Chapter 5

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

Mirjam A. Lips, Hanno Pijl, Jan B. van Klinken, Gerrit H. de Groot, Ignace M. Janssen I, Bert Van Ramshorst, Bart A. Van Wagensveld, Dingeman J. Swank, Fracois Van Dielen, Johannes W.A. Smit

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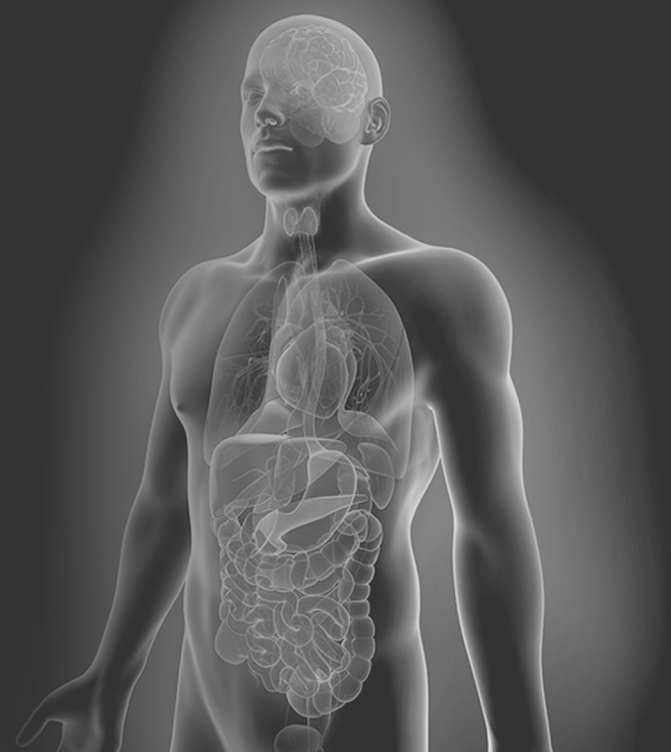

ABSTRACT

Objective

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

Methods

We included 12 lean, 27 obese females with normal fasting glucose (NGT) and 27 obese females with type 2 diabetes mellitus (T2DM). Weight loss was achieved by restrictive treatment (Gastric Banding (GB) or high–protein-low-calorie (VLCD)) or by Roux-en-Y-Gastric Bypass (RYGB). Fasting serum leptin, thyroid stimulating hormone (TSH), triiodothyronine (T3), *reverse* T3 (rT3) and free levothyroxine (fT4) concentrations were measured at baseline, and 3 weeks and 3 months after start of the interventions.

Results

Obesity was associated with higher TSH, T3 and rT3 and normal fT4 in all subjects as compared to controls. After 3 weeks, calorie restriction and RYGB induced a decline in TSH and a rise in rT3 and fT4. The increase in rT3 correlated with serum Il-8 and Il-6 levels. After three months, fT4 and rT3 returned to baseline levels, whereas TSH and T3 were persistently decreased as compared with baseline. No differences in the effects on thyroid hormone parameters between the interventions or between NGT and T2DM were observed at any time-point.

Conclusions

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

INTRODUCTION

Thyroid hormone plays an important role in energy metabolism (1;2). In obese subjects, thyroid stimulating hormone (TSH) and serum triiodothyronine (T3) serum concentrations are generally higher 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 TRH in obesity has been described (3-6). The increase in resting energy expenditure, diminishing the available energy for accumulation in fat, is thought to be an adaptive response to chronic nutrient surfeit (7;8).

Most studies on weight loss in obesity report a decrease in serum TSH and T3 levels. This may be explained by a decline in serum leptin levels which consequently causes a fall in serum TSH and T3 levels (9-11). 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 rT3 levels during calorie restriction and fasting (11-13). Differences between studies on the effect of weight loss on serum rT3 may be explained by differences in study population, weight loss intervention and time-course of measurements. Increased serum rT3 levels may be related to increased type 3 deiodinase (D3), which is enhanced by inflammatory pathways, or decreased type 2 deiodinase (D2) activity. Active weight loss strategies, i.e. calorie restriction per se or gastro-intestinal surgery in general may all cause transient systemic stress, insulin resistance and tissue injury to a certain degree and may therefore lead to differential effects on D3 activity (14-16).

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 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. We therefore performed a prospective study comparing the effects of restrictive weight loss strategies (VLCD and gastric banding) with RYGB on thyroid hormone metabolism. In addition, as this has not been studied before, 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 included obese female subjects eligible for both restrictive (VLCD and GB) and RYGB treatment. 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 longstanding obesity and failed weight loss attempts as the surgical patients. As RYGB is reported to have superior metabolic effects in subjects with T2DM (as compared to GB), GB is not a preferred surgical treatment for obese T2DM patients in the Netherlands. We therefore 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 their calorie intake was comparable throughout the study, we included subjects who underwent VLCD and GB 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 as compared to lean controls. All diabetic subjects were treated with oral medication only (metformin, sulfonylureum derivatives, or thiazolidinediones). Body weight of all subjects had been stable for at least 3 months prior to inclusion, and none of the subjects reported the use of weight loss medications. 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 to the obese subjects; with a BMI in between 20-25 kg/m^2 and a normal plasma glucose concentration in fasting condition.

Ethics

The 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 (NTC01167959).

Study design

Subjects were studied within a month before surgery or the initiation of the VLCD and again between 2 and 3 weeks and 3 months after surgery or imitation of the VLCD. Subjects were admitted to the clinical research center after an overnight (10h) fast. Anthropometric measurements were taken and bioelectric impedance analysis (QuadscanBodystat® United Kindom) was performed. A canula was inserted into an antecubital vein and a fasting blood sample was taken. Blood was collected in a SST® Gel and Clot Activator tube (Becton and Dickinson) and a vacutainer on EDTA. All blood samples, and serum samples when clotted, were centrifuged promptly (2000 g at 4 °C, for 10 minutes) and subsequently plasma or serum was divided in separate plastic tubes and frozen (-80 °C) until assay.

Interventions

RYGB

During RYGB, a 25 milliliters gastric pouch was created and connected to a 100cm Roux-en-Y limb. Gastric banding entailed placement of a standard silicone LapBand® (Inamed, Allegan, Santa Barbara, CA) around the stomach to create a 15 milliliters pouch. On the first day after surgery, 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 would hand in an intake diary of the postoperative period allowing us to make an estimation of their caloric intake.

Restrictive interventions

Gastric Banding

Gastric banding entailed placement of a standard silicone LapBand® (Inamed, Allegan, Santa Barbara, CA) around the stomach to create a 15 milliliters pouch. On the first day after surgery, 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 soluble powder for preparation of shakes, omelet, soups and bread. Each sachet contained ~90 kcal (~18 g protein, ~2.5-5 g carbohydrates, 0.5-2 g fat). Subjects were allowed 4-5 sachets a day and an additional choice of selected vegetables (600 kcal/day

in total) during the first 3 weeks. Up to 2 months patients were allowed more additional vegetables, 1 piece of fruit an 1 portion of light dairy produce (800 ckal/day in total). After two months, patients were allowed a light evening meal (replacing 1 Prodimed sachet) on intermittent days (1000 ckal/day in total). For the evening meal, they could choose from several a prescribed recipes.

Concomitant medication

At the day of operation or start of the VLCD, all blood glucose lowering agents were discontinued to avoid hypoglycemia. Only metformin treatment was reinstalled if fasting blood glucose levels remained above 7 mmol after intervention. If subjects, at baseline, used prescriptions such as antihypertensive or cholesterol lowering medication, these were continued throughout the whole time course of the study.

Assays

Serum glucose was measured using a Modular P800 chemistry analyzer of Roche Diagnostics (Mannheim, Germany) with a coefficient of variation (CV) of 1.7%. Insulin was measured with an immunometric assay on an automated Immulite 2500 (Siemens, Breda, The Netherlands) with an intra-assay CV 6-7.5%. HbA1c was measured in whole blood samples using a fully automated High Performance Liquid Chromatography Integra 800 analyzer of Roche Diagnostics (Mannheim, Germany) with a coefficient of variation (CV) of <0.6%. Serum free T4 (FT4) and thyroid stimulating hormone (TSH) were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (Roche, Almere, The Netherlands). The intra-assay CVs were respectively 1.6-2.2 % and 1.3-5.0 %. Serum T3 was measured with a fluorescence polarization Immunoassay on an AxSym system (Abbott, Abbott Park, IL, USA). The CV was 2.5-9.0 %. Leptin was measured by radioimmunoassay (RIA) (Leptin HL-81K, Millipore, Billerica USA) with an inter-assay precision of 3.6–6.2% and an intra-assay precision of 3.4-8.3%. Serum reverse T3 was measured using a commercially available RIA (ALPCO diagnostics, The Netherlands) with an inter- assay precision of 3,0% and an intra-assay precision of 3,9%. Interleukin-8 (IL-8) and interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor-necrosis factor α (TNF-α)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 ± standard deviation of the mean. Differences between subject groups (NGT vs. T2DM) and lean controls at baseline and the effect of intervention after three weeks and three months were

compared with a mixed-effects model, with the patient groups and diabetes as fixed effects and the subject specific deviances modelled with random intercepts. To analyze whether baseline values and trends of change over time within the different groups were similar between groups we applied a stepwise Mixed Model reduction procedure. The Bonferroni posthoc test was used to correct for multiple testing. Correlation between parameters was calculated with Pearsons correlation coefficient. A p-value of <0.05 was considered statistically significant. Graphs were computed in Prism Graph Pad 5.

RESULTS

Subject characteristics

The baseline characteristics of all participants are given in table **1** (restrictive versus malabsorptive) and in table 2 (T2DM versus NGT). All obese subjects and healthy controls were Caucasian females, with a mean age of 49.4 ± 0.6 yrs. We included 32 subjects with T2DM and 30 NGT obese individuals. Eight subjects left the study because they were not able to comply with the VLCD (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 very low calorie diet, whereas the 11 NGT subjects were treated with gastric banding. Within the RYGB group 16 subjects were NGT, 15 subjects had T2DM.

According to protocol, all diabetic subjects discontinued their glucoregulatory drugs at the day of operation or start of the diet, except for metformin, which was continued if fasting blood glucose levels remained above 7 mmol after intervention (27% (n=4) of subjects after RYGB vs. 17 % (n=2) of subjects after VLCD, p=ns).

According to protocol patients expanded their intake after operation (RYGB and GB) from a liquid diet for 5 days (<600 kcal/day) towards a more solid diet (700-800 kcal/day) in 3 weeks. Thereafter, patients followed the intake guidelines of The Dutch Obesity clinic, containing up to 1000 ckal a day. Retrospective assessment of postoperative calorie intake showed a mean intake of 700 kcal/day in the second and third week post-surgery and up to 1000 kcal in the second and third month post-surgery, which was similar to the calorie intake in the diet group. None of the subjects reported any problems adhering to the VLCD or the prescribed meal plan.

Table 1 - Effects of restrictive and RYGB intervention on anthropometrics biochemical values and thyroid hormones in obese subject with NGT and DM.

	Restrictive treatment@			RYGB		
	Before	**After 3 weeks**	**After 3 months**	**Before**	**After 3 weeks**	**After 3 months**
BMI (kg/m²) All	**41.5 ± 5.2**	**39.0 ± 4.9***	**36.5 ± 5.4***	**43.9 ± 3.7**	**40.6 ± 3.7***	**36.6 ± 3.8***
NGT (11/16)$	43.1 ± 3.0	40.5 ± 2.9*	38.4 ± 3.2*	44.2 ± 3.3	40.9 ± 3.6*	37.1 ± 3.8*
T2DM (12/15)$	40.2 ± 6.4	37.7 ± 6.0*	34.7 ± 6.6*	43.5 ± 4.2	40.5 ± 4.0*	36.1 ± 3.8*
Weight (kg) All	**115 ± 16**	**109 ± 16**	**101 ± 15***	**125 ± 13**	**116 ± 13**	**105 ± 13***
NGT (11/16)	118 ± 13	113 ± 14	106 ± 14*	128 ± 9	119 ± 10	108 ± 11*
T2DM (12/15)	112 ± 18	105 ± 17	97 ± 16*	121 ± 16	112 ± 15	101 ± 13*
Homa-ir (%)All	**3.8 ± 2.8**	**1.4 ± 1.2***	**1.9 ± 1.7**	**3.3 ± 2.8**	**2.6 ± 2.1**	**1.4 ± 1.0**
NGT (11/16)	2.6 ± 2.1	1.6 ± 1.6	2.4 ± 2.0	2.3 ± 1.8	1.9 ± 1.8	1.1 ± 0.9
T2DM (12/15)	4.9 ± 2.9	1.2 ± 0.8*	1.3 ± 0.9*	4.4 ± 3.3	3.3 ± 2.1#	1.8 ± 1.0*
Fasting glucose (mmol/l) All	**6.7 ± 2.6**	**5.0 ± 0.9**	**5.5 ± 0.9**	**6.9 ± 2.6**	**5.7 ± 1.5**	**5.3 ± 0.9**
NGT (11/16)	5.0 ± 0.6	4.9 ± 0.6	5.2 ± 0.8	5.0 ± 0.6	4.8 ± 0.8	4.9 ± 0.53
T2DM (12/15)	8.4 ± 2.6	5.2 **± 1.2***	5.9 ± 1.0*	8.9 ± 2.4	6.7 ± 1.5*	5.8 ± 1.2*
Fasting insulin (mU/l) All	**12.6 ±8.3**	**5.8 ± 4.8**	**7.7 ± 6.2**	**10.3 ± 7.4**	**9.8 ± 7.8**	**5.8 ± 3.9**
NGT (11/16)	11.5 ± 8.7	6.9 ± 6.3	10.0 ± 7.2	9.8 ± 7.4	8.8 ± 9.3#	4.9 ± 4.0*#
T2DM (12/15)	13.5 ± 8.1	4.9 ± 2.9*	5.0 ± 3.5*	10.8 ± 7.6	10.9 ± 6.2#	6.9 ± 3.9*
TSH (mU/L) All	**2.6 ± 1.3**	**2.1 ± 1.1***	**1.8 ± 0.9***	**3.4 ± 1.7**	**2.5 ± 1.4***	**2.0 ± 1.1***
NGT (11/16)	2.4 ± 1.5	1.8 ± 1.3 *	1.5 ± 0.9 *	3.5 ± 1.9	2.8 ± 1.5*	2.1 ± 1.1 *
T2DM (12/15)	2.9 ± 1.1	2.3 ± 0.9	2.0 ± 0.9**	3.2 ± 1.5	2.1 ± 1.1**	1.8 ± 1.0**
T3 (nmol/L) All	**1.7 ± 0.6**	**1.5 ± 0.5**	**1.5 ± 0.5***	**1.9 ± 0.3**	**1.8 ± 0.3**	**1.6 ± 0.2***
NGT (11/16)	2.0 ± 0.7	1.9 ± 0.6	1.7 ± 0.4 *	1.9 ± 0.3	1.7 ± 0.3 *	1.6 ± 0.2 *
T2DM (12/15)	1.5 ± 0.4	1.2 ± 0.3**	1.2 ± 0.3**	1.9 ± 0.3	1.9 ± 0.3	1.7 ± 0.3*
fT4 (pmol/L) All	**14.6 ± 3.4**	**16.1 ± 3.5***	**15.1 ± 3.3**	**15.3 ± 2.8**	**17.0 ± 3.3***	**14.6 ± 2.3**
NGT (11/16)	14.9 ± 4.2	16.8 ± 4.6 *	15.7 ± 4.2	14.7 ± 2.5	16.4 ± 3.8 *	14.5 ± 2.6
T2DM (12/15)	14.3 ± 2.7	15.5 ± 2.3	14.5 ± 2.3	16.0 ± 3.1	17.7 ± 2.6*	14.7 ± 2.1
revT3 (ng/ml) All	**0.29 ± 0.13**	**0.33 ± 0.14***	**0.28 ± 0.12**	**0.28 ± 0.09**	**0.33 ± 0.08***	**0.27 ± 0.07**
NGT (11/16)	0.26 ± 0.09	0.32 ± 0.11 *	0.27 ± 0.08	0.27 ± 0.06	0.33 ± 0.08*	0.27 ± 0.06
T2DM (12/15)	0.32 ± 0.15	0.35 ± 0.17	0.29 ± 0.15	0.30 ± 0.12	0.33 ± 0.09	0.28 ± 0.08
T3/rT3 All	**6.9 ± 3.0**	**5.2 ± 2.2***	**6.2 ± 2.3**	**7.4 ± 2.6**	**5.8 ± 2.1***	**6.5 ± 2.1**
NGT (11/16)	8.2 ± 3.2	6.3 ± 2.1 *	7.0 ± 2.0	7.4 ± 2.3	5.5 ± 2.3**	6.4 ± 2.4
T2DM (12/15)	5.6 ± 2.3	4.0 ± 1.6 *	5.2 ± 2.4	7.3 ± 3.0	6.1 ± 2.0	6.6 ± 1.7

	Restrictive treatment®			RYGB		
	Before	After 3 weeks	After 3 months	Before	After 3 weeks	After 3 months
Leptin (μg/L) All	**56.2 ± 25.2**	**33.0 ± 18.4***	**33.2 ± 18.5***	**81.6 ± 44.2**	**49.1 ± 29.7***	**34.0 ± 33.6***
NGT (11/16)	70.6 ± 25.4	45.0 ± 14.5 **	42.6 ± 15.5*	96.5 ± 45.0	55.9 ± 30.7 **	34.2 ± 28.0**
T2DM (12/15)	42.9 ± 16.8	22.0 ± 14.5 **	21.6 ± 15.5**	65.7 ± 38.6	41.8 ± 27.8**	33.7 ± 39.8**#
Il-8 (pg/ml) All	**7.5 ± 2.5**	**10.1 ± 3.7**	**9.3 ± 2.8**	**9.2 ± 3.1**	**14.1 ± 5.0**	**11.1 ± 3.9**
NGT (11/16)	7.5 ± 2.1	9.3 ± 3.7	8.7 ± 2.4	8.5 ± 3.6	13.8 ± 4.8	11.1 ± 3.1
T2DM (12/15)	7.5 ± 2.8	10.9 ± 3.6	9.9 ± 3.1	9.9 ± 2.6	14.4 ± 5.3	11.1 ± 4.6
Il-6 (pg/ml) All	**2.1 ± 1.7**	**1.8 ± 1.2**	**1.6 ± 0.9**	**2.3 ± 1.3**	**1.9 ± 1.1**	**2.0 ± 1.3**
NGT (11/16)	1.2 ± 0.7	1.3 ± 0.6	1.4 ± 0.8	1.9 ± 0.7	1.6 ± 0.8	1.3 ± 0.6
T2DM (12/15)	2.8 ± 1.9	2.3 ± 1.3	1.9 ± 0.9	2.7 ± 1.6	2.3 ± 1.2	2.7 ± 1.4
Il-10 (pg/ml) All	**5.1 ± 4.5**	**5.6 ± 9.0**	**5.5 ± 6.9**	**2.4 ± 2.0**	**7.4 ± 17.4**	**4.8 ± 9.1**
NGT (11/16)	4.3 ± 3.5	6.8 ± 12.9	4.5 ± 5.6	2.3 ± 1.7	11.6 ± 24.1	6.9 ± 12.7
T2DM (12/15)	5.9 ± 5.1	4.4 ± 4.2	6.4 ± 8.2	2.6 ± 2.3	3.3 ± 2.8	2.6 ±1.6
TNF-α (pg/ml) All	**6.9 ± 2.5**	**8.1 ± 3.1**	**6.6 ± 3.0**	**8.4 ± 2.0**	**11.4 ± 3.7**	**8.8 ± 3.0**
NGT (11/16)	7.5 ± 2.6	8.9 ± 3.1	7.6 ± 2.5	7.6 ± 1.6	9.7 ± 2.2	7.5 ± 2.9
T2DM (12/15)	6.5 ±2.5	7.4 ± 3.0	5.8 ± 3.2	9.3 ±2.1	13.0 ± 4.1	10.0 ± 2.5

Values are presented as mean ± SD. The effect of intervention after three weeks and three months were compared with a mixed-effects model. with the patient groups and diabetes as fixed effects and the subject specific deviances modelled with random intercepts. The Bonferroniposthoc test was used to correct for multiple testing. @ NGT underwent GB, T2DM underwent VLCD. $ (left column) are the parameters tested: between parentheses the number of subjects undergoing restrictive treatment (column 2-4) and RYGB (column 5-7). * = p<0.05 / ** = p< 0.001; significant effect of intervention within group as compared to baseline; # = significant different effect of intervention (restriction or RYGB) between NGT and T2DM subjects.

Weight loss

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

Baseline thyroid hormone parameters

Fasting plasma levels of TSH, T3, fT4, rT3, T3/rT3 and leptin are shown in table 1/table 2. At baseline, no differences were observed between patients in restrictive or RYGB groups. TSH was higher in all obese subjects than in lean controls. FT4 was comparable between obese subjects and lean controls. T3 levels were higher in obese NGT subjects

Table 2 - Baseline characteristics of study groups.

	NGT (30)	T2DM (32)	Controls (12)	P value
Age (yrs)	47.7 ± 6.4	51.0 ± 7.1	49.2 ± 6.22	p=ns
BMI (kg/m²)	43.8 ± 3.2	42.0 ± 5.5	21.7 ± 1.6	p<0.001 T2DM/NGTvscontrols
HbA1c (mmol/mol)	36.1 ± 7.8	49.6 ± 12.0	31.9 ± 2.5	p<0.001 T2DMvs controls/NGT
Homa-ir (%)	2,4 ± 1,9	4.6 ± 3,4	0,3 ± 0,1	p<0.001 T2DM/NGTvscontrols
Fasting glucose (mmol/l)	5.0 ± 0.6	8.7 ± 2.5	4.7 ± 0.3	p<0.001 T2DMvscontrols/NGT
Fasting insulin (mU/l)	10.5 ± 7.9	12.0 ± 7.8	1.6 ± 0.2	p<0.001 T2DM/NGTvs controls
revT3 (ng/ml)	0.27 ± 0.08	0.31 ± 0.13	0.22 ± 0.05	p<0.05 T2DM vs controls
T3/revT3	7.8 ± 2.7	6.6 ± 2.8	7.7 ± 1.8	p=ns
Leptin (μg/L)	86.0 ± 39.8	55.6 ± 32.5	9.9 ± 5.0	p<0.001 T2DMvsNGTvs controls
Il-8 (pg/ml)	8.1 ± 3.0	8.9 ± 2.9	7.3 ± 2.1	p=ns
Il-6 (pg/ml)	1.6 ± 0.8	2.8 ± 1.7	0.7 ± 0.8	p=ns
Il-10 (pg/ml)	3.2 ± 2.8	4.1 ± 4.2	1.7 ± 1.0	p=ns
TNF-α (pg/ml)	7.6 ± 2.0	8.0 ± 2.7	5.8 ± 1.3	p=0,05 T2DM/NGTvs controls

Values are presented as mean ± SD. Differences between subject groups (NGT vs T2DM) and lean controls at baseline were compared with a mixed-effects model with the patient groups and diabetes as fixed effects and the subject specific deviances modelled with random intercepts. The Bonferroniposthoc test was used to correct for multiple testing. *A p-value <0.05* was considered statistically significant.

and rT3 levels were higher in obese T2DM subjects as compared to lean controls. T3/rT3 ratio was not statistically different between the different patient groups at baseline; however there was a trend towards a decreased T3/rT3 ratio in T2DM subjects. Leptin levels were significantly higher in obese subjects than in controls. Obese NGT subjects had higher leptin levels than obese T2DM subjects. There was a trend towards enhanced interleukin-8 (Il-8) and interleukin-6 (Il-6) levels in T2DM subjects as compared to NGT subjects and lean controls. TNF-α level was enhanced in obese subjects as compared to lean controls and interleukin-10 (Il-10) levels were similar in all groups.

3-week effects on thyroid hormone parameters

Three weeks after the initiation of the different interventions (table 1), weight loss and the decline in leptin levels were similar in all treatment groups. A comparable and significant decrease in TSH levels were observed in the restriction and RYGB groups. T3 levels decreased after both interventions, but the effect did not reach statistical significance. Furthermore, both after RYGB and restriction, a significant increase in fT4

and rT3 levels, and a decrease in T3/rT3 levels were observed. After both interventions there was a trend towards enhanced Il-8 levels. Il-6, Il-10 and TNF-α 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 restriction,. The increase in fT4 and rT3 levels did not reach significance in T2DM subjects after restriction.

There was no significant correlation between the decrease in TSH levels and changes in BMI or Leptin. However, the increase in rT3 levels showed a significant positive correlation with the increase in Il-8 levels in all obese subjects (r=0.433 / p=0.002) and with Il-6 levels (r=0.341 / p=0.016), which was not observed with Il-10 and TNF-α.

3 months effects on thyroid hormone parameters

After three months, there was a persistent decline in TSH and T3 levels in the restriction and RYGB groups, whereas fT4 and rT3 levels returned to baseline levels. T3/rT3 ratio increased as compared with the 3-weeks timepoint in both groups, but did not reach baseline levels. Three months after intervention, the decline in rT3 levels as compared with 3 weeks correlated with a decline in Il-8 levels (r=0.335 / p=0.018), but not with Il-6, Il-10 and TNF-α. Notably, the effects on thyroid hormone parameters induced by either calorie restriction or RYGB were comparable in NGT and T2DM subjects.

Effects of intervention in pooled analyses

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. Therefore, we analysed our data with a reduction mixed model, to assess whether analysis of the pooled data set was allowed (i.e. if the changes in thyroid hormones and TSH were the same irrespective of intervention type). This was indeed true for TSH, fT4, rT3 and T3/rT3 (table3/figure 1). Within the complete patients group, there was a significant ($p<0.001$) increase in rT3 and fT4 levels 3 weeks after the start of the interventions and a return to baseline levels after three months. TSH levels decreased significantly after three weeks and three months. T3/rT3 levels decreased after three weeks, and slightly increased thereafter again. However, this ratio was still decreased after three months as compared to baseline. In contrast, according to this model changes in levels of T3 were not comparable between groups and between different interventions. As such, we did not perform a pooled analysis of all T3 data.

Table 3 - Combined effects of both restrictive and RYGB weight loss intervention in all obese subjects (pooled data).

Obese	1.Baseline	2.After 3 weeks	3.After three months	p-value
TSH (mU/L)	**3.1 ± 1.6**	**2.3 ± 1.3 ****	**1.9 ± 1.0****	**p<0.001 1-2 & 1-3**
T3 (nmol/L)	1.8 ± 0.5	1.7 ± 0.4	1.6 ± 0.3	-
fT4 (pmol/L)	**15.0 ± 3.1**	**16.6 ± 3.4 ****	**14.8 ± 2.8**	**p<0.001 1-2**
revT3 (ng/ml)	**0.29 ± 0.1**	**0.33 ± 0.1****	**0.27 ± 0.1**	**p<0.001 1-2**
T3/rT3	**7.2 ± 2.8**	**5.5 ± 2.2 ****	**6.3 ± 2.2 ****	**p<0.001 1-2 & 1-3**

To analyse whether baseline values and trends of change over time within the different groups were similar between groups we applied a stepwise Mixed Model reduction procedure. Reduction was not allowed for T3. The Bonferroniposthoc test was used to correct for multiple testing. Values are presented as mean ± SD. *A p-value <0.05* was considered statistically significant. *=p<0.05/**=p<0.001; significant effect of intervention within group as compared to baseline.

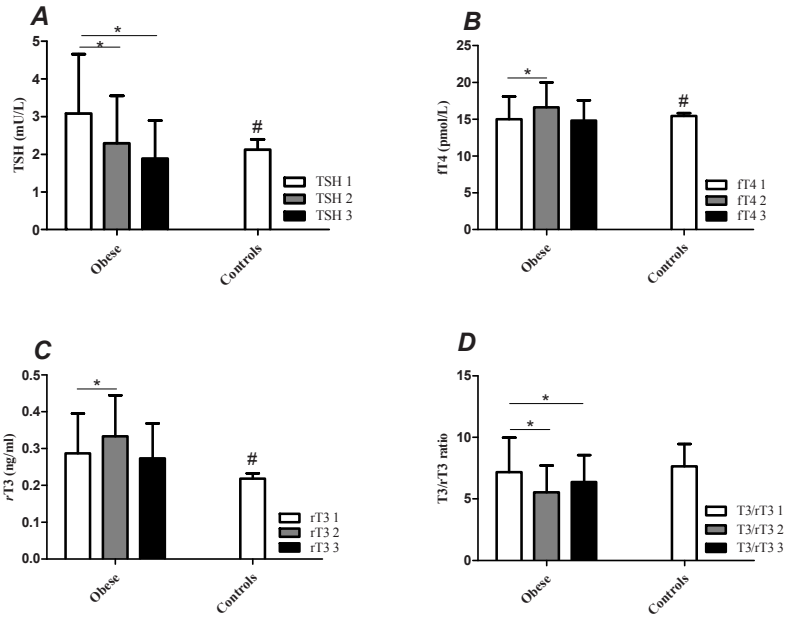

Figure 1. Effects of intervention on TSH (A), fT4 (B), rT3 (C) and T3/rT3 ratio (D) in the pooled obese subject group at baseline (white bar, 1), three weeks (grey bar, 2) and three months(black bar, 3) after intervention as compared to lean controls at baseline.

Values are presented as mean ± SD. First, we applied a stepwise Mixed Model reduction procedure to analyze whether baseline values and trends of change over time within the different groups were similar between groups. Then the same model calculated the significance of changes over time within the pooled group. The Bonferroniposthoc test was used to correct for multiple testing. A p-value <0,05 was considered statistically significant. * = p<0,05 significant effect of intervention within group as compared to baseline. # = p<0,05 significant difference of obese group as compared to controls at baseline.

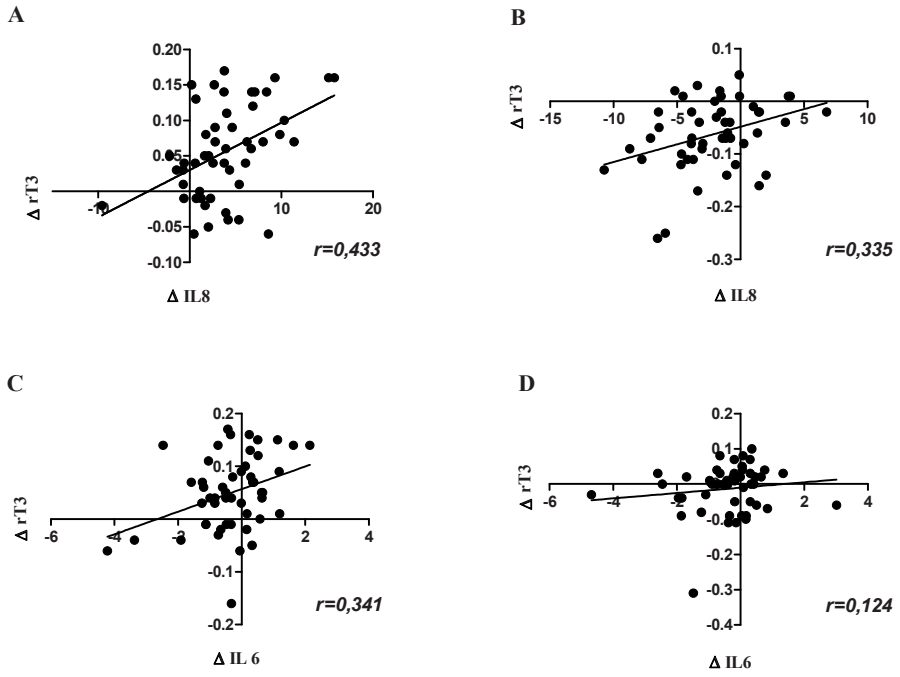

Figure 2. Correlation between change in IL-8 and IL-6 levels and rT3 level after 3 weeks and three months.

Correlation between change in IL-8 and rT3 at three weeks (A) and three months (B), and correlation between change in IL-6 and rT3 at three weeks (C) and at three months (D). Changes are calculated as compared to beseline. Correlations are calculated with Pearsons correlation coëfficient. r = correlation co-efficient.

DISCUSSION

This is the first study to compare the time course of the effects of RYGB with those 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 three weeks after the interventions, when only little weight was lost and effects are probably related to the surgical procedure, peri-operative stress and starvation; and three months after these interventions, when weight loss was prominent.

We showed that, at baseline, obesity was associated with enhanced TSH, T3 and rT3 levels and normal fT4 levels as compared to lean controls. T2DM in obese subjects was associated with a further increase in rT3 levels, and as compared to NGT obese subjects, relatively decreased T3 levels. We found that both calorie restriction and RYGB, induce a rapid decline in serum TSH levels and a rise in rT3 and fT4 levels. With prolonged weight loss, again irrespective of weight loss strategy, TSH and T3 decreased significantly, whereas the transiently increased fT4 and rT3 levels returned to baseline levels. The changes in serum rT3 levels showed a positive significant correlation with serum Il-6 and Il-8 levels. Importantly, whereas subtle differences between NGT and T2DM obese subjects were present at baseline, both subjects groups showed a comparable response to the two different weight loss strategies. In aggregate, these data indicate that obesity with or without T2DM is associated with enhanced TSH, T3 and rT3 levels, whereas fT4 levels are not different from controls, which is in line with the existent literature (4-6;19-21).

The relatively elevated serum rT3 levels together with normal fT4 levels in obese subjects at baseline are not easy to explain. Chronic over-nutrition is suggested to decrease rT3 catabolism through enhanced peripheral D1 and reduced peripheral D2 expression (22). However, decreased D2 activity is unlikely, as we also observed increased T3 levels. Likewise, increased D1 expression would lead to an increased conversion of T4 to T3. However, we did not find a difference in T3/T4 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 enhancement in rT3 levels (23). An alternative explanation would be an induction of D3 activity, coinciding with low grade systemic inflammation in obesity (24-26). Indeed, we found higher serum Il-6 and Il-8 levels in obese subjects as compared with controls. Although insignificant, the incline in rT3, Il-8 and Il-6 levels was most pronounced in T2DM subjects, coinciding with a relative decrease in T3 levels as compared to 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 we observed at baseline.

Irrespective of the weight loss strategy, 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 starting the intervention, points to peripheral effects as well. The concomitant increase in rT3, fT4 and decreased T3/rT3 ratio, implies 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 activity (28). Decreased D1 activity however would lead to lower instead of higher rT3 levels. Therefore, the increased fT4 levels and decreased T3/rT3 ratios 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 Il-8, 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 il-8 levels was similar in all treatment groups and of a transient nature. This suggests that irrespective of tissue injury induced by surgery, a transient catabolic state induced by peri-operative starvation or calorie 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 il-8 levels had returned to baseline levels. This suggests that the upregulation of the thyroid axis as described before 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 three months, suggesting a new equilibrium between TSH and peripheral thyroid hormone metabolism.

Interestingly, and as alluded to before, changes in levels of TSH, T3, rT3 were comparable between groups and interventions as analysed by mixed model. In contrast, changes in T3 levels were, although not significantly different, not comparable between groups. We do not have an exact explanation for his 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, 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. Eenhanced lipolysis and circulating free fatty acids during the acute phase response in severe fasting decreases the abundance of thyroid hormone binding proteins (THBP) (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 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 suppression of TSH secretion by activation of AMPK (30;31). However, despite the use of metformin by T2DM subjects at baseline (26 of 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 seen 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 re-arrangementsbrought about by this procedure.

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