therapy is often expected to cause weight loss due to its effect on resting energy expenditure. However, despite levothyroxine-induced enhancement of resting energy expenditure, fat mass loss is rarely seen after levothyroxine substitution therapy. The mechanism behind this conundrum is unknown.

Aim: The aim of the study was to assess the effect of levothyroxine therapy on hunger sensations and *ad libitum* food intake in individuals with hypothyroidism.

Design and setting: Prospective cohort study of 18 newly diagnosed hypothyroid women (thyroid-stimulating hormone (TSH) >10 mU/L). Participants were investigated at diagnosis, after normalization of TSH (<4.0 mU/L), and after 6 months of successful treatment. Eighteen age and body mass index-matched healthy controls were also included.

Intervention: Hypothyroid individuals were treated with levothyroxine according to European Thyroid Association guidelines.

Main outcomes: Changes in hunger sensation were assessed using visual analog scales (cm) before and during a standardized mixed meal test, and food intake was measured during a subsequent *ad libitum* meal (g).

Results: After 6 months of levothyroxine therapy, mean resting energy expenditure was increased by 144 kcal/day (10%) ($P < 0.001$). Weight loss was comprised of 0.8 kg fat-free mass while fat mass remained unchanged. Fasting hunger sensation increased from a mean of 4.5 (s.d. 2.2) cm to 5.5 (s.d. 2.2) cm ($P = 0.047$). The numerical increase in *ad libitum* meal intake did not reach statistical significance.

Conclusion: Our data suggest that levothyroxine-induced hunger may be a culprit in the lack of fat mass loss from levothyroxine therapy.

Key Words
- levothyroxine
- hunger
- food intake
- hypothyroidism
- body composition

Introduction

Hypothyroidism is a common endocrine disease, affecting about 3% of the general population (1), with levothyroxine substitution therapy being the standard treatment (2, 3). Hypothyroidism is associated with increased body mass index (BMI) (4, 5), and several factors have been proposed to contribute to this association such as fluid retention (6), reduced resting energy expenditure (REE) (7), and reduction in physical activity (8). Despite the common assumption that hypothyroidism leads to overweight/obesity, studies point to a similar body composition between hypothyroid individuals and healthy controls (9, 10). When initiating levothyroxine therapy, people are often told to anticipate improvements in symptoms, energy expenditure, and, thus, body weight loss. However, the evidence of substantial levothyroxine therapy-induced weight loss is – at best – scarce (especially when it comes to loss of fat mass). Hoogwerf and colleagues followed 18 individuals with hypothyroid for 24 months after initiating levothyroxine substitution therapy and found that body weight decreased modestly after 6 months but increased to baseline levels by 24 months of treatment (11). In another prospective study, Gjedde *et al.* investigated the effect of levothyroxine therapy for 2 months in 11 individuals with autoimmune hypothyroidism and observed a mean weight loss from baseline of 75.4 kg (s.d. 14.3) to 72.5 kg (s.d. 12.9) (7). As expected, the intervention was associated with a significant increase in REE amounting to 326 kcal/24 h, but a paradoxical and significant increase in body fat mass accompanied by a 4.5 kg loss of lean body mass was also observed. Further, Karmisholt *et al.* reported a virtually unchanged fat mass despite a significant increase in REE and self-reported physical activity after the initiation of levothyroxine therapy (12).

The mechanism behind the lack of fat mass reduction from levothyroxine therapy has, to our knowledge, not been directly addressed. Notably, the abovementioned studies did not measure appetite and food intake, and thus, we hypothesized that levothyroxine substitution therapy – despite its REE-increasing effect – may induce mechanisms preserving or even increasing body fat mass and that levothyroxine-induced increase in REE is accompanied by increased appetite and food intake, as mechanisms responsible for the lack of fat mass loss.

Here, we evaluated appetite and satiety sensations as well as caloric intake during an *ad libitum* meal test in newly diagnosed hypothyroid individuals before and during the initial months of levothyroxine treatment. As multiple factors influence hunger and food intake, we also investigated gallbladder motility, gastric emptying (11), and a range of hormones and peptides related to appetite and satiety.

Materials and methods

Study design

The study's primary endpoints were changes from baseline hunger and *ad libitum* food intake after 6 months of levothyroxine therapy in newly diagnosed hypothyroid women. The participants were subjected to three identical experimental days performed at the start of levothyroxine substitution therapy (visit 1), after ~1 month of treatment following normalization of thyroid-stimulating hormone (TSH) (i.e. < 4 mU/L) (visit 2), and after at least 6 months of substitution therapy with TSH < 4 mU/L (visit 3). Levothyroxine therapy was initially evaluated every 4 weeks until the participants were euthyroid and then every 3 months. Key secondary endpoints were changes in REE, body composition, and gastric and gallbladder emptying. For comparison, 18 healthy age and BMI-matched women were recruited as controls and subjected to a single experimental day without levothyroxine treatment.

Ethical approval, registration, and study conduct

The study was approved by the Research Ethics Committee of the Capital Region of Denmark (Reg. no. H-15001954), registered with Clinicaltrials.gov (Registration no. NCT02993562), and conducted according to the latest revision of the Declaration of Helsinki.

Consent from each participant was obtained after fully explaining the purpose and nature of all procedures used.

Participants

Participants with hypothyroidism were recruited through outpatient clinics in the Capital Region of Denmark and from the Copenhagen General Population Study (13). Inclusion criteria for hypothyroid participants included female gender, age 20-70 years, and diagnosis of hypothyroidism with serum TSH $\geq$ 10 mU/L, confirmed on two separate occasions.

Exclusion criteria included hypothyroidism from thyroid cancer, thyroid surgery, pituitary disease, treatment with amiodarone or lithium, and competing diseases or conditions judged incompatible with participation in the study by the investigators. Healthy controls were recruited via advertising, and their eligibility criteria were similar to hypothyroid participants except for no diagnosis of hypothyroidism and TSH within the normal range (0.4–4 mU/L).

Experimental procedures

Before each visit, participants received written instructions regarding their individual caloric needs according to World Health Organization guidelines corresponding to 120–185 KJ/kg weight ([14]). They were encouraged to eat in accordance with these and the Danish National Health Authority guidelines for food content, recommending a diversified plant-rich diet with less meat. Participants were instructed to abstain from strenuous exercise for 24 h, fast from midnight prior to each experimental day, and arrive at the hospital by nonstrenuous transportation. Procedures on experimental days are outlined in Fig. 1. At the start of each visit, participants' body composition was examined using dual x-ray absorptiometry after voiding. The scanner discriminated between fat mass, fat-free mass, and bone mass. Then participants were placed in a semirecumbent position in a hospital bed, and an intravenous catheter was inserted in an antecubital vein for blood sampling. During the initial 30 min, fasting (baseline) measurements (time points −30, −15, and −10 min) were conducted, including blood sampling, appetite evaluation, REE, and gallbladder volume measurement. At time point 0 min, participants ingested a liquid mixed meal (125 mL nutritional drink, Nutridrink Compact, Danone Nutricia, Schiphol, Netherlands) containing 300 kcal (11.6 g fat, 37.1 g carbohydrates, and 11.8 g protein) with 100 mL water and 1.5 g acetaminophen (for assessment of gastric emptying) over 5 min. Appetite sensations (hunger, satiety, fullness, and prospective food intake) were evaluated by visual analog scales (VAS) at time points −30, −15, −10, 15, 30, 50, 70, 90, 120, 150, 180, 210, and 240 min. Blood samples primarily related to hunger and satiety (ghrelin, glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), and gastrin), gastric emptying (acetaminophen) and glucose homeostasis (glucose, insulin, and C-peptide) was collected at the same time points, while blood samples for thyroid status (TSH, free thyroxine and total triiodothyronine) and the satiety hormone leptin, was collected at time point −30 min. Plasma glucose was centrifuged and analyzed immediately after sampling, while blood samples for later analysis were centrifuged for 20 min at 1200 *g* and 4°C. Gallbladder volume was measured by ultrasound (Pro Focus Ultrasound System, Class I type B, BK Medical, Herlev, Denmark) using the ellipsoid method ([15]) before the standardized mixed meal and at time points 30, 50, 90, 150, and 210 min. REE was measured after 20 min of resting at time points −10, 70, and 210 min by indirect calorimetry (CCM Express, Medgraphics, St. Paul, MN, USA) for 12 min. An *ad libitum* pasta Bolognese meal was served in one bowl containing 1 kg pasta Bolognese (1440 Kcal) with 500 mL of water at time point 270 min. Participants were instructed to eat and drink until they felt comfortably full. Food intake was measured by weighing before and after the meal. At each visit, participants were outfitted with an Actiheart Pedometer (CamNtech Ltd., Cambridgeshire, UK) for the following 2–5 days. The Actiheart Pedometer calculates the vertical movement of the body on a 0–200 scale based on Actiheart activity counts. In relation to each participant visit, 24 h of consecutive Actiheart measurements were used for estimating participant activity. At each visit, approximately 24 h of consecutive activity measurements were used when available.
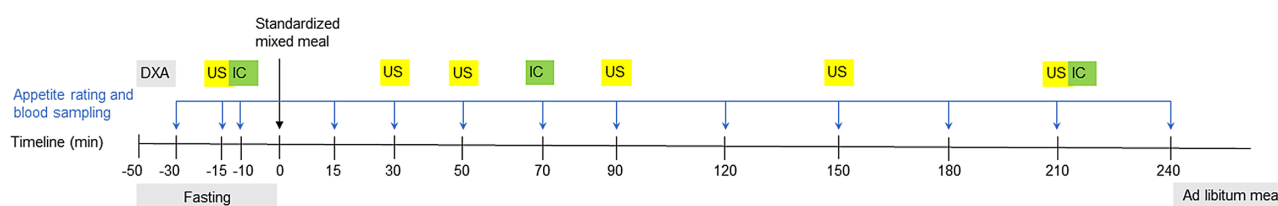

Figure 1

Experimental procedures on study days. Overview of the experimental procedures on study days. Eighteen newly diagnosed hypothyroid women were investigated on 3 similar experimental days; at the start of levothyroxine substitution therapy (visit 1), after normalization of thyroid-stimulating hormone (visit 2), and after at least 6 months of treatment (visit 3). Eighteen female controls, matched for age and body mass index, were subjected to a single experimental day for comparison. DXA, dual absorptiometry scan; IC, indirect calorimetry; US, ultrasound of the gallbladder.

Blood sample analyses

Serum TSH was analyzed by sandwich chemiluminescence immunoassay (reference range 0.35–4.0 mU/L, CV 19.6% at 0.29 mU/L). Free thyroxine (reference range 11.5–22.7 pmol/L, CV 23% at 11.7 pmol/L) and total triiodothyronine (reference range 1.0-2.6 nmol/L, CV 29.6% at 1.07 nmol/L) were analyzed by competitive immune analysis assay using chemiluminescence technology on ADVIA Centaur XP from Siemens (Siemens Healthcare GmbH). Plasma glucose was analyzed bedside on YSI 2900 Biochemistry analyzer (Xylem Inc. White Plains, NY, USA). Plasma C-peptide was measured using a two-sided electrochemiluminescence immunoassay (Roche Diagnostics). Total ghrelin was measured by radioimmunoassay (RIA) (Millipore). Serum insulin was measured using two-sided electrochemiluminescence (Roche/Hitachi Modular Analytics, Roche Diagnostics). Plasma leptin was analyzed on Human Leptin Immunoassay from R&D Systems (Bio-Techne Corporation, Minneapolis, MN, USA). The concentration of CCK in plasma was measured using an in-house RIA, antibody no. 92128 (16). Plasma gastrin was measured using an in-house RIA (antibody no. 2604) as previously described (17, 18). Plasma GLP-1 was measured using antiserum no. 89390 (19). Plasma acetaminophen was analyzed using enzymatic determination and absorption photometry, Siemens Atellica (Siemens Healthcare).

Calculations and statistics

The power of the study was based on Gregersen *et al.* (20) demonstrating that 17 participants were sufficient for the detection of a 500 kJ change in *ad libitum* food intake, corresponding to 83 g of pasta Bolognese, and Flint *et al.* demonstrating that 18 participants were required to establish a difference of 10 mm in hunger and satiety evaluated by VAS in a paired study design (21). The area under the curve (AUC) was calculated using the trapezoidal rule. Gallbladder maximum ejection fraction was determined as the difference in gallbladder volume when fasting (time point –10 min) to minimum volume divided by fasting volume. Insulin resistance was calculated using the Homeostasis Model Assessment calculator (https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-mrc-units/DTU/software/homa). Gaussian and non-Gaussian-distributed data were analyzed using parametric and nonparametric tests, respectively. According to distribution, comparisons between visits

(visits 1, 2, and 3 of the included participants) were conducted by related samples *t*-test or Wilcoxon rank-sum test, and comparisons between hypothyroid participants and healthy controls were conducted by the Student's *t*-test or the Wilcoxon–Mann–Whitney test (the single visit of the healthy controls was compared to participants' visits 1 and 3 separately). Multiple imputations were used to replace missing values in the appetite ratings (1.4% of cases) (IBM SPSS version 22). All Actiheart measures showing mean heart rate below 30 beats per min or above 190 beats per min were considered invalid and excluded from calculations, resulting in 10 to 70% of measurements excluded at each visit. AUC values were compared by repeated measures analysis of variance (ANOVA), and fasting values were calculated as the mean of values from time points –30, –15, and –10 min. Due to the limited size of this study, significance levels were not corrected for multiple testing. All statistics were performed using GraphPad Prism 9.1.0.

Results

Participant characteristics and therapy

Of the 18 newly diagnosed hypothyroid women included (Table 1), 13 were treatment-naive, and five had initiated treatment of 25–50 μg levothyroxine daily 1–2 weeks before visit 1 due to severe symptoms. At visit 1, 15 participants were overtly hypothyroid (TSH ≥ 10 mU/L with free thyroxine < 11 pmol/L), and three participants were subclinically hypothyroid (TSH ≥ 10 mU/L with normal free thyroxine). Though we aimed for TSH within the reference range (<4.0 mU/L) from visit 2 and onward, one person had TSH of 4.7 mU/L at visit 2, and another person had TSH of 4.4 mU/L at visit 3. Free levothyroxine was above the lower limit of the reference range (11.5–22.7 pmol/L) for all participants at both visits 2 and 3 (Table 2). During the study, one participant

Table 1 Baseline characteristics of participants.

	Hypothyroid women (*n* = 18)	**Healthy female controls** (*n* = 18)
Age (years)	44.4 (6.4)	45.2 (13.1)
BMI (kg/m^2)	29.0 (13.4)	29.4 (5.5)
TSH[a] (mU/L)	46.9 (12.4, 83.8)	1.6 (1.0, 2.2)
Free T4[a] (pmol/L)	9.2 (7.3, 10.7)	14.1 (13.5, 15.2)

Data are means with standard deviations or medians[a] with interquartile ranges. BMI, body mass index; T4, thyroxine; TSH, thyroid-stimulating hormone.

Table 2 Participant characteristics and primary outcomes.

	Participants visit 1[i]	Participants visit 2[j]	Participants visit 3[k]	Healthy controls[l]
Thyroid				
TSH[a] (mU/L)	46.9 (12.4, 83.8)[j, k]	1.2 (0.2, 3.2)[i]	1.2 (0.5, 2.1)[i]	1.6 (1.0, 2.2)
Free thyroxine[a] (pmol/L)	9.2 (7.3, 10.7)[j, k]	18.5 (16.9, 22.5)[i]	16.0 (18.9, 17.7)[i, l]	14.1 (13.5, 15.2)[k]
Total triiodothyronine (mmol/L)	1.1 (0.4)[j, k]	1.6 (0.4)[i]	1.5 (0.4)[i]	1.5 (0.3)
Body composition				
Weight (kg)	83.2 (17.0)[j]	82.4 (16.6)[i]	82.2 (16.9)	80.8 (11.4)
FM (kg)	33.7 (11.9)	33.9 (11.9)	33.6 (11.8)	30.4 (10.1)
FFM (kg)	46.8 (6.4)[j, k]	45.9 (5.7)[i]	46.0 (6.2)[i]	47.7 (3.5)
Energy expenditure				
REE, fasting (kcal/day)	1380 (171)[j, k]	1519 (258)[i]	1524 (225)[i]	1,490 (101)
REE/FFM (kcal/kg/day)	29.8 (4.0)[j, k]	33.1 (3.6)[i]	33.2 (2.4)[i, l]	31.3 (2.5)[k]
Physical activity				
24-h activity score (0–200 scale) movement	7.1 (21.8)[j, k]	10.0 (26.5)[j, k]	8.5 (22.1)[i, j]	Not measured
Appetite sensations, fasting				
Hunger (cm)	4.5 (2.2)[k]	5.1 (1.9)	5.5 (2.2)[i, l]	3.7 (2.5)[k]
Fullness (cm)	2.5 (1.3)	2.7 (1.4)	2.5 (1.7)	2.5 (1.9)
Satiety (cm)	3.5 (1.9)	3.4 (1.1)	3.3 (1.6)	4.0 (1.9)
Prospective food intake (cm)	5.7 (1.9)	5.9 (1.4)	6.4 (1.6)	5.2 (1.8)
Appetite sensations, AUC				
Hunger (cm × min)	2976 (807)	3064 (871)	3234 (911)	2692 (809)
Satiety (cm × min)	1772 (640)	1807 (602)	1681 (757)	1843 (544)
Fullness (cm × min)	1537 (603)	1698 (661)	1564 (780)	1572 (689)
Prospective food intake (cm × min)	3377 (691)	3477 (633)	3562 (814)	3120 (734)
Ad libitum **food intake**				
Pasta Bolognese[a] (g)	367 (274, 484)	363 (246, 473)	395 (270, 474)	453 (369, 524)
Water[a] (mL)	500 (396, 500)	485 (409, 500)	500 (408, 500)	500 (379, 500)

Data are presented as mean (s.d.) or median[a] (first and third quartiles) according to Gaussian distribution. According to distribution, comparisons between participant's visits were conducted by related samples *t*-test or Wilcoxon rank-sum test, and comparisons between participants and healthy controls were conducted by the Student's *t*-test or the Wilcoxon–Mann–Whitney test. Significant differences (*P* < 0.05) when compared to visit 1, 2, 3, or healthy controls are indicated by [i], [j], [k], and [l], respectively.
FFM, fat-free mass; FM, fat mass; REE, resting energy expenditure; TSH, thyroid-stimulating hormone.

started a diet, two restarted smoking between visits 2 and 3, and one became hyperthyroid, probably due to the presence of thyroid receptor antibodies but returned to euthyroidism at visit 3 without antithyroid medication. The mean daily dose of levothyroxine at visit 3, not including the latter participant, was 931 (s.d. 218) µg per week, corresponding to 1.6 µg per kg body weight per day.

Appetite sensation scores

The participants' mean sensation of hunger during fasting increased from visit 1 to visit 2 (4.5 (s.d. 2.2) vs 5.1 (s.d. 1.9), *P* = 0.023) and remained increased at visit 3 (5.5 (s.d. 2.2), *P* = 0.047) compared to visit 1; the healthy controls' mean hunger sensation was significantly lower when compared to visit 3 of the participants (3.7 (s.d. 2.5), *P* = 0.022) (Table 2, Fig. 2A). The change in hunger was not significant when calculated as AUC for the entire visit (Table 2). Satiety, fullness, and

prospective food intake of participants did not change significantly from visit 1 and were not significantly different compared to the healthy controls at visit 1 or 3 (Table 2, Fig. 2B, C, and D).

Ad libitum food intake

Between visits 1 and 3, *ad libitum* food intake increased numerically from a median of 367 g to 395 g, though this was not statistically significant (*P* = 0.59) (Table 2, Fig. 3A). Standard deviations (155 g and 138 g, respectively) were larger than expected. There was no significant difference in *ad libitum* food intake between healthy controls and participants (*P* = 0.40, and *P* = 0.32 for visits 1 and 3, respectively). Water intake during *ad libitum* meals was not significantly different between participants and healthy controls. In 60% of meals, participants drank all the 500 mL water provided (Table 2).
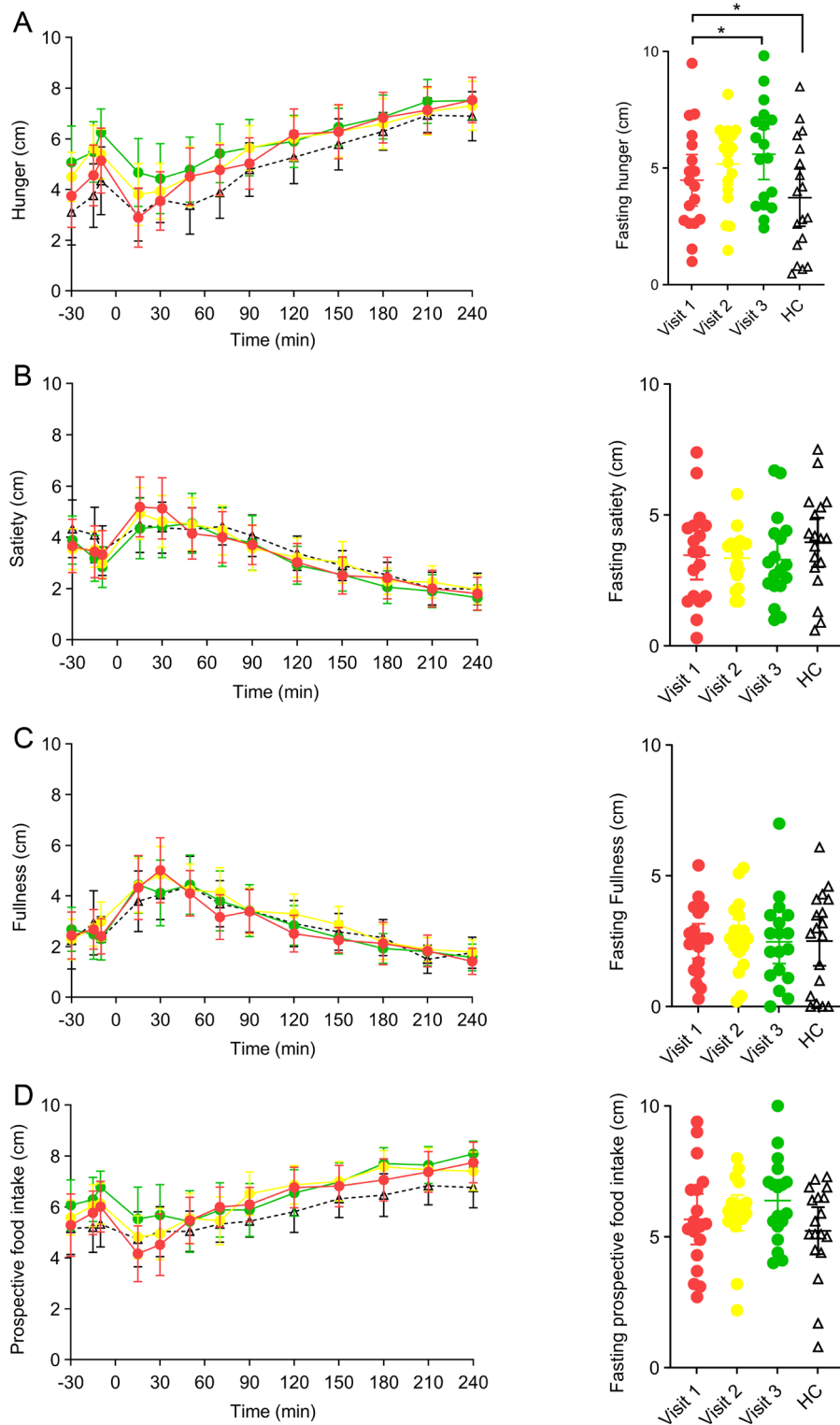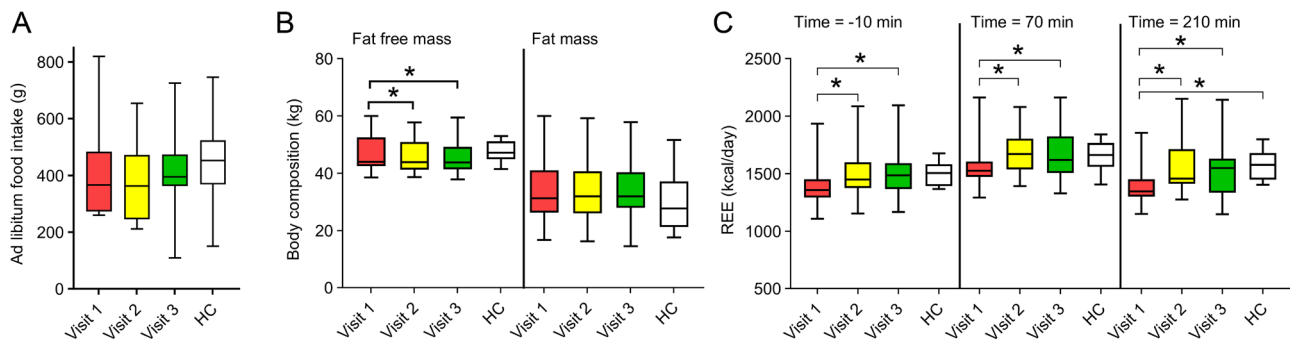
Figure 2
Appetite-related measures. Visual analog score (cm) of hunger (A), satiety (B), fullness (C), and prospective food intake (D) during a standardized mixed meal test performed in hypothyroid women at visit 1 (at diagnosis; red circles/curves), visit 2 (after 4 weeks treatment with levothyroxine and normalization of thyroid-stimulating hormone; yellow circles/curves) and visit 3 (after at least 6 months of levothyroxine treatment; green circles/curves) and in healthy controls (white triangles, dashed curves). Fasting values are mean of time points, −30, −15, and −10 min. The standardized mixed meal was ingested at time 0 min. Data are presented as means in the graphs to the left and as individual values to the right, both with 95% CIs. *, $P < 0.05$.

Figure 3

Food intake, body composition, and resting energy expenditure. *Ad libitum* food intake (A), body composition (B), and REE (C) in hypothyroid women (*n* = 18) at visit 1 (at diagnosis; red boxes), visit 2 (after 4 weeks treatment with levothyroxine and normalization of thyroid-stimulating hormone; yellow boxes) and visit 3 (after at least 6 months of levothyroxine treatment; green boxes) and in healthy controls (*n* = 18) (white boxes). REE was measured three times during each visit (10 min before and 70 and 210 min after ingestion of a standardized mixed meal). The *ad libitum* meal was served 240 min after the standardized mixed meal was initiated. Data are presented as medians with 25th and 75th percentiles and hinges marking minimum to maximum. *, *P* < 0.05.

Bodyweight and body composition

Bodyweight tended to decrease between visits 1 and 3 by a mean of 1.1 kg (s.d 2.9, *P* = 0.12), ranging from a 9.4 kg weight loss in a participant who had a smoking relapse to a 2.4 kg weight gain (Table 2). The weight change was composed of a significant reduction in fat-free mass, as fat mass remained unchanged during the study (Table 2, Fig. 3B). Between visits 2 and 3, weight and fat-free mass did not change. The body composition of the healthy controls was not significantly different compared to participants at visits 1 and 3 (Table 2).

Energy expenditure and physical activity

Both fasting and the two postprandial measurements (i.e. time points –10, 70, and 210 min, respectively) of REE increased from visit 1 to visits 2 and 3 (Fig. 3C). Fasting REE (time point –10 min) increased significantly from a mean of 1380 kcal/day to 1,519 kcal/day at visit 2 (*P* = 0.006) and remained significantly higher at visit 3 (1524 kcal/day) (Table 2). There was no significant difference in fasting REE or fasting REE/fat-free mass between participants and healthy controls at visit 1. Still, healthy controls had significantly lower REE/fat-free mass than participants at visit 3 (*P* = 0.029) but not significantly lower REE (*P* = 0.56) (Table 2, Fig. 3C). The physical activity level for participants increased from visit 1 to visit 2 (*P* < 0.001) and remained significantly elevated at visit 3 (*P* = 0.024). Unfortunately, the Actiheart sensors produced a fluctuating and declining amount of data. Twenty-four hours of Actiheart data was not available in 16.6% of participant visits, and

physical activity was not measured in healthy controls. Consequently, we chose only to conclude that physical activity did not seem to change and not make conclusions on the numerical increase in physical activity (Table 2).

Gallbladder volume and emptying

Participants' fasting gallbladder volume decreased from a mean of 30.5 (s.d. 14.9) mL at visit 1 to 26.6 (s.d. 15.9) mL at visit 2 (*P* = 0.19) and 23.4 (s.d. 9.5) mL at visit 3 (*P* = 0.034). Fasting gallbladder volume was significantly larger in participants at visit 1 compared to healthy controls (*P* = 0.042), but this difference vanished after levothyroxine therapy at visit 3 (*P* = 0.49) (Fig. 4A). Maximum ejection fraction of the gallbladder was unchanged between all visits, but the minimum volume decreased in participants between visits 1 and 2 by 2.4 (s.d. 4.4) mL (*P* = 0.035) and between visits 1 and 3 by 3.6 (s.d. 8.3) mL (*P* = 0.086). We observed no difference in maximum ejection fraction or minimum gallbladder volume when comparing participants at visits 1 and 3, respectively, to healthy controls (Table 3, Fig. 4A, B, and C). When investigated as AUC, there were no changes from levothyroxine therapy and no differences between participants and healthy controls (Table 3, Fig. 4D).

Gastric emptying per acetaminophen absorption

Before the standardized mixed meal, in both participants and healthy controls, serum acetaminophen concentrations were below the assay detection level of 0.0132 mmol/L. Serum concentrations during the
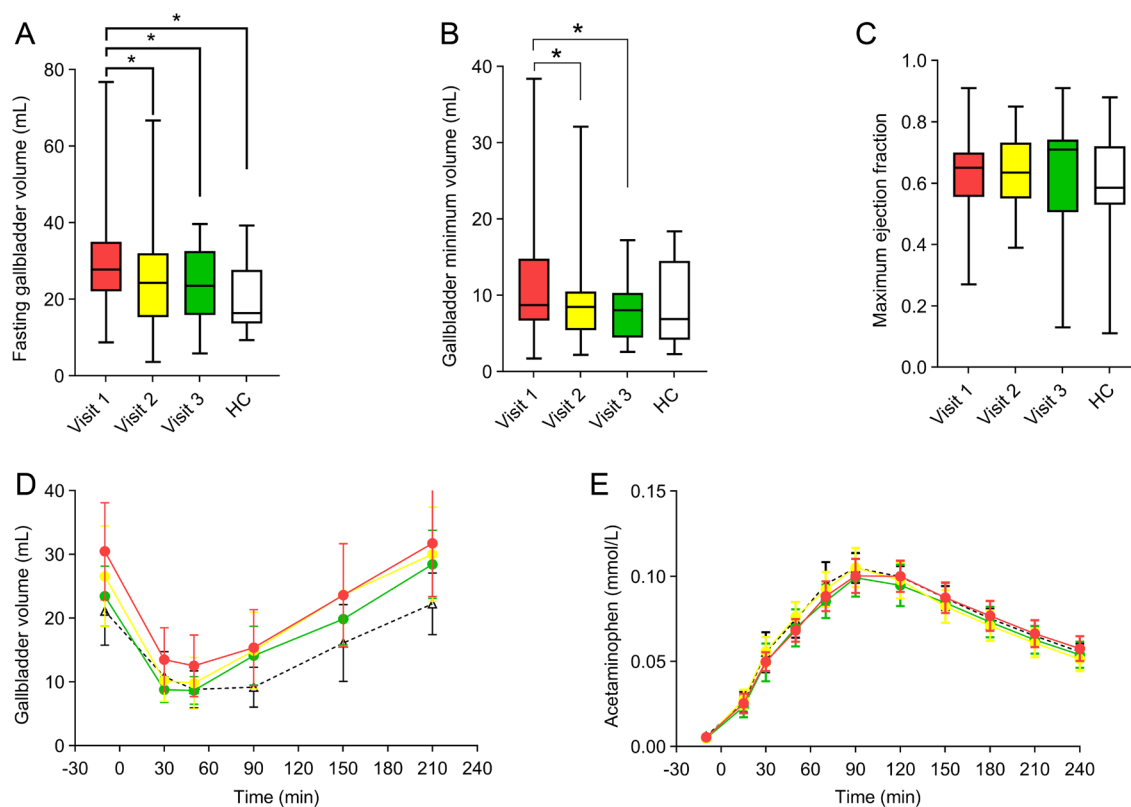
Figure 4
Gallbladder and gastric motility. Fasting gallbladder volume (A), minimum measured gallbladder volume (B), maximum ejection fraction (C), all-visit gallbladder volume (D), and plasma acetaminophen (E) during a standardized mixed meal test in hypothyroid women ($n = 18$) at visit 1 (at diagnosis; red boxes/circles/curves), visit 2 (after 4 weeks treatment with levothyroxine and normalization of thyroid-stimulating hormone; yellow boxes/circles/curves) and visit 3 (after at least 6 months of levothyroxine treatment; green boxes/circles/curves) and in healthy controls ($n = 18$) (white boxes/triangles, dashed curves). The standardized mixed meal was ingested at time 0 min. Data in A–C are medians with 25th and 75th percentiles and hinges marking min and maximum, and in D and E, data are means with 95% CI. *, $P < 0.05$.

standardized mixed meal test were, at all participant visits, similar to healthy controls (Table 3, Fig. 4E), suggesting that gastric emptying was not influenced by levothyroxine therapy.

Hunger-related hormones

Fasting levels of CCK were unchanged in hypothyroid participants during the study, but they exhibited elevated fasting CCK concentrations at visit 1 and 3 when compared to healthy controls (both $P < 0.001$) (Table 3). AUCs for CCK during mixed meal tests were similar between visits in both participants and healthy controls (Table 3, Fig. 5A). Concentrations of plasma gastrin were determined in 17 individuals, as one participant presented with hypergastrinemia. Poststudy examination revealed that this was due to achlorhydria. Fasting gastrin levels increased by a mean of 1.8 mmol/L

(s.d. 2.1, $P = 0.003$) from visit 1 to visit 2 but decreased by 1.2 mmol/L (s.d. 2.3, $P = 0.036$) at visit 3 (Table 3). AUCs for gastrin during mixed meal tests were similar between visits in both participants and healthy controls (Table 3, Fig. 5B). Ghrelin concentrations in the fasted state were similar between visits in both participants and healthy controls, and levels also decreased similarly in response to the mixed meal test (Table 3, Fig. 5C). Fasting GLP-1 levels and postprandial GLP-1 (AUC) were similar at all participant visits. Healthy controls exhibited lower fasting GLP-1 levels compared to participants at visit 1 ($P = 0.012$) but similar levels at visit 3. Postprandial GLP-1 levels were similar between visits in both participants and healthy controls at both visit 1 and 3 (Table 3, Fig. 5D). Leptin concentrations, measured once each visit, were similar between visits, and no differences between participants and healthy controls were observed.

Table 3 Physiological processes and hormones related to food intake and glycemic control.

	Participants visit 1[i]	Participants visit 2[j]	Participants visit 3[k]	Healthy controls[l]
Gallbladder and gastric emptying				
Gallbladder, fasting volume (mL)	30.5 (14.8)[j, k, l]	26.6 (15.9)[i]	23.4 (9.5)[i]	21.1 (10.1)[i]
Gallbladder, minimum volume (mL)	11.6 (8.8)[j]	9.2 (6.5)[i]	8.0 (3.9)	8.5 (5.5)
Gallbladder, max. ejection fraction	0.63 (0.14)	0.64 (0.12)	0.62 (0.22)	0.60 (0.18)
Gallbladder, volume AUC (mL × min)	4277 (2694)	4012 (2552)	3594 (1398)	2945 (1531)
Acetaminophen, AUC (mmol/L × min)	16.4 (0.9)	16.4 (1.0)	15.9 (1.1)	16.6 (0.9)
Hormones related to food intake				
CCK, fasting (pmol/L)	1.11 (0.64)[l]	1.05 (0.65)	1.04 (0.58)[l]	0.38 (0.35)[i, k]
CCK, AUC (pmol/L × min)	519 (75)	536 (66.5)	578 (80)	544 (80)
Gastrin, fasting (pmol/L)	10.5 (4.9)[j]	12.3 (3.9)[i, k]	11.1 (4.4)[j]	9.3 (4.7)
Gastrin, AUC (pmol/L × min)	3972 (354.4)	4260 (360.7)	4183 (337.0)	3525 (373.0)
Ghrelin, fasting (pmol/L)	460.7 (384.2)	407.3 (329.3)	408.4 (284.3)	568.9 (345.1)
Ghrelin, AUC (pmol/L × min)	113,325 (27,317)	105,872 (23,604)	102,318 (21,363)	119,032 (23,826)
GLP-1, fasting (pmol/L)	11.7 (3.6)[l]	11.1 (3.3)	10.3 (5.0)	8.5 (3.4)[i]
GLP-1, AUC (pmol/L × min)	3907 (324.1)	3830 (287.3)	4183 (506.5)	4020 (291.3)
Leptin, fasting (mmol/L)	32.1 (21.2)	37.4 (26.2)	37.7 (24.9)	26.4 (22.4)
Glucose and insulin				
Glucose, fasting (mmol/L)	5.1 (0.5)	5.1 (0.4)	5.2 (0.5)	5.1 (0.3)
Glucose, AUC (mmol/L × min)	2775 (2637, 3072)[k]	2865 (2568, 3079)	2907 (2,616, 3,108)[i]	2811 (2742, 3093)
Insulin, fasting (pmol/L)	66.07 (2.5)	67.12 (1.6)	74.39 (5.4)	59.1 (9.6)
Insulin, AUC (pmol/L × min)	50,982 (7938)	48,497 (7746)	53,035 (9777)	37,606 (5554)
HOMA2-IR	1.24 (0.70)	1.26 (0.66)	1.40 (0.78)	1.11 (0.59)

Data are presented as mean (s.d.) or median* (first and third quartiles) according to Gaussian distribution. According to distribution, comparisons between participant visits were conducted by related samples *t*-test or Wilcoxon rank test, and comparisons between participants and healthy controls were conducted by the Student's *t*-test or the Wilcoxon–Mann–Whitney test. A significant difference ($P < 0.05$) between present value and visit 1, 2, 3, or healthy controls is indicated by [i], [j], [k], or [l], respectively. AUC, area under the curve; CCK, cholecystokinin; GLP-1 glucagon-like peptide 1; HOMA2, Homeostasis Model Assessment 2; IR, insulin resistance.

Glucose and insulin

While fasting plasma glucose did not change significantly in participants after levothyroxine therapy, AUC for plasma glucose increased significantly from visit 1 to visit 3 ($P = 0.048$) (Table 3, Fig. 6A and B). When compared to the healthy controls, there were no significant differences in fasting plasma glucose or AUC for plasma glucose. In participants, fasting insulin levels and postprandial insulin responses were similar at the three visits and comparable to the fasting and postprandial responses in the healthy controls (Table 3, Fig. 5C). Insulin resistance, calculated as homeostasis model assessment (HOMA2-IR), remained similar at all visits for participants and was comparable to HOMA2-IR in the healthy controls (Table 3, Fig. 5D).

Post hoc power calculation

Due to a larger-than-expected s.d. of the primary outcome, *ad libitum food* intake, we conducted a post hoc calculation of the required number of participants to reach statistical significance with the observed s.d. Assuming an *ad libitum* food intake change from visits 1 to 3 corresponding to 50% of the 144 kcal/day increase in REE, the mean increase in pasta Bolognese would be 50 g after 6 months of levothyroxine treatment. Using the observed s.d. of 103, $\alpha = 0.05$, and power = 80% in a paired design such as this, we would require 35 participants for statistical significance, suggesting that the present study is underpowered and that the lack of statistical significance of the increased *ad libitum* food intake after levothyroxine therapy may represent at statistical type 2 error.

Discussion

In this study, we investigated the influence of levothyroxine therapy on hunger and food intake in previously untreated hypothyroid individuals. We found a significant increase in fasting hunger sensation after initiation of levothyroxine therapy, but the observed increase in *ad libitum* food intake and sensation of hunger during the mixed meal test did not reach statistical significance. The participant's body composition changed only by a decrease in fat-free mass, while fat mass was unaffected by levothyroxine therapy despite a significant
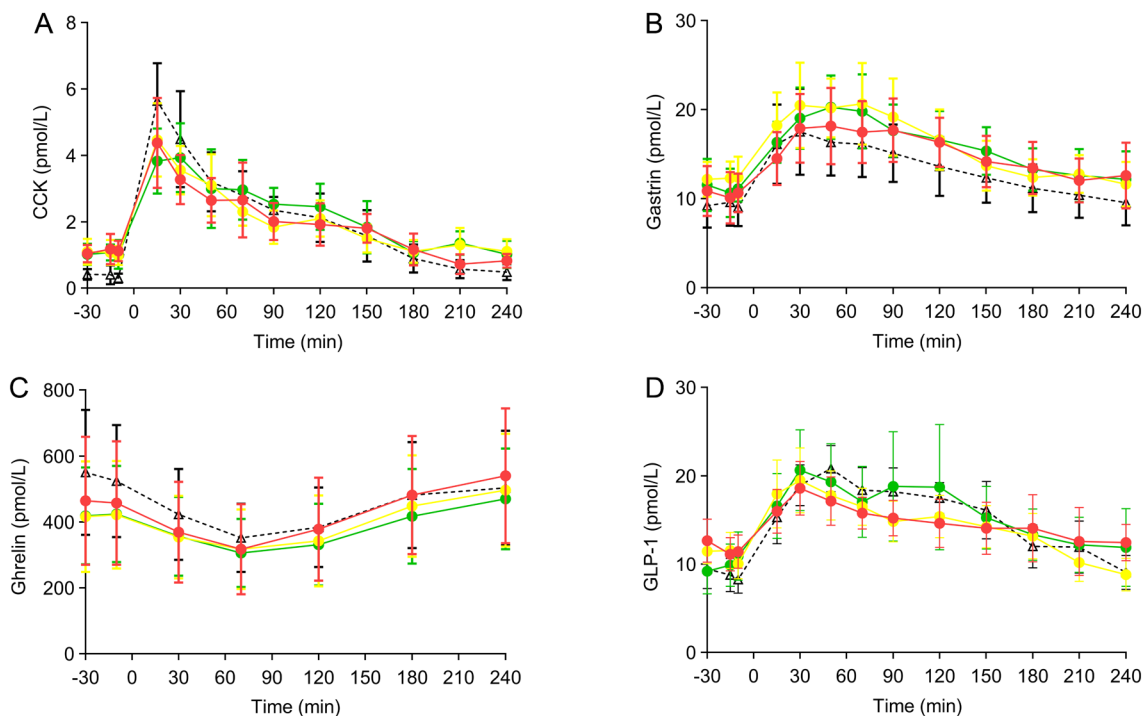
Figure 5

Hunger and satiety-related hormones. Cholecystokinin (A), gastrin (B), ghrelin (C), and glucagon-like peptide 1 (D) concentrations during a standardized mixed meal test in hypothyroid women ($n = 18$) at visit 1 (at diagnosis; red boxes/circles/curves), visit 2 (after 4 weeks treatment with levothyroxine and normalization of thyroid-stimulating hormone; yellow boxes/circles/curves) and visit 3 (after at least 6 months of levothyroxine treatment; green boxes/circles/curves) and in healthy controls ($n = 18$) (white boxes/triangles, dashed curves). The standardized mixed meal was ingested at time 0 min. Data are means with 95% CIs. CCK, cholecystokinin; GLP-1, glucagon-like peptide 1.
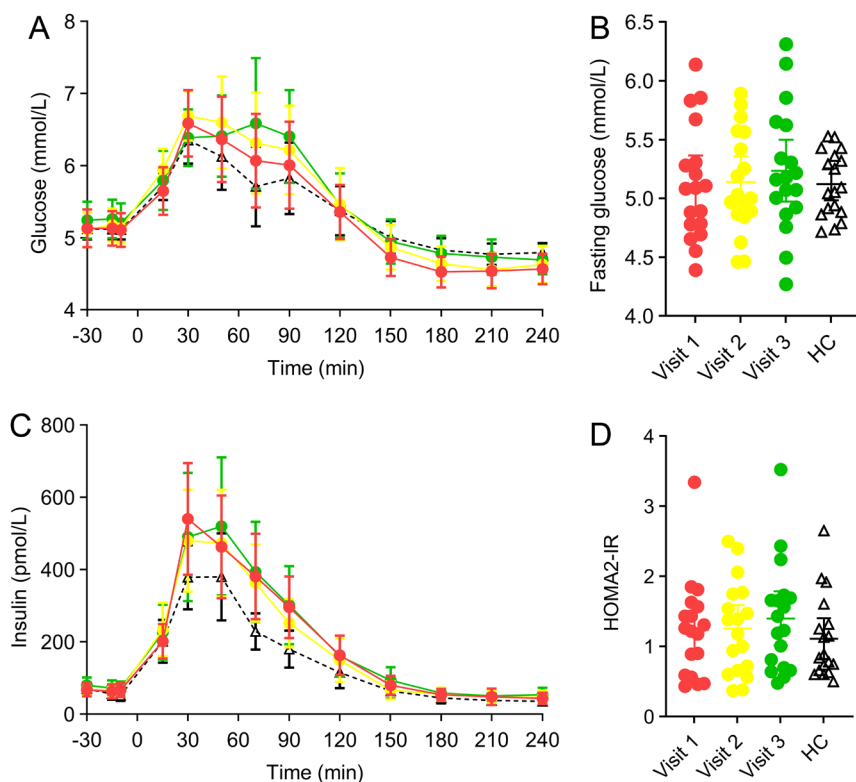
Figure 6

Glucose control and insulin resistance. Plasma glucose (A), fasting plasma glucose (B), and serum insulin (C) concentrations and homeostatic model assessment of insulin resistance (D) during a standardized mixed meal test in hypothyroid women ($n = 18$) at visit 1 (at diagnosis; red boxes/circles/curves), visit 2 (after 4 weeks treatment with levothyroxine and normalization of thyroid-stimulating hormone; yellow boxes/circles/curves) and visit 3 (after at least 6 months of levothyroxine treatment; red boxes/circles/curves) and in healthy controls ($n = 18$) (white boxes/triangles, dashed curves). The standardized mixed meal was ingested at time 0 min. Data are presented as means in the graphs to the left and as individual values to the right, Both with 95% CIs. HC, healthy controls; HOMA2-IR, homeostatic model assessment of insulin resistance.

144 kcal/day increase in fasting REE. Any increase in energy expenditure should, if not countered by an increase in food intake or decrease in physical activity, lead to weight loss. In the present study, a numerical (non-statistically significant) increase in *ad libitum* food intake was observed after initiation of levothyroxine therapy, which, combined with the increase in fasting hunger, may indicate a possible counterbalance to REE-mediated combustion of fat.

The included hypothyroid participants all had high serum TSH at diagnosis (>10 mU/L) beyond the discussion of whether to treat or not (2, 3). As such, changes from levothyroxine therapy should be evident in these participants. The strength of the present study is the standardized, systematic design for evaluating changes in hunger sensations, food intake, and metabolic parameters. However, due to the larger-than-expected observed s.d. of the primary endpoint, *ad libitum* food intake, the study turned out to be underpowered, suggesting that the lack of statistical significance of the increased *ad libitum* food intake after levothyroxine therapy may represent a statistical type 2 error. The menstrual cycle has been reported to influence food choice and food intake (22), which may have contributed to the greater-than-expected s.d. in this group of females only (as visit 1 had to be performed immediately after diagnosis, we could not schedule study days according to menstrual cycle and, therefore, we decided to perform study days at random in relation to menstrual cycle). The sample size calculation was made from previous studies comprising young, healthy men aged 19–36 years (mean 25 years) and with normal BMI (mean 22.7 kg/m^2) (23).

To our knowledge, this is the first study to directly investigate changes in appetite sensations and food intake during a levothyroxine-induced transition from a hypothyroid to a euthyroid state. Giménez-Palop *et al.* investigated self-reported food intake before and after treatment in both hyperthyroid and hypothyroid individuals. The hypothyroid participants did not change their self-reported food intake significantly, but the untreated hyperthyroid participants consumed more food before returning to euthyroidism (24). Two studies have investigated changes in REE, body composition by bioelectrical impedance, and self-reported food intake during the initial treatment period for Graves hyperthyroidism (25, 26). In both studies, REE was increased, and participants reported increased food intake before initiating antithyroid treatment. It is conceivable that an increase in REE is followed by an increase in food intake. However, it appears that in hyperthyroidism, the compensatory increase in food intake is inadequate, leading to weight loss (27).

Interestingly, after at least 6 months of levothyroxine therapy (visit 3) in the present study, REE per fat-free mass reached a level in participants that was significantly higher than for the healthy controls, which may allude to the greater fasting hunger sensation observed in the participants. Blundell *et al.* have suggested that fat-free mass, due to its close relation to the resting metabolic rate, is a major determinant of energy intake and report that resting metabolic rate is a predictor of fasting hunger levels (28, 29). This matches our findings of increased hunger sensation after an increase in REE from levothyroxine therapy. Further, thyroid hormones are speculated to have a direct orexigenic effect on the central nervous system (30), which may affect hunger and food intake. Both of these notions support that levothyroxine treatment may be responsible for the increased hunger observed in our study.

Physical activity is an essential determinant of body weight, and we evaluated physical activity to understand better possible changes in body weight, composition, and energy requirements. We observed increasing physical activity from visit 1 to visits 2 and 3, which, combined with the levothyroxine-induced increase in REE, would be expected to reduce fat mass. Nevertheless, fat mass was unaffected, supporting the notion that levothyroxine therapy increases energy intake.

There is a well-established association between hypothyroidism and symptoms that can be related to reduced gastrointestinal motility (31). Most investigations show signs of increased gastric emptying time in hypothyroid individuals (32, 33, 34, 35), but others have found no difference in gastric emptying in participants during euthyroidism and hypothyroidism (35). Studies have demonstrated an improvement in symptoms of impaired gastric emptying from levothyroxine therapy (34, 36) and improved gastric emptying half-time measured by radioactive isotope scanning (32, 37) and electrogastrography (34). In the present study, using the acetaminophen absorption test for evaluation of gastric emptying, we found no evidence of changed gastric emptying in hypothyroid participants or related to the initiation of levothyroxine therapy. These findings should be interpreted with caution due to the inaccuracy of the acetaminophen absorption method for evaluation of gastric emptying when compared to gold standard scintigraphy (38).

Compared to healthy controls, we observed a significantly greater fasting gallbladder volume in

hypothyroid participants, which normalized following levothyroxine therapy. The relationship between hypothyroidism and clinical gallbladder manifestations has been investigated by Laukkarinen *et al.* reporting an increased risk of gallstones in untreated hypothyroid participants compared with healthy controls (39). The same group has also demonstrated that thyroxine causes relaxation of the sphincter of Oddi in pigs and in the human sphincter of Oddi specimens (40), possibly underlying the decreased gallbladder volume observed in the present study.

CCK is best known for its stimulating effects on the release of digestive enzymes from the pancreas and gallbladder contraction, but it also has been shown to suppress hunger (41, 42). Studies on the relationship between CCK and thyroid disease are scarce. Nakazawa *et al.* investigated changes in gastric motility, CCK, ghrelin, and GLP-1 in dogs during hypothyroidism, euthyroidism, and hyperthyroidism (43). They reported that changes in thyroid hormone concentrations did not alter gastrointestinal transit time or concentrations of CCK. To our knowledge, CCK levels in hypothyroid participants and the effect of levothyroxine on CCK secretion in humans have not been studied previously. In the present study, fasting CCK levels were found elevated in hypothyroid participants, but we did not find any significant changes in CCK levels in response to levothyroxine therapy. The elevated fasting CCK levels in hypothyroid participants may be implicated in their increased fasting gallbladder volume.

We investigated gastrin due to its regulatory effect on gastric acid secretion. In the present study, fasting gastrin levels increased significantly in our hypothyroid participants following levothyroxine therapy. Hypogastrinemia in hypothyroidism, with improvement from levothyroxine therapy, has been reported previously (44), and the positive correlation between gastrin levels and thyroid hormones has also been confirmed in hyperthyroidism (45, 46).

The satiety hormone GLP-1 (47) did not change from levothyroxine therapy in the present study. To our knowledge, no studies have evaluated changes in GLP-1 in humans from levothyroxine therapy. Concentrations of the hunger-promoting hormone ghrelin were unchanged in this study, confirming a study by Canpolat *et al.* investigating circulating ghrelin concentrations during the initial treatment of subclinical hypothyroidism (34). Similarly, the abovementioned study in dogs by Nakazawa *et al.* did not show changes in GLP-1 or ghrelin from levothyroxine therapy (43). Opposing this,

Braclik *et al.* reported a significant increase in ghrelin concentrations in nine hypothyroid participants treated with levothyroxine (48). In humans, leptin levels are correlated to obesity (49), and the hormone is an established satiety modulator (50), but the relationship between leptin and thyroid function is not fully understood (51). Plasma concentrations of leptin did not change significantly in response to levothyroxine therapy in our hypothyroid participants, which is in line with previous observations (48).

Taken together, the hunger and satiety hormone data described herein do not explain changes in hunger following levothyroxine therapy, but several other appetite-regulating hormones or factors may be involved; also a direct effect from energy expenditure and food intake, as suggested by Blundell *et al.* (29), may play a role and warrants further studies.

After 6 months of levothyroxine therapy and reestablishment of euthyroidism, our participants exhibited reduced postprandial glucose tolerance as compared to baseline (overt hypothyroidism), whereas no differences in insulin levels or HOMA2-IR were observed. Other studies have reported conflicting findings. Pasandideh *et al.* reported no change in HOMA-IR after 8 weeks of levothyroxine treatment (52), while Handisurya *et al.* described improved insulin sensitivity (assessed by euglycemic–hyperinsulinemic clamp) but increased HbA1c and serum insulin after normalization of the hypothyroid state (53). We speculate that the reduced postprandial glucose tolerance observed in the present study may relate to levothyroxine-induced gluconeogenesis as previously described (54, 55).

Conclusion

Levothyroxine therapy in newly diagnosed hypothyroid women induced a significant increase in REE without reducing fat mass after 6 months of treatment. While hunger was increased after thyroid hormone replacement, the observed increase in *ad libitum* food intake did not reach statistical significance, likely due to a greater than expected variation in participants' *ad libitum* food intake.

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