CLINICAL STUDY

Roux-en-Y gastric bypass and calorie restriction induce comparable time-dependent effects on thyroid hormone function tests in obese female subjects

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Abstract

Objective: Obesity and weight loss influence thyroid hormone physiology. The effects of weight loss by calorie restriction vs Roux-en-Y gastric bypass (RYGB) in obese subjects have not been studied in parallel. We hypothesized that differences in transient systemic inflammation and catabolic state between the intervention types could lead to differential effects on thyroid hormone physiology.

Design and methods: We recruited 12 lean and 27 obese females with normal fasting glucose (normal glucose tolerant (NGT)) and 27 obese females with type 2 diabetes mellitus (T2DM) for this study. Weight loss was achieved by restrictive treatment (gastric banding or high-protein-low-calorie diet) or by RYGB. Fasting serum leptin, TSH, triiodothyronine (T₃), reverse T₃ (rT₃), and free thyroxine (fT₄) concentrations were measured at baseline and 3 weeks and 3 months after the start of the interventions.

Results: Obesity was associated with higher TSH, T₃, and rT₃ levels and normal fT₄ levels in all the subjects when compared with the controls. After 3 weeks, calorie restriction and RYGB induced a decline in TSH levels and a rise in rT₃ and fT₄ levels. The increase in rT₃ levels correlated with serum interleukin 8 (IL8) and IL6 levels. After 3 months, fT₄ and rT₃ levels returned to baseline levels, whereas TSH and T₃ levels were persistently decreased when compared with baseline levels. No differences in the effects on thyroid hormone parameters between the interventions or between NGT and T2DM subjects were observed at any time point.

Conclusions: In summary, weight loss directly influences thyroid hormone regulation, independently of the weight loss strategy used. The effects may be explained by a combination of decreased leptin levels and transient changes in peripheral thyroid hormone metabolism.

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Introduction

Thyroid hormone plays an important role in energy metabolism (1, 2). Serum concentrations of TSH and triiodothyronine (T₃) are generally higher in obese subjects than in normal-weight subjects. Although the exact mechanism is unknown, this effect may be partially explained by a central effect of increased serum leptin levels. In addition, an enhanced responsiveness of TSH to thyrotrophin-releasing hormone in obesity has been described (3, 4, 5, 6). The increase in resting energy expenditure, diminishing the available energy for accumulation in fats, is thought to be an adaptive response to chronic nutrient surfeit (7, 8).

Most studies on weight loss in obesity have reported a decrease in serum TSH and T₃ levels. This may be

explained by a decline in serum leptin levels, which consequently leads to a decrease in serum TSH and T₃ levels (9, 10, 11). In addition, alterations in peripheral thyroid metabolism may also contribute to decreased serum T₃ levels. Indeed, some, but not all, studies have reported an increase in reverse T₃ (rT₃) levels during calorie restriction and fasting (11, 12, 13). Differences between studies on the effect of weight loss on serum rT₃ levels may be explained by differences in the study population, weight loss intervention, and time course of measurements. Increased serum rT₃ levels may be related to increased type 3 deiodinase (D3) activity, which is enhanced by inflammatory pathways, or decreased D2 activity. Active weight loss strategies, i.e. calorie restriction *per se* or gastrointestinal surgery in general, may cause transient systemic stress, insulin

resistance, and tissue injury to a certain degree and may, therefore, lead to differential effects on D3 activity (14, 15, 16).

No head-to-head comparison has been made between the effects of restrictive and surgical weight loss strategies on thyroid hormone physiology. In view of the extensive metabolic effects of Roux-en-Y gastric bypass (RYGB) (17), apart from calorie restriction *per se*, we hypothesized that restrictive weight loss strategies and bariatric surgery differ in their effects on thyroid hormone function tests. Therefore, we carried out a prospective study comparing the effects of restrictive weight loss strategies (VLCD and gastric banding (GB)) with those of RYGB on thyroid hormone metabolism. In addition, as such a study had not been carried out earlier, we also compared the effects of weight loss on thyroid hormones in equally obese NGT and type 2 diabetes mellitus (T2DM) subjects.

Subjects and methods

Subjects

We recruited obese female subjects eligible for both restrictive (VLCD and GB) and RYGB treatments. Subjects eligible for GB or RYGB were recruited from the waiting lists of several hospitals in The Netherlands. They had been screened previously by a multidisciplinary team of the Nederlandse Obesitaskliniek (Dutch Obesity Clinic) according to the international criteria as described by Fried *et al.* (18). Subjects eligible for dietary treatment were recruited after referral by their general practitioner or internist. They fulfilled the same criteria for age, BMI, co-morbidity, history of long-standing obesity, and failed weight loss attempts as the surgical patients. As RYGB is reported to have superior metabolic effects in subjects with T2DM (when compared with GB), GB is not a preferred surgical treatment for obese DM patients in The Netherlands. Therefore, we included a group of T2DM subjects who fulfilled the criteria for bariatric intervention and treated them with a very-low-calorie diet (VLCD). As the effects of GB result primarily from calorie restriction and the calorie intake was comparable throughout the study, we included subjects who underwent VLCD and GB treatments in the calorie restriction treatment group. Exclusion criteria were smoking, age > 65 years, and any chronic disease other than diabetes, hypertension, and dyslipidemia, including thyroid diseases and psychiatric illness.

The subjects had either normal fasting glucose (NGT) or T2DM according to WHO standards. Our NGT subjects had normal fasting glucose at baseline, and postprandial glucose levels were equal in NGT subjects when compared with those in lean controls. All diabetic subjects were treated with oral medication only (metformin, sulfonylurea derivatives, or thiazolidinediones). Body weight of all the subjects had been stable

for at least 3 months prior to inclusion into the study, and none of the subjects reported the use of weight loss medications. The participants were allowed to use oral blood glucose-lowering tablets, cholesterol-lowering statins, and antihypertensive medication.

Control subjects were recruited via an advertisement in the university and hospital newspaper. They were all healthy females, age matched with the obese subjects, with a BMI between 20 and 25 kg/m² and a normal plasma glucose concentration in fasting condition.

Ethical approval

The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all subjects provided written informed consent before participation. Clinical trial registration was performed at ClinicalTrials.gov (NCT01167959).

Study design

The subjects were examined a month before surgery or initiation of the VLCD treatment and again between 2 and 3 weeks and 3 months after surgery or initiation of the VLCD treatment. The subjects were admitted to the clinical research center after an overnight (10 h) fast. Anthropometric measurements were taken and bioelectrical impedance analysis (Quadscan Bodystat, Douglas, Isle of Man, UK) was performed. A cannula was inserted into an antecubital vein and a fasting blood sample was collected. Blood was collected in a SST Gel and Clot Activator tube (Becton and Dickinson, Franklin Lakes, NJ, USA) and a vacutainer containing EDTA. All blood samples, and serum samples when clotted, were centrifuged promptly (2000 *g* at 4 °C for 10 min), and subsequently plasma or serum was collected into separate plastic tubes and frozen (−80 °C) until assay.

Interventions

RYGB During RYGB, a 25 ml gastric pouch was created and connected to a 100 cm Roux-en-Y limb. GB entailed the placement of a standard silicone LapBand (Inamed, Allegan, Santa Barbara, CA, USA) around the stomach to create a 15 ml pouch. On the first day after surgery, the patients were prescribed a clear liquid diet for 4–5 days. For the first 3 months after surgery, a staged meal progression was prescribed, containing liquids and ground or pureed protein sources and vegetables. On their return visit, they were required to hand in an intake diary of the postoperative period, allowing us to make an estimation of their caloric intake.

Restrictive interventions *Gastric banding.* GB entailed the placement of a standard silicone LapBand (Inamed, Allegan) around the stomach to create a 15 ml pouch. On the first day after surgery, the patients were

prescribed a clear liquid diet for 4–5 days. For the first 3 months after surgery, a staged meal progression was prescribed as described above.

Very-low-calorie diet. Commercially available Prodimed (Prodimed Benelux BV, Valkenswaard, The Netherlands) is a high-protein-low-calorie (VLCD) meal replacement plan, with an average calorie intake of 700 kcal/day. The meal replacement plan consists of sachets of a soluble powder for the preparation of shakes, omelet, soups, and bread. Each sachet contains ~90 kcal (~18 g protein, ~2.5–5 g carbohydrates, and 0.5–2 g fat). The subjects were allowed to consume four to five sachets a day and an additional choice of selected vegetables (600 kcal/day in total) during the first 3 weeks. Up to 2 months, the patients were allowed to consume more additional vegetables, one piece of fruit, or one portion of light dairy produce (800 kcal/day in total). After 2 months, the patients were allowed to consume a light evening meal (replacing one Prodimed sachet) on intermittent days (1000 kcal/day in total). For the evening meal, they could choose any of the several prescribed recipes.

Concomitant medication

On the day of surgery or initiation of the VLCD treatment, the use of all blood glucose-lowering agents was discontinued to avoid hypoglycemia. Only metformin treatment was started again if fasting blood glucose levels remained above 7 mmol after the interventions. If the subjects, at baseline, used prescriptions such as antihypertensive or cholesterol-lowering medication, their use was continued throughout the whole time course of the study.

Assays

Serum glucose levels were measured using a Modular P800 chemistry analyzer of Roche Diagnostics with a coefficient of variation (CV) of 1.7%. Insulin levels were measured using an immunometric assay on an automated Immulite 2500 (Siemens, Breda, The Netherlands) with an intra-assay CV of 6–7.5%. HbA1c levels were measured in whole-blood samples using a fully automated HPLC Integra 800 analyzer of Roche Diagnostics with a CV <0.6%. Serum free T₄ (fT₄) and TSH levels were measured using a chemiluminescence immunoassay with a Modular Analytics E-170 system (Roche). The intra-assay CV were 1.6–2.2 and 1.3–5.0% respectively. Serum T₃ levels were measured using a fluorescence polarization immunoassay on an AxSym System (Abbott). The CV was 2.5–9.0%. Leptin levels were measured using a RIA kit (Leptin HL-81K, Millipore, Billerica, MA, USA) with an inter-assay precision of 3.6–6.2% and an intra-assay precision of 3.4–8.3%. Serum rT₃ levels were measured using a commercially available RIA kit (ALPCO

Diagnostics, The Netherlands) with an inter-assay precision of 3.0% and an intra-assay precision of 3.9%. Interleukin 8 (IL8), IL6, IL10, and tumor necrosis factor (TNF) levels were measured using a commercially available kit (Mesoscale Discovery (MSD), Maryland, USA) with a CV of 4.8%.

Statistical analysis

Data were analyzed using SPSS 17.0. Data are presented as means $\pm$ S.E.M. Differences between the subject groups (NGT vs T2DM) and lean controls at baseline and the effect of interventions after 3 weeks and 3 months were compared using a mixed-effects model, with the patient groups and diabetes as fixed effects and the subject-specific deviances modeled with random intercepts. To analyze whether baseline values and trends of change over time within the different groups were similar between the groups, we used a stepwise mixed model reduction procedure. The Bonferroni's *post hoc* test was used to correct for multiple testing. Correlation between the parameters was calculated using Pearson's correlation coefficient. A *P* value <0.05 was considered statistically significant. Graphs were computed in Prism GraphPad 5 (La Jolla, CA, USA).

Results

Subject characteristics

The baseline characteristics of all the participants are given in Table 1 (restrictive vs malabsorptive) and in Table 2 (T2DM vs NGT). All obese subjects and healthy controls were Caucasian females, with a mean age of 49.4 ± 0.6 years. We included 32 subjects with T2DM and 30 obese NGT individuals. Eight subjects left the study because they were not able to comply with the rules of the VLCD treatment ($n=2$), because of logistic issues ($n=3$), and because of mild postoperative complications ($n=3$). In the restrictive group, the 12 T2DM subjects were on the VLCD treatment, whereas the 11 NGT subjects were treated with GB. Within the RYGB group, 16 subjects had NGT and 15 subjects had T2DM.

According to the protocol, all diabetic subjects discontinued using their glucoregulatory drugs on the day of surgery or initiation of the dietary treatment, except for metformin, the use of which was continued if fasting blood glucose levels remained above 7 mmol after the interventions (27% ($n=4$) of the subjects after RYGB vs 17% ($n=2$) of the subjects after the VLCD treatment, $P=NS$).

According to the protocol, the patients increased their intake after surgery (RYGB and GB) from a liquid diet for 5 days (<600 kcal/day) toward a more solid diet (700–800 kcal/day) in 3 weeks. Thereafter, the patients followed the intake guidelines of The Dutch Obesity clinic, consuming up to 1000 kcal a day. Retrospective

Table 1 Effects of restrictive and RYGB interventions on anthropometric and biochemical values and thyroid hormone levels in obese subjects with NGT and DM. Values are presented as mean $\pm$ s.d. The effects of interventions after 3 weeks and 3 months were compared using a mixed-effects model, with the patient groups and diabetes as fixed effects and the subject-specific deviances modeled with random intercepts. The Bonferroni's *post hoc* test was used to correct for multiple testing.

	Restrictive treatment ^a			RYGB		
	Before	After 3 weeks	After 3 months	Before	After 3 weeks	After 3 months
BMI (kg/m ²)						
All	41.5 $\pm$ 5.2	39.0 $\pm$ 4.9*	36.5 $\pm$ 5.4*	43.9 $\pm$ 3.7	40.6 $\pm$ 3.7*	36.6 $\pm$ 3.8*
NGT (11/16) ^b	43.1 $\pm$ 3.0	40.5 $\pm$ 2.9*	38.4 $\pm$ 3.2*	44.2 $\pm$ 3.3	40.9 $\pm$ 3.6*	37.1 $\pm$ 3.8*
DM (12/15) ^b	40.2 $\pm$ 6.4	37.7 $\pm$ 6.0*	34.7 $\pm$ 6.6*	43.5 $\pm$ 4.2	40.4 $\pm$ 4.0*	36.1 $\pm$ 3.8*
Weight (kg)						
All	115 $\pm$ 16	109 $\pm$ 16	101 $\pm$ 15*	125 $\pm$ 13	116 $\pm$ 13	105 $\pm$ 13*
NGT (11/16)	118 $\pm$ 13	113 $\pm$ 14	106 $\pm$ 14*	128 $\pm$ 9	119 $\pm$ 10	108 $\pm$ 11*
DM (12/15)	112 $\pm$ 18	105 $\pm$ 17	97 $\pm$ 16*	121 $\pm$ 16	112 $\pm$ 15	101 $\pm$ 13*
HOMA-IR (%)						
All	3.8 $\pm$ 2.8	1.4 $\pm$ 1.2*	1.9 $\pm$ 1.7	3.3 $\pm$ 2.8	2.6 $\pm$ 2.1	1.4 $\pm$ 1.0
NGT (11/16)	2.6 $\pm$ 2.1	1.6 $\pm$ 1.6	2.4 $\pm$ 2.0	2.3 $\pm$ 1.8	1.9 $\pm$ 1.8	1.1 $\pm$ 0.9
DM (12/15)	4.9 $\pm$ 2.9	1.2 $\pm$ 0.8*	1.3 $\pm$ 0.9*	4.4 $\pm$ 3.3	3.3 $\pm$ 2.1 ^c	1.8 $\pm$ 1.0*
Fasting glucose (mmol/l)						
All	6.7 $\pm$ 2.6	5.0 $\pm$ 0.9	5.5 $\pm$ 0.9	6.9 $\pm$ 2.6	5.7 $\pm$ 1.5	5.3 $\pm$ 0.9
NGT (11/16)	5.0 $\pm$ 0.6	4.9 $\pm$ 0.6	5.2 $\pm$ 0.8	5.0 $\pm$ 0.6	4.8 $\pm$ 0.8	4.9 $\pm$ 0.53
DM (12/15)	8.4 $\pm$ 2.6	5.2 $\pm$ 1.2*	5.9 $\pm$ 1.0*	8.9 $\pm$ 2.4	6.7 $\pm$ 1.5*	5.8 $\pm$ 1.2*
Fasting insulin (mU/l)						
All	12.6 $\pm$ 8.3	5.8 $\pm$ 4.8	7.7 $\pm$ 6.2	10.3 $\pm$ 7.4	9.8 $\pm$ 7.8	5.8 $\pm$ 3.9
NGT (11/16)	11.5 $\pm$ 8.7	6.9 $\pm$ 6.3	10.0 $\pm$ 7.2	9.8 $\pm$ 7.4	8.8 $\pm$ 9.3 ^c	4.9 $\pm$ 4.0 ^{a,c}
DM (12/15)	13.5 $\pm$ 8.1	4.9 $\pm$ 2.9*	5.0 $\pm$ 3.5*	10.8 $\pm$ 7.6	10.9 $\pm$ 6.2 ^c	6.9 $\pm$ 3.9*
TSH (mU/l)						
All	2.6 $\pm$ 1.3	2.1 $\pm$ 1.1*	1.8 $\pm$ 0.9*	3.4 $\pm$ 1.7	2.5 $\pm$ 1.4*	2.0 $\pm$ 1.1*
NGT (11/16)	2.4 $\pm$ 1.5	1.8 $\pm$ 1.3*	1.5 $\pm$ 0.9*	3.5 $\pm$ 1.9	2.8 $\pm$ 1.5*	2.1 $\pm$ 1.1*
DM (12/15)	2.9 $\pm$ 1.1	2.3 $\pm$ 0.9	2.0 $\pm$ 0.9 [†]	3.2 $\pm$ 1.5	2.1 $\pm$ 1.1 [†]	1.8 $\pm$ 1.0 [†]
T ₃ (nmol/l)						
All	1.7 $\pm$ 0.6	1.5 $\pm$ 0.5	1.5 $\pm$ 0.5*	1.9 $\pm$ 0.3	1.8 $\pm$ 0.3	1.6 $\pm$ 0.2*
NGT (11/16)	2.0 $\pm$ 0.7	1.9 $\pm$ 0.6	1.7 $\pm$ 0.4*	1.9 $\pm$ 0.3	1.7 $\pm$ 0.3*	1.6 $\pm$ 0.2*
DM (12/15)	1.5 $\pm$ 0.4	1.2 $\pm$ 0.3 [†]	1.2 $\pm$ 0.3 [†]	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3	1.7 $\pm$ 0.3*
FT ₄ (pmol/l)						
All	14.6 $\pm$ 3.4	16.1 $\pm$ 3.5*	15.1 $\pm$ 3.3	15.3 $\pm$ 2.8	17.0 $\pm$ 3.3*	14.6 $\pm$ 2.3
NGT (11/16)	14.9 $\pm$ 4.2	16.8 $\pm$ 4.6*	15.7 $\pm$ 4.2	14.7 $\pm$ 2.5	16.4 $\pm$ 3.8*	14.5 $\pm$ 2.6
DM (12/15)	14.3 $\pm$ 2.7	15.5 $\pm$ 2.3	14.5 $\pm$ 2.3	16.0 $\pm$ 3.1	17.7 $\pm$ 2.6*	14.7 $\pm$ 2.1
rT ₃ (ng/ml)						
All	0.29 $\pm$ 0.13	0.33 $\pm$ 0.14*	0.28 $\pm$ 0.12	0.28 $\pm$ 0.09	0.33 $\pm$ 0.08*	0.27 $\pm$ 0.07
NGT (11/16)	0.26 $\pm$ 0.09	0.32 $\pm$ 0.11*	0.27 $\pm$ 0.08	0.27 $\pm$ 0.06	0.33 $\pm$ 0.08*	0.27 $\pm$ 0.06
DM (12/15)	0.32 $\pm$ 0.15	0.35 $\pm$ 0.17	0.29 $\pm$ 0.15	0.30 $\pm$ 0.12	0.33 $\pm$ 0.09	0.28 $\pm$ 0.08
T ₃ /rT ₃						
All	6.9 $\pm$ 3.0	5.2 $\pm$ 2.2*	6.2 $\pm$ 2.3	7.4 $\pm$ 2.6	5.8 $\pm$ 2.1*	6.5 $\pm$ 2.1
NGT (11/16)	8.2 $\pm$ 3.2	6.3 $\pm$ 2.1*	7.0 $\pm$ 2.0	7.4 $\pm$ 2.3	5.5 $\pm$ 2.3 [†]	6.4 $\pm$ 2.4
DM (12/15)	5.6 $\pm$ 2.3	4.0 $\pm$ 1.6*	5.2 $\pm$ 2.4	7.3 $\pm$ 3.0	6.1 $\pm$ 2.0	6.6 $\pm$ 1.7
Leptin (μ g/l)						
All	56.2 $\pm$ 25.2	33.0 $\pm$ 18.4*	33.2 $\pm$ 18.5*	81.6 $\pm$ 44.2	49.1 $\pm$ 29.7*	34.0 $\pm$ 33.6*
NGT (11/16)	70.6 $\pm$ 25.4	45.0 $\pm$ 14.5 [†]	42.6 $\pm$ 15.5*	96.5 $\pm$ 45.0	55.9 $\pm$ 30.7 [†]	34.2 $\pm$ 28.0 [†]
DM (12/15)	42.9 $\pm$ 16.8	22.0 $\pm$ 14.5 [†]	21.6 $\pm$ 15.5 [†]	65.7 $\pm$ 38.6	41.8 $\pm$ 27.8 [†]	33.7 $\pm$ 39.8 ^{†,c}
IL8 (pg/ml)						
All	7.5 $\pm$ 2.5	10.1 $\pm$ 3.7	9.3 $\pm$ 2.8	9.2 $\pm$ 3.1	14.1 $\pm$ 5.0	11.1 $\pm$ 3.9
NGT (11/16)	7.5 $\pm$ 2.1	9.3 $\pm$ 3.7	8.7 $\pm$ 2.4	8.5 $\pm$ 3.6	13.8 $\pm$ 4.8	11.1 $\pm$ 3.1
DM (12/15)	7.5 $\pm$ 2.8	10.9 $\pm$ 3.6	9.9 $\pm$ 3.1	9.9 $\pm$ 2.6	14.4 $\pm$ 5.3	11.1 $\pm$ 4.6
IL6 (pg/ml)						
All	2.1 $\pm$ 1.7	1.8 $\pm$ 1.2	1.6 $\pm$ 0.9	2.3 $\pm$ 1.3	1.9 $\pm$ 1.1	2.0 $\pm$ 1.3
NGT (11/16)	1.2 $\pm$ 0.7	1.3 $\pm$ 0.6	1.4 $\pm$ 0.8	1.9 $\pm$ 0.7	1.6 $\pm$ 0.8	1.3 $\pm$ 0.6
DM (12/15)	2.8 $\pm$ 1.9	2.3 $\pm$ 1.3	1.9 $\pm$ 0.9	2.7 $\pm$ 1.6	2.3 $\pm$ 1.2	2.7 $\pm$ 1.4
IL10 (pg/ml)						
All	5.1 $\pm$ 4.5	5.6 $\pm$ 9.0	5.5 $\pm$ 6.9	2.4 $\pm$ 2.0	7.4 $\pm$ 17.4	4.8 $\pm$ 9.1
NGT (11/16)	4.3 $\pm$ 3.5	6.8 $\pm$ 12.9	4.5 $\pm$ 5.6	2.3 $\pm$ 1.7	11.6 $\pm$ 24.1	6.9 $\pm$ 12.7
DM (12/15)	5.9 $\pm$ 5.1	4.4 $\pm$ 4.2	6.4 $\pm$ 8.2	2.6 $\pm$ 2.3	3.3 $\pm$ 2.8	2.6 $\pm$ 1.6
TNF α (pg/ml)						
All	6.9 $\pm$ 2.5	8.1 $\pm$ 3.1	6.6 $\pm$ 3.0	8.4 $\pm$ 2.0	11.4 $\pm$ 3.7	8.8 $\pm$ 3.0
NGT (11/16)	7.5 $\pm$ 2.6	8.9 $\pm$ 3.1	7.6 $\pm$ 2.5	7.6 $\pm$ 1.6	9.7 $\pm$ 2.2	7.5 $\pm$ 2.9
DM (12/15)	6.5 $\pm$ 2.5	7.4 $\pm$ 3.0	5.8 $\pm$ 3.2	9.3 $\pm$ 2.1	13.0 $\pm$ 4.1	10.0 $\pm$ 2.5

* $P < 0.05$ /[†] $P < 0.001$; significant effect of interventions within groups when compared with baseline.

^aNGT subjects who underwent GB and DM subjects who underwent VLCD treatment.

^b(Left column) parameters tested: in parentheses is the number of subjects who underwent restrictive treatment (columns 2–4) and RYGB (columns 5–7).

^cSignificantly different effect of interventions (restriction or RYGB) between NGT and DM subjects.

assessment of the postoperative calorie intake showed a mean intake of 700 kcal/day in the second and third weeks after surgery and up to 1000 kcal in the second and third months after surgery, which was similar to the calorie intake in the dietary treatment group. None of the subjects reported any problems due to adherence to the VLCD or the prescribed meal plan.

Weight loss

At baseline, no differences in BMI were observed between the subject groups. After 3 weeks and 3 months, weight loss was comparable between the RYGB and restrictive groups, and no differences were observed between NGT and T2DM subjects (Table 1).

Baseline thyroid hormone parameters

Fasting plasma levels of TSH, T_3 , fT_4 , rT_3 , T_3/rT_3 , and leptin are given in Tables 1 and 2. At baseline, no differences were observed between patients in the restrictive or RYGB groups. TSH levels were higher in all the obese subjects than in the lean controls. fT_4 levels were comparable between obese subjects and lean controls. T_3 levels were higher in obese NGT subjects and rT_3 levels were higher in obese T2DM subjects than in the lean controls. The $T_3:rT_3$ ratio was not statistically different between the different patient groups at baseline; however, there was a trend toward a decreased $T_3:rT_3$ ratio in T2DM subjects. Leptin levels were significantly higher in obese subjects than in the controls. Obese NGT subjects had higher leptin levels than obese T2DM subjects. There was a trend toward increased IL8 and IL6 levels in T2DM subjects when compared with the levels in NGT subjects and lean controls. $TNF\alpha$ levels were enhanced in obese subjects when compared with those in the lean controls, and IL10 levels were similar in all the groups.

Three-week effects on thyroid hormone parameters

Three weeks after the initiation of the different interventions (Table 1), weight loss and decline in leptin levels were similar in all the treatment groups. A comparable and significant decrease in TSH levels was observed in the restrictive and RYGB groups. T_3 levels decreased after both the interventions, but the effect did not reach statistical significance. Furthermore, after both RYGB and restrictive interventions, a significant increase in fT_4 and rT_3 levels and a decrease in T_3/rT_3 levels were observed. After both the interventions, there was a trend toward enhanced IL8 levels. IL6, IL10, and $TNF\alpha$ levels were not significantly affected by the interventions.

Small differences in within-group effects were observed between NGT and T2DM subjects after the interventions. The decline in TSH levels did not reach significance in T2DM subjects after the restrictive interventions. The increase in fT_4 and rT_3 levels did not reach significance in T2DM subjects after the restrictive interventions.

There was no significant correlation between the decrease in TSH levels and changes in BMI or leptin levels. However, the increase in rT_3 levels showed a significant positive correlation with the increase in IL8 levels in all the obese subjects ($r=0.433/P=0.002$) and with IL6 levels ($r=0.341/P=0.016$), which was not observed with IL10 and $TNF\alpha$ levels.

Three-month effects on thyroid hormone parameters

After 3 months, there was a persistent decline in TSH and T_3 levels in the restrictive and RYGB groups, whereas fT_4 and rT_3 levels returned to baseline levels. The $T_3:rT_3$ ratio increased when compared with that at the 3-week time point in both the groups, but did not reach baseline levels. Three months after the

Table 2 Baseline characteristics of the study groups. Values are presented as mean $\pm$ s.d. Differences between the subject groups (NGT vs T2DM) and lean controls at baseline were compared using a mixed-effects model with the patient groups and diabetes as fixed effects and the subject-specific deviances modeled with random intercepts. The Bonferroni's *post hoc* test was used to correct for multiple testing.

	NGT (30)	DM (32)	Controls (12)	P value
Age (years)	47.7 $\pm$ 6.4	51.0 $\pm$ 7.1	49.2 $\pm$ 6.22	NS
BMI (kg/m ²)	43.8 $\pm$ 3.2	42.0 $\pm$ 5.5	21.7 $\pm$ 1.6	<0.001, T2DM/NGT vs controls
HbA1c (mmol/mol)	36.1 $\pm$ 7.8	49.6 $\pm$ 12.0	31.9 $\pm$ 2.5	<0.001, T2DM vs controls/NGT
HOMA-IR (%)	2.3 $\pm$ 1.9	5.1 $\pm$ 3.4	0.3 $\pm$ 0.1	<0.001, T2DM/NGT vs controls
Fasting glucose (mmol/l)	5.0 $\pm$ 0.6	8.7 $\pm$ 2.5	4.7 $\pm$ 0.3	<0.001, T2DM vs controls/NGT
Fasting insulin (mU/l)	10.5 $\pm$ 7.9	12.0 $\pm$ 7.8	1.6 $\pm$ 0.2	<0.001, T2DM/NGT vs controls
rT_3 (ng/ml)	0.27 $\pm$ 0.08	0.31 $\pm$ 0.13	0.22 $\pm$ 0.05	<0.05, DM vs controls
T_3/rT_3	7.8 $\pm$ 2.7	6.6 $\pm$ 2.8	7.7 $\pm$ 1.8	NS
Leptin (μ g/l)	86.0 $\pm$ 39.8	55.6 $\pm$ 32.5	9.9 $\pm$ 5.0	<0.001, T2DM vs NGT vs controls
IL8 (pg/ml)	8.1 $\pm$ 3.0	8.9 $\pm$ 2.9	7.3 $\pm$ 2.1	NS
IL6 (pg/ml)	1.6 $\pm$ 0.8	2.8 $\pm$ 1.7	0.7 $\pm$ 0.8	NS
IL10 (pg/ml)	3.2 $\pm$ 2.8	4.1 $\pm$ 4.2	1.7 $\pm$ 1.0	NS
$TNF\alpha$ (pg/ml)	7.6 $\pm$ 2.0	8.0 $\pm$ 2.7	5.8 $\pm$ 1.3	0.05, T2DM/NGT vs controls

A P value <0.05 was considered statistically significant.

interventions, the decline in rT_3 levels when compared with that during 3 weeks correlated with a decline in IL8 levels ($r=0.335/P=0.018$), but not with IL6, IL10, and TNF α levels. Notably, the effects on thyroid hormone parameters induced by either calorie restriction or RYGB were comparable in NGT and T2DM subjects.

Effects of interventions on a pooled analysis

Because the different weight loss strategies used in this study elicited comparable changes in thyroid hormone parameters, we hypothesized that these changes occurred irrespective of the weight loss strategy used. Therefore, we analyzed our data using a reduction mixed model, to assess whether the analysis of the pooled data set was allowed (i.e. whether the changes in thyroid hormone and TSH levels were the same irrespective of the intervention type). This was indeed true for TSH, fT_4 , rT_3 , and T_3/rT_3 levels (Table 3 and Fig. 1). Within the complete patient group, there was a significant ($P<0.001$) increase in rT_3 and fT_4 levels 3 weeks after the start of the interventions and a return to baseline levels after 3 months. TSH levels decreased significantly after 3 weeks and 3 months. The $T_3:rT_3$ ratio decreased after 3 weeks and slightly increased thereafter. However, this ratio was still decreased after 3 months when compared with that at baseline. By contrast, according to this model, changes in T_3 levels were not comparable between the groups and between different interventions. As such, we did not carry out a pooled analysis of all the T_3 data.

Discussion

This is the first study to compare the time course of the effects of RYGB with that of the effects of purely restrictive weight loss strategies on the thyroid hormone axis in obese females with or without T2DM. Although weight loss is associated with central effects of leptin on TSH secretion, we hypothesized that RYGB and restrictive weight loss strategies differ in the induction of transient systemic inflammation, which may influence thyroid hormone physiology independent of weight

loss. Therefore, the subjects in this study were examined 3 weeks after the interventions, when only little weight was lost and effects were probably related to the surgical procedure, perioperative stress, and starvation, and 3 months after these interventions, when weight loss was prominent.

We showed that, at baseline, obesity was associated with enhanced TSH, T_3 , and rT_3 levels and normal fT_4 levels when compared with lean controls. T2DM in obese subjects was associated with a further increase in rT_3 levels and, when compared with obese NGT subjects, relatively decreased T_3 levels. We found that both calorie restriction and RYGB induce a rapid decline in serum TSH levels and a rise in rT_3 and fT_4 levels. With prolonged weight loss, again irrespective of the weight loss strategy used, TSH and T_3 levels decreased significantly, whereas the transiently increased fT_4 and rT_3 levels returned to baseline levels. The changes in serum rT_3 levels showed a significantly positive correlation with serum IL6 and IL8 levels. Importantly, whereas subtle differences between obese NGT and T2DM subjects were present at baseline, both the subject groups showed a comparable response to the two different weight loss strategies. In brief, these data indicate that obesity with or without T2DM is associated with enhanced TSH, T_3 , and rT_3 levels, whereas fT_4 levels are not different from those of the controls, which is in line with the existent literature (4, 5, 6, 19, 20, 21).

The relatively elevated serum rT_3 levels together with normal fT_4 levels in obese subjects at baseline are not easy to explain. Chronic overnutrition is suggested to decrease rT_3 catabolism through enhanced peripheral D1 expression and reduced peripheral D2 expression (22). However, decreased D2 activity is unlikely, as we also observed increased T_3 levels. Likewise, increased D1 expression would lead to an increased conversion of T_4 to T_3 . However, we did not find a difference in the $T_3:T_4$ ratio between obese subjects and controls. Furthermore, given the low biological D1 activity in humans, altered D1 activity is unlikely to be solely responsible for the increase in rT_3 levels (23). An alternative explanation would be an induction of D3 activity, coinciding with low-grade systemic inflammation in obesity (24, 25, 26). Indeed, we found higher serum IL6 and

Table 3 Combined effects of both restrictive and RYGB weight loss intervention in all obese subjects (pooled data). To determine whether baseline values and trends of change over time within the different groups were similar between the groups, we used a stepwise mixed model reduction procedure. Reduction was not allowed for T_3 . The Bonferroni's *post hoc* test was used to correct for multiple testing. Values are presented as mean $\pm$ s.d.

Obese	Baseline	After 3 weeks	After 3 months	P value
TSH (mU/l)	3.1 $\pm$ 1.6	2.3 $\pm$ 1.3 [†]	1.9 $\pm$ 1.0 [†]	<0.001, 1–2 and 1–3
T_3 (nmol/l)	1.8 $\pm$ 0.5	1.7 $\pm$ 0.4	1.6 $\pm$ 0.3	–
fT_4 (pmol/l)	15.0 $\pm$ 3.1	16.6 $\pm$ 3.4 [†]	14.8 $\pm$ 2.8	<0.001, 1–2
rT_3 (ng/ml)	0.29 $\pm$ 0.1	0.33 $\pm$ 0.1 [†]	0.27 $\pm$ 0.1	<0.001, 1–2
T_3/rT_3	7.2 $\pm$ 2.8	5.5 $\pm$ 2.2 [†]	6.3 $\pm$ 2.2 [†]	<0.001, 1–2 and 1–3

A P value <0.05 was considered statistically significant. * $P<0.05$ /[†] $P<0.001$; significant effect of interventions within groups when compared with baseline.

Figure 1 Effects of interventions on TSH (A), fT₄ (B), and rT₃ (C) levels and T₃:rT₃ ratio (D) in the pooled obese subject group at baseline (white bar, 1) and 3 weeks (gray bar, 2) and 3 months (black bar, 3) after interventions when compared with those in the lean controls at baseline. Values are presented as mean $\pm$ s.d. Differences in effects between the interventions were evaluated using a mixed model analysis. The Bonferroni's *post hoc* test was used to correct for multiple testing. A *P* value <0.05 was considered statistically significant. **P*<0.05, significant effect of interventions within groups when compared with baseline. #*P*<0.05, significant difference in the obese group when compared with controls at baseline.

IL8 levels in obese subjects than in the controls. Although insignificant, the increase in rT₃, IL8, and IL6 levels was most pronounced in T2DM subjects, coinciding with a relative decrease in T₃ levels when compared with the levels in obese NGT subjects. Increased D3 activity would indeed lead to lower T₃ levels and increased rT₃ levels and, therefore, might explain the relatively elevated serum rT₃ levels that we observed at baseline.

Irrespective of the weight loss strategy used, TSH and T₃ levels decreased with weight loss. Although reduced leptin levels, by lowering TSH synthesis, may contribute to lower serum T₃ levels (27), the fact that fT₄ levels increased 3 weeks after the start of the interventions points to peripheral effects as well. The concomitant increase in rT₃ and fT₄ levels and decreased T₃:rT₃ ratio imply alterations in peripheral deiodination during the acute phase of the interventions. Short-term effects of starvation and low-energy diets *per se* are associated with decreased D1 and D2 activities (28). Decreased D1 activity, however, would lead to lower instead of higher rT₃ levels. Therefore, the increased fT₄ levels and decreased T₃:rT₃ ratio may be best explained by a combination of decreased D2 activity and increased D3 activity. Increased D3 activity may be supported by the fact that we found a correlation between changes in rT₃ and circulating IL8 levels, which suggests that at least, in part, inflammation-mediated D3 activity may have contributed to the effect.

Importantly, the short-term rise in rT₃, fT₄, and IL8 levels was similar in all the treatment groups and of a transient nature. This suggests that irrespective of tissue injury induced by surgery, a transient catabolic state induced by perioperative starvation or caloric restriction is associated with suppressed TSH synthesis and enhanced D3 and/or decreased D2 activation. Three months after the start of the interventions, a persistent decrease in TSH and T₃ levels was observed, whereas rT₃, fT₄, TSH, and IL8 levels had returned to baseline levels. This suggests that the upregulation of the thyroid axis as described earlier in obesity is reversed by weight loss. In addition to the decrease in TSH and T₃ levels, the transient alterations in peripheral thyroid hormone metabolism stabilize at their initial level after 3 months, suggesting a new equilibrium between TSH levels and peripheral thyroid hormone metabolism.

Interestingly, and as alluded to before, changes in TSH, T₃, and rT₃ levels were comparable between the groups and interventions as indicated by the mixed model. By contrast, changes in T₃ levels were, although not significantly different, not comparable between the groups. We do not have an exact explanation for this finding; however, subtle differences in T₃ levels at baseline may be responsible for this effect. As for the other parameters, we can conclude that all three intervention methods (GB, VLCD, and RYGB) induce comparable effects in both NGT and T2DM subjects.

A limitation of our study was that we were not able to measure deiodinase activity directly in human tissues. In addition, we cannot rule out several additional interactions with the pituitary–thyroid axis that may have impacted our results. Enhanced lipolysis and circulating free fatty acids during the acute-phase response during severe fasting decrease the abundance of thyroid hormone-binding proteins (THBPs) (24, 29). This phenomenon, however, has been only observed in the face of sepsis in critical illness and strict prolonged fasting, which was not the case in our subjects. Moreover, an acute fall in THBP levels cannot explain the decrease in total T₃ levels. Furthermore, although a unifying theory on the effects of metformin on thyroid function tests is currently missing, its use has been associated with the suppression of TSH secretion by the activation of AMPK (30, 31). However, despite the use of metformin by T2DM subjects at baseline (26 of the 32 subjects), TSH levels were comparably enhanced in NGT and T2DM subjects. As the majority of the obese population undergoing RYGB is female, we decided not to include male subjects in any of the groups to prevent possible gender differences confounding our results. This, however, implicates that our results apply to the female population only.

In conclusion, we have shown that both restrictive weight loss strategies and RYGB induce similar changes in thyroid hormone physiology, which can be explained by changes in the peripheral conversion of thyroid hormone. Although subtle differences were observed,

changes in T2DM subjects mimic the effects observed in NGT subjects. Our data clearly show that in contrast to our hypothesis, calorie restriction induces effects on thyroid function tests that are mimicked by RYGB, whereas apparently no additional effects are induced by the gastrointestinal rearrangements brought about by this procedure.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M A Lips was responsible for study concept and design, acquisition of the data, analysis and interpretation of the data, drafting of the manuscript, and statistical analysis; H Pijl was responsible for study concept and design and study supervision; J B van Klinken was responsible for the statistical analysis; G H de Groot was responsible for study concept and design; I M Janssen/B Van Ramshorst/B A Van Wagenveld/D J Swank/F Van Dielen were responsible for the acquisition of data; J W A Smit was responsible for the analysis and interpretation of data and critical revision of the manuscript for important intellectual content.

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