Chapter 6

Autonomic nervous system activity in diabetic and healthy obese and the effect of distinct weight loss strategies

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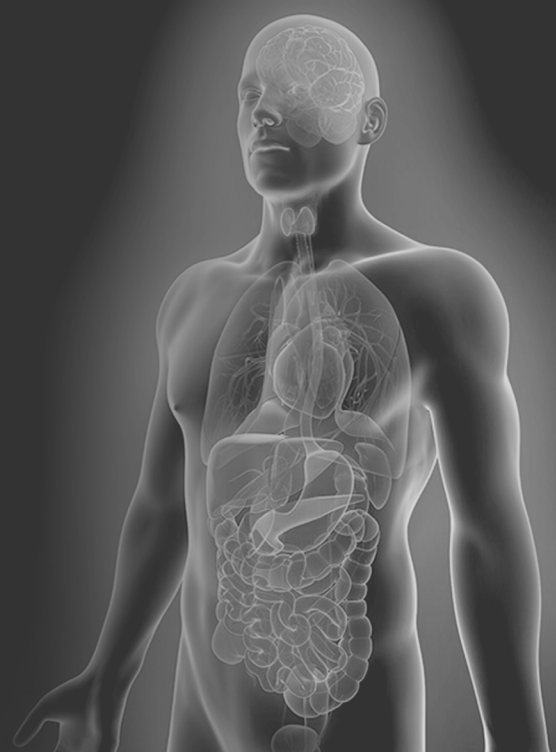

ABSTRACT

Objective

Obesity and type 2 diabetes mellitus (T2DM) are reported to be associated with relative over-activity of the sympathetic nervous system (SNS), which is reversible by weight loss. However, direct effects of weight loss by calorie restriction versus Roux-en-Y-Gastric Bypass (RYGB) on SNS overactivity were not studied in parallel. This study compared the effects of RYGB versus restrictive weight loss in obese patients with normal glucose tolerance (NGT) and with T2DM on SNS function as measured by heart rate variability (HRV).

Methods

Lean (n=12), obese NGT (n=27) and T2DM (n=27) subjects were included. Weight reduction in NGT subjects was achieved by Gastric Banding (GB) or RYGB and in T2DM subjects by RYGB or high protein very-low-calorie diet (VLCD). HRV analysis was performed and blood samples were taken at baseline, 3 weeks and 3 months after intervention.

Results

At baseline, T2DM subjects showed SNS overactivity and NGT subjects showed similar, but non-significant, findings as compared to lean controls. Weight loss after three weeks was comparable in all treatment groups, whereas after three months weight loss was most in VLCD and RYGB subjects. RYGB and VLCD treatment reduced SNS activity within three weeks in T2DM patients. After three months, restoration to normal ANS activity was evident for all groups, except for the NGT-GB group.

Conclusion

We can conclude that SNS over-activity is more pronounced in obese T2DM subjects as compared to NGT subjects. Reduction of SNS over-activity coincides with weight loss with the time-course of reduction dependent on the type of intervention. Surgery or caloric restriction may transiently induce SNS over-activity but do not prevent a direct restoration of sympathovagal balance.

INTRODUCTION

Both obesity and Type 2 Diabetes Mellitus (T2DM) are marked by abnormal metabolic profiles (1). The pathogenesis of obesity and T2DM is incompletely understood, but likely involves complex adaptations to chronic over-nutrition and inactivity (2). The autonomic nervous system (ANS) has a role in the regulation of long and short term energy balance, and ANS deregulation is implicated in the pathogenesis of obesity and T2DM (3). However, debate exists on the initiation of the pathogenic process; whether ANS deregulation is a pathogenic factor in the development of T2DM or if, conversely, chronic hyperglycaemia and hyperinsulinemia lead to ANS dysfunction.

Obesity and its early complications (i.e. insulin resistance and impaired fasting glucose), are associated with overstimulation of the sympathetic nervous system (SNS) and decreased tone of the parasympathetic nervous system (PNS) (4;5). Long-term complications of T2DM are associated with chronic SNS over-activation (6). Furthermore, ANS dysfunction is also present in subjects preceding the onset of T2DM, suggesting that the development of ANS impairment parallels the development of obesity, hyperinsulinemia and insulin resistance (7-9) or has a pathogenic role in the development of diabetes (10;11). Once T2DM has developed, chronic hyperglycemia and persistent increase in sympathetic activity down regulates peripheral b-adrenergic receptors (12;13), resulting in inability of the SNS to enhance energy expenditure (14).

Several trials showed that caloric restriction and weight loss have a beneficial influence on ANS dysfunction (15). However, to the best of our knowledge, no trials were performed to compare the effects of restrictive and "metabolic" weight loss strategies in NGT and T2DM obese subjects in parallel. Importantly, several factors including severe calorie restriction, surgery- and anaesthesia induced stress and hyperinsulinemia after Roux-en-Y-Gastric-Bypass (RYGB) are suggested to have a stimulatory effect on the ANS (16).

ANS function can be assessed by heart rate variability (HRV) measurement, based on the interval variation between heart beats. Measures of heart rate variability in both the time and frequency domain are reliable parameters to index the sympathetic-parasympathetic nervous system balance (17).

The aim of this study was to assess the direct effect of surgery and caloric restriction and the effect of weight loss in groups of obese subjects with and without T2DM on ANS function as measured by HRV. We hypothesized, that RYGB would have different sub-acute effects on ANS dysfunction as compared to calorie restriction per se.

Furthermore, we hypothesized that recovery of ANS function would depend both on preoperative metabolic status and on type of intervention.

SUBJECTS AND METHODS

Subjects

We included obese females eligible for both dietary and surgical treatment. Subjects eligible for surgical treatment were recruited from the waiting lists of several Dutch bariatric surgery centers. They had been screened previously by a multidisciplinary team of the Nederlandse Obesitaskliniek (Dutch Obesity Clinic) to establish if they fulfilled the international criteria for bariatric surgery (18). Subjects eligible for dietary treatment were recruited after referral by their general practitioner or internist. They fulfilled the same criteria as surgical patients for BMI, co-morbidity, history of longstanding obesity and failed weight loss attempts. Exclusion criteria were smoking, age>65 years and any chronic disease other than diabetes, including psychiatric illness.

The subjects had either normal fasting glucose (NGT) or T2DM according to WHO standards. All diabetic subjects were treated with oral medication only (metformin or sulfonylurea derivatives). Subjects who reported the use of weight loss medications within 90 days prior to enrollment in the study were excluded. Body weight of all subjects had been stable for at least 3 months prior to inclusion. Participants were allowed to use cholesterol lowering statins and antihypertensive medication.

Control subjects were recruited via an advertisement. 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.

Study design

Initially, we intended to include obese NGT and subjects with T2DM, who would have GB or RYGB to systematically compare the physiological effects of these interventions. However, since RYGB was reported to have superior metabolic effects in subjects with T2DM surgeons were reluctant to treat these subjects with GB (19). Instead, we chose to include 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 gastric banding

presumably result primarily from calorie restriction, we reasoned that a VLCD might mimic the effects of GB.

Subjects were studied (after an overnight (10h) fast) within a month before surgery and again between 2 and 3 weeks after surgery. All oral glucose-lowering agents were discontinued 48 hours. A canula was inserted into an antecubital vein and a fasting blood sample was taken. Subjects were then given 266 milliliters of a standardized fluid meal (Nutridrink®, 400 kcal; 49 energy % carbohydrate (48,9 g), 35% lipids (15,4 g), 16% protein (15,9 g)). Blood samples were drawn at the start of drinking (t=0) and 5, 10, 20, 30, 60, 90, 120, 150,180 minutes postprandial. 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.

Surgery

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, Allergan, Santa Barbara, CA) around the stomach to create a 15 milliliters pouch. Patients increased their postoperative intake from a liquid diet for 5 days (<600 kcal/day) towards a more solid diet (700-800 kcal/day) after 3 weeks. Thereafter, patients followed the guidelines of The Dutch Obesity Clinic, ingesting up to 1000-1200 kcal/day.

Very low calorie diet (VLCD)

Commercially available Prodimed® (Prodimed Benelux BV, Valkenswaard, The Netherlands) is a high-protein-low-calorie meal replacement plan (VLCD), with an average calorie intake of 600 kcal/day. The meal replacement plan consists of sachets (~90 kcal each of which ~18 g protein, ~2.5-5 g carbohydrates, 0,5-2 g fat) soluble powder for preparation of meals. 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 1 piece of fruit an 1 portion of light dairy produce (800-1000 ckal/day in total). After two months, patients were allowed a light evening meal from prescribed recipes on intermittent days (1000-1200 ckal/day in total).

Use of 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/L after intervention.

Assays

All samples were analyzed after one freeze-thaw cycle. 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 using a immuno-fluorometric assay on a Primus Ultra2 analyser with a CV of 0,5-0,8%. Leptin was measured using a radioimmunoassay (RIA) (Milipore, St Charles Missouri USA) with a CV of 3,4-4,7%.

Assessment of insulin resistance

Insulin resistance was calculated with the Homeostatic model assessment (HOMA2) (20) with the computer application downloaded from http://www.dtu.ox.ac.uk/homacalculator/download.php.

Heart rate variability measurement

When patients had been in supine position for a minimum of 15 minutes and before or at least 30 minutes after blood sampling, a 5-min continuous recording 12-lead electrocardiogram (ECG) registration was made using the CardioPerfect ECG recording system (Cardiocontrol, Rijswijk, The Netherlands). Subjects were instructed to relax, to breathe regularly, not to speak and to stay awake. Each registration was screened for artefacts, and subsequently analyzed for HRV parameters according to the internationally accepted guidelines (17), using Kubios HRV software vs 2 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). These parameters were all in the time domain: mean RR-interval; the square root of the mean squared difference of successive RR intervals (RMSSD; estimate of short-term components of HRV); the delta standard deviations of RR intervals, SD1 and SD2, and the ratio of SD1 over SD2 (balance of PNS/SNS tone) (17). The ECG-recordings were also subjected to power spectrum analysis using the same software, but as these analyses showed the same outcome, these parameters are not further reported.

Statistical analysis

Data were analyzed using SPSS 17.0. Data are presented as means ± standard deviation. Differences between subjects groups and lean controls at baseline were calculated using Univariate ANOVA. The effects of the different interventions 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 Bonferroni posthoc test was used to correct for multiple testing. A p-value <0,05 was considered statistically significant. Graphs were developed in Prism Graph Pad 5.

RESULTS

Subject characteristics

All obese subjects and healthy controls were Caucasian females, with a mean age of 49,4 ± 0,6 yrs (table 1). We included 32 subjects with T2DM and 30 NGT obese individuals. Eight subjects dropped out during the course of the study because they were not able to comply with the VLCD group (n=2), because of practical issues (n=3); and because of postoperative complications (n=3).

Table 1 - Baseline characteristics of studygroups.

	NGT (27)	T2DM (27)	Controls (11)	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/NGT vs controls
Weight (kg)	124.3 ± 11.7	117.2 ± 17.1	64.4 ± 7.2	p<0.001 T2DM/NGTvs controls
HbA1c (mmol/mol)	36.1 ± 7.8	49.6 ± 12.0	31.9 ± 2.5	p<0.001 T2DM vs controls/NGT
Homa-ir (%)	1.3 ± 0.9	1.6 ± 1.1	0.2 ± 0.0	p<0.001 T2DM/NGTvs controls
Fasting glucose (mmol/l)	5.0 ± 0.6	8.7 ± 2.5	4.7 ± 0.3	p<0.001 T2DM vs controls/NGT
Fasting insulin (mU/l)	10.5 ± 7.9	12.0 ± 7.8	1.6 ± 0.2	p<0.001 T2DM/NGTvs controls
Leptin (μg/L)	86.0 ± 39.8	55.6 ± 32.5	9.9 ± 5.0	p<0.001 T2DM/NGTvs controls
AUC glucose (mmol/l/3h)	1037 ± 167.8	1843 ± 546	952 ± 32.8	p<0.001 T2DM vs controls/NGT
AUC insulin (mU/l/3h)	7344 ± 5741	5821 ± 2819	2278 ± 961	p<0.001 T2DM/NGTvs controls

Values are presented as mean ± SD. Differences between groups at baseline are calculated with Univariate ANOVA. LSD correction was applied to correct for multiple testing.

Baseline characteristics of studygroups are shown in table 1. All parameters showed the expected differences between lean controls, obese NGT and obese T2DM subjects. Average time from diagnosis of T2DM at baseline was 3.7 ± 2.5 years in both T2DM groups.

Weight loss and intake

Average weight loss at 3 weeks was comparable after either intervention. After three months, RYGB and VLCD had lost more weight (approximately 15%) than NGT-GB (table 3a,b). According to protocol patients expanded their intake after operation from a liquid diet for 5 days (<600 ckal/day) towards a more solid diet (700-800 ckal/day) after 3 weeks. Thereafter, patients followed the intake guidelines of The Dutch Obesity clinic, containing up to 1200 ckal a day. Calorie intake in the diet group was similar to the surgically treated patients.

Medication use

All diabetic subjects discontinued their oral antidiabetics at the day of operation or start of the diet as per protocol. Metformin treatment was reinstalled in a similar percentage of patients in both diabetic groups (RYGB 27%, VLCD 17%). The use of other drugs such as statins and antihypertensive drugs was slightly higher in the diabetic subjects. At baseline, statins were used by 60% of T2DM patients and 25% of NGT patients. Of T2DM patients 50% of used antihypertensives (diuretics 7, ACE-inhibitors 5, b-blokckers 6) against 33% in NGT patients (diuretics 4, ACE-inhibitors 3, b-blockers 4). Use of medication known to affect HRV parameters was non-significantly different in both groups, and was continued throughout the entire study period.

To test the possible effect of concomitant medication with a possible influence on ANS tone, the statistical analyses was done separately for patients using or not using medication. These analyses showed similar results and therefore the data of the entire population is presented.

Baseline characteristics of HRV parameters

At baseline, T2DM subjects had a lower average RR-interval compared to NGT subjects and lean controls (table 2). RMSSD was decreased in T2DM subjects as compared to healthy controls ($p<0.05$) and no significant decrease was observed as compared to NGT subjects ($p= 0.053$. SD1 and SD2 were lower in T2DM subjects compared to lean controls (respectively $p= 0.035$ and $p=0.005$) and, again, no significant decrease was observed as compared to NGT subjects ($p=0.053$ and $p=0.067$).

Effects of intervention within groups

The effects of intervention within individual study groups are presented in table 3a and 3b.

Table 2 - Baseline HRV parameters of studygroups.

	NGT (27)	T2DM (27)	Controls (11)	P value
Av. RR Interval (ms)	945.4 ± 118.7	842.8 ± 126.3*	975.8 ± 118.7	p<0.05 T2DM vs NGT / controls
RMSSD (ms)	37.0 ± 21.7	26.1 ± 13.8	37.6 ± 13.9	p<0.05 T2DM vs NGT / controls
SD1 (ms)	26.2 ± 15.4	18.5 ± 9.7	26.6 ± 9.9	p<0.05 T2DM vs NGT / controls
SD2 (ms)	54.8 ± 21.1	46.3 ± 25.8	67.9 ± 22.6	p<0.05 T2DM vs NGT / controls
SD1/SD2	0.47 ± 0.2	0.43 ± 0.2	0.40 ± 0.1	p=ns

Values are presented as mean ± SD. Differences between groups at baseline are calculated with Univariate ANOVA. LSD correction was applied to correct for multiple testing.

NGT subjects after GB: No significant changes were induced by GB, however SD1/SD2 ratio and the RR-interval tended to increase after three months.

NGT subjects after RYGB: In NGT subjects, RYGB increased all HRV parameters gradually, with differences reaching significance after three months.

T2DM subjects after RYGB: In T2DM subjects, RR-interval increased three weeks after RYGB. SD1, SD1/SD2 and RMSSD increased significantly after three months.

T2DM subjects after VLCD: VLCD directly increased RR-interval, RMSSD, SD1, SD2 and SD1/SD2. These effects remained significant at 3 months.

Effects of intervention between groups

RR-interval was prolonged in T2DM subjects directly after RYGB and VLCD, whereas the effect of RYGB in NGT subjects was significant only after three months. RMSSD increased in T2DM subjects directly after VLCD, whereas after three months, RMSSD increased to the same extent in subjects following RYGB.

SD1, SD2 and SD1/SD2 ratio increased within three weeks in T2DM subjects after VLCD. After three months, SD1 increased to a comparable extend in RYGB subjects. In T2DM subjects, VLCD increased SD2 to a larger extend than RYGB. In NGT subjects, RYGB increased SD2 to a larger extent than GB. After three months; the effect on SD1/SD2 was comparable in RYBG and VLCD subjects, but less in GB subjects.

Correlations

Glucose / Insulin: There was a correlation between fasting glucose and mean RR (-0.48, p=0,000), RMSSD (0.442; p=0.001), SD1 (0.441; p=0,001) and SD2 (0.423; p=0.002) for

Table 3a - Effects of intervention after three weeks and three months in NGT patients on anthropometrics, biochemical values and HRV parameters within and between study groups.

	1. NGT-GB (n=11)			2. NGT-RYGB (n=16)		
	Before	**After 3 weeks**	**After 3 months**	**Before**	**After 3 weeks**	**After three months**
Weight (kg)	118.6 ± 12.9	113.1 ± 14.0*	106.6 ± 14.0*	128.2 ± 9.3	119.4 ± 10.2*	108.1 ± 10.9*
BMI (kg/m²	43.1 ± 3.0	40.5 ± 2.9*	38.4 ± 3.2*	44.2 ± 3.3	40.9 ± 3.6*	37.1 ± 3.8*
% Weight loss		4.8 ± 2.6	10.2 ± 3.4		6.9 ± 3.0	15.7 ± 4.6#
Glucose (mmol/l)	5.0 ± 0.6	4.9 ± 0.6	5.2 ± 0.8	5.0 ± 0.6	4.8 ± 0.8	4.9 ± 0.53
Insulin (mU/l)	11.5 ± 8.7	6.9 ± 6.3	10.0 ± 7.2	9.8 ± 7.4	8.8 ± 9.3#	4.9 ± 4.0*
Homa-ir (%)	1.5 ± 1.1	0.1 ± 0.1*	0.2 ± 0.1*	1.3 ± 0.9	0.2 ± 0.2*	0.1 ± 0.1*
Leptin (µg/L)	70.6 ± 25.4	45.0 ± 14.5	42.6 ± 15.5	96.5 ± 45.0	55.9 ± 30.7	34.2 28.0
Av. RR Interval (ms)	960.5 ± 112.3	970.3 ± 110.0	1014.3 ± 155.5	934.4 ± 125.9	963.3±139.6*	1058.0±170.9*
RMSSD (ms)	42.7 ± 20.6	49.9 ± 25.7	44.8 ± 23.0	32.7 ± 22.1	37.9 ± 25.3	57.8 ± 44.3*
SD1 (ms)	30.3 ± 14.6	35.4 ± 18.2	31.7 ± 16.6	23.2 ± 15.7	26.8 ± 17.9	41.0 ± 31.4*
SD2 (ms)	59.3 ± 21.5	59.3 ± 17.2	52.3 ± 18.4	51.5 ± 20.9	61.6 ± 30.5	68.6±23.9*#
SD1/SD2	0.53 ± 0.1	0.59 ± 0.2	0.61 ± 0.2	0.42 ± 0.2	0.44 ± 0.2	0.58 ± 0.4*

Values are presented as mean ± SD. Differences within group over time and in effect between the interventions were evaluated by Mixed Model Analysis with individual "baseline values" and "T2DM/NGT" as covariates and patient-groups (intervention) as fixed factor. Bonferroni correction was used to correct for multiple testing. *A p-value <0.05* was considered statistically significant. * = significant effect of intervention within group (versus baseline); # = significantly different effect between two interventions in either NGT or T2DM group.

Table 3b - Effects of intervention after three weeks and three months in T2DM patients on anthropometrics, biochemical values and HRV parameters within and between study groups.

	3. T2DM -RYGB (n=15)			4. T2DM -VLCD (n=12)		
	Before	**After 3 weeks**	**After 3 months**	**Before**	**After 3 weeks**	**After three months**
Weight (kg)	121.4 ± 15.9	112.5 ± 15.1*	100.9 ± 13.4*	112.0 ± 17.7*	105.3± 16.7*	97.2 ± 16.1*
BMI (kg/m²	43.5 ± 4.2	40.4 ± 4.0*	36.1 ± 3.8*	40.2 ± 6.4	37.7 6.0*	34.7 ± 6.6*
% Weight loss		7.3 ± 1.7	16.9 ± 2.1		6.0 ± 1.5	14.6 ± 3.3
Glucose (mmol/l)	8.9 ± 2.4	6.7 ± 1.5*	5.8 ± 1.2*	8.4 ± 2.6	5.2 ± 1.2*	5.9 ± 1.0*
Insulin (mU/l)	10.8 ± 7.6	10.9 ± 6.2	6.9 ± 3.9*	13.5 ± 8.1	4.9 ± 2.9*#	5.0 ± 3.5*
Homa-ir (%)	1.6 ± 1.1	0.2 ± 0.1*	0.1 ± 0.1*	1.9 ± 1.1	0.1 ± 0.1*	0.1 ± 0.1*
Leptin (µg/L)	65.7 ± 38.6	41.8 ± 27.8	33.7 ± 39.8	42.9 ± 16.8	22.0 ± 14.5	21.6 ± 15.5
Av. RR Interval (ms)	847.6±133.0	943.3 ± 137.7*	966.9± 170.9	837.2 ± 123.0	917.5± 123.1*	930.1 ± 125.2*
RMSSD (ms)	27.7 ± 15.9	30.1 ± 14.1	36.0 ± 15.7*	24.4 ± 11.3	44.1 ± 17.9*#	39.0 ± 16.3*
SD1 (ms)	19.6 ± 11.2	21.6 ± 10.0	25.5 ± 11.2*	17.3 ± 8.0	30.5 ± 12.5*#	31.3 ± 15.6*
SD2 (ms)	48.6 ± 28.5	43.5 ± 15.7	44.3 ± 19.0	43.7 ± 23.2	57.9 ± 27.1*#	60.2 ± 31.2* #
SD1/SD2	0.44 ± 0.2	0.49 ± 0.2	0.60 ± 0.2*	0.40 ± 0.1	0.60 ± 0.3*	0.58 ± 0.4*

Values are presented as mean ± SD. Differences within group over time and in effect between the interventions were evaluated by Mixed Model Analysis with individual "baseline values" and "T2DM/NGT" as covariates and patient-groups (intervention) as fixed factor. Bonferroni correction was used to correct for multiple testing. *A p-value <0.05* was considered statistically significant. * = significant effect of intervention within group (versus baseline); # = significantly different effect between two interventions in either NGT or T2DM group.

all obese subjects. AUC glucose also correlated in the T2DM subjects with mean RR (-0,441; p=0,021); RMSSD (0.570; p=0.002); SD1 (0.570; p=0,002); SD2 (0.389; p=0.045).

DISCUSSION

This is the first study to extensively evaluate the effect of RYGB compared to restrictive weight loss strategies on ANS function in matched groups of obese NGT and obese T2DM subjects. Subjects were studied within three weeks after surgery, when weight loss was limited; to compare the effect of the gastrointestinal rearrangement induced by RYGB to the effect of caloric restriction *per se*. Subjects were studied three months after surgery to evaluate the effect of marked weight loss. The principal finding of this study is in line with our hypothesis: SNS over-activity is more pronounced in obese subjects with T2DM as compared to obese subjects with a normal glucose tolerance. SNS over-activity decreases with weight loss, with the time course of the effect depending on the type of intervention. Three months after intervention, this resulted in a comparable increase of PNS activity and restoration of the sympathovagal balance in subjects after RYGB or VLCD, whereas there was no effect in GB subjects.

ANS dysfunction in obesity and T2DM has generally been associated with sympathetic activation (4;6;21), which, at least to some extent, is reversible by weight loss (4;22;23) Two reciprocal hypotheses explain the possible pathophysiological mechanisms involved. In short, the hyperinsulinemic or insulin resistant state in obesity could cause ANS dysfunction, or conversely, pre-existing enhanced sympathetic tone causes hyperinsulinemia and insulin resistance.

In insulin sensitive patients, postprandial hyperinsulinemia coincides with a transient SNS dominance enhancing REE to counteract hyperglycemia (24-26). At baseline, ANS function in our obese NGT subjects was not significantly different from lean controls, despite their hyperinsulinemic but normoglycemic state. Furthermore, SNS activity did not correlate to the enhanced insulin levels in the obese subjects. These observations do not support the notion that hyperinsulinemia *per se* increases SNS activity. However, parameters of PNS activity correlated negatively with fasting and postprandial glucose in T2DM subjects. These observations suggests that insulin resistance alone may be related to unopposed over-activity of the SNS, and that it is the subsequent rise in glucose levels in T2DM that impairs b-adrenergic signaling, reducing ANS responsiveness to variations in metabolic rate (12;27). Taken together, and concordant with our baseline results, this suggests a gradual increase in SNS activation during evolution from obesity

with a limited degree of hyperinsulinemia, towards T2DM and development of overt SNS hyperactivity (9).

Conversely, some observations suggest a pathogenic role for SNS over-activity in the development and progression of insulin resistance (10;28). SNS activation increases lipolysis, thereby enhancing free fatty acid release and inhibiting glucose transport. As well, adipose tissue derived leptin causes sympathetic activation through a hypothalamic pathway that enhances resting energy expenditure(REE) (27). Selective Leptin resistance in obesity (29) however, counteracts its metabolic effects, whereas the activating effect of Leptin on the SNS is preserved (28;30;31). As hyperinsulinemia, insulin resistance and hyperleptinemia are all hallmarks of obesity, it is difficult to define the initiator of this vicious circle. As we suggested, a combination of the two existent hypotheses may be true. Limited hyperinsulinemia activates the SNS, and subsequent hyperglycemia leads to neuronal damage to the unmyelinated fibers of the ANS (32;33), whereas with progression of the disease also myelinated nerve fibers will be damaged.

Our hypothesis that ANS dysfunction was reversible by weight loss, relative to the degree of weight loss and type of weight loss intervention, was confirmed by the results of our different treatment groups. Moreover, none of the HRV parameters of the study groups three weeks or months after intervention showed statistical significant differences with lean controls, suggesting a return to normal levels.

Subjects after RYGB showed a delay in PNS response as compared to subject after VLCD. Indeed, intestinal rearrangements after RYGB enhance postprandial insulin release in the face of decreased postprandial glucose levels, suggesting a transient reduction in insulin sensitivity during the direct postoperative period that can be ascribed to transient increases in inflammation, tissue injury or perioperative starvation (34). In contrast, caloric restriction by VLCD or GB directly decreased fasting and postprandial glucose and insulin levels, probably by a reduction in endogenous glucose production (35). A comparable effect was achieved 3 months after RYGB, when insulin resistance was decreased following the loss in adipose tissue. From these observations, we suggest that a decline in insulin resistance *per se* is important for the recovery of autonomic function.

It has been suggested that calorie restriction or surgical stress have stimulating effects on the SNS (16). The overall increase in PNS activity in our subjects after VLCD and RYGB,

however, does *not* support an important effect of caloric restriction or surgical stress. Furthermore, anaesthesia during surgery has profound suppressive effects on SNS or total ANS activity (36). It is unknown when these effects fade, since most studies only registered HRV during anaesthesia induction and recovery (36). We observed a gradual increase and decrease in respectively PNS and SNS activity tone in our RYGB patients even after three weeks. This contradicts a prolonged repressive effect of anaesthesia, or suggests an effect that is only transient and does allow a continuous gradual increase in PNS over SNS activity. Also, we cannot rule out that mild postoperative complications such as pain and anxiety could have confounded the results in the surgical groups. However, although a small effect may have been present, as mentioned above, the gradual but persistent increase and decrease in respectively PNS and SNS activity tone in our RYGB subjects contradicts a strong effect of these complications.

Postprandial (but not fasting) GLP-1 hypersecretion, enhancing glucose stimulated insulin release, has been observed after RYGB, and was present in our subjects as well (data not shown (37)). Rodent studies suggest a clear stimulating effect of GLP-1 administration on SNS activity (38). Postprandial GLP-1 hypersecretion after RYGB, but not after restrictive weight loss, may therefore induce a stimulatory effect on SNS activity postprandial after RYGB. The fact that we did not find correlations between GLP-1 levels and HRV parameters, neither a stimulatory effect of RYGB on SNS activity in the fasting state suggests that GLP-1 did not affect our results. However, we cannot exclude an effect of GLP-1 on postprandial HRV, and this should be explored in future studies.

This study has some weaknesses. First of all, only female subjects were included, 80% of which were postmenopausal. This implicates that the results of this study apply only to female subjects. Moreover we cannot rule out that differences in menstrual cycle (menopausal state) could have affected HRV measurements. However, we expected the a priori effect on HRV parameters which the interventions would cause to be much larger and as such to rule out possible influences due to menstrual cycle/menopausal state. Unfortunately, due to the large impact of GB and RYGB procedures, randomization of interventions was not allowed in our protocol. Moreover, two different interventions have been used in the calorie restriction group, which may have biased the effects one way or another. However, no clear evidence exists on metabolic effects of gastric banding apart from those effects due to weigh loss. Therefore we suggest our comparison of these restrictive ways of weight loss against RYGB is valuable to at least some extend.

For obvious reasons, we were unable to restrict to obese subjects not using any medication affecting autonomic regulation, such as beta-blockers and ACE-inhibitors**,** since these are frequently prescribed for concomitant hypertension in this study population. beta-blockers are known to affect heart rate and ANS tone, although the actual changes on HRV parameters are generally modest (39). Although we realize that we had a relatively small sample size, we performed our statistical analyses separately for patients with or without using medication. These analyses showed similar results and suggest that our findings are robust. We recognize, however, that we cannot completely rule out possible effects of the medication on our findings.

We conclude that SNS over-activity is more pronounced in obese T2DM subjects, as compared to obese NGT subjects. This suggests a gradual increase in SNS activation caused by obesity, which may be associated with progressive insulin resistance and hyperinsulinemia. This seems a dual process by *hyperinsulinemia*, impairing b-adrenergic signaling, and *hyperglycemia* causing actual nerve fiber damage. Transient SNS over-activity may be induced by surgically induced decrease in insulin sensitivity, but does not prevent gradual and possibly lasting restoration of autonomic nervous system tone after surgery. In general, reduction of SNS over-activity coincides with weight loss, with the time-course of reduction dependent on the type of intervention.

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