Hans Christian August Eickhoff

IMPACT OF BARIATRIC SURGERY ON CONTROL OF TYPE 2 DIABETES – NEUROENDOCRINE MECHANISMS AND CLINICAL SIGNIFICANCE

Doctoral Thesis in Health Sciences, branch of Medicine, speciality in Surgery (Surgery), supervised by Professor Doutor Francisco José Franqueira de Castro e Sousa, MD, PhD, and presented to the Faculty of Medicine of the University of Coimbra

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UNIVERSIDADE DE COIMBRA

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IMPACT OF BARIATRIC SURGERY ON CONTROL OF TYPE 2 DIABETES – NEUROENDOCRINE MECHANISMS AND CLINICAL SIGNIFICANCE

O IMPACTO DA CIRURGIA BARIÁTRICA NO CONTROLO DA DIABETES TIPO 2 – MECANISMOS NEURO-ENDÓCRINOS E IMPLICAÇÕES CLÍNICAS

Doctoral Thesis in Health Sciences, branch of Medicine, speciality in Surgery (Surgery), supervised by Professor Doutor Francisco José Franqueira de Castro e Sousa, MD, PhD, and presented to the Faculty of Medicine of the University of Coimbra

Coimbra, 2014

All experimental research was conducted at the Laboratory for Experimental Research, the Institute of Physiology, and the Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Coimbra, Portugal. The clinical study was accomplished at the Obesity Center of Hospital de Santiago, Setúbal, Portugal.

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Eickhoff H., Louro T. M., Matafome P.N., Vasconcelos F., Seiça R.M., Castro Sousa F., 2014. Amelioration of glycemic control by sleeve gastrectomy and gastric bypass in a lean animal model of type 2 diabetes: restoration of gut hormone profile. Obes Surg, DOI: 10.1007/s11695-014-1309-8 [Epub ahead of print].

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ABBREVIATIONS

AHEAD	Action for Health in Diabetes [study]
ANOVA	Analysis of variance
ASA	American Society of Anesthesiologists
AUC	Area under the curve
BMI	Body mass index | *índice de massa corporal*
BPD	Bilio-pancreatic diversion
BPD-DS	Bilio-pancreatic diversion with duodenal switch
CCK	Cholecystokinin
DIO	Diet-induced obesity
DJB	Duodenal-jejunal bypass
DJE	Duodenal-jejunal exclusion
ECG	Electrocardiogram
FBG	Fasting blood glucose
FMUC	Faculty of Medicine of the University of Coimbra
FPG	Fasting plasma glucose
GB	Gastric bypass | *bypass gástrico*
GHS-R	Growth hormone secretagogue receptor
GIP	Glucose-dependent insulinotropic polypeptide *(initially denominated gastric inhibitory peptide)*
GIR	Glucagon-insulin ratio
GJB	Gastro-jejunal bypass
GK	Goto-Kakizaki
GKC	Goto-Kakizaki rats in control group | *ratos Goto-Kakizaki no grupo controlo*
GKGB	Goto-Kakizaki rats submitted to gastric bypass | *ratos Goto-Kakizaki submetidos a bypass gástrico*

GKSG	Goto-Kakizaki rats submitted to sleeve gastrectomy | *ratos Goto-Kakizaki submetidos a gastrectomia vertical*
GKSS	Goto-Kakizaki rats submitted to sham surgery | *ratos Goto-Kakizaki submetidos a cirurgia simulada*
GLP-1	Glucagon-like peptide-1 | *peptídeo semelhante à glucagina-1*
HbA1c	Glycated hemoglobin
HOMA	Homeostasis model assessment
HOMA2	Updated homeostasis model assessment | *modelo de avaliação da homeostase modificado*
HSD	Honest significant difference
IAPP	Islet amloid polypeptide
IBILI	Institute for Biomedical Imaging and Life Sciences
IFG	Impaired fasting glucose | *alteração da glicémia do jejum*
IGT	Impaired glucose tolerance
II	Ileal interposition
II-DSG	Ileal interposition plus diverted sleeve gastrectomy
II-SG	Ileal interposition plus sleeve gastrectomy
IR	Insulin resistance | *resistência à insulina*
IT	Ileal transposition
MDJB	Modified duodenal-jejunal bypass
MRI	Magnetic resonance imaging
NAFLD	Nonalcoholic fatty liver disease
NPY	Neuropeptide Y
OXM	Oxyntomodulin
PG	Plasma glucose
PYY	Peptide tyrosine tyrosine | *peptídeo tirosina tirosina*
QUICKI	Quantitative insulin sensitivity check index
SEM	Standard error of the mean

SG	Sleeve gastrectomy | *gastrectomia vertical*
SGDJB	Sleeve gastrectomy with duodenal-jejunal bypass
SOS	Swedish Obese Subjects [study]
T2D	Type 2 diabetes | *diabetes tipo 2*
UCD-T2DM	University of California at Davis type 2 diabetes mellitus
WIC	Wistar rats in control group | *ratos Wistar no grupo controlo*
Y2R	Y2 receptor
ZDF	Zucker diabetic fatty rat
ZFR	Zucker fatty rat

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SUMMARY

SUMMARY

Background: Bariatric and metabolic surgery is an accepted treatment for obese patients with type 2 diabetes (T2D). However, the potential of gastrointestinal surgery regarding glycemic control in non-severely obese diabetic patients has yet to be defined. Along with weight loss itself, changes in gut hormone profiles after surgery play an important role in diabetes remission. Pathophysiology of T2D includes insulin resistance and insufficient insulin secretion, possibly modified by surgical procedures. Excessive or inadequate glucagon secretion promoting hepatic gluconeogenesis and glycogenolysis is also believed to contribute to hyperglycemia in diabetic patients. In the present experimental study, we explored the effect of established bariatric procedures, with and without duodenal exclusion, on glycemic control and pancreatic and gut hormone profile in Goto-Kakizaki (GK) rats, a lean animal model of T2D. Data obtained from our experimental model were supplemented by the results of a clinical study on the effects of sleeve gastrectomy (SG) on impaired fasting glucose (IFG) and T2D in obese patients.

Methods: Forty 12- to 14-week-old GK rats were randomly assigned to four groups: control group (GKC), sham surgery (GKSS), sleeve gastrectomy (GKSG), and gastric bypass (GKGB). Ten age-matched Wistar rats served as a non-diabetic control group (WIC). Glycemic control and plasma lipids were assessed at the beginning of the observation period and four weeks after surgery. Fasting and mixed meal-induced plasma levels of insulin, glucagon, ghrelin, glucagon-like peptide-1 (GLP-1), and peptide tyrosine tyrosine (PYY) were measured. In the clinical study, SG as stand-alone treatment for severe or morbid obesity was performed in 23 patients with T2D or IFG. No post-operative complications occurred and patients were dismissed from hospital on day 2 after surgery. Body mass index (BMI), fasting plasma glucose (FPG) and fasting insulin were determined before and up to 24 months after surgery. Insulin resistance and beta-cell function were calculated using the modified homeostasis model assessment (HOMA2).

Results: Glycemic control was improved in GKSG and GKGB groups. Fasting insulin and glucagon levels in WIC rats were similar to GKC or GKSS. Fasting glucagon levels were highest in GKGB. No significant meal-induced variations

of glucagon levels were observed in WIC, GKC or GKSS rats, whereas in groups GKSG and GKGB a significant rise occurred 30 minutes after a mixed meal, maintained up to 60 minutes. Both GKSG and GKGB rats showed an elevated glucagon-insulin ratio at 60 minutes in comparison to all other groups. Mixed meal-induced gut hormone profiles in WIC rats were significantly different from GKC and GKSS. After SG and gastric bypass (GB), GK rats showed a similar postprandial decrease in ghrelin as observed in non-diabetic WIC rats. Following both surgical procedures, a significant meal-induced increase in PYY and GLP-1 could be demonstrated. In the clinical study, BMI, FPG and fasting insulin improved significantly as early as 3 months after surgery. A three-fold increase in pre-operative insulin resistance (3.05) decreased to near-normal values (1.14) during the same period. Interestingly, overall beta-cell function diminished at 12 months of follow-up (79.6%), in comparison to pre-operative values (117.8%). Patients with a markedly reduced preoperative beta-cell function (<40%) did not achieve a complete remission after surgery.

Conclusions: SG and GB induced a similar improvement in overall glycemic control in lean diabetic rodents. GKC and GKSS rats showed similar glucagon levels in comparison to non-diabetic WIC rats, without variation after a mixed meal. GKSG or GKGB groups improved their overall glucose metabolism, albeit an augmented post-procedural glucagon secretion. Meal-induced profiles of ghrelin, GLP-1, and PYY in GK rats were significantly modified by SG and GB and became similar to non-diabetic Wistar rats. In obese patients with T2D or IFG, commonly characterized by an increased insulin resistance and insulin secretion, SG induced remission through reduction of insulin resistance. Our data does not support the hypothesis that duodenal exclusion and early contact of food with the ileal mucosa alone may explain changes in gut hormone profile in GK rats after gastrointestinal surgery. In our experimental study, an augmented glucagon secretion after surgery did not contribute to an impaired glucose tolerance. Pre-operative insulin resistance and beta-cell function calculated by mathematical models like HOMA2 might have prognostic value and should be studied in patients undergoing metabolic surgery.

RESUMO

Introdução: A cirurgia bariátrica e metabólica representa uma opção terapêutica válida em doentes obesos com diabetes tipo 2 (T2D). No entanto, o seu potencial no controlo glicémico em doentes sem obesidade severa ou mórbida não está, ainda, completamente esclarecido. Para além da perda de peso, as alterações no perfil de hormonas digestivas têm um papel importante na remissão da T2D. A patofisiologia da T2D é caracterizada pela resistência hepática e periférica à insulina e a deficiente secreção de insulina, sendo possível a sua modulação através de intervenções cirúrgicas gastrointestinais. Um fator que ainda pode contribuir para a hiperglicémia em doentes diabéticos consiste no aumento da glicogenólise e gluconeogénese hepáticas devido à secreção excessiva ou inadequada de glucagina. No presente estudo experimental avaliámos o efeito de técnicas cirúrgicas estabelecidas no tratamento da obesidade, com ou sem exclusão duodenal, no controlo glicémico e perfil hormonal intestinal e pancreático em ratos Goto-Kakizaki (GK), um modelo animal não obeso da T2D. Os resultados do estudo animal foram complementados por um estudo clínico que avaliou os efeitos da gastrectomia vertical calibrada na alteração da glicémia do jejum (IFG) e na T2D em doentes obesos.

Métodos: Quarenta ratos GK, com 12 – 14 semanas de idade, foram distribuídos de forma randomizada por 4 grupos: grupo controlo (GKC), cirurgia simulada (GKSS), gastrectomia vertical (GKSG), e *bypass* gástrico (GKGB). Dez ratos Wistar da mesma idade formaram o grupo controlo não-diabético (WIC). O controlo glicémico e os lipídeos plasmáticos foram avaliados no início do período de observação e quatro semanas após a cirurgia. Determinaram-se os níveis plasmáticos, em jejum e após uma refeição líquida mista, de insulina, glucagina, grelina, peptídeo semelhante à glucagina-1 (GLP-1) e peptídeo tirosina tirosina (PYY). No estudo clínico, 23 doentes obesos, portadores de T2D ou IFG, foram submetidos a gastrectomia vertical (SG) calibrada por via laparoscópica; não houve complicações pós-operatórias e todos os doentes tiveram alta hospitalar no segundo dia após a intervenção cirúrgica. Determinaram-se o índice de massa corporal (BMI), a glicémia e a insulinémia em jejum antes e até 24 meses depois da operação. A resistência à insulina

(IR) e a função da célula beta foram calculados usando o modelo de avaliação da homeostase modificado (HOMA2).

Resultados: O controlo glicémico melhorou nos grupos GKSG e GKGB. A insulinémia e a glucaginémia em jejum foram semelhantes nos grupos WIC e GKC ou GKSS. A glucaginémia foi mais elevada no grupo GKGB. Nos grupos WIC, GKC ou GKSS não se observaram alterações pós-prandiais significativas da glucagina. No entanto, ocorreram subidas significativas aos 30 minutos após a refeição líquida tanto no grupo GKSG como no GKGB, mantida até aos 60 minutos. Os ratos dos grupos GKSG e GKGB apresentaram uma razão glucagina-insulina elevada aos 60 minutos, em comparação com todos os outros. Os perfis pós-prandiais das hormonas do tubo digestivo foram significativamente diferentes nos ratos Wistar quando comparados com os GKC e GKSS. Após SG e *bypass* gástrico (GB), os ratos GK apresentaram uma descida pós-prandial da grelina, semelhante aos WIC não diabéticos. Observaram-se ainda subidas pós-prandiais significativas do PYY e GLP-1 após ambas as técnicas cirúrgicas.

No estudo clínico, o BMI, a glicémia e a insulinémia em jejum melhoraram significativamente aos 3 meses do pós-operatório. A IR, aumentada até 300% no pré-operatório, desceu no mesmo período até valores próximos da normalidade (114%). Globalmente, a função da célula beta diminuiu aos 12 meses de catamnese (79.6%), em comparação com os valores pré-operatórios (117.8%). Doentes com uma função reduzida da célula beta no pré-operatório (<40%) não obtiveram remissão completa após a intervenção cirúrgica.

Conclusão: Em roedores diabéticos não obesos, a SG e o GB permitiram uma melhoria semelhante no controlo glicémico global. Os ratos dos grupos GKC e GKSS apresentaram níveis semelhantes de glucagina quando comparados a ratos Wistar não diabéticos; estes valores não se modificaram após uma refeição mista. Os grupos GKSG e GKGB melhoraram o controlo global da glicémia, apesar de uma secreção aumentada de glucagina. Os perfis pós-prandiais de grelina, GLP-1 e PYY nos ratos GK foram significativamente diferentes após SG e GB tornando-se semelhantes aos dos ratos Wistar não-diabéticos. Em doentes obesos com T2D ou IFG, geralmente caracterizados por um incremento da secreção de insulina e da IR, a SG induziu a remissão

através de uma diminuição da IR. Os nossos resultados não sustentam a teoria que a exclusão duodenal e o contacto precoce de alimentos com a mucosa ileal explicam, por si só, as alterações do perfil das hormonas digestivas em ratos GK após cirurgia gastrintestinal. No estudo experimental, o aumento pós-operatório dos níveis plasmáticos de glucagina não contribuiu para a diminuição da tolerância à glicose. A IR e a função da célula beta pré-operatórias, calculadas através de modelos matemáticos como a HOMA2, podem ter valor prognóstico e devem ser avaliadas em doentes submetidos a cirurgia metabólica.

PRELIMINARY NOTE

The initial conception of our research project was laid out in 2007 and accepted as project for a doctoral thesis by the Faculty of Medicine of the University of Coimbra (FMUC) in 2008. Due to unforeseen difficulties regarding the reproduction of the local breeding colony of Goto-Kakizaki (GK) rats belonging to the Institute of Physiology of FMUC, in the context of relocation from the Center for Experimental Research at the University Hospital of Coimbra to new facilities at the Institute for Biomedical Imaging and Life Sciences (IBILI), preliminary experimental studies began in late 2010. Since then, a considerable amount of research on the subject of gut hormones and enteroendocrine effects of surgery has been published. For practical reasons and to increase the scientific substance, all relevant content of papers published to date of final outline of our thesis (July 2014) will be reflected in the corresponding sections of the manuscript, including the section on “BACKGROUND AND CURRENT STATE OF RESEARCH”.

INTRODUCTION

INTRODUCTION

According to recent data, 382 million people suffer from diabetes worldwide, many of them undiagnosed. Type 2 diabetes accounts for most cases and, besides genetic predisposition, sedentary lifestyle, overweight, and poor dietary habits represent main contributing factors. Until 2035, a 55% increase in prevalence is expected (International Diabetes Federation, 2013). Diabetes imposes a major burden on health costs and leads to premature death due to complications like cardiovascular disease and diabetic nephropathy with subsequent renal insufficiency. Other non-lethal consequences of diabetes include debilitating retinopathy and blindness or peripheral neuro-vascular disease and amputation.

Conservative measures including behavioral weight loss programs and intensive life-style interventions have a favorable short-term effect on body weight and glycemic control in obese patients with type 2 diabetes if compared to standard care (Unick et al., 2011) and also improve glucose-stimulated insulin secretion and beta-cell function associated with augmented incretin secretion (Solomon et al., 2010). However, the long-term results of the Look AHEAD (Action for Health in Diabetes) trial turned out to be rather disappointing with only half of the patients obtaining a weight-loss over 5% of initial body weight, after 8 years of follow-up (The Look AHEAD Research Group, 2014); impact on diabetes control and glycated hemoglobin levels was reduced (Unick et al., 2013). Regarding the primary endpoint of the study, cardiovascular morbidity and mortality was similar in both groups (intensive lifestyle intervention or usual diabetes support and education) and the trial was interrupted after a mean follow-up of 9.6 years and 5,145 enrolled patients (The Look AHEAD Research Group, 2014).

Surgical treatment of obesity has been shown to be more effective than medical treatment, not only regarding weight loss, but also concerning control of comorbidities like type 2 diabetes (T2D). The Swedish Obese Subjects (SOS) study enrolled prospectively 2,010 patients submitted to bariatric surgery and 2,037 contemporaneously matched controls treated conservatively. After 10 years of follow-up, the incidence of diabetes was 7% in the surgical group and 24% in the group submitted to conservative treatment ($p<0.001$). Recovery from

pre-existing diabetes was more frequent in the surgical group (36% *vs.* 13%), underscoring the favorable effects of bariatric surgery on diabetes control (Sjöström et al., 2004). Overall mortality during a follow-up period up to 15 years was lower in the surgical group with an adjusted cumulative hazard ratio of 0.71 (Sjöström et al., 2007).

In specialized centers, the beneficial effect of bariatric surgery, particularly gastric bypass (GB), on glycemic control in obese patients with T2D or impaired fasting glucose (IFG) has been recognized for decades (Pories et al., 1995, 1987). Remission was observed in nearly all patients with impaired fasting glucose (97.8%) and in most patients with T2D (82.9%). Similar or even better results regarding diabetes control were obtained after bilio-pancreatic diversion (BPD) with remission rates close to 100% (Marinari et al., 2006; Scopinaro, 2006; Scopinaro et al., 1998).

An extensive and systematic meta-analysis including thousands of patients confirmed the effect of bariatric surgery on weight loss and glycemic control (Buchwald et al., 2004). Addressing specifically the question of diabetes remission after bariatric surgery, a more recent meta-analysis concluded that a complete resolution of the clinical manifestations of diabetes, e. g. fasting glycemia <100 mg/dl and glycated hemoglobin (HbA1c) <6% without medication, occurred in 78.1% of patients (Buchwald et al., 2009). Diabetes was improved or resolved in 86.6% and remission was maintained for more than 2 years in 74.6%. BPD revealed to be the most effective surgical technique (95.1% resolved), followed by GB (80.3% resolved), vertical banded gastroplasty (79.7% resolved), and laparoscopic adjustable gastric banding (56.7% resolved).

In many patients, improvement of glycemic control occurs rather early after GB, within hours or days, before any significant weight loss has been achieved (Pories et al., 1995; Smith et al., 1996) and was thought to be related to neuro-endocrine mechanisms due to the bypass of the gastric antrum and duodenum. Analyzing their series of 608 patients submitted to GB in 2001, including 146 diabetic patients and 152 with impaired glucose tolerance (IGT), Pories and Albrecht postulated the hypothesis that, in vulnerable individuals, T2D might be caused by an overstimulation of the foregut and pancreas by incretins like

glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP) (Pories and Albrecht, 2001).

More recently, superobese patients with T2D or IGT, submitted to sleeve gastrectomy (SG) as first stage of bilio-pancreatic diversion with duodenal switch (BPD-DS), were shown to ameliorate glucose control at 18 months of follow-up, without any bypass of the duodenum and without completing the second stage of surgery (Silecchia et al., 2006). In another study, including 33 obese or superobese patients, diabetes remission rates after SG alone reached 84.6% after 3 years and 76.9% after 5 years, but 9 patients were submitted to the second stage of BPD-DS and excluded from further analysis (Abbatini et al., 2013).

SG and GB represent distinct surgical techniques regarding the exclusion of the duodenum and proximal jejunum, hypothetically implied in diabetes remission (Pories and Albrecht, 2001). Interestingly, recent data suggests that both techniques are similarly effective in treating T2D in severely and morbidly obese patients, at least in the short term (Cutolo et al., 2012; de Gordejuela et al., 2011; Jiménez et al., 2012; Keidar et al., 2013; Nocca et al., 2011; Zhang et al., 2013). Notwithstanding, a meta-analysis including five randomized controlled trials on diabetes remission demonstrated that GB was more effective than SG at the cost of an increased complication rate, but many analyzed parameters were only available from 2 or 3 studies (Li et al., 2013). A more extensive review and meta-analysis including 33 studies and 1375 patients did not show any significant differences in short-term diabetes remission after both techniques (Yip et al., 2013). Furthermore, recent randomized controlled trials comparing medical and surgical treatment of T2D in obese patients demonstrated superior results after SG, GB, and BPD in comparison to medical treatment (Kashyap et al., 2013; Leslie et al., 2012; Mingrone et al., 2012; Palikhe et al., 2014; Samat et al., 2013; Schauer et al., 2012).

Less is known regarding the efficacy of surgical approaches in controlling T2D in patients that are simply overweight (body mass index [BMI] 25 – 30 kg/m^2) or suffer from mild obesity (BMI 30 – 35 kg/m^2). In a small series from Italy, nine diabetic patients with a BMI of 28 – 35 kg/m^2 were submitted to SG, with favorable results regarding diabetes control in comparison to a control group,

one year after surgery (Abbatini et al., 2012). Two studies from Chile including 30 and 31 patients with mild obesity submitted to GB showed similar results with remission rates of 65% and 93.6%, after 2 and 3 years of follow-up (Boza et al., 2011a; Lanzarini et al., 2013). However, two studies from Brazil revealed conflicting results after GB. A less favorable outcome was observed in 27 diabetic patients with class I obesity (BMI 30 – 35 kg/m^2) achieving remission only in 48.1% of cases after a mean follow-up of 20 months (de Sa et al., 2011). In another study in the same population, 88% of 66 patients achieved remission (HbA1c <6.5%) after a median follow-up of 5 years (R. V Cohen et al., 2012). In diabetic Asian patients with a BMI <35 Kg/m^2, remission rates after GB were merely about 55% (fasting plasma glucose (FPG) <110 mg/dl and HbA1c <6%), even with a markedly improved insulin resistance, after a follow-up of 2 years (Lee et al., 2011b). Similar results were obtained in Indian patients with class I obesity and, after 5 years of follow-up, diabetes remission according to ASA criteria was maintained in 57.7% of patients (Lakdawala et al., 2013). BPD in 30 diabetic patients with mild obesity or simply overweight showed slightly better results and diabetes remission (defined as HbA1c <6%) was obtained in 67% (Scopinaro et al., 2011).

Diabetes resolution after bariatric surgery is associated with reduction in hepatic and peripheral insulin resistance after gastric banding, gastric bypass or sleeve gastrectomy (Ballantyne et al., 2006; Benaiges et al., 2013; Rizzello et al., 2010; Wickremesekera et al., 2005). However, gut hormones and the enteroinsular axis play a renowned role in insulin secretion, and in obese patients neuroendocrine mechanisms controlling hunger and satiety seem to be affected by bariatric surgical procedures (le Roux et al., 2006). Nonetheless, the effect of gastrointestinal surgery on type 2 diabetes in non-severely or morbidly obese patients and underlying changes in gut hormone profiles remains to be further elucidated.

BACKGROUND AND CURRENT STATE OF RESEARCH

BACKGROUND AND CURRENT STATE OF RESEARCH

Short overview on the pathophysiology of type 2 diabetes

Criteria for the diagnosis of diabetes include HbA1c levels ≥6.5%, FPG ≥126 mg/dL, 2-h plasma glucose (PG) after an 75g oral glucose tolerance test ≥200mg/dL, or random PG ≥200mg/dL in a subject with clinical manifestations of hyperglycemia (American Diabetes Association, 2014). Individuals with IFG (FPG levels 100 -125 mg/dL) or IGT (2-h PG levels 140 – 199 mg/dL) are considered at an increased risk for the development of diabetes.

Whereas type 1 diabetes is characterized by an absolute insulin deficiency due to progressive beta-cell destruction, patients with T2D present with an insufficient pancreatic insulin secretion in response to a given plasma glucose level to maintain normoglycemia in the presence of hepatic and peripheral insulin resistance (DeFronzo, 1992). Insulin resistance represents a key feature of T2D and is associated with accumulation of hepatic fat deposits (Seppälä-Lindroos et al., 2002), reduced suppression of serum free fatty acids (FFA) by insulin, and intramyocellular triglyceride content in the skeletal muscle (Guo, 2007). In obese patients, pro-inflammatory cytokine expression and suppression of anti-inflammatory proteins like adiponectin ads to insulin resistance and nonalcoholic fatty liver disease (NAFLD) (Asrih and Jornayvaz, 2013). Progression of the condition leads to beta-cell failure, frequently referred to as exhaustion of the beta-cell, and mechanisms like glucotoxicity, lipotoxicity and islet amyloid polypeptide (IAPP) deposition have been implicated in deterioration of insulin secretion (DeFronzo, 2009).

Loss of the incretin effect due to changes in incretin levels and action like resistance to GIP or reduced secretion of GLP-1 plays an additional role in the development of type 2 diabetes (Holst et al., 2009). Furthermore, the beneficial effect of both hormones on beta-cell proliferation and neogenesis, as well as the prevention of apoptosis, seems to be impaired in diabetic patients. Excessive or inadequate secretion of glucagon from pancreatic alpha-cells promoting hepatic gluconeogenesis and glycogenolysis possibly also contributes to impaired glucose tolerance in type 2 diabetes (Gastaldelli et al., 2000; Jiang and Zhang, 2003).

Endocrine function of the gut

Gut hormones from enteroendocrine cells of the gastrointestinal mucosa play an important role in the regulation of energy homeostasis and glucose metabolism. Besides acting on the regulation of satiety and hunger via neuroendocrine pathways, gut-derived peptide hormones control gastric emptying and stimulate post-prandial insulin release from pancreatic beta-cells (Bloom et al., 2005; Drucker, 2007).

The increased oral glucose tolerance in comparison to intravenous administration has been described since the late 19th century (Creutzfeldt, 1979). However, only the availability of a radioimmunoassay for insulin determination in the 1960's allowed to demonstrate an intensified insulin response after an oral glucose load in comparison to intravenous administration (Dupre, 1964; McIntyre et al., 1964). The insulinotropic effect of a peptide with a "glucagon-like immunoreactivity" stimulated by oral glucose intake was described (Samols et al., 1966, 1965), eventually derived from the gut as it was still present in patients submitted to pancreatectomy (Samols and Marks, 1967).

Gastric inhibitory peptide (GIP)[1] was isolated in 1970 (Brown et al., 1970) and its origin from the duodenal K-cells was demonstrated (Buffa et al., 1975; Polak et al., 1973). In both rats (Turner et al., 1973) and humans (Dupre et al., 1973), it stimulated insulin secretion, but administration of exogenous GIP would stimulate glucagon secretion as well (Taminato et al., 1977), apparently in a dose-dependent fashion at basal glucose concentrations (J. J. Meier et al., 2003). In obese patients, fat together with glucose stimulated the release of immunoreactive GIP more than glucose alone, and even more so in subjects with impaired glucose tolerance that also presented with elevated fasting levels of GIP (Creutzfeldt et al., 1978). Apparently, in patients with T2D, GIP would not increment insulin release as it would in non-diabetic subjects (Elahi et al., 1994), having a defective receptor in patients with T2D being proposed to explain the reduced insulinotropic effect in these patients (Holst et al., 1997). However, whereas the continuous infusion of GIP in diabetic patients would only produce a weak response regarding insulin secretion, the response to a

[1] Later denominated "glucose-dependent insulinotropic polypeptide" due to its effects on insulin secretion.

bolus administration of GIP seems to remain preserved, making a defective receptor unlikely (Meier et al., 2004).

In 1981, a clinical study was published that showed a post-prandial elevation of enteroglugacon from the distal gut in obese patients that had undergone jejuno-ileal or biliopancreatic bypass (Sarson et al., 1981). It could be demonstrated in extracts from rat gut that, after neutralization and immune-absorption of GIP, more than 50% of the incretin effect remained, indicating the presence of additional gut hormones that stimulate insulin secretion (Ebert et al., 1983). Further studies determined the potent insulinotropic effect of the 31 amino acids containing form of GLP-1 in rats (Mojsov et al., 1987) and man (Kreymann et al., 1987). GLP-1 is found in intestinal L-cells with an increasing concentration in the distal jejunum, ileum and colon (Eissele et al., 1992) and was recognized as a new incretin hormone with distinguished properties (Orskov, 1992).

A marked elevation of GLP-1 was shown 20 years after jejuno-ileal bypass (Näslund et al., 1998), confirming the findings of the earlier study published in 1981 by Sarson *et al*. Besides effects on glucose homeostasis, GLP-1 inhibits gastric acid secretion (O'Halloran et al., 1990) and gastric emptying (Nauck et al., 1997). It also acts on the central nervous system in specific areas of the hypothalamus by inhibiting food intake and mediating satiety in rats (Turton et al., 1996) and humans (Gutzwiller et al., 1999b). In diabetic patients, it does not only decrease energy intake and enhance satiety, but also controls postprandial plasma glucose levels (Gutzwiller et al., 1999a). Furthermore, administration of GLP-1 to healthy volunteers reduced the postprandial increase in serum triglycerides, besides delaying gastric emptying as described before (Meier et al., 2006). In overweight and obese humans, meal-induced secretion of GLP-1 was more pronounced after a meal with high fat content in comparison to high content in carbohydrates and associated with short-term control of food intake (Gibbons et al., 2013). Additionally, GLP-1 modulates plasma ghrelin in the postprandial period preventing an immediate decrease in ghrelin levels after the meal and suppressing the subsequent increase in the late postprandial period (Hagemann et al., 2007). In an animal model, enhanced protein-mediated GLP-1 secretion after duodenal administration of zein hydrolysate was inhibited through vagal denervation, whereas the effect of jejunal and ileal administration

was maintained, highlighting the different mechanisms of GLP-1 secretion throughout the gastrointestinal tract (Hira et al., 2009).

Peptide tyrosine tyrosine (PYY) was initially isolated from porcine upper small intestine (Tatemoto and Mutt, 1980) and an inhibitory effect on exocrine pancreatic secretion was described in cats (Tatemoto, 1982). The molecular structure of PYY is similar to neuropeptide Y (Tatemoto et al., 1982) and further studies identified PYY in L-cells of the distal small bowel and, in increasing concentrations, in the colon and rectum (Adrian et al., 1985; Lundberg et al., 1982). PYY is secreted in response to food intake, particularly with a high lipid content (Adrian et al., 1985). In the central nervous system, subtype PYY_{3-36} interacts as an agonist with the Y2 receptor (Y2R) on neuropeptide Y (NPY) neurons in the arcuate nucleus of the hypothalamus inhibiting food intake (Batterham et al., 2002). In humans, functional magnetic resonance imaging could demonstrate activation of specific brain areas after exogenous administration of PYY_{3-36} and $GLP\text{-}1_{7-36}$, similar to those observed after regular food intake (Batterham et al., 2007; De Silva et al., 2011). Recent research in mice suggested that PPY acts also on GLP-1 secretion through activation of peripheral Y2R (Chandarana et al., 2013).

Besides release from intestinal L-cells, PYY is also co-stored with glucagon in pancreatic alpha-cells. *In vivo* experiments in a mouse model showed no effect on basal plasma levels of insulin or glucagon, but stimulated secretion of both hormones was inhibited (Böttcher et al., 1989). PYY restrains the glucose-stimulated insulinotropic action of GIP in dogs (Guo et al., 1989), but insulinostatic effects of PYY on glucose-stimulated insulin secretion through inhibition of the cyclic AMP cascade were also observed in isolated mouse islets (Nieuwenhuizen et al., 1994). Hence, a combined effect of direct intra-islet action of PYY and indirect processes mediated through other gut hormones is plausible.

Similar to PYY, oxyntomodulin (OXM) is another enterohormone derived from proglucagon and secreted from L-cells in the distal ileum (Bataille et al., 1981). Actions of OXM include inhibition of basal and postprandial gastric acid secretion, gastroduodenal motility and gastric emptying (Schjoldager et al., 1989). Furthermore, OXM reduces food intake in rats (Dakin et al., 2001) and

humans (Cohen et al., 2003). In obese non-diabetic volunteers, preprandial subcutaneous administration of OXM resulted in significant weight loss over a four-week study period (Wynne et al., 2005). A cumulative effect on food intake during the simultaneous administration of PPY has been demonstrated (Field et al., 2010). Besides a decrease in energy intake, OXM promoted an increase in activity related energy expenditure in a small randomized controlled trial including 15 healthy overweight and obese volunteers (Wynne et al., 2006). Recent research suggested that dual activation of the GLP-1 receptor and the glucagon receptor by OXM is involved in weight loss and maintenance of glucose homeostasis after extrinsic administration in mice (Du et al., 2012; Kosinski et al., 2012; Pocai, 2014).

In the late 1990s, a new gastrointestinal hormone was identified (Kojima et al., 1999) that binded to the previously described orphan growth hormone secretagogue receptor (GHS-R) in the pituitary gland and the hypothalamus (Howard et al., 1996). Ghrelin, an acylated peptide of 28 amino acids, represents a growth-hormone secretagogue and is secreted mainly from the gastric fundus and, to a far lesser extent, throughout the remaining gastrointestinal tract (Date et al., 2000; Kojima et al., 1999). Its exogenous administration induces an increase in food intake and adiposity in rats through activation of the growth hormone receptor on NPY neurons in the arcuate nucleus of hypothalamus (Tschöp et al., 2000; Wren et al., 2000). Alongside with effects on the central regulation of satiety and hunger, ghrelin acts indirectly on gastric acid secretion via stimulation of the vagus nerve (Date et al., 2001). In analogy to observations in rodents, intravenous ghrelin infusion increases food intake in man (Wren et al., 2001). Generally, plasma ghrelin is decreased in obese patients if compared to lean controls (Tschöp et al., 2001), particularly in patients with insulin resistance (McLaughlin et al., 2004). Somewhat conversely, obese patients with NAFLD, a condition associated with hepatic insulin resistance, exibit higher ghrelin levels together with more advanced fibrosis and steatohepatitis (Estep et al., 2011).

Initial studies in rats showed an upregulation of ghrelin secretion in the fasting state and after insulin administration (Toshinai et al., 2001) indicating a working feedback mechanism for the stimulation of food intake. However, contradicting

research in the isolated rat stomach found that insulin and amino acids decreased ghrelin secretion, whereas lipids had no effect and acetylcholine increased secretion significantly (Shrestha et al., 2009). In healthy human volunteers a similar inhibition of ghrelin secretion after insulin-induced hypoglycemia was demonstrated (Broglio et al., 2004a; Leonetti et al., 2004).

Apparently, ghrelin has an effect on glucose metabolism inducing hyperglycemia and a decrease in serum insulin levels in healthy lean volunteers (Broglio et al., 2001). The particular importance of ghrelin in maintaining glucose homeostasis is supported by the discovery of the islet ghrelin cell (Wierup et al., 2002) and reinforced by the description of GHS-R expression in pancreatic beta-cells (Dezaki et al., 2008). Besides stimulation of GHS-R by circulating ghrelin, a paracrine effect on insulin secretion was proposed, apparently in a dose-dependent fashion. In isolated mouse islets, ghrelin in very high concentrations increased glucose-stimulated insulin release, whereas low concentrations inhibited insulin secretion (Salehi et al., 2004). However, most studies report an inhibitory effect of ghrelin on insulin release that, in a physiological context, acts in a reciprocal association with insulin (Dezaki et al., 2008; Peng et al., 2012; Tong et al., 2010; Wierup et al., 2014). Thus, high fasting plasma ghrelin levels are linked to low fasting insulin levels and the post-prandial increase in insulin secretion is accompanied by a decrease in circulating ghrelin. Apparently, inhibitory effects on insulin secretion are additionally mediated by suppression of GLP-1-induced insulin release by exogenous and endogenous islet-derived ghrelin (Damdindorj et al., 2012).

Less attention has been paid to the enteroendocrine function of cholecystokinin (CCK), a gastrointestinal peptide secreted from duodenal and jejunal I-cells. Its anorectic effects are mediated through receptors on the vagus nerve and activation of specific areas in the brain stem and hypothalamus. Apparently, long-term weight control is independent from the CCK1 and CCK2 receptor as receptor-deficient mice showed a comparable daily food intake, weight gain and glucose homeostasis in comparison to wild-type animals. Yet, exogenous administration of CCK would reduce food intake in wild-type and CCK2 receptor-deficient mice, thus indicating a role for the CCK1 receptor in the regulation of short-term satiety (Kopin et al., 1999). However, recent research

questions the role of the CCK2 receptor in long-regulation of hunger and satiety as receptor-deficient mice developed hyperphagia, obesity and impairment of glucose homeostasis (Clerc et al., 2007).

The importance of CCK-mediated intra-duodenal fat hydrolysis on GLP-1 secretion has been shown in healthy volunteers that received an intra-duodenal fat infusion with or without an irreversible inhibitor of gastro-intestinal lipases (orlistat). In conclusion, the selective blockade of the CCK1 receptor abolished the effect of fat hydrolysis on GLP-1 secretion (Beglinger et al., 2010). An analogous effect of CCK1 receptor blockage on the secretion of ghrelin and PPY had been previously observed using a similar study design (Degen et al., 2007).

Enteroendocrine effects of bariatric and metabolic surgery

For many years, the effect of gastrointestinal surgery on body weight and comorbidities has been mainly attributed to restriction of caloric intake through reduction of gastric capacity or malabsorption due to bypassing of gastrointestinal segments. However, isolated early reports suggested that endocrine mechanisms associated with surgical procedures contribute to outcome after surgery and resolution of T2D (Ackerman, 1981; Halverson et al., 1982; Pories et al., 1987; Sarson et al., 1981). Later on, the ascent of laparoscopic surgery, the epidemic-like spread of obesity, and the subsequent expansion of surgical treatment for obesity, together with the diffusion of knowledge on gut hormones, led to a significant increase in research on the enteroendocrine effects of gastrointestinal surgery for obesity and their contribution to outcome after treatment, particularly regarding diabetes remission.

In general, the incretin effect of gastrointestinal hormones reflects the augmented insulin secretion after an oral glucose load in comparison to isoglycemic intravenous glucose administration defined by: $[(\text{InsulinAUC}_{\text{oral}} - \text{InsulinAUC}_{\text{isoglycose IV}}) / \text{InsulinAUC}_{\text{oral}} \times 100\%]$[2]. In obese diabetic patients, the incretin effect on insulin secretion is markedly impaired in comparison to obese

[2] AUC = area under the curve.

non-diabetic controls. Yet, one month after GB, a more than fourfold increase in the incretin effect was observed, alongside with amelioration of fasting blood glucose and a significantly augmented glucose-stimulated GLP-1 secretion. In comparison to the non-diabetic obese control group, the enhanced incretin effect in operated diabetic patients reached comparable values (Laferrère et al., 2007)

A postoperative decrease in fasting plasma GIP levels in obese diabetic patients was observed after GB and associated with normalization of blood glucose and fasting insulin, indicating a role for incretin metabolism in the amelioration of glucose homeostasis after duodenal exclusion (Rubino et al., 2004). In non-diabetic obese patients, a similar reduction in fasting GIP levels occurred after GB, whereas fasting GLP-1 levels remained unchanged. In other studies, postprandial GLP-1 response increased significantly after GB in comparison to preoperative values (Borg et al., 2006) and lean or obese controls (le Roux et al., 2006). Effects of GB on GLP-1 secretion were maintained even 10 years after surgery (Dar et al., 2012). In obese patients with T2D, the impaired GLP-1 response to a standardized test meal was still significant in comparison to patients with normal or impaired glucose tolerance, six weeks after GB, but recovered after one year of follow-up (Morínigo et al., 2006a). However, a peak plasma level of GLP-1 was observed 30 minutes after a mixed liquid meal, as early as 7 days after GB in obese diabetic patients, being more pronounced on subsequent observations at 30 and 90 days after surgery (Umeda et al., 2011). Analogous observations regarding glucose metabolism, plasma insulin and plasma GIP levels were described after BPD, as soon as one week after surgery. Fasting and glucose-stimulated GLP-1 levels increased simultaneously (Guidone et al., 2006).

Sleeve gastrectomy, a surgical technique without duodenal exclusion, produced similar results regarding GLP-1 secretion in comparison to patients submitted to GB (Nannipieri et al., 2013; Peterli et al., 2012, 2009; Romero et al., 2012). In another study in obese diabetic patients submitted to SG, the first phase of insulin secretion after an intravenous glucose tolerance test improved early after surgery and secretion of GLP-1 and PYY was increased. However, patients with longstanding T2D (>10 years) did not experience the same improvement in

glucose homeostasis and secretion of gut hormones (Basso et al., 2011). Interestingly, in Asian patients with a BMI of 31.5±3.2 kg/m2 and longstanding type 2 diabetes submitted to either SG or GB, a far superior rate of diabetes resolution was observed after GB in comparison to SG, albeit a similar decrease in BMI and enhancement of meal-induced GLP-1 secretion after both surgical procedures (Lee et al., 2011a).

After GB in obese human patients, whether diabetic or not, a sharp and statistically significant postprandial rise of PYY could be observed if compared to lean or obese controls (Borg et al., 2006; Korner et al., 2006, 2005; le Roux et al., 2006; Morínigo et al., 2006b). Obese patients submitted to SG, together with partial enterectomy and omentectomy (Santoro et al., 2008, 2006), or SG alone (Basso et al., 2011; Dimitriadis et al., 2013; Karamanakos et al., 2008) experienced similar changes in PYY after surgery. Comparing SG and GB to medical treatment, fasting and post-prandial PYY increased similarly after both surgical techniques, whereas conservative treatment had no influence on plasma PYY levels (Valderas et al., 2010). An increase in fasting and meal-induced PYY levels has also been observed after BPD-DS, a surgical technique that incorporates SG (Hedberg et al., 2011).

Markedly reduced fasting ghrelin levels were observed after GB in comparison to obese and normal-weight controls or to patients with diet-induced weight loss (Cummings et al., 2002; Frühbeck et al., 2004a, 2004b; Tritos et al., 2003). No differences were observed between diabetic or non-diabetic obese patients regarding low preoperative ghrelin levels and postoperative decrease (Geloneze et al., 2003). Meal-related and diurnal fluctuations of plasma ghrelin seem to be suppressed after surgery (Cummings et al., 2002). Comparing pre- and postprandial ghrelin levels, Korner and coworkers could demonstrate an increased postprandial suppression of plasma ghrelin in patients submitted to GB in comparison to obese controls achieving a profile similar to lean controls (Korner et al., 2006, 2005). Yet, contradictory results indicate an unchanged fasting plasma ghrelin in weight-stable patients and even an increase in patients experiencing active weight loss after GB (Borg et al., 2006; Faraj et al., 2003; Holdstock et al., 2003; Nijhuis et al., 2004). Minigastric bypass (MGB) represents a variation of GB with a longer gastric pouch and a lateral gastro-

jejunostomy. Apparently, no effect on fasting plasma ghrelin levels is exerted, but excess weight loss correlated negatively with high preoperative fasting ghrelin levels (Liou et al., 2008).

Hypothetically, transient decrease of fasting ghrelin after GB might be attributable to vagus nerve dysfunction after surgery, as ghrelin is produced mainly in the gastric fundus and secretion depends on vagal stimulation (Sundbom et al., 2007). However, pre- and postprandial plasma levels are not necessarily associated with a successful surgical outcome regarding weight loss or satiety after GB (Christou et al., 2005). Comparable results regarding the missing predictive value of fasting plasma ghrelin levels for post-surgical weight loss were decribed after gastric banding (Busetto et al., 2006).

One year after BPD, fasting ghrelin levels were increased in comparison to pre-operative values (Adami et al., 2004), thus indicating probably a new steady-state. In another study regarding patients submitted to BPD, plasma ghrelin levels also increased after surgery, but circadian pulsatility was disrupted, in a similar fashion as observed in patients submitted to GB (Cummings et al., 2002).

A sustained decrease in fasting ghrelin levels was observed after SG in comparison to preoperative values (Langer et al., 2005). In a comparative study, fasting ghrelin levels decreased and postprandial ghrelin was markedly suppressed after SG, whereas no significant changes were demonstrated after GB (Karamanakos et al., 2008). Similarly, fasting ghrelin levels were significantly suppressed after BPD-DS, if compared to obese patients treated with vertical gastroplasty or diet that experienced increased fasting ghrelin plasma levels (Kotidis et al., 2006). In another study, resection of the gastric fundus added to standard GB suppressed fasting ghrelin in a fashion similar to SG, whereas ghrelin levels increased in the group submitted to standard GB. Moreover, meal-stimulated secretion of GLP-1 and PYY was more pronounced after GB with fundus resection (Chronaiou et al., 2012).

However, at least the initial effect of bariatric surgery on improvement of insulin sensitivity and beta-cell function in obese non-diabetic or diabetic patients might be due to caloric restriction and not related to changes in secretion of

gastrointestinal hormones, as demonstrated in studies involving controls and patients submitted to GB (Isbell et al., 2010) and BPD-DS (Plourde et al., 2014). Conversely, a postprandial increase in PYY and GLP-1 was shown 2 – 3 weeks after GB, not observed in an age- and BMI-matched control group after a 7-day low-calorie diet (Evans et al., 2012), albeit results may have been biased because dietary changes in patients in the GB group lasted for already 2 – 3 weeks.

Pancreatic hormones and metabolic surgery

As outlined above, hepatic and peripheral insulin resistance represents a key feature of T2D and even more so in obese patients. Progression of disease leads to beta-cell failure due glucotoxicity, lipotoxicity and deposition of IAPP. Loss of incretin function plays an additional role. Consequently, patients with IGT or overt T2D can present with high, normal or low insulin plasma levels, depending on disease stage and contribution of the different factors. Plasma glucagon levels tend to be high due to inadequate or excessive release from pancreatic alpha-cells and might contribute to impairment of glucose tolerance.

In obese patients, whether diabetic or not, high preoperative fasting plasma insulin levels related to insulin resistance decrease significantly after all types of bariatric surgical procedures including gastric banding, sleeve gastrectomy, gastric bypass, and biliopancreatic diversion (Adami et al., 2004; Ballantyne et al., 2006; Benaiges et al., 2013; Faria et al., 2013; Gumbs et al., 2005; Mari et al., 2006; Pontiroli et al., 2002; Pories et al., 2010, 1992, 1987; Ramos et al., 2006; Rizzello et al., 2010; Wickremesekera et al., 2005). Mixed-meal induced insulin secretion tends to be augmented after surgery (Falkén et al., 2011; le Roux et al., 2006; Peterli et al., 2009). Reduced hepatic insulin resistance and increased hepatic insulin-induced glucose uptake in obese diabetic and non-diabetic patients after both GB and SG has been demonstrated by positron emission tomography (PET) during an euglycemic hyperinsulinemic clamp (Immonen et al., 2014). Besides improvement in insulin sensitivity and reduction of insulin resistance, an augmented beta-cell glucose sensitivity after BPD in

obese diabetic patients indicates major changes in beta-cell function (Camastra et al., 2007).

Less is known regarding the influence of surgical procedures on glucagon secretion. In general, fasting glucagon is not affected after surgery, whereas meal-induced response is initially incremented and decreases after one year to preoperative values (Falkén et al., 2011).

Research in animal models and humans

Goto-Kakizaki rats derived originally from glucose intolerant Wistar rats submitted to repetitive selective inbreeding (Goto et al., 1976) and have been studied extensively as a model for T2D in non-obese humans. They represent similar features in comparison to the human pancreatic phenotype in patients with T2D (Portha et al., 2009; Seiça et al., 2003a). In hyperglycemic conditions, the isolated pancreas of GK rats presents with a blunted pancreatic insulin response after stimulation by the incretin hormones GIP and GLP-1 in comparison to the pancreas of non-diabetic Wistar rats (Edholm et al., 2009).

In GK rats, duodenal exclusion without gastric resection ameliorated glycemic control in comparison to sham operated controls, as demonstrated by an oral glucose tolerance test, three weeks after surgery. No statistically significant effect on fasting insulin and GIP (Rubino and Marescaux, 2004) or glucose-stimulated insulin, GIP and GLP-1 was observed (Pacheco et al., 2007). Comparing gastro-jejunal bypass (GJB) to gastro-jejunostomy without duodenal exclusion in the same animal model, it could be shown that animals submitted to surgery without duodenal exclusion would not experience an ameliorated glucose control in comparison to sham surgery or non-operated controls (Rubino et al., 2006). A later study in GK rats could show an increase in GLP-1 secretion after GJB, associated with higher mRNA insulin expression in pancreatic islets (de Luis et al., 2012). Duodenal-jejunal bypass (DJB) also enhanced beta-cell area and decreased associated fibrosis in comparison to sham-operated animals (Speck et al., 2011). Gastric bypass carried out in a fashion similar to humans including reduction of gastric volume in addition to DJB promoted enhanced expression of the GLP-1 receptor in pancreatic islet,

besides augmented fasting GLP-1 levels and reduced insulin resistance (Liu et al., 2011). Apparently, DJB in GK rats also up-regulates hepatic insulin signaling pathways and down-regulates key regulatory enzymes of gluconeogenesis (Sun et al., 2013).

However, ileal transposition (IT), a surgical technique without duodenal exclusion, showed amelioration of glucose control in operated GK rats, alongside with a statistically significant rise in GLP-1 after an oral glucose load in comparison to sham operated animals (Patriti et al., 2005). Additionally, an increased proglucagon expression was demonstrated in the transposed ileal segment (Patriti et al., 2007). Comparing duodenal-jejunal exclusion (DJE) to IT in the same animal model, a similar post-operative glucose-stimulated increase in GLP-1 secretion and improvement of glucose tolerance was observed after both surgical techniques. Interestingly, improved glucose tolerance was reversible in rats submitted to DJE through administration of Exendin-[9-39], a GLP-1 receptor antagonist (Kindel et al., 2009). Similar results regarding glucose homeostasis and glucose-stimulated GLP-1 secretion were obtained in another study in GK rats comparing BPD without gastric resection to animals submitted to IT. No significant differences between groups were observed (Zhang et al., 2011). Besides an increment in GLP-1 secretion, IT in GK rats promoted myocardial glucose uptake, protein expression of insulin signaling pathways, and protein levels of membrane glucose transporter 4 (Yan et al., 2012).

In the same rodent model, the effect of SG on glucose homeostasis and fasting plasma ghrelin was compared to the effect of a modified duodenal-jejunal bypass (MDJB), a technique with duodenal exclusion similar to DJE. Interestingly, SG provided better results regarding glucose metabolism and reduced fasting plasma ghrelin levels (Li et al., 2009). Combination of duodenal exclusion and SG in GK rats showed a more pronounced GLP-1 secretion after an oral glucose load in comparison to SG alone. However, both surgical techniques obtained similar results regarding decrease of fasting ghrelin and glucose-stimulated increase of PYY in comparison to controls (Sun et al., 2012).

Research in obese University of California at Davis type 2 diabetes mellitus (UCD-T2DM) rats, a recent polygenic rodent model for T2D (Cummings et al.,

2008), revealed a metabolic effect of SG regarding glucose homeostasis, even without bypassing any segment of the digestive tract. Further investigation revealed also an increase in nutrient-stimulated GLP-1 and PPY secretion, alongside with decreased fasting plasma insulin and ghrelin (Cummings et al., 2012).

Other rodent models used to study the effects of bariatric and metabolic surgery include strains with a single genetic defect like the obese Zucker fatty rat (ZFR) due to a simple autosomal recessive gene on chromosome 5. A diabetic substrain of the model was obtained by selective inbreeding for impairment of glucose homeostasis and denominated Zucker diabetic fatty rat (ZDF) (Srinivasan and Ramarao, 2007). Albeit a model with a single genetic defect might not correctly represent the polygenic nature of human T2D, male ZDF rats are characterized by progression to overt diabetes together with insulin resistance and beta-cell apoptosis. After DJB, a decrease in food intake and weight gain has been observed, alongside with a meal-induced suppression of acylated ghrelin, in the presence of increased fasting plasma ghrelin levels (Rubino et al., 2005).

Insulin sensitivity in ZDF rats submitted to GB was significantly improved in comparison to pair-fed controls or animals with ad libitum access to food, in the absence of significant changes in visceral or subcutaneous fat assessed by magnetic resonance imaging (MRI). Dextrose-stimulated GLP-1 secretion and glucose/intralipid-stimulated secretion of PYY was significantly enhanced after surgery (Meirelles et al., 2009). In the same animal model, SG with IT, a technique without exclusion of the proximal small intestine, induced a similar weight loss and improvement in glucose homeostasis in comparison to GB (Boza et al., 2011b). However, SG alone also improved glucose homeostasis in ZDF rats, even without associated IT (Kadera et al., 2013; Lifante et al., 2012). Apparently, SG in ZDF acts through reduction in food intake, suppression of plasma ghrelin and increase in glucose-stimulated secretion of GLP-1, GIP and PYY, whereas DJB ameliorated glucose homeostasis without significant changes in weight gain or effect on mentioned gut hormones. Interestingly, postprandial glucagon response was suppressed after DJB, and addition of DJB

to SG further improved glucose tolerance and decreased weight gain (Patel et al., 2013).

In a non-diabetic rodent model with diet-induced obesity (DIO), the transposition of a segment of the distal ileum to the proximal jejunum was able to promote a reduced food intake and weight gain in adult Long-Evans rats. In response to an oral gavage with D-Glucose, increased GLP-1 levels were observed in comparison to sham operated animals. Additionally, gavage with an intralipid solution produced an increased stimulated PYY secretion. Post mortem studies showed an overexpression of preglucagon mRNA and PYY mRNA in the transposed segment (Strader et al., 2005). In a later study, an improvement in glucose tolerance after IT was shown in low dose streptozotocin-induced diabetic rats and non-diabetic controls with a similar increase in gut hormones as described before (Strader et al., 2009). In the same animal model, SG and GB enhanced mixed-meal stimulated GLP-1 secretion. Insulin sensitivity assessed by hyperinsulinemic euglycemic clamp studies was improved after both surgical techniques (Chambers et al., 2011).

In DIO Sprague-Dawley rats, gastric bypass produced an increase in plasma PYY levels, however without affecting GLP-1 plasma levels (Suzuki et al., 2005). In the same model, weight loss or weight stabilization in comparison to an age-matched control group correlated to post-operative decrease in plasma ghrelin (Stylopoulos et al., 2005). Nonetheless, gut hormones in DIO rats were affected by SG alone and impaired postprandial GLP-1 secretion was restored to levels similar to those observed in lean controls (Al-Sabah et al., 2014). In other models for DIO using Wistar and Long-Evans rats, no improvement of insulin sensitivity was observed after DJB (Kindel et al., 2010), but an enhanced beta-cell secretory capacity was demonstrated (Araujo et al., 2012).

In a non-obese non-diabetic rodent model using Wistar rats, an increase in fasting PYY and GLP-1 was observed in animals submitted to jejuno-ileal bypass (le Roux et al., 2006) or BPD (Borg et al., 2007). After BPD in the same animal model, adaptive processes towards modulation of food intake were accompanied by an increased NPY expression in brain tissue (Nadreau et al., 2006). Comparing SG alone to SG plus IT in non-obese non-diabetic Sprague-Dawley rats, a similar expression of pancreatic GLP-1 producing cells was

observed after both procedures, but only animals submitted to SG plus IT showed and enhancement of ileal GLP-1 production (Johannessen et al., 2013). In the same rodent model, the length of the transposed ileum determined the amplitude of effects on gut hormones and glucose metabolism (Ramzy et al., 2014).

Based on the results of IT and its effects on enteroendocrine pathways and glucose homeostasis in animal research, de Paula and coworkers started an experimental clinical study in 39 diabetic patients with a body mass index from 23.4 – 35 kg/m2 (DePaula et al., 2008), not considered for bariatric surgery by international guidelines ("Gastrointestinal surgery for severe obesity: National Institutes of Health Consensus Development Conference Statement.," 1992). Patients were submitted to ileal interposition (II)[3] plus SG (II-SG) or II plus diverted SG (II-DSG) with a direct anastomosis between the sectioned duodenum and the transposed ileal segment. After a mean follow-up of 7 months, 86.9% achieved an acceptable glycemic control and the remaining 13.1% improved. In a later publication, both surgical techniques proved to have a significant effect on postprandial plasma levels of gastrointestinal hormones. After a mean follow-up of 16 months (range 14 – 22 months), meal-stimulated secretion of GIP, GLP-1 and PYY increased, whereas plasma ghrelin levels were suppressed (DePaula et al., 2009). As expected, insulin sensitivity and glucose-stimulated beta-cell function improved significantly after both procedures (De Paula et al., 2011; Vencio et al., 2011). An update on results of the same center was published in 2012. After a mean follow-up of 39.1 months, 78.3% of 202 patients achieved a HbA1c <6.5%, without medication (DePaula et al., 2012). Another study from a different surgical center evaluated the results of II-SG in non-obese and obese patients with type 2 diabetes and showed remission (defined as a HbA1c level <6.5%) in 80% of patients, after a mean follow-up of 13 months (Tinoco et al., 2011). Using the same surgical technique (II-SG) in diabetic Indian patients, weather obese or not, diabetes remission was obtained in 47% of 42 patients after 6 months (Kota et al., 2012b). Results of II-DSG were markedly better with remission rates of 70.5% in 32 patients after a mean follow-up of 13.1 months (Kota et al., 2012a).

[3] Comparable to IT.

Based on experimental results of DJB in a lean animal model of type 2 diabetes, a small clinical study was conducted in 35 diabetic patients without substantial excess weight (BMI 28.4±2.9 kg/m^2). However, diabetes remission defined as HbA1c <7% off medication occurred in only 40% after 12 month of follow-up, without any significant weight loss (R. Cohen et al., 2012). Albeit an amelioration of glucose homeostasis in some patients, DJB without gastric resection did not show a beneficial effect on beta-cell function or insulin resistance in another study from the same group (Klein et al., 2012).

AIMS AND SCOPE OF INVESTIGATION

AIMS AND SCOPE OF INVESTIGATION

In the present study, we evaluate the effect of sleeve gastrectomy and gastric bypass on glycemic control and plasma gut hormone profiles in a lean rodent model of type 2 diabetes. Both surgical techniques represent safe and established approaches for the treatment of severe and morbid obesity in current clinical practice, but are not yet broadly used in lean patients or patients with mild obesity or simply overweight. Gastric bypass serves as a model that includes duodenal exclusion, whereas sleeve gastrectomy comprises no bypass of the gastrointestinal tract. Results are compared to lean non-diabetic rats.

Additionally, a clinical study in obese patients with impaired fasting glucose and type 2 diabetes submitted to sleeve gastrectomy highlights the role of insulin resistance in the pathophysiology of glucose metabolism and mechanisms of post-surgical recovery.

Our investigation shall contribute to the understanding of changes in gut hormones profiles after accepted bariatric surgical techniques with or without duodenal exclusion and their future clinical usefulness in non-severely or morbidly obese patients with type 2 diabetes.

MATERIAL AND METHODS

MATERIAL AND METHODS[4]

1. Experimental Study

1.1. Animals

Twelve- to 14-week-old male GK rats and age-matched Wistar rats were obtained from local breeding colonies at the Laboratory for Experimental Research, Faculty of Medicine, University of Coimbra, Coimbra, Portugal [Figure.1].

Figure 1 12- to 14-week-old male Goto-Kakizaki rats

All animals were maintained in a controlled environment with day-night cycles of 12 hours, a temperature of 22 – 24 ºC, and a relative humidity of 50 – 60 percent. Animals had ad libitum access to water and standard rat chow supplied by Charles River, SAFE, France. In the first post-operative days, operated animals had additional access to a mixed liquid diet (Forticare®) supplied by Nutricia, Danone Group, France.

The study protocol regarding the use of laboratory animals was approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra, Coimbra, Portugal. After completing the study, 30 days after surgery, all animals were sacrificed by cervical displacement, according to the procedures approved by the Institutional Animal Care and Use Committee.

[4] Adapted from published articles or manuscripts submitted for publication.

1.2. Study Protocol and Surgical Techniques

Forty non-obese diabetic GK rats were randomly assigned to the following groups: control (GKC), sham surgery (GKSS), sleeve gastrectomy (GKSG), and gastric bypass (GKGB). Wistar rats (n=10) served as an additional non-diabetic control group (WIC). Before surgery or entering the control group, venous blood was sampled from the tail vein for determination of blood glucose level, glycated hemoglobin, cholesterol, and triglycerides, after an overnight fast, and, without previous fasting, for random glycemia.

Animals assigned to surgery (GKSS, GKSG, GKGB) were operated under intra-muscular anesthesia using ketamine (75 mg / kg bodyweight, Pfizer Inc., New York, NY, USA) and chlorpromazine (3 mg / kg bodyweight, Laboratórios Vitória, Amadora, Portugal). After shaving the abdomen, skin was prepped with a 4% polividone-iodine solution (MEDA Pharma, Lisbon, Portugal). A midline incision was performed and the abdominal cavity entered. Surgery was performed accordingly to the randomization of animals. After completing surgery and rinsing the abdomen with sterile saline, meloxicam (1 mg / kg bodyweight, Boehringer Ingelheim GmbH, Ingelheim, Germany) in sterile saline was left in the peritoneal cavity for post-operative analgesia. The abdominal wall was closed in all animals by continuous mass closure with a 3/0 short-term absorbable suture of braided and coated polyglycolic acid (Safil Quick®, B Braun, Aesculap AG, Tuttlingen, Germany). Post-operative care included subcutaneous analgesia with meloxicam (1 mg / kg bodyweight) and oral antibiotics (doxycycline, 5 mg / kg bodyweight, Pfizer Inc., New York, NY, USA) mixed to drinking water during 5 days. Animals dying from post-operative complications (n=12) were replaced to maintain the original number of animals in groups.

Blood samples were repeated at four weeks for the same parameters as described above. Three to four days later, after an overnight fast, all animals were fed 3 cc of a mixed liquid diet (Forticare®) supplied by Nutricia, Danone Group, France. Blood samples were drawn by intra-cardiac puncture in the anesthetized animal before and after the oral fed at 10, 20, 30, 40, and 60 minutes for the dosage of plasma concentrations of the following pancreatic and

gut hormones: insulin, glucagon, GLP-1, PYY, and ghrelin. Animals were sacrificed afterwards as described above.

1.2.1 Sham Surgery

In animals assigned to sham surgery, the stomach was mobilized and a 3 cm incision in anterior gastric wall was performed. To resemble more complex surgical techniques, an interval of 20 minutes was observed before closing the gastric incision with a continuous extra-mucosal technique using a 4/0 mid-term absorbable synthetic glyconate monofilament thread (Monosyn®, B Braun, Aesculap AG, Tuttlingen, Germany).

1.2.2 Sleeve Gastrectomy

Sleeve gastrectomy was performed by dissection of the greater curvature including the lower part of the stomach and the aglandular forestomach with ligation of the short gastric vessels accordingly to a technique described before (de Bona Castelan et al., 2007; Lopez et al., 2009) [Figure 2A].

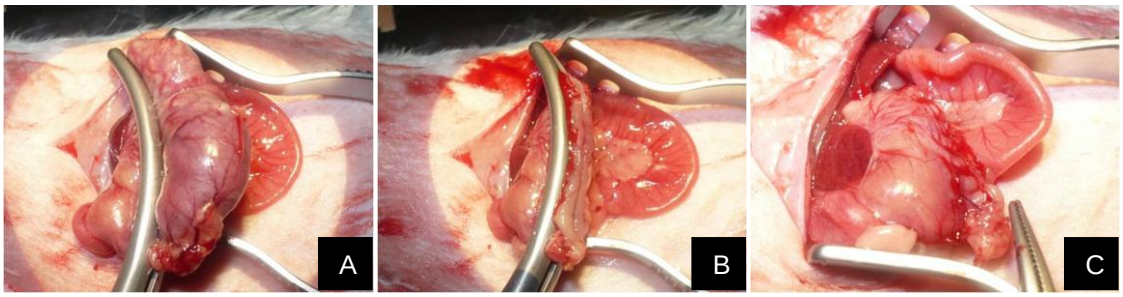

Figure 2 Rat GKSG#5: Sleeve gastrectomy

The stomach was sectioned over a bulldog clamp to fashion the gastric sleeve. The operative specimen, including most of the forestomach, was removed [Figure 2B]. The gastric wound was closed with a continuous invaginating extra-mucosal suture with a 4/0 mid-term absorbable synthetic glyconate monofilament thread as described above (Monosyn®, B Braun, Aesculap AG, Tuttlingen, Germany) [Figure 2C].

1.2.3 Gastric Bypass

Gastric bypass was done in a similar fashion as in humans and described in rats (Meguid et al., 2004). The mid portion of the stomach of animals allocated to gastric bypass was freed from adhesions on the greater and lesser curvature. Horizontal section of the stomach was performed leaving only a small rim of glandular stomach on the proximal part [Figure 3A]. The distal stomach was closed using a 4/0 mid-term absorbable monofilament suture (Monosyn®, B Braun, Aesculap AG, Tuttlingen, Germany), in an invaginating extra-mucosal technique [Figure 3B].

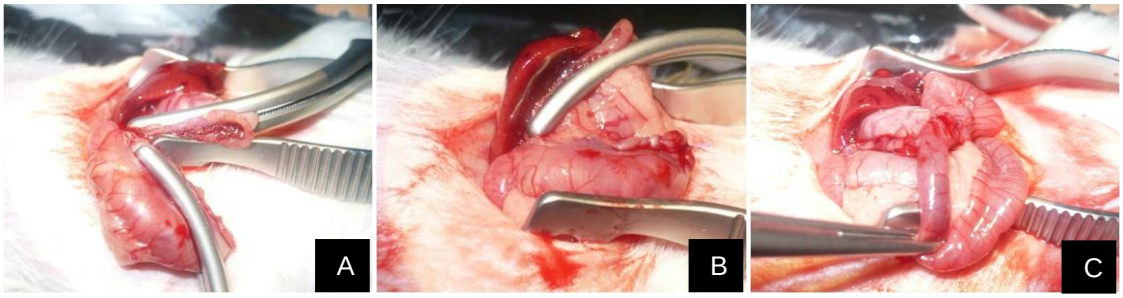

Figure 3 Rat GKGB#4 Gastric bypass

Beginning at the lesser curvature, the proximal stomach was closed in a similar fashion, leaving a small opening close to the greater curvature to perform the gastro-jejunal anastomosis. The angle of Treitz was identified and 10 cm were measured on the proximal jejunum. The jejunal loop was brought up to the proximal stomach and a 2 cm incision was made on the antimesenteric border of the bowel wall. The gastro-jejunal anastomosis was performed in an end-to-side fashion with a extramucosal continuous suture using a 5/0 mid-term absorbable synthetic monofilament suture of glyconate (Monosyn®, B Braun, Aesculap AG, Tuttlingen, Germany) [Figure 3C]. No mesenteric defects were closed.

1.3. Glucose, Lipids, Glycated Hemoglobin and Hormones

Glycemia was determined in the tail vein through a glucose-oxidase reaction using a commercially available glucometer with appropriate test strips (Glucometer Elite, Bayer SA, Portugal). Serum cholesterol and triglycerides were determined using a portable analyzer (Accutrend GCT, Roche

Diagnostics, F. Hoffmann-La Roche Ltd., Switzerland). Glycated hemoglobin was measured using a Siemens DCA 2000+ Analyzer (Siemens AG, Germany).

GLP-1, PYY, ghrelin and glucagon were analyzed by competitive enzyme-linked immunosorbent assays (Wako Pure Chemical Industries Ltd., Osaka, Japan and Peninsula Laboratories, LLC, Bachem Group, San Carlos, CA, USA), using specific antibodies that bind to sample and biotinylated tracer, respectively. A 96-well microtiter plate coated with a secondary antibody capturing the primary antibody bound to sample or tracer was subsequently submitted to a reaction of captured tracer with HRP-conjugated streptavidin, which produces a colored or fluorescent product after adding a substrate.

Plasma insulin was determined in a similar fashion using a non-competitive solid phase two-site enzyme immunoassay based on the sandwich technique (Mercodia AB, Uppsala, Sweden) where insulin in the sample reacts to specific antibodies bound to the microtiter plate and peroxidase-conjugated antibodies in a solution producing a colored solution together with a substrate.

Duplicate samples of all gut hormones were measured using a microplate reader with Gen5^{TM} software (Synergy HT, Biotek, Winooski, VT, USA).

Insulin sensitivity was assessed by a mathematical model (quantitative insulin sensitivity check index [QUICKI]) calculating the reciprocal index of the sum of logarithms for plasma glucose in mg/dL and plasma insulin in µU/mL as described elsewhere (Katz et al., 2000; Muniyappa et al., 2009). The area under the curve (AUC) was calculated for post-prandial insulin and glucagon secretion using the trapezoidal method.

2. Clinical Study

2.1 Patients

From February 2008 to July 2010, SG as a stand-alone treatment for severe or morbid obesity was performed in 10 patients with T2D and 13 patients with IFG as defined by American Diabetes Association criteria (blood sugar ≥ 126 mg/dl or ≥ 100 mg and ≤ 125 mg/dl, respectively, or on antidiabetic medication). Six patients were on oral antidiabetics. 19 patients were female (82.6%) and mean age was 45.3±2.1 years (20 – 62 years). All patients were assessed preoperatively by a multidisciplinary team including surgeon, nutritionist and psychologist. Other medical specialties were involved accordingly to clinical requirements. Pre-operative work-up included ECG, chest radiography, upper endoscopy, abdominal ultrasound and blood testing with endocrine screening. After signing an informed consent, patients were submitted to laparoscopic SG. Peri-operatively, insulin was administered subcutaneously to control blood sugar levels. No post-operative complications occurred and all patients were dismissed from hospital on a liquid diet on day 2 after surgery.

Patients were reassessed at the outpatient department at day 9 and 1, 3, 6, 12, and 24 months after surgery. A soft diet was started in the third week after surgery and a solid diet 2 – 3 weeks later. At one month, subcutaneous insulin accordingly to blood sugar levels could be discontinued in all patients. BMI, fasting glycemia and fasting insulin (assessed by standard laboratorial methods in venous blood) were registered before and 3, 12 and 24 months after surgery. Patients with fasting blood glucose (FBG) <100 mg/dl without medication were considered in remission. Mean preoperative BMI was 41.4±1.1 kg/m2 (range 33.4[5] – 52.4 kg/m2), FBG was 122.9±6.7 mg/dl (range 97[6] – 254 mg/dl) and fasting insulin was 22.6±2.4 µIU/ml (range 4.6 – 41.8 µIU/ml). Beta-cell function and insulin resistance were calculated from fasting glucose and insulin using the updated homeostasis model assessment (HOMA) calculator version 2.2, available from the Diabetes Trials Unit website (http://www.dtu.ox.ac.uk) (Levy et al., 1998; Matthews et al., 1985). Twenty-two out of 23 patients were

[5] One patient with BMI < 35 kg/m2 at date of surgery due to standard pre-operative diet.
[6] One patient on oral antidiabetics with fasting glycemia < 100 mg/dl.

available for follow up at 3 months, 20 out of 23 patients at 12 months, and 18 out of 23 patients at 24 months.

2.2 Surgical Technique

Laparoscopic SG was carried out under general anesthesia, eventually combined with a thoracic epidural catheter, in a standardized fashion using a 4-port technique and a Nathanson liver retractor inserted through a separate epigastric incision.

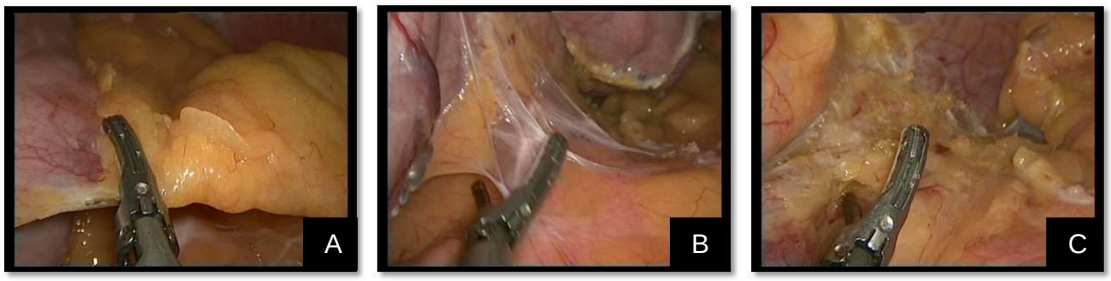

Figure 4 Patient 9: Gastric mobilization

During surgery, special attention was given to a complete mobilization of the gastric fundus and the His' angle, with complete dissection of retrogastric adhesions [Figure 4A – 4C].

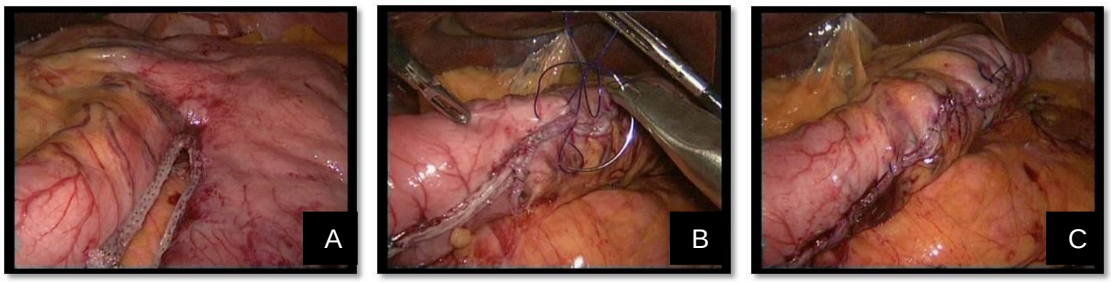

Figure 5 Patient 9: Gastric transection and oversewing of the staple line

The stomach was transected over a 36F bougie beginning 4 – 6 cm from the pylorus using an endoscopic stapler with 6 cm cartridges and adequate staple height regarding the thickness of the gastric wall [Figure 5 A]. The staple line was oversewn with a running suture using a 2/0 mid-term absorbable synthetic glyconate monofilament thread in all patients [Figure 5B, 5C]. A suction drain was left routinely in the beginning of the experience.

3. Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM) regarding the number of experiments described. The Shapiro-Wilk test was used to assess if samples were extracted from a variable that follows a normal distribution. For samples extracted from normally distributed variables, the Student's t-test was used to compare means of two independent samples. For non-normally distributed variables, the non-parametric Mann-Whitney test was used to reject or keep the null hypothesis that there were no differences in means of groups (H_0: $\mu_1 = \mu_2$). Differences were considered statistically significant at a level of 95% ($p < 0.05$).

To reduce type I errors induced by repeated t-tests, analysis of variance (ANOVA) was employed to describe differences between more than two groups regarding the same variable or repeated measures of a variable in the same group, if normal distribution and homogeneity of variance assessed by the modified Levene's test could be assumed. The Tukey's HSD (honest significant difference) test was used to perform post-hoc comparisons between samples. Variables that presented either with non-normal distribution or heteroscedasticity were analyzed using the non-parametric Kruskal-Wallis and post-hoc analysis including multiple pairwise comparisons using the Steel-Dwass-Critchlow-Fligner procedure test (Adams et al., 2009; Ferreira et al., 2012; Kruskal and Wallis, 1952; Van Hecke, 2010).

RESULTS

RESULTS[7]

1. Experimental Study

1.1. Weight and Lipids

Wistar rats were heavier than age-matched GK rats [Table 1]. Animals in control groups (WIC, GKC) or submitted to sham surgery (GKSS) sustained statistically significant weight gain during the observation period. No significant weight changes occurred in the groups submitted to SG (GKSG) or GB (GKGB), if compared to pre-operative data [Table 1].

Pre-operative values for serum cholesterol were similar in all groups. Rats submitted to gastric bypass experienced a small but statistically significant amelioration of pre-operative values ($p < 0.005$), whereas rats submitted to SG did not sustain any significant alteration [Table 1]. Multiple comparison by ANOVA with post-hoc analysis revealed that, in comparison to both control groups (WIC, GKC) and GKSS, post-operative serum cholesterol was significantly lower in GKGB.

All groups sustained a decrease of serum triglycerides during the observation period [Table 1]. However, only in rats submitted to SG the amelioration of serum triglycerides was statistically significant if compared to pre-operative values ($p < 0.05$). The comparison of serum triglycerides between groups at the end of the observation period showed no statistically significant difference [Table 1].

1.2. Glucose Metabolism

Fasting glycemias at baseline were similar in all groups (78±2.1 - 106±13.0 mg/dl), except for GKSS. Due to maturation of young animals, WIC presented with significantly lower fasting glycemias (72±3.9 mg/dl) than GK rats in all groups (106±4.9 - 136±9.2 mg/dl) at the end of the observation period. Random glycemias at the end of the observation period were also lower in WIC (87±2.4

[7] Adapted from published articles or manuscripts submitted for publication.

mg/dl) in comparison to GK rats ($p<0.02$), except for rats submitted to SG (106±12.3 mg/dl) [Table 1].

Table 1 Experimental study in 50 rats: Weight, blood glucose, glycated hemoglobin and lipids at baseline and four weeks after surgery

	WIC	GKC	GKSS	GKSG	GKGB
Weight (g) baseline	328±11.9[a]	256±9.0	270±13.4	277±7.3	284±9.2
Weight (g) 4 weeks	388±9.1***[b]	308±8.0***	330±11.5**	296±17.8	303±12.5
Fasting glycemia (mg/dl) baseline	78±2.1[c]	102±11.3	110±10.9	97±8.3	106±13.0
Fasting glycemia (mg/dl) 4 weeks	72±3.9[d]	106±4.9	111±5.7	112±5.3*	136±9.2*
Random glycemia (mg/dl) 4 weeks	87±2.4[e]	169±30.4	153±20.5	106±12.3	129±13.2
HbA1c (%) baseline	3.5±0.1[d]	4.8±0.3	4.8±0.3	5.1±0.3	5.0±0.3
HbA1c (%) 4 weeks	3.3±0.1[b]	5.0±0.2	4.6±0.2	3.8±0.1***[f]	4.0±0.2*[g]
Cholesterol (mg/dl) baseline	161±2.1	159±1.6	164±1.2	161±1.4	163±1.9
Cholesterol (mg/dl) 4 weeks	162±1.4	164±1.4	162±1.5	158±1.9	156±1.1***[h]
Triglycerides (mg/dl) baseline	123±5.5	128±11.2	142±18.3	134±8.8	123±4.2
Triglycerides (mg/dl) 4 weeks	115±5.0	114±6.4	116±12.0	106±6.3*	108±11.6

WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). All values are given with standard error of the mean (SEM). The Mann-Whitney test or Student's t-test were used to assess for differences between baseline and values at the end of the observation period (*$p<0.05$, **$p<0.01$, ***$p<0.005$). The Kruskal-Wallis test or ANOVA were used to assess for intergroup differences ([a]$p<0.05$ in comparison to GKC, GKSG, GKGB; [b]$p<0.05$ in comparison to GKC, GKSS, GKSG, GKGB; [c]$p<0.05$ in comparison to GKSS; [d]$p\leq0.005$ in comparison to GKC, GKSS, GKSG, GKGB; [e]$p<0.02$ in comparison to GKC, GKSS, GKGB; [f]$p<0.05$ in comparison to GKC, GKSS; [g]$p<0.05$ in comparison to GKC; [h]$p<0.05$ in comparison to WIC, GKC, GKSS).

Overall glycemic control assessed by determination of HbA1c was significantly improved in GK rats submitted to SG ($p < 0.005$) or GB ($p < 0.05$), when compared to pre-operative values [Table 1]. Wistar rats also improved their levels of HbA1c during the observation period, but without statistical significance. No amelioration was observed in either sham operated or control group GK rats. Comparing overall glycemic control at the end of the observation period between groups, GKSG rats showed statistically significant improvement in comparison to GKC and GKSS [Table 1]. No significant difference in comparison to animals submitted to GB was observed. Despite of statistically significant amelioration of overall glycemic control in GK rats submitted to either

SG or GB, animals in these two groups did not achieve a glycemic control comparable to WIC ($p < 0.05$).

1.3. Insulin and Insulin Sensitivity

Fasting plasma insulin levels were similar in Wistar and GK rats, whether submitted to surgery or not. Meal induced plasma insulin concentrations at 20 minutes were significantly higher in GK rats (all groups) in comparison to WIC ($p<0.05$). At 30 minutes, plasma insulin was highest in GKSG (17.48±0.75 µg/L; $p<0.05$ in comparison to all other groups) and lowest in GKGB (9.21±0.39 µg/L). Similar results were observed at 40 minutes. However, at 60 minutes both GKSG and GKGB presented with lower plasma insulin than other groups (5.95±0.28 and 4.77±0.63 µg/L; $p<0.05$). Time series revealed a significant rise in comparison to baseline at 20 minutes in all groups ($p<0.05$), but whereas GKC and GKSS maintained similar values throughout all following timepoints, a significant reduction in plasma insulin concentration was observed in WIC, GKSG and GKGB at 60 minutes in comparison to the previous timepoint ($p<0.05$) [Figure 6].

Total insulin secretion during the first 60 minutes after a liquid mixed-meal expressed as AUC was highest in GKC (614±18.3 µg L^{-1} h; $p<0.05$ in comparison to GKSS, GKGB) and lowest in GKGB (396±15.3 µg L^{-1} h; $p<0.05$ in comparison to all other GK). Differences between Wistar and GK rats in all groups were not statistically significant [Table 2].

Insulin sensitivity was assessed using QUICKI as described above. GK rats in the control group and submitted to sham surgery presented with a lower insulin sensitivity (0.2601 ± 2.5^{-3} and 0.25 ± 5.3^{-3}, respectively) than Wistar rats (0.284 ± 1.9^{-3}; $p<0.05$). GKSG and GKGB had slightly improved indexes in comparison to GKC and GKSS, and no significant differences in comparison to Wistar rats were observed [Table 2].

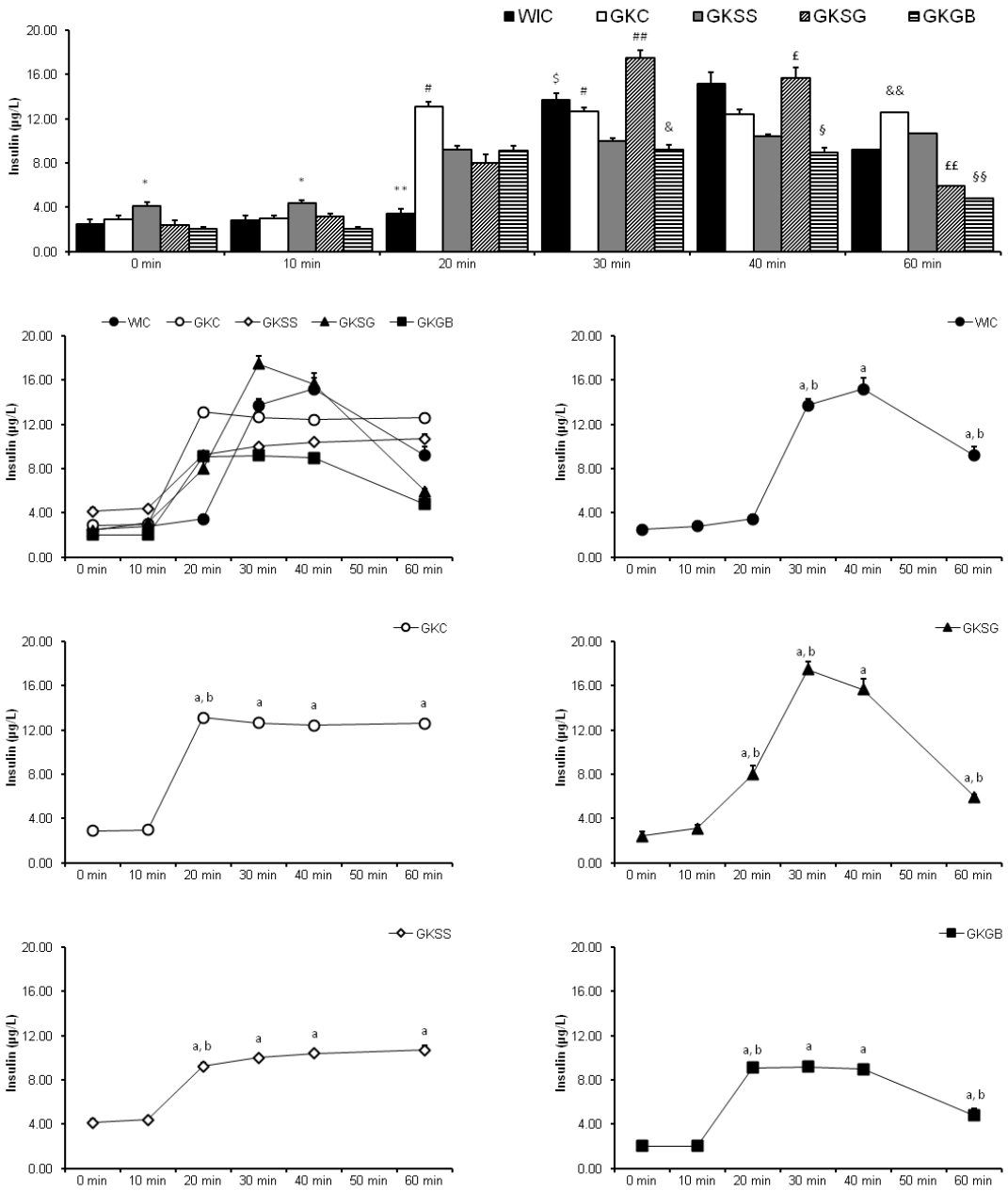

Figure 6 Insulin – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKGB; **p<0.05 in comparison to GK (all subgroups); $^{\#}$p<0.05 in comparison to GKSS, GKSG, GKGB; $^{\$}$p<0.05 in comparison to GKSG, GKGB; $^{\#\#}$p<0.05 in comparison to all other groups; $^{\&}$p<0.05 in comparison to WIC, GKC, GKSG; $^{£}$p<0.05 in comparison to GKSS, GKGB; §p<0.05 in comparison to WIC, GKC; $^{\&\&}$p<0.05 in comparison to WIC, GKSG, GKGB; $^{££}$p<0.05 in comparison to GKC, GKSS; §§p<0.05 in comparison to WIC, GKC, GKSS. ^{a}p<0.05 in comparison to baseline; ^{b}p<0.05 in comparison to previous timepoint.

1.4. Glucagon and Glucagon-Insulin Ratio

GK rats submitted to gastric bypass presented with higher fasting plasma glucagon than all other groups (405.86±30.14 pg/mL; $p<0.05$ in comparison to GKC, GKSG) and maintained this pattern at 10 and 20 minutes after the test meal. However, at 30 minutes plasma glucagon increased significantly in rats submitted to SG (676.04±18.01 pg/mL), as well as, to a lesser extent, in rats submitted to GB (652.37±39.69 pg/mL). Hormone levels remained stable at a high level in both groups up to 60 minutes ($p<0.05$ in comparison to WIC, GKC, GKSS for GKSG; $p<0.05$ in comparison to GKC, GKSS for GKGB). In all control groups, a slight and not statistically significant increase in plasma glucagon was observed at 30 minutes, without any further change [Figure 7]. Total glucagon secretion during the postprandial period described as AUC was significantly higher in GKSG (29.9±0.8 ng mL^{-1} h) and GKGB (31.5±1.7 ng mL^{-1} h) in comparison to GKC (15.0±3.0 ng mL^{-1} h) and GKSS (18.1±2.0 ng mL^{-1} h) [Table 2].

Table 2 Experimental study in 50 rats: Insulin sensitivity, plasma insulin, and glucagon at four weeks after surgery

	WIC	GKC	GKSS	GKSG	GKGB
Insulin sensitivity (QUICKI) 4 weeks	$0.284±1.9^{-3\,a}$	$0.2601±2.5^{-3}$	$0.25±5.3^{-3}$	$0.2678±6.4^{-3}$	$0.2659±4.4^{-3}$
Insulin AUC (µg L^{-1} h) 4 weeks	532±29.3	614±18.3[b]	521±12.6	592±19.6[c]	396±15.3[d]
Glucagon AUC (ng ml^{-1} h) 4 weeks	22.9±2.7	15.0±3.0	18.1±2.0	29.9±0.8[a]	31.5±1.7[a]

WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass), AUC (= area under the curve). All values are given with standard error of the mean (SEM). The Kruskal-Wallis test was used to assess for intergroup differences ([a]$p<0.05$ in comparison to GKC, GKSS; [b]$p<0.05$ in comparison to GKSS; [c]$p<0.05$ in comparison to GKGB; [d]$p<0.05$ in comparison to GKC, GKSS, GKSG).

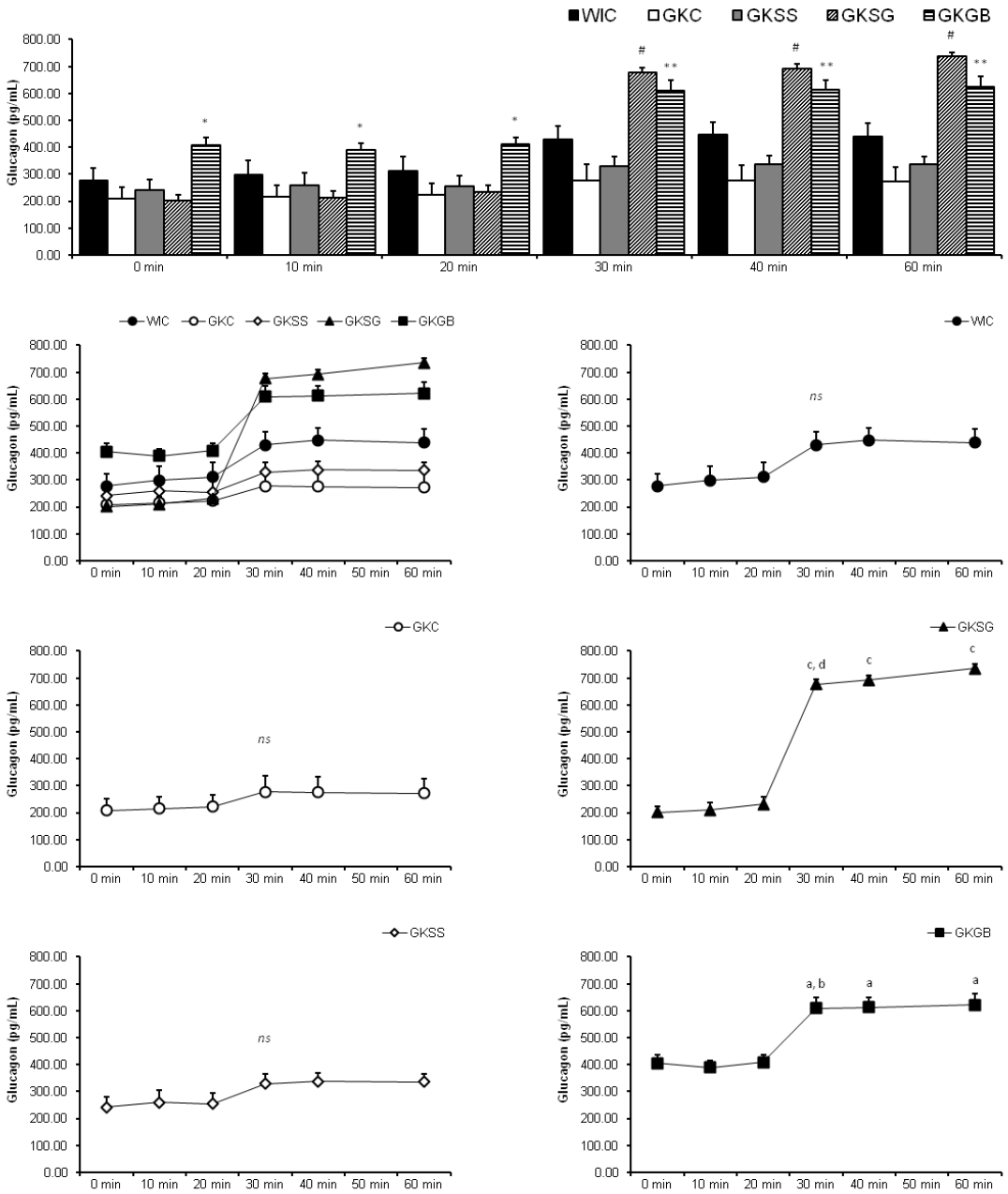

Figure 7 Glucagon – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKC, GKSG; #p<0.05 in comparison to WIC, GKC, GKSS; **p<0.05 in comparison to GKC, GKSS. [a]p<0.05 in comparison to baseline; [b]p<0.05 in comparison to previous timepoint; [c]p<0.01 in comparison to baseline; [d]p<0.01 in comparison to baseline.

Calculation of glucagon-insulin ratio (GIR) revealed higher values for GKGB in comparison to GKC and GKSS at baseline ($p<0.05$) and to GKC, GKSS and GKSG ($p<0.05$) at 10 minutes after the test meal. However, a significant decrease in GIR occurred at 20 minutes ($p<0.05$). At this timepoint, WIC maintained a stable GIR (1.0-4±1.68-5) in comparison to previous timepoints, significantly higher ($p<0.05$) than GIR in GK rats (all groups). However, at all following timepoints up to 60 minutes GKGB showed an increased GIR in comparison to all control groups ($p<0.05$). GK rats submitted to sleeve gastrectomy showed a significant increase in GIR at 60 minutes (1.26-4±6.1-6), achieving a ratio similar to GKGB, and higher than other groups ($p<0.05$ in comparison to WIC, GKC, GKSS) [Figure 8].

1.5. Ghrelin

Regarding fasting ghrelin, no differences between groups were observed. However, after administration of a mixed liquid meal as described above, GK rats submitted to SG or GB sustained a significant decrease in plasma ghrelin, 30 minutes after the test meal that was maintained up to 60 minutes, without any further significant changes. WIC showed a similar pattern, whereas GKC and GKSS sustained no significant change in plasma ghrelin at the defined time points [Figure 9].

Ten and 20 minutes after the test meal, no difference in plasma ghrelin were observed between groups. At 30 min, WIC, GKSG, and GKGB showed a significant decrease in comparison to GKC. At 40 minutes, plasma ghrelin levels were significantly lower in GKSG in comparison to WIC, GKC and GKSS. Both GKSG and GKGB expressed significantly lower plasma ghrelin levels in comparison to control groups (WIC, GKC), 60 minutes after the test meal [Figure 9].

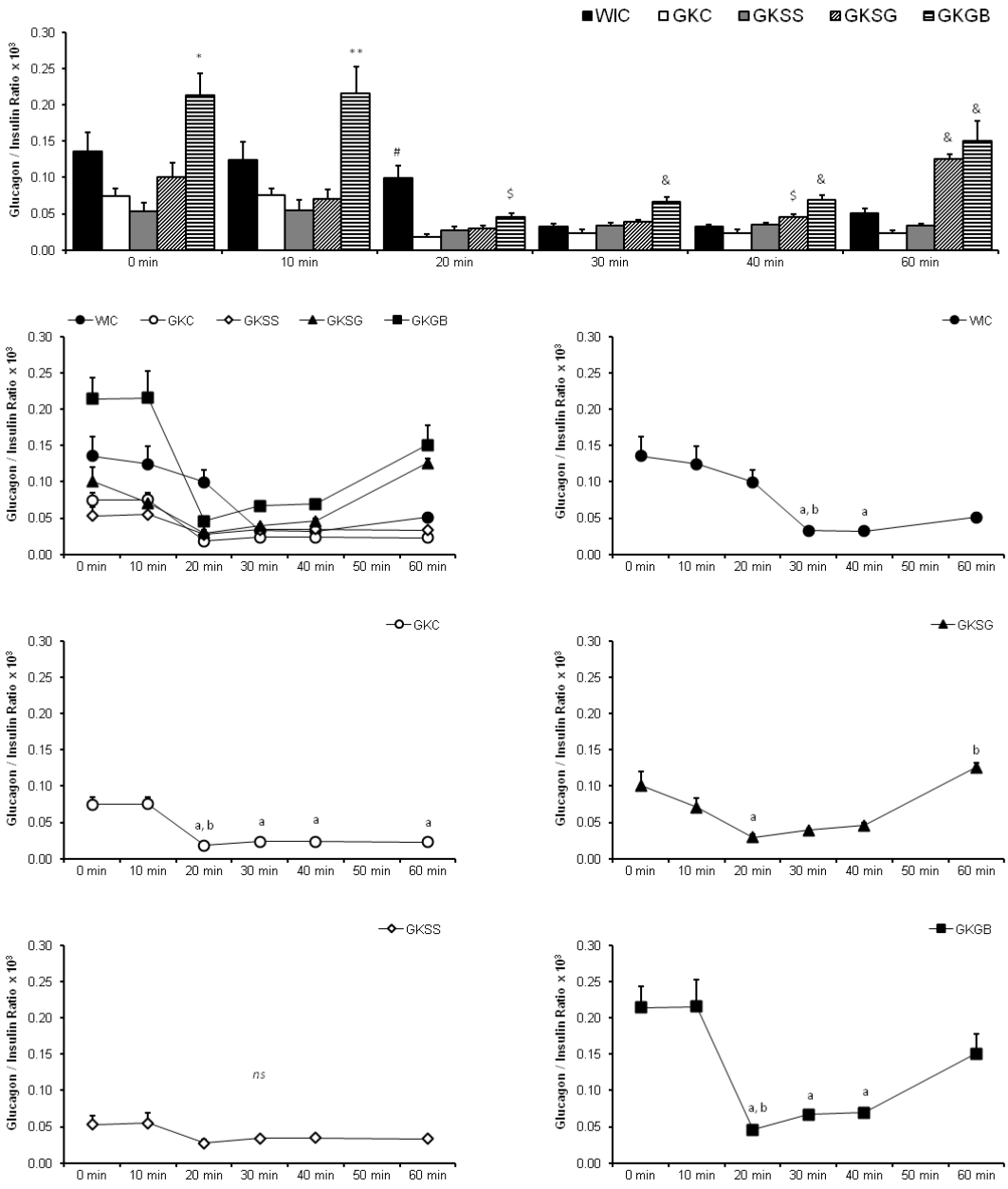

Figure 8 Glucagon-insulin ratio – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKC, GKSS; **p<0.05 in comparison to GKC, GKSS, GKSG; #p<0.05 in comparison to GK (all subgroups); $p<0.05 in comparison to GKC; &p<0.05 in comparison to WIC, GKC, GKSS. ap<0.05 in comparison to baseline; bp<0.05 in comparison to previous timepoint.

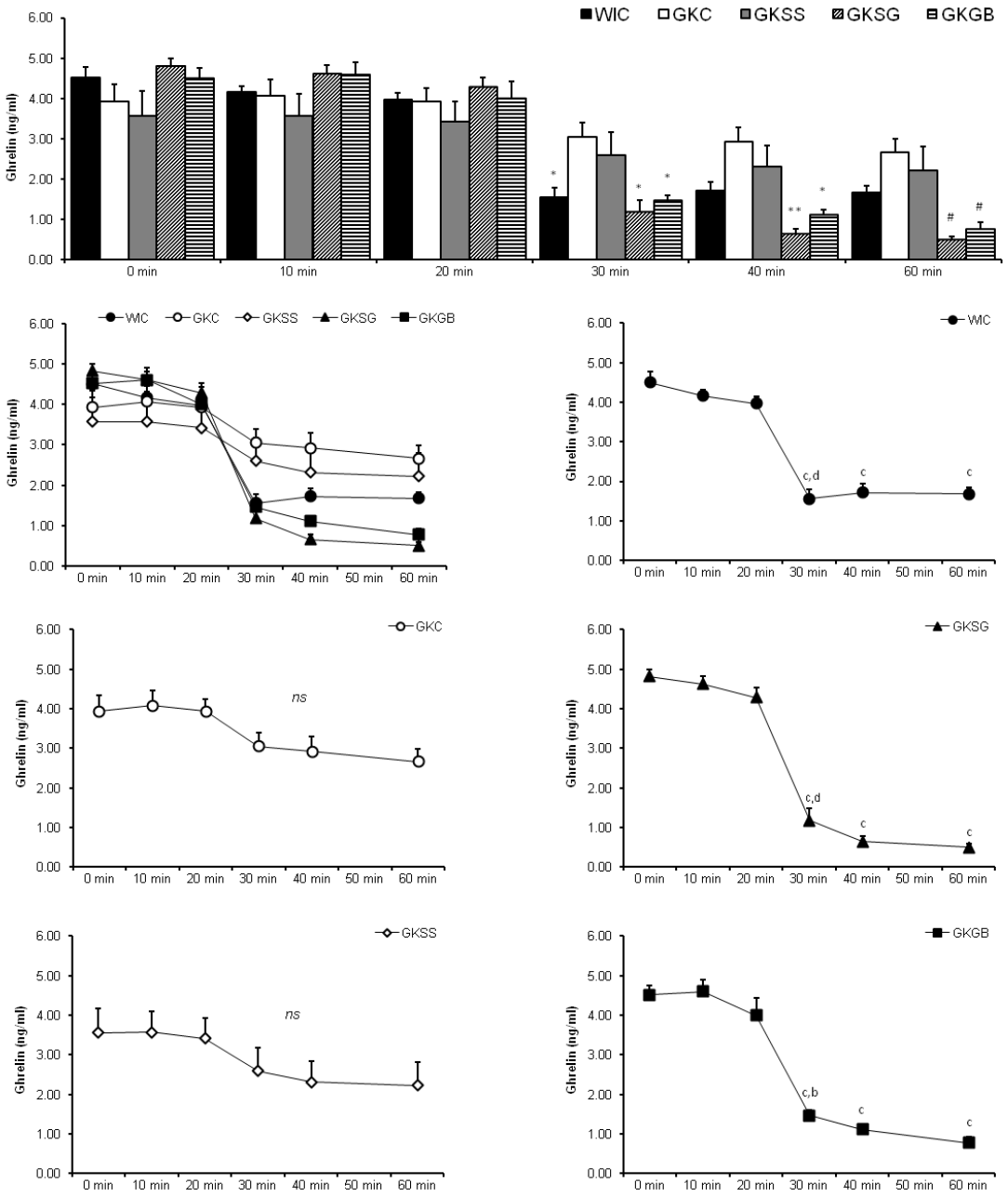

Figure 9 Ghrelin – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKC; **p<0.05 in comparison to WIC, GKC, GKSS; $^{\#}$p<0.05 in comparison to WIC, GKC. ^{b}p<0.05 in comparison to previous timepoint; ^{c}p<0.005 in comparison to baseline; ^{d}p<0.005 in comparison to previous timepoint.

1.6. Peptide Tyrosine Tyrosine

Wistar rats presented with significantly higher fasting PYY levels than GK rats, independent of allocated treatment. Postprandial, there was a significant rise of plasma PYY in WIC, 30 minutes and 60 minutes after the test meal. GK rats in both control groups showed a slow rise in postprandial PYY, beginning at 40 minutes. However, GKGB showed a significant rise in PYY levels at all time points, starting as early as ten minutes postprandially. GKSG showed a similar pattern, starting 20 minutes after the test meal [Figure 10].

No significant differences in plasma PYY were observed between WIC and GKGB, ten minutes after the test meal. At 20 minutes, GK rats in both control groups (GKC, GKSS) had significantly lower plasma PPY levels than all other groups. At this time point, no differences were observed between WIC and GKSG or GKGB. GK rats in both control groups (GKC, GKSS) maintained significantly lower plasma PYY at all time points from 20 minutes onwards, in comparison to WIC and GK rats allocated to SG or GB. However, GKGB showed significantly higher levels in postprandial plasma PYY than GKSG, up to 60 min after the test meal [Figure 10].

1.7. Glucagon-like Peptide-1

Fasting levels of GLP-1 were higher in GK rats in comparison to Wistar rats, particularly in animals submitted to SG or GB ($p<0.05$). After administration of the test meal, a significant rise in plasma GLP-1 was observed in WIC, as early as ten minutes postprandial, with another significant increment during the next ten minutes. GKSG experienced a similar rise in GLP-1 after the test meal, starting a little later at 20 min, whereas GKGB sustained a significant increment in plasma GLP-1 at 30 min, reaching a plateau thereafter. Only a very small meal-induced rise in plasma GLP-1 was observed in GK rats in both control groups [Figure 11].

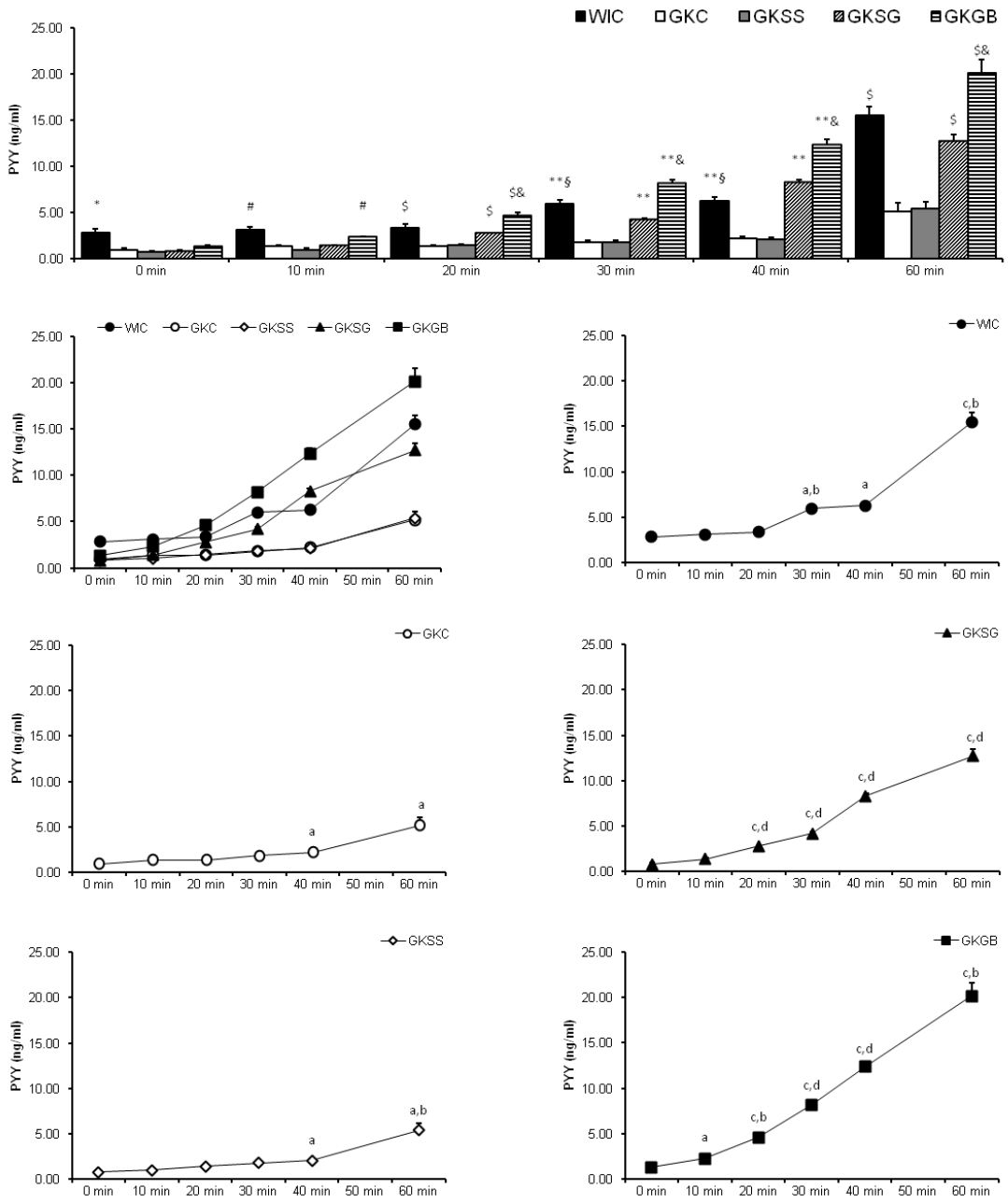

Figure 10 PYY – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKC, GKSS, GKSG, GKGB; $^{\#}$p<0.05 in comparison to GKC, GKSS, GKSG; $^{\$}$p<0.05 in comparison to GKC, GKSS; $^{\&}$p<0.05 in comparison to GKSG; §p<0.05 in comparison to GKSG, GKGB; **p<0.05 in comparison to all other groups. ^{a}p<0.05 in comparison to baseline; ^{b}p<0.05 in comparison to previous timepoint; ^{c}p<0.005 in comparison to baseline, ^{d}p<0.005 in comparison to previous timepoint.

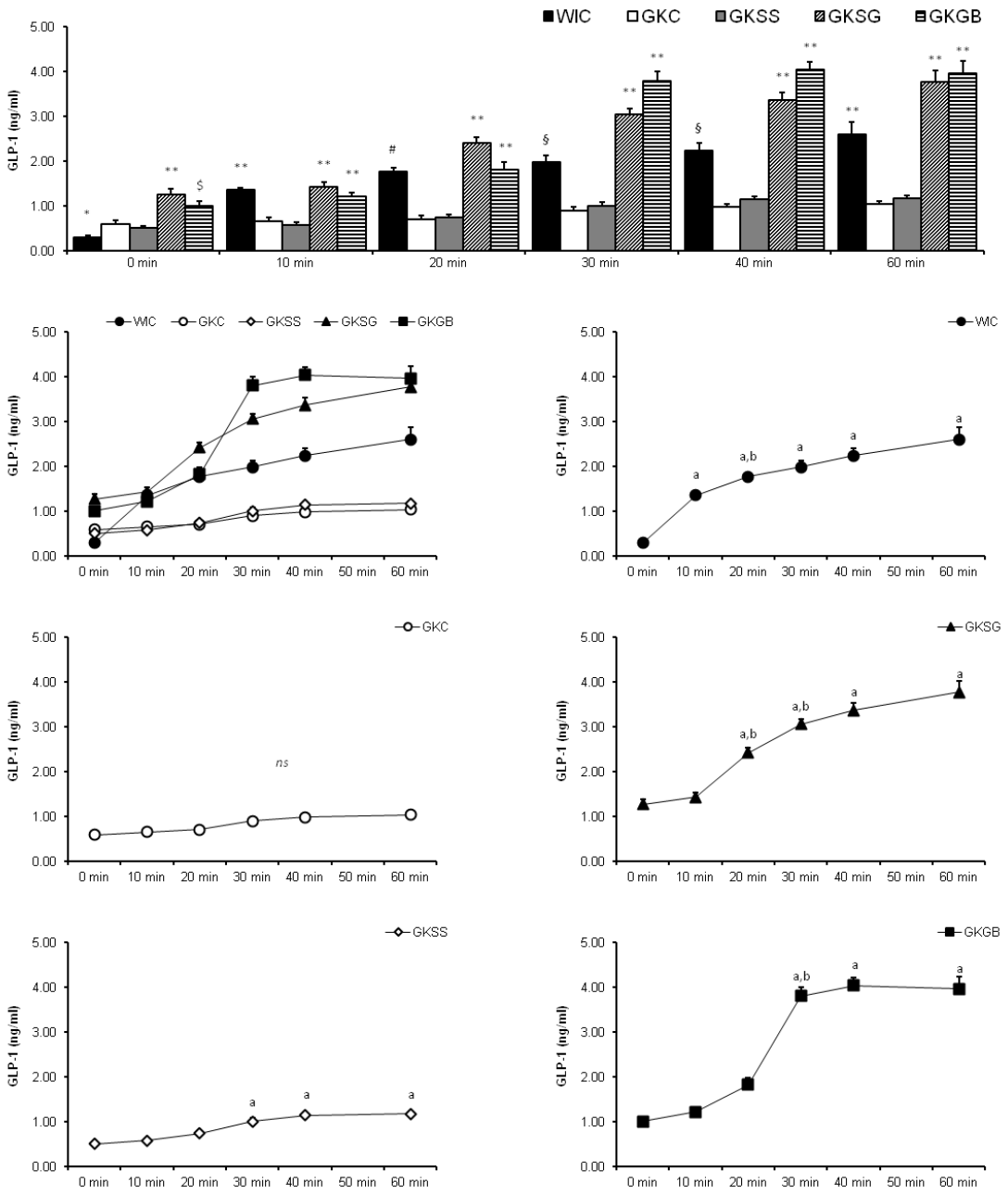

Figure 11 GLP-1 – meal-induced response. WIC (= Wistar rats – control), GKC (= Goto-Kakizaki rats – control), GKSS (= Goto-Kakizaki rats – sham surgery), GKSG (= Goto-Kakizaki rats – sleeve gastrectomy), GKGB (= Goto-Kakizaki rats – gastric bypass). Kruskal-Wallis test with post-hoc analysis: *p<0.05 in comparison to GKSS, GKSG, GKGB; **p<0.05 in comparison to GKC, GKSS; $p<0.05 in comparison to GKSS; #p<0.05 in comparison to GKC, GKSS, GKSG; §p<0.05 in comparison to all other groups. ap<0.05 in comparison to baseline; bp<0.05 in comparison to previous timepoint.

Plasma levels of GLP-1 reached similar values in Wistar and GKSG or GKGB as soon as ten minutes postprandial. GLP-1 levels in these three groups were significantly higher in comparison to GK rats in both control groups at all time points postprandial. Plasma GLP-1 was significantly higher in GKSG and GKGB when compared to Wistar rats, 30 and 40 minutes after the test meal. However, no more significant differences could be observed between these three groups, 60 minutes postprandial [Figure 11].

2. Clinical study

After SG, a significant decrease in BMI occurred, as early as 3 month after surgery (BMI 33.0±1.0 kg/m2). Maximum weight loss occurred until one year after surgery (BMI 27.8±0.7 kg/m2) and remained stable at 2 years of follow-up (BMI 28.3±0.8 kg/m2). Post-operative BMI was significantly lower than preoperative BMI at all time points (p<0.001, ANOVA with post-hoc analysis) [Figure 12].

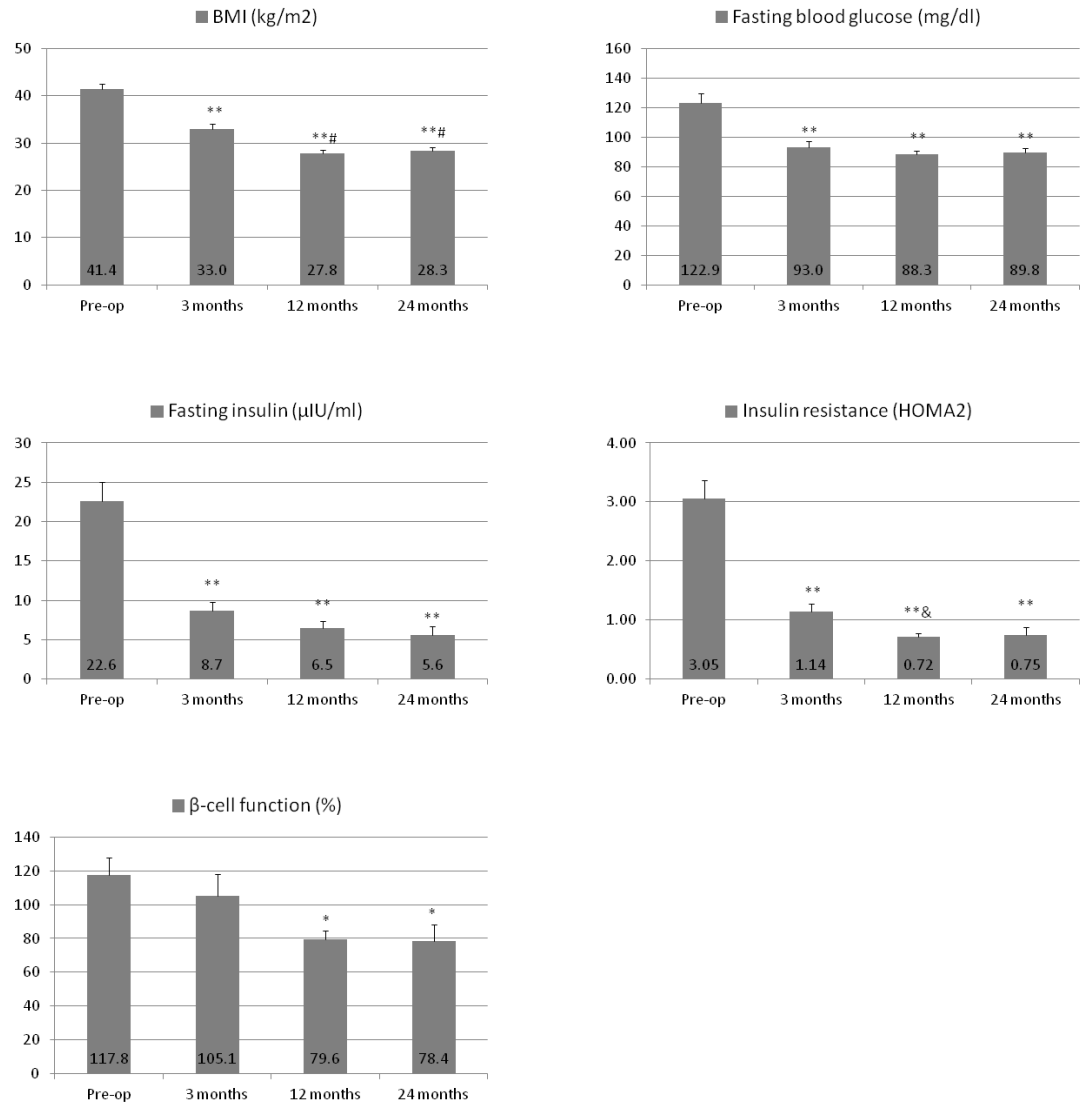

Figure 12 Clinical study in 23 patients submitted to sleeve gastrectomy: Pre- and post-operative body mass index, fasting blood glucose, fasting insulin, insulin resistance, and beta-cell function. BMI (= body mass index). ANOVA and Tukey HSD post-hoc analysis (Kruskal-Wallis test with post-hoc analysis for fasting insulin and insulin resistance): *p<0.05 in comparison to baseline; **p<0.001 in comparison to baseline; $^{\#}$p<0.01 in comparison to results at 3 months; $^{\&}$p<0.05 in comparison to results at 3 months.

Fasting blood glucose decreased from 122.9±6.7 mg/dl to 93.0±4.0 mg/dl (3 months), 88.3±2.7 mg/dl (12 months), and 89.8±2.9 mg/dl (24 months). Differences between all post-operative time points and pre-operative values were statistically significant ($p<0.001$, ANOVA with post-hoc analysis), but after 3 months of follow-up, fasting glycemias remained stable, despite continuing weight loss until 12 months of follow-up [Figure 12]. In 87.5% of patients, fasting blood glucose normalized completely to <100 mg/dl. Concomitantly, fasting insulin fell from 22.6±2.4 µIU/ml pre-operatively to 8.7±1.1 µIU/ml at 3 months, 6.5±0.9 µIU/ml at 1 year, and 5.6±1.1 µIU/ml at 2 years. Statistical analysis showed a significant decrease as early as 3 months after surgery, without any significant changes thereafter ($p<0.001$, Kruskal-Wallis-Test) [Figure 12].

Mean pre-operative beta-cell function calculated by the updated homeostasis model assessment using a computer program (HOMA2), was slightly above normal (117.8±9.9%), but three patients presented with a beta-cell function <40%, whereas in all other patients function was >70%. During follow-up, mean beta-cell function decreased slightly but not significantly to 105.1±13.2% at 3 months.

Table 3 Body mass index, beta-cell function and insulin resistance in three patients with impaired pre-operative beta-cell function

	P1 *pre-op.*	**P1** *2 years*	**P2** *pre-op.*	**P2** *2 years*	**P3** *pre-op.*	**P3** *2 years*
BMI (kg/m^2)	33.9*	26.0	45.1	29.3	36.6	29.7
FBG (mg/dl)	137	109	143	82	254	118
Fasting insulin (µIU/ml)	5.6	4.3	8.1	9.9	14.0	9.8
Beta-cell function (%)	33.3	43.8	39.8	66.5	21.7	50.6
Insulin resistance	0.8	0.6	1.2	0.4	2.4	0.9

BMI (= body mass index), FBG (= fasting blood glucose), P1/2/3 (= patient 1/2/3). *Patient 1 presented with a BMI < 35 kg/m2 at surgery due to standard pre-operative low-calorie diet. Patients available for follow-up: 22/23 at 3 months; 20/23 at 12 months; 18/23 at 24 months.

A significant decline in beta-cell function ($p<0.05$) was observed at 1 year (79.6±5.0%) and at 2 years (78.4±9.8%) [Figure 12]. Interestingly, there was a

slight improvement in patients with a very low pre-operative beta-cell function [Table 3]. Insulin resistance dropped sharply during the first three months from 3-fold increased mean pre-operated values (3.05±0.31) to 1.14±0.13 ($p<0.001$). Another slight decrease was observed during the first year after surgery (0.72±0.06, $p<0.05$), stabilizing thereafter (0.75±0.13) [Figure 12]. Before surgery, 69.6% of patients presented with a markedly increased insulin resistance (>2.0) that decrease to normal or below-normal values (<1.0) in 86.7%.

DISCUSSION

DISCUSSION[8]

In our lean animal model of T2D, overall glycemic control assessed by HbA1c levels improved after SG and GB. Surprisingly, meal-induced insulin secretion diminished after surgery, particularly in rats submitted to GB. However, the reduction in insulin secretion was accompanied by increased insulin sensitivity. Postprandial glucagon secretion was significantly enhanced after surgical procedures, both in comparison to GKC and GKSS rats, as well as non-diabetic Wistar rats. In GKGB, GIR started to increase 30 minutes after the test meal and remained high throughout the observation period. GKSG showed a similar high GIR at 60 minutes. Meal-induced plasma levels of ghrelin, PYY, and GLP-1 were significantly modified by surgical interventions. Although there was no difference in fasting plasma ghrelin levels between groups, WIC and GK rats submitted to either SG or GB sustained a similar postprandial decrease in plasma ghrelin. Wistar rats presented with different levels in fasting PYY and GLP-1 in comparison to GK rats. However, meal induced response was similar in Wistar and in GK rats submitted to SG or GB. GK rats in the control group (GKC) or submitted to sham surgery (GKSS) sustained no improvement of glucose metabolism during the observation period and were characterized by reduced insulin sensitivity, the absence of a significant meal-reduced suppression of ghrelin, as well as a flattened meal-induced increase of PYY and GLP-1.

The results of our study showed no amelioration in fasting glucose in animals submitted to both SG or GB (which incorporates duodenal exclusion) and contrast with previous findings regarding the effects of GB, duodenal exclusion or SG on fasting glucose in GK rats (Donglei et al., 2012; Liu et al., 2011; Pacheco et al., 2007; Rubino and Marescaux, 2004). Nevertheless, other studies could not find significant differences in fasting glucose in GK rats submitted to GJB or other forms of duodenal exclusion in comparison to controls, which is consistent with our results (de Luis et al., 2012; Kindel et al., 2009). However, our study could show amelioration in overall glycemic control

[8] Adapted from published articles or manuscripts submitted for publication.

demonstrated by improved glycated hemoglobin levels in GK rats submitted to SG or GB, not assessed in any of the previous studies.

Analogies regarding beta-cell dysfunction in human T2D and GK rats include reduction in beta-cell mass, alterations in microenvironment in islets, and multiple functional deficits (Portha et al., 2009; Seiça et al., 2003b). As expected, GK rats in the present study showed an impaired metabolic control in comparison to Wistar rats, with significantly worse levels of HbA1c at the beginning and at the end of the observation period. However, rats submitted to SG or GB improved their HbA1c levels significantly, although meal induced insulin secretion (expressed as AUC) did not change (GKSG) or even diminished (GKGB). A similar reduction in insulin secretion has been observed after GJB (Weng et al., 2013). Fasting insulin levels do not seem to be affected by surgery (Donglei et al., 2012). Nevertheless, other studies reported conflicting results after a prolonged follow-up (Speck et al., 2011; Trung et al., 2013). Possibly, the improved insulin sensitivity observed in our study might have contributed to amelioration of glucose metabolism. Furthermore, a decrease in insulin resistance and enhanced glycemic control has been also described after dietary restriction in GK rats, together with modulation of systemic and muscle oxidative stress markers (Rodrigues et al., 2011).

Glucagon secreted from pancreatic α-cells acts as a counterregulatory hormone for insulin and promotes elevation of blood glucose by hepatic glycogenolysis and gluconeogenesis (Jiang and Zhang, 2003). Consequently, a role in the pathophysiology of T2D has been proposed (Quesada et al., 2008; Unger and Orci, 1975; Unger, 1985). Besides secretion in response to low plasma glucose levels, plasma concentration of glucagon is modulated by insulin at an intra-islet level inhibiting secretion at high insulin levels (Bansal and Wang, 2008). Interestingly, the results of our study showed stable levels of plasma glucagon during the postprandial period with a slight rise at 30 minutes in WIC, GKC and GKSS, following a significant increase in plasma insulin at 20 minutes in GK rats (at 30 minutes in WIC). Apparently, no inhibition of glucagon secretion did occur. In rats submitted to SG or GB, a significant increase in plasma glucagon followed a rise in plasma insulin, but with a descending glucagon-insulin ratio that recovered at 60 minutes. Although an increased fasting plasma glucagon

and a delayed glucose-stimulated suppression has been described in humans with T2D (Knop et al., 2012), meal-induced or glucose-stimulated glucagon secretion increased after SG, albeit an improved glucose tolerance (Laferrère et al., 2008; Salehi et al., 2011). A similar postprandial rise in glucagon has been observed in humans with type 1 diabetes, not submitted to surgery, whereas glucagon in β-cell competent individuals remained stable (Cooperberg and Cryer, 2009), suggesting other stimuli for glucagon secretion besides hypoglycemia or decrease of insulin secretion during hypoglycemia.

Impaired pathways regarding the inhibitory effect of hyperglycemia on glucagon secretion, referred to as α-cell resistance, have been identified in studies of α-cell lines (Xu et al., 2006). Interestingly, a switch-off signal from zinc atoms, whether bound to insulin or not, also seems to be involved in initiating glucagon release from the alpha-cell, as could be shown in streptozotocin-induced diabetic Wistar rats (Zhou et al., 2007). The removal of somatostatin-mediated inhibition of glucagon secretion via pancreatic delta-cells by sympathetic activation may further contribute to inadequate glucagon secretion (Hauge-Evans et al., 2010). As suggested by present data, the multifactorial regulation of glucagon secretion and its importance regarding glucose metabolism need to be further elucidated.

The results of our study refer to meal-induced plasma levels of insulin and glucagon regarding a specific type of liquid diet and results may vary with carbohydrate content (Gutniak et al., 1986). Analysis of glucose metabolism was carried out rather early after surgery and might not reflect changes after a more prolonged follow-up. As such, data should be interpreted with caution regarding the long-term impact of bariatric and metabolic surgical procedures on plasma glucagon. However, our data questions the role of hyperglucagonemia in glucose intolerance in a lean animal model of type 2 diabetes and suggests that the role of glucagon in glucose metabolism after bariatric and metabolic surgery deserves further studies.

In obese humans, reported effects of bariatric surgery on fasting ghrelin are contradictory indicating either a reduction after surgery (Cummings et al., 2002; Frühbeck et al., 2004b), or unchanged fasting plasma ghrelin levels in weight-

stable patients. In patients experiencing active weight loss after gastric bypass surgery, even an increase in fasting ghrelin was observed (Borg et al., 2006; Faraj et al., 2003; Holdstock et al., 2003).

In an animal model, GK rats submitted to either SG alone or SG with duodenal-jejunal bypass (SGDJB) showed significantly reduced fasting ghrelin in comparison to controls, two and 16 weeks after surgery (Sun et al., 2012). Similar results were reported by Trung and coworkers regarding fasting levels of des-acyl ghrelin in GK rats and diet-induced obese Wistar rats after sleeve gastrectomy (Trung et al., 2013). No meal-induced measurements were made in both studies.

In contrast, our study did not show any differences in fasting ghrelin between Wistar and GK rats, independent of allocated treatment. Also in a non-diabetic animal model on a high-fat diet, fasting ghrelin levels were similar after GB in comparison to pair-fed controls. However, a significant meal-induced suppression was observed in rats submitted to GB (Shin et al., 2010). Our data showed a significant meal-induced decrease in plasma ghrelin in Wistar rats, not observed in control or sham operated GK rats. Interestingly, a similar postprandial suppression of plasma ghrelin was present in GK rats submitted to SG or GB. Apparently, the dynamic meal-induced response in plasma ghrelin is the distinguishing feature of operated GK rats in comparison to GK controls. As this dynamic response was also present in non-diabetic Wistar rats, our data might indicate that SG and GB restored an apparent defect in meal-induced suppression of plasma ghrelin in GK rats. A recent clinical study showed an analogous association between postprandial ghrelin suppression and postoperative weight loss, insulin sensitivity, and peak GLP-1 secretion in obese diabetic patients submitted to gastric bypass (Samat et al., 2013). Hence, fasting plasma ghrelin levels seem to represent a subordinate factor regarding the effects of surgery.

Ileal interposition in a rodent model of T2D with mild obesity (UCD-T2DM rats) showed ameliorated glucose tolerance and reduced fasting glucose levels, eight months after surgery. Stimulated PYY levels by an oral lipid load were significantly higher than in sham operated animals (Cummings et al., 2010). In another study, SG and SGDJB in GK rats had similar effects on fasting and

stimulated PYY levels, two and 16 weeks after surgery. A significant increase was observed in comparison to sham operated animals and control group at all time points (Sun et al., 2012). In contrast, our study showed no increase in fasting PYY after either SG or GB. Meal-induced PYY levels were significantly increased in GK rats submitted to GB or SG in comparison to sham-operated animals or controls. However, postprandial peak PYY levels after GB were significantly higher than after SG.

In a clinical study, including 58 normal-weight, overweight, or mildly obese diabetic patients submitted to SG with an additional intestinal procedure (ileal interposition or ileal interposition with diversion of the duodenum), improved glycemic control after surgery was observed in over 90% of cases. Hormonal evaluation before and after a mixed meal showed a significant increase in plasma PYY at all time points (fasting, 30 minutes, 60 minutes, 120 minutes). Also fasting and meal-stimulated GLP-1 levels increased significantly after both operations (DePaula et al., 2009). Nonetheless, the specific contribution of either component of the surgical procedure (SG or ileal interposition) regarding changes in gut hormone profiles and glycemic control could not be assessed.

In our lean animal model of T2D, SG alone would increment meal-induced plasma levels of GLP-1 without duodenal diversion or ileal interposition. Similar results were obtained after GB. In diabetic rodents with mild obesity (UCD-T2DM rats), ileal interposition without SG would increment meal-induced response in plasma GLP-1 (Cummings et al., 2010). Interestingly, glucose-stimulated GLP-1 levels in GK rats were significantly higher after SG with duodenal-jejunal bypass than after SG alone, but no measurements after a mixed meal were obtained. Moreover, overall glycemic control was not enhanced by SGDJB in comparison to SG alone (Sun et al., 2012).

In non-diabetic obese patients no significant differences between SG and GB regarding the postoperative meal-induced increment in PYY and GLP-1 could be found (Peterli et al., 2012). To our knowledge, no similar studies have been carried out in non-severely or morbidly obese diabetic patients. A recent study in obese diabetic patients compared the effects of BPD with a long alimentary limb and SG on glycemic control and gut hormones. No significant differences regarding diabetes control and glucose-stimulated increment in GLP-1 and PYY

were found between groups (Tsoli et al., 2013). However, treatment arms were not randomized and indication for surgical intervention was based on BMI (all patients with BMI > 50 kg/m2 were assigned to open BPD).

As could be shown in the present study, and somewhat intriguingly, SG and GB showed similar meal-induced responses in gut hormones like PYY and GLP-1 secreted by L-cells present in the distal ileum. Hence, mechanisms other than direct contact of food with the intestinal mucosa are probably involved in the secretion of gut hormones, yet to be explored. However, the importance of intra-duodenal fat hydrolysis on GLP-1 secretion was documented in healthy volunteers that received an intra-duodenal fat infusion with or without a irreversible inhibitor of gastro-intestinal lipases (orlistat). Moreover, the effect of intra-duodenal fat hydrolysis was more pronounced after the infusion of long-chain fatty acids and apparently mediated by cholecystokinin as the selective blockade of the CCK-1 receptor would abolish the effect of fat hydrolysis on GLP-1 secretion (Beglinger et al., 2010).

In general, gastrointestinal surgical procedures like SG or GB improve diabetes control and represent an accepted therapeutic option, at least in obese patients (American Diabetes Association, 2014; Dixon et al., 2011). They have a well-documented effect on the secretion of gut hormones that, by themselves, are believed to exert a favorable influence on glucose homeostasis. In diabetic patients, the subcutaneous administration of GLP-1 analogues improves glycemic control, supposedly through stimulation of glucose-dependent insulin secretion and suppression of glucagon secretion (Shyangdan et al., 2010). Furthermore, symptomatic hyperinsulinism after GB was controlled by administration of Exendin 9-39, a GLP-1 receptor antagonist, indicating a significant role for the insulinotropic action of GLP-1 after bariatric surgery (Salehi et al., 2014, 2011). Moreover, the favorable effects of IT on glucose tolerance after oral glucose administration in diet-induced obese Long-Evans rats was reversible after administration of Exendin 9-39, supporting evidence of direct effects of GLP-1 on glucose homeostasis (Gaitonde et al., 2012).

As shown in the present study, meal-induced secretion of GLP-1 is enhanced after surgery in GK rats and becomes similar to secretion in non-diabetic Wistar rats. However, concerning pancreatic hormone secretion, improvement of

glucose homeostasis was not associated with an enhanced meal-induced insulin secretion. Furthermore, a study on the effect of SG on weight loss and glucose tolerance in GLP-1 receptor-deficient mice and wild-type controls showed that post-procedural improvement was similar in both groups (Wilson-Pérez et al., 2013). Consequently, other factors than direct stimulation of insulin secretion through surgically enhanced GLP-1 plasma levels are possibly involved in ameliorated glycemic control (Vidal and Jiménez, 2013). However, reduced meal-stimulated increase in GLP-1 secretion was associated with failed weight loss after GB (de Hollanda et al., 2014). Thus, GLP-1 secretion seems to be associated rather indirectly with improvement of glucose tolerance after metabolic surgery and not through direct stimulation of insulin secretion.

A recent and intriguing approach to further clarify the improvement of glucose homeostasis after GB refers to reprogramming of intestinal glucose metabolism, including up-regulation of glucose transporter-1 and enhanced basolateral glucose uptake. Apparently, increased metabolic needs of the intestine and exposure of the Roux limb to undigested nutrients render the gut a major tissue for glucose disposal (Saeidi et al., 2013). Furthermore, modulation of inflammatory responses through gut microbiota may contribute to reduced insulin sensitivity in diabetic subjects and probiotics are under investigation concerning their potential to change the course of disease (Gomes et al., 2014; Tilg and Moschen, 2014). However, the true contribution of these new concepts to the understanding of the pathophysiology of T2D and diabetes remission after surgery remains to be elucidated and was not targeted by our research plan.

The results of our experimental study refer to postprandial gut hormone profiles in anesthetized rodents and may not reflect entirely normal physiology. As many drugs used for induction or maintenance of anesthesia, ketamine and chlorpromazine used in the present study produce anticholinergic side effects. It has already been shown that acetylcholine enhances ghrelin secretion in the isolated rat stomach and in humans (Broglio et al., 2004b; Shrestha et al., 2009) and its inhibition might alter plasma ghrelin levels. However, as all animals in the present study were submitted to the same anesthetic protocol, no influence on observed differences between groups must be assumed.

Our clinical study showed that obese patients with impaired fasting glucose or T2D improved their metabolic control after SG and achieved a sustained weight loss up to 24 months of follow-up. High preoperative plasma insulin levels decreased significantly as early as 3 months after surgery, without any significant further change during follow-up. In parallel, insulin resistance improved to normal or near-normal values during the first 3 months, dropping further until the end of the first year. Despite enhanced glycemic control, beta-cell function declined 1 year after surgery and stabilized afterwards.

Patients with a prolonged history of IFG or T2D eventually develop beta-cell insufficiency characteristic of full-blown disease. However, as shown in the present study, before surgery most patients have very high insulin levels associated with insulin resistance. No pancreatic insufficiency is present and insulin secretion is high, although not high enough to overcome insulin resistance to maintain blood sugar levels in the normal range. In his original Banting lecture, Reaven conceived the term “syndrome X” for a cluster of cardiovascular risk factors linked to insulin resistance (Reaven, 1988). Apparently, low insulin sensitivity or insulin resistance could antecede impairment of glucose tolerance (Martin et al., 1992), as well as cardiovascular diseases or even cancer by many years (Facchini et al., 2001). More recently, the pathophysiology of insulin resistance has been associated with cardiometabolic and cardiovascular disease through mitochondrial dysfunction and formation of reactive oxygen species (Kim et al., 2008). In turn, reactive oxygen species emission and oxidative damage to key signaling mediators from mitochondrial dysfunction and nutrient overload are related to insulin signaling and the development of NAFLD (Besse-Patin and Estall, 2014).

In our obese patients with T2D or IFG, insulin resistance returned to normal or near normal after 3 months, and glycemic control was restored. A pronounced decrease in insulin resistance up to 60 days after SG in patients with T2D was also observed by Rizzello and coworkers (Rizzello et al., 2010), although beta-cell function and long-term results had not been studied. They hypothesized that restoration of insulin sensitivity was due to early hormonal modifications in the gastro-intestinal tract itself, involving GLP-1, ghrelin and PYY. For GLP-1, a direct effect of endovenous administration on post-prandial glycemia has been

shown. However, this effect could have been due to delayed gastric emptying, increased first-phase insulin secretion and suppression of hepatic glucose output (Juris J Meier et al., 2003). Insulin resistance is associated with triglyceride accumulation in muscle (Perseghin et al., 1999) and liver (Utzschneider and Kahn, 2006). Furthermore, the release of non-esterified fatty acids, glycerol, hormones, and proinflammatory cytokines from adipose tissue adds to reduced insulin sensitivity (Kahn et al., 2006). Regular aerobic exercise and a moderately energy restricted diet in obese individuals ameliorate NAFLD and insulin resistance (Bhat et al., 2012). Mobilization of triglyceride accumulation in muscle and liver during weight loss after surgery might contribute to improvement of insulin resistance. An analogous observation was made in insulin resistant obese patients with T2D submitted to a moderately hypocaloric very-low-fat diet that improved hepatic glucose metabolism with normalization of hepatic insulin resistance and reduction of intrahepatic lipid content assessed by magnetic resonance spectroscopy (Petersen et al., 2005).

Our study showed a significant improvement of insulin resistance after surgically induced weight loss, followed by reduced insulin secretion. Operated patients experienced a decline in beta-cell function that occurred at a later stage of postoperative recovery and is preceded by improvement of glycemic control and insulin resistance. Insulin resistance and hyperinsulinemia have little influence on beta-cell dysfunction (Mari et al., 2011), and the majority of patients is characterized by a compensatory augmented preoperative beta-cell function (Prentki and Nolan, 2006). However, a small number of patients did actually present with a deteriorated beta-cell function with rather low insulin levels and did not obtain full remission after surgery, suggesting a partially irreversible decrease in beta-cell mass, possibly due to increased apoptosis (Butler et al., 2003) and linked glucolipotoxicity (Prentki et al., 2002), as well as inflammatory mechanisms (Akash et al., 2013). Two of these patients did show a normal insulin resistance and the third patient revealed additionally an increased insulin resistance. All three patients ameliorated glycemic control after surgery associated with a decrease in insulin resistance and an improvement in beta-cell function, but only one of these patients achieved normoglycemia. Similar observations have been made after GB in obese patients with T2D; beta-cell glucose sensitivity did not return to normal, one

year after surgery (Nannipieri et al., 2011). Thus, prognosis of remission of T2D after bariatric surgery is probably linked to preoperative beta-cell function.

Several studies associated favorable outcome regarding diabetes remission after bariatric or metabolic surgery to clinical and laboratorial parameters like higher BMI, lower age, shorter duration of diabetes, lower levels of HbA1c or FPG, and no requirement for insulin (Casella et al., 2011; Hayes et al., 2011; Huang et al., 2011). However, these factors probably reflect rather indirectly the relative significance of insulin resistance and deterioration of beta-cell function for impairment of glucose homeostasis in individual patients. Recent data support the prognostic value of pre-operative C-peptide assessment for predicting successful outcome after surgery (Lee et al., 2013, 2012). Furthermore, even patients with clinical remission of T2D may not have entirely recovered beta-cell function after surgery (Dutia et al., 2014).

Relative improvement of beta-cell function in patients with an impaired preoperative beta-cell function is possibly due to an increased postoperative meal-induced secretion of gastrointestinal hormones like GLP-1 which has shown to exert a stimulating effect on insulin secretion (Jørgensen et al., 2013) and, hypothetically, on beta-cell survival. Further studies in diabetic patients with beta-cell dysfunction could additionally clarify the hypothesis that success of surgery in this subgroup of patients depends on surgical technique. It has already been shown that BPD or BPD-DS are particularly efficient in obese patients with T2D (Buchwald et al., 2009; Marinari et al., 2006), whereas II-SG could apparently improve diabetic control in non-obese patients (De Paula et al., 2010). Stimulation of L-cells in the distal small intestine through early contact with food and subsequent secretion of gastrointestinal hormones like GLP-1 and PYY is probably highest with these surgical techniques and could be responsible for improved glycemic control (Strader et al., 2005). However, the specific effect on beta-cell function in the subgroup of patients with beta-cell insufficiency remains to be further elucidated.

Limitations to our clinical study include sample size and the presence of drop-outs. Selection bias regarding surgical technique was avoided since all bariatric patients with T2D or IGF treated by the author during the above-mentioned period were included in the study. Statistical methods were adequate to validate

observed differences in studied parameters. However, the study of patient cohorts from other centers would further consolidate our observations. Benefits from a longer follow-up period include the possibility of identifying specific patient groups whose results hold up over time. Our study favors the prognostic value of preoperative assessment of beta-cell function and insulin resistance through mathematical models, possibly facilitating the stratification of prognosis after surgery. Consequently, it would be interesting to study patients submitted to GB or BPD/BPD-DS to improve our knowledge regarding the role of insulin resistance and beta-cell function in the context of other surgical techniques and eventually permit to tailor the procedure to an individual patient.

CONCLUSION

CONCLUSION

Both SG and GB improved overall glycemic control and insulin sensitivity in GK rats, a lean animal model of T2D. Regarding secretion of pancreatic hormones, no significant differences in total meal-induced insulin secretion were observed between Wistar rats and GK rats in all groups. GK rats showed similar glucagon levels in comparison to non-diabetic Wistar rats, without any variation in response to a mixed meal. However, GK rats submitted to SG and GB showed a significant rise in glucagon, 30 minutes after a mixed meal, as well as an increased glucagon-insulin ratio at 60 minutes, albeit a significant amelioration of overall glycemic control. Apparently, an augmented post-procedural glucagon secretion after surgery does not contribute to an impaired glucose tolerance. In face of our results, the role of glucagon on glycemic control after bariatric surgery deserves further investigation.

Sham operated or control group GK rats showed a distinct postprandial gut hormone profile if compared to non-diabetic Wistar rats. Meal-induced gut hormone profiles in GK rats submitted to either SG or GB showed significant post-operative modifications and were similar to Wistar rats. Gastrointestinal surgical procedures modulated both suppressed postprandial ghrelin secretion and flattened meal-induced response of PYY and GLP-1, characteristic for lean diabetic GK rats. Duodenal exclusion alone does not seem to play a significant role in post-surgical amelioration of T2D and restoration of gut hormone profile. Mechanisms other than the direct contact of food with the intestinal mucosa should be taken into account to explain the effect of surgery on postprandial gut hormone secretion.

The results of our clinical study suggest that in most obese patients, T2D is preceded by an enhanced beta-cell function in response to an increased insulin resistance. Later, as disease progresses, augmented beta-cell function cannot compensate for insulin resistance anymore and IFG or overt T2D arise. At this stage, the disease remains reversible by reduction of insulin resistance through bariatric surgical techniques like SG, possibly due to reduction of intracellular lipid load and improvement of mitochondrial dysfunction. Apparently, prognosis concerning diabetes remission is dubious in patients with impaired preoperative beta-cell function, but other surgical approaches, namely BPD-DS or IT, may

yield favorable results in this subgroup of patients that, however, still warrant confirmation.

Taken together, the results of our study demonstrate that important changes in gut hormone profiles occur together with amelioration of glucose homeostasis after bariatric and metabolic surgical procedures, even in the absence of significant obesity, whereas the modulation of pancreatic hormone secretion seems to play a subordinate role. Sleeve gastrectomy, a technique without duodenal exclusion, is effective in a lean animal model and in obese humans with T2D. Preoperative beta-cell function and insulin resistance are likely to represent important prognostic factors for outcome after surgery that should be assessed in all patients with T2D or IFG proposed for metabolic surgery to permit stratification and evaluation of surgical efficacy.

In the context of our data, the mechanisms of modulation of pathophysiological pathways for insulin resistance through gastrointestinal surgery deserve further investigation and shall represent an interesting field for future research.

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