Diminished counterregulatory responses to meal-induced hypoglycemia 4 years after RYGB

de Heide, Loek J.M.; van den Broek, Merel; van Dijk, Gertjan; Emous, Marloes; van Beek, André P.

Published in:
Obesity Surgery

DOI:
10.1007/s11695-020-05035-9

Publication date:
2021

Obesity Surgery (2021) 31:597–602
https://doi.org/10.1007/s11695-020-05035-9

ORIGINAL CONTRIBUTIONS

Diminished Counterregulatory Responses to Meal-Induced Hypoglycemia 4 Years After RYGB

Loek J. M. de Heide[1] · Merel van den Broek[1] · Gertjan van Dijk[2] · Marloes Emous[1] · André P. van Beek[3]

Received: 8 May 2020 / Revised: 1 October 2020 / Accepted: 6 October 2020 / Published online: 16 October 2020

Abstract

Purpose Post-bariatric hypoglycemia is a complication of bariatric surgery, especially Roux-en-Y gastric bypass (RYGB). The counterregulatory hormonal and sympathetic neural responses were measured during a previously reported meal test in which 48% had an almost asymptomatic hypoglycemic event.

Materials and Methods Forty-four randomly selected patients 4 years after RYGB. A liquid meal test (MMT) after overnight fasting. Based on the glucose nadir during the MMT, patients were divided in a hypo group (glucose < 3.3 mmol/L) and a non-hypo group (glucose ≥ 3.3 mmol/L). Cortisol, epinephrine, norepinephrine, blood pressure, and heart rate were measured up to 180 min after ingestion of the meal. Incremental areas under the curve (iAUC), peak, and delta hormone responses after the glucose nadir were calculated. Parameters were compared between the hypo and non-hypo groups.

Results A total of 21/44 (48%) had an almost asymptomatic hypoglycemic event. Cortisol and epinephrine responses in the hypo group were not increased compared to the non-hypo group, and there were no signs of increased sympathetic nerve activity. Peak and delta cortisol were lower in the hypo compared to the non-hypo group. Norepinephrine was higher in the hypo group especially in the time frame 60–120 and 120–180 min after start of the meal.

Conclusion No increase in epinephrine and a lower cortisol response to hypoglycemia were observed compared to normoglycemia during a meal test in patients after RYGB. Norepinephrine levels were higher in the hypo group. These findings may suggest that possible recurrent hypoglycemia after RYGB results in blunting of counterregulatory responses indicative of hypoglycemia-induced autonomic failure.

Clin Trial Register ID ISRCTN 11738149.

Keywords Bariatric surgery · RYGB · Mixed meal test · Hypoglycemia · Cortisol · Catecholamines · Counterregulation

Introduction

Roux-en-Y gastric bypass surgery (RYGB) effectively and rapidly induces improvement in glucose metabolism and even remission of diabetes mellitus 2 which is mainly attributed to the gastrointestinal hormones, especially glucagon-like polypeptide-1 (GLP-1) [1].

The potential downside, however, is the development of postprandial hyperinsulinemic hypoglycemia (PHH) also called post-bariatric hypoglycemia (PBH). Depending on the definition and diagnostic modality, the prevalence varies widely from 0.2 with yearly hospital admission rate to 75% in studies with blinded continuous glucose monitoring [2–5]. No more than one in five of these episodes was noticed. It is hypothesized that this lack of symptoms during PBH is the result of hypoglycemia unawareness.

Hypoglycemia unawareness can develop in strictly regulated type 1 diabetes (T1D) and is characterized by a lack of adrenergic warning symptoms during a hypoglycemia.

It is postulated that recurrent hypoglycemic episodes induce blunting of the adrenergic and hypothalamic–pituitary–adrenal (HPA)–responses to hypoglycemia diminishing the

✉ Loek J. M. de Heide
l.de.heide@mcl.nl

[1] Center for Obesity North-Netherlands (CON), Medical Center Leeuwarden, H. Dunantweg 2, 8934 AD Leeuwarden, The Netherlands

[2] GELIFES-Neurobiology, Department of Behavioral Neuroscience, University of Groningen, Groningen, The Netherlands

[3] Department of Endocrinology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

counterregulation, a phenomenon known as hypoglycemia-associated autonomic failure (HAAF) [6].

In our recent study, with randomly selected patients on average 4 years after RYGB surgery, 48% had a hypoglycemic event (< 3.3 mmol/L) during a mixed meal test (MMT) [7]. Remarkably, none of the patients experienced adrenergic symptoms and few neuroglycopenic symptoms.

The aim of this study was to investigate whether those patients who developed hypoglycemia had a diminished adrenergic and HPA axis activation indicative of HAAF.

Materials and Methods

Subjects

The present study included patients, aged between 18 and 75 years, who underwent a primary RYGB. Patients were excluded if they had diabetes mellitus at the time of the study or reported symptomatic hypoglycemia in daily life. For details concerning the study patient recruitment, we refer to the paper by van den Broek et al. [8]. The study population was representative of the entire surgical cohort ($n = 550$) in terms of age, sex, comorbidities before surgery, preoperative weight, and postoperative weight at the time of the study. The study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Medical Ethical Review Board Leeuwarden. Written informed consent has been obtained from each patient.

Surgical Procedure

The primary RYGB consisted of a biliopancreatic limb of 80 cm, an alimentary limb of 150 cm, and a pouch with an estimated volume of 30 to 60 cc.

Study Protocol

The MMT was performed after an overnight fast of minimally 8 h. The test meal consisted of 200-mL Ensure® Plus (Abbott Laboratories, North Chicago, IL, USA) containing 300 kcal, 12.5-g protein, 40.4-g carbohydrate, 9.84-g fat, and 154.86-g water. The patients had to finish this test meal within 10 min. Blood samples, via an indwelling catheter in an antecubital vein, were collected before the test meal and at 10, 20, 30, 60, 90, 120, 150, 180, and 210 min after the start. Blood pressure and heart rate were assessed at all time points.

Analytic Procedures

Analyses of glucose and cortisol were performed immediately after the last blood withdrawal on a Cobas analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Glucose: P module (hexokinase reaction), cortisol: Modular E170 module (competition assay).

Blood for the analyses of epinephrine and norepinephrine was collected in EDTA tubes before the test meal and at 10, 20, 30, 60, 120, and 180 min after the start. Plasma was isolated immediately after withdrawal and stored at − 80 °C until analyzed. Analyses were performed by high-performance liquid chromatography (HPLC) with electrochemical detection. The limits of detection (LoD) of norepinephrine, epinephrine, normetanephrine, and metanephrine were 15.6 pg/mL, 62.5 pg/mL, 125 pg/mL, and 125 pg/mL, respectively.

Statistical Analysis

Results were presented as median (IQR) unless otherwise specified. Changes over time were graphically presented with a mean ± SEM. Epinephrine and norepinephrine concentrations that fell below the detection limit were converted into the limit of quantification in order to perform the statistical analyses.

The patients were divided into two groups depending on the occurrence of hypoglycemia during the MMT. The hypo group consisted of patients with a postprandial glucose nadir concentration < 3.3 mmol/L, and in the non-hypo group, postprandial glucose concentration remained ≥ 3.3 mmol/L.

The start of the ingestion of the mixed meal was compared between groups with a Mann–Whitney *U* test.

Hormone and sympathetic neural responses were calculated by assessing the area under the curves relative to baseline from 0 to 180 min ($iAUC_{0–180}$) and (to investigate temporal effects) from 0 to 60 min, 60 to 120 min, and 120 to 180 min. Peak hormone responses after the glucose nadir as well as delta peak minus fasting hormones were also calculated. Differences between the two groups were calculated with the Mann–Whitney *U* test. Statistical analyses were performed in IBM SPSS Statistics for Windows, version 24.0. Due to the exploratory nature of this study, the statistical significance was not interpreted as hypothesis-confirming but rather as hypothesis-generating.

Results

Study Population

Fifty-one patients entered the MMT. Due to voluntary withdrawal ($n = 1$), meeting an exclusion criterion ($n = 1$), and problems with blood withdrawal ($n = 5$), the analyses of plasma glucose and cortisol were complete in 44 patients. Due to an insufficient amount of blood volume, norepinephrine, epinephrine, and normetanephrine could only be determined in 38 patients. Hence, data of 38 patients were available for complete case analysis.

Patient characteristics were described in detail by Emous et al.: mean age 47, on average 48 months after surgery, BMI 31.2 kg/m^2, TWL 33.1% [7]. Start of the mixed meal ingestion was similar in both groups (with: 09:20 [08:52; 09:37], without: 09:00 [08:40; 09:20], $p = 0.129$).

Twenty-one of 44 (47.7%) had a hypoglycemia (< 3.3 mmol/L) during the MMT. The hypoglycemia started at 30, 60, 90, or 120 min in two, ten, eight, and one patients, respectively. In 18 patients with hypoglycemia, catecholamines could be measured. Twelve had a glucose nadir between 3.2 and 2.2 mmol/L and 6 below 2.2. mmol/L. Eight patients had hypoglycemia at one time point, 4 at two, 5 at three, and 1 at four during the MMT. The glucose concentrations over time during the MMT are presented in Fig. 1.

Fasting (Table 1)

The hypo group had a significantly lower glucose concentration compared to the non-hypo group ($p = 0.015$). Cortisol, norepinephrine, and epinephrine were not different between the two groups ($p = 0.22$, $p = 0.20$, $p = 0.133$).

Postprandial (Fig. 1, Tables 2 and 3)

The incremental area under the curve (iAUC) of plasma epinephrine and cortisol was not significantly different. The iAUC of plasma norepinephrine over the whole 180 min ($NE_{0–180}$) was significantly higher ($p = 0.002$) in the hypo group (Table 2). The iAUC $NE_{60–120}$ ($p = 0.002$) and iAUC $NE_{120–180}$ ($p = 0.001$) mainly contributed to these effects.

Both peak (significant) and delta cortisol (borderline significant) were lower in the hypo group versus the non-hypo group. Peak and delta epinephrine were not different. Norepinephrine peak was not different, but delta norepinephrine was significantly higher in the hypo group.

Blood Pressure and Heart Rate (Fig. 1)

There were no significant differences in both parameters between both groups in iAUC.

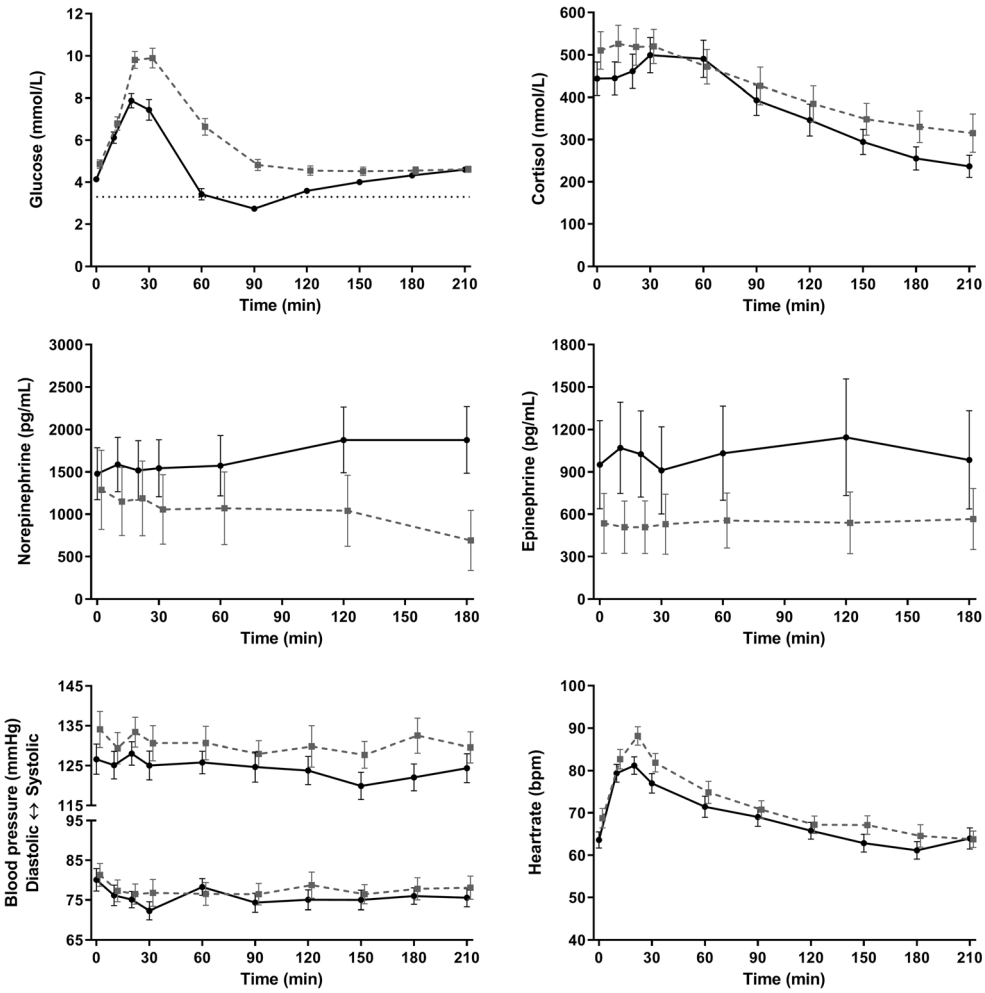

Fig. 1 Concentrations of glucose, the counterregulatory hormones, blood pressure, and heart rate during a mixed meal test in post-RYGB patients with and without hypoglycemia. Data are mean ± SEM. Black solid line: patients with postprandial hypoglycemia (glucose nadir concentration < 3.3 mmol/L). $n = 21$ for glucose, cortisol, blood pressure, and heartrate; $n = 19$ for norepinephrine; $n = 18$ for epinephrine

Table 1 Fasting concentrations of glucose and the counterregulatory hormones in post-RYGB patients with hypoglycemia and without hypoglycemia

	With hypoglycemia	Without hypoglycemia	*p**
Glucose (mmol/L)	*n* = 21; 4.2 [3.8; 4.5]	*n* = 23; 4.7 [4.0; 5.6]	0.015
Cortisol (nmol/L)	*n* = 21; 393 [288; 541]	*n* = 23; 476 [342; 640]	0.22
Epinephrine (pg/mL)	*n* = 18; 431 [63; 1651]	*n* = 20; 88 [63; 978]	0.133
Norepinephrine (pg/mL)	*n* = 19; 1492 [60; 2847]	*n* = 20; 63 [16; 2348]	0.20

Data are median [IQR]

*Analysis with the Mann–Whitney *U* test

Discussion

In this randomly selected population of patients on average 4 years after RYGB, 48% had mostly asymptomatic hypoglycemia (< 3.3 mmol/L) during a MMT, without a response of the counterregulatory hormones cortisol and epinephrine and with no sympathetic nerve response of blood pressure and heart rate. Norepinephrine (NE) levels were higher in the group with hypoglycemia. Our results suggest an impaired adrenal epinephrine and cortisol counterregulatory response to post-ingestive hypoglycemia indicative of HAAF. Both peak and delta cortisol were lower in the hypo group, suggesting less responsiveness of the hypothalamic–pituitary–adrenal axis to hypoglycemia, possibly due to previously experienced hypoglycemic events in daily life. The glucose cutoff level of 3.3 mmol/L during the MMT is based on the recommendation from our review of the literature on PBH (level of evidence III, grade C) [2]. Our results are in line with several other studies. Goldfine et al. also observed asymptomatic hypoglycemia during a MMT in 3 out of 9 RYGB patients, 2–4 years after surgery [9]. Blood pressure and heart rate were also not different. The counterregulatory hormones were not measured. Abrahamsson et al. found a diminished hypoglycemia symptom score and lower counterregulatory hormone response after RYGB compared to before surgery during a stepwise hyperinsulinemic hypoglycemic clamp test in 12 patients [10]. Also, peak systolic blood pressure and peak heart rate were lower. The patients in this study, however, were symptomatic during the hypoglycemic episode. This can be explained by the difference in induction of hypoglycemia and the shorter interval between surgery and the MMT, 4–5 versus 48 months in our patients. Gasser et al. found a prevalence of 13% of hypoglycemia after a solid food meal in 12 patients 1 year after RYGB. A total of 40% of them were asymptomatic [11]. Together with our study, these data suggest a progressive loss of hypoglycemic symptoms over time after RYGB. A similar decrease in counterregulatory response to hypoglycemia was observed after vertical banded gastroplasty, suggesting that the type of bariatric surgery is not causing the diminished response [12]. In healthy

Table 2 Incremental AUC for cortisol, epinephrine, and norepinephrine

iAUC	With hypoglycemia	Without hypoglycemia	*p**
Cortisol: nmol*min/mL			
0–60 min	− 795 [− 2415; 4290]	− 1965 [− 3255; 1155]	0.245
60–120 min	− 2640 [− 7980; 3240]	− 4770 [− 7125; − 465]	0.487
120–180 min	− 6855 [− 12,465; − 3390]	− 5775 [− 13,125; − 3725]	0.909
0–180 min	− 9445 [− 21,240; 5280]	− 13,050 [− 21,870; − 1050]	0.570
Epinephrine: pg*min/mL			
0–60 min	0 [− 4852; 1852]	0 [− 2101; 1184]	0.904
60–120 min	0 [− 11,905; 17,750]	0 [− 3474; 4874]	0.861
120–180 min	− 387 [− 12,676; 7036]	0 [− 4793; 3627]	0.476
0–180 min	− 49 [− 27,639; 15,494]	0 [− 6790; 10,975]	0.717
Norepinephrine: ng*min/mL			
0–60 min	3135 [− 1051; 10,256]	0 [− 14,465;471]	0.039
60–120 min	10,215 [− 138; 23,519]	− 267 [− 26,303; 0]	0.002
120–180 min	17,953 [− 269; 42,388]	−361 [− 30,670; 0]	0.001
0–180 min	28,640 [− 530; 71,199]	−818 [− 74,872; 0]	0.002

Data are median [IQR], cortisol *n* = 21 vs 23, epinephrine and norepinephrine *n* = 18 vs 20 in the hypo vs non-hypo group

*Analysis with the Mann–Whitney *U* test

Table 3 Cortisol, epinephrine, and norepinephrine peak after the glucose nadir and delta peak minus fasting responses in both groups during the MMTT

	With hypoglycemia	Without hypoglycemia	*p**
Peak cortisol (nmol/L)	424 [246; 594]	546 [429; 672]	0.025
Delta cortisol (nmol/L)	− 32 [− 147; 97]	36 [0; 97]	0.052
Peak epinephrine (pg/mL)	214 [33; 1417]	222 [25; 1609]	0.677
Delta epinephrine (pg/mL)	4 [− 87; 376]	1 [0; 323]	0.600
Peak norepinephrine (pg/mL)	2145 [383; 3658]	242 [6; 2413]	0.101
Delta norepinephrine (pg/mL)	400 [57; 907]	0 [0; 256]	0.039

Data are median [IQR], cortisol $n = 21$ vs 23, epinephrine and norepinephrine $n = 18$ vs 20 in the hypo vs non-hypo group

*Analysis with the Mann–Whitney *U* test

subjects, an antecedent hypoglycemic episode results in a decrease in both symptoms and counterregulatory hormone response to a subsequent hypoglycemia [13]. It is postulated that this can lead to a vicious circle in which successive hypoglycemic events induce less counterregulation and warning signs leading to progression of hypoglycemia unawareness. Hypoglycemia unawareness caused by antecedental hypoglycemic episodes has also been described in patients with an insulinoma [14]. We postulate that a comparable phenomenon is present in patients after RYGB. This could explain the discrepancy between the low incidence of self-reported hypoglycemic symptoms and the high prevalence of hypoglycemia in continuous glucose monitoring registrations [3, 5]. Blood pressure and heart rate did not increase during or after the hypoglycemia, suggesting a diminished sympathetic nerve response. As shown in Fig. 1, heart rate did increase in the first 30 min after the ingestion of the meal, suggesting a functional sympathetic nerve response to early dumping which occurs typically in this time frame [15]. Norepinephrine levels were higher in the hypo group compared to the non-hypo group. This is in accordance with findings from hypoglycemia clamp studies in healthy volunteers in which a preceding hypoglycemia also did not change norepinephrine levels [16–18]. Plasma norepinephrine is derived from spillover of sympathetic nerves. Only a very small portion of norepinephrine reaches the bloodstream as most is metabolized rapidly after reuptake into the nerve terminals [19]. Furthermore, norepinephrine levels from blood samples out of an antecubital vein are influenced by the local sympathetic activity and may not be representative of the sympathetic nerve activity in the whole body [19]. Cortisol levels were not low at the start of the study due to the fact that blood was withdrawn during insertion of the venous canula, and there is also a physiological decline in cortisol during the day. However, the average cortisol concentration of 400 nmol/L at the start was not so high that an increase in response to hypoglycemia (which in normal volunteers reaches on average 700 nmol/L) would not be recognized [20].

The strength of our study is that this is a random selection of patients, who were comparable to a large surgical cohort ($n = 550$) in terms of age, sex, comorbidities before surgery, and pre- and postoperative weight, providing a proper reflection of the post-RYGB population [21]. No one in this cohort reported adrenergic or neuroglycopenic symptoms in a questionnaire of late dumping symptoms [22]. Limitations of this study however also need to be mentioned. The induction of hypoglycemia was not set up for the purpose of studying catecholamines and cortisol response and can therefore not be compared to results from stepwise hypoglycemia clamp studies. With only limited time points of measurements of blood glucose, we cannot define how long patients remained in the hypoglycemic range and whether this would have been sufficiently long to induce a counterregulatory response. However, 6 of the 18 patients with our definition of hypoglycemia had a glucose nadir below 2.2 mmol/L, and 10 were at least 30 min in the hypoglycemic range. Without information on glucose levels during the days before the MMT, continuous glucose monitoring before the MMT would have been more informative for our hypothesis. Due to lack of samples, we were not able to measure catecholamines at 90 and 150 min. It is possible that a difference in the adrenergic responses was present at these time points. A post hoc power calculation showed that with the large standard deviations of the various measurements of epinephrine and norepinephrine, the number of patients was too low to reach 80% power. It cannot be excluded that differences would be present with larger numbers of patients. However, based on other hypoglycemia studies, a much higher response of the catecholamines could have been expected. We were not able to measure glucagon or growth hormone. Some studies also found a blunting of glucagon response after a preceding hypoglycemia and after RYGB. Growth hormone only plays a role after 3 or more hours of hypoglycemia. There were relatively more females in the group with hypoglycemia. This could be considered a confounding factor as females have a lower catecholamine response to hypoglycemia compared to men [22].

In conclusion, during a MMT in randomly selected RYGB patients on average 4 years after surgery, there was no cortisol and epinephrine response to a meal-induced hypoglycemia and there were no signs of increase in sympathetic nerve

activity. These results may support the hypothesis that some RYGB patients with postprandial hypoglycemia suffer from hypoglycemia unawareness and impaired counterregulatory responses to hypoglycemic episodes, indicative of HAAF.

Acknowledgments We thank Jan Bruggink for technical assistance in the measurements of the catecholamines.

Data Availability All data used in the "Results" section of this manuscript can be made available on request to the corresponding author.

Compliance with Ethical Standards

The study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Medical Ethical Review Board Leeuwarden. Written informed consent has been obtained from each patient.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Batterham RL, Cummings DE. Mechanisms of diabetes improvement following bariatric/metabolic surgery. Diabetes Care. 2016;39:893–901.
2. Emous M, Ubels FL, van Beek AP. Diagnostic tools for post-gastric bypass hypoglycaemia. Obes Rev. 2015;16:843–56.
3. Marsk R, Jonas E, Rasmussen F, et al. Nationwide cohort study of post-gastric bypass hypoglycaemia including 5,040 patients undergoing surgery for obesity in 1986-2006 in Sweden. Diabetologia. 2010;53:2307–11.
4. Kellogg TA, Bantle JP, Leslie DB, et al. Postgastric bypass hyperinsulinemic hypoglycaemia syndrome: characterization and response to a modified diet. Surg Obes Relat Dis. 2008;4:492–9.
5. Kefurt R, Langer FB, Schindler K, et al. Hypoglycemia after Roux-en-Y gastric bypass: detection rates of continuous glucose monitoring (CGM) versus mixed meal test. Surg Obes Rel Dis. 2015;11:564–9.
6. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. N Engl J M. 2013;369:362–72.
7. Emous M, van den Broek M, Wijma R, et al. Prevalence of hypoglycaemia in a random population after Roux-en-Y gastric bypass after a meal test. Endocr Connect. 2019;8:969–78.
8. van den Broek M, de Heide LJM, Wijma RB, et al. Satiety and gastrointestinal hormones during a mixed meal tolerance test after gastric bypass surgery: association with plasma amino acid concentrations. Surg Obes Relat Dis. 2018;14:1106–17.
9. Goldfine AB, Mun EC, Devine E, et al. Patients with neuroglycopenia after gastric bypass have exaggerated incretin and insulin secretory response to a mixed meal. J Clin Endocrinol Metab. 2007;92:4678–85.
10. Abrahamsson N, Börjesson JL, Sundbom M, et al. Gastric bypass reduces symptoms and hormonal responses in hypoglycemia. Diabetes. 2016;65:2667–75.
11. Gasser M, Meier C, Herren S, et al. Is testing for postprandial hyperinsulinemic hypoglycemia after gastric bypass necessary? Clin Nutr. 2019;38:444–9.
12. Gulstrand M, Ahren B, Wredling R, et al. Alteration of the counterregulatory response to insulin-induced hypoglycemia and of cognitive function after massive weight reduction in severely obese subjects. Metabolism. 2003;52:900–7.
13. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. Diabetes. 1991;40:223–6.
14. Mitrakou A, Fanelli C, Veneman T, et al. Reversibility of unawareness of hypoglycemia in patients with insulinomas. N Engl J of Med. 1993;329:834–9.
15. van der Kley FG, Vecht J, Lamers CBH, et al. Diagnostic value of dumping provocation in patients after gastric surgery. Scand J Gastroenterol. 1996;31:1162–6.
16. Moheet A, Kumar A, Eberly LE, et al. Hypoglycemia-associated autonomic failure in healthy humans: comparison of two vs three periods of hypoglycemia on hypoglycemia-induced counterregulatory and symptom response 5 days later. J Clin Endocrinol Metab. 2014;99:664–70.
17. Davis SN, Mann S, Galassetti P, et al. Effects of differing durations of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia in normal humans. Diabetes. 2000;49:1897–903.
18. Arbelaez AM, Powers WJ, Videen TO, et al. Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition: a mechanism for hypoglycemia-associated autonomic failure. Diabetes. 2008;57:470–5.
19. Goldstein DS, Eisenhofer G, Kopin IJ. Sources and significance of plasma levels of catechols and their metabolites in humans. J Pharmacol Exp Ther. 2003;305:800–11.
20. Davis SN, Shavers C, Costa F, et al. Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. J Clin Invest. 1996;98:680–92.
21. Emous M, Wolffenbuttel BHR, Totte E, et al. The short- to midterm symptom prevalence of dumping syndrome after primary gastric-bypass surgery and its impact on health-related quality of life. Surg Obes Rel Dis. 2017;13:1489–500.
22. Amiel SA, Maran A, Powrie JK, et al. Gender differences in counterregulation to hypoglycaemia. Diabetologia. 1993;36:460–4.