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Gastric bypass surgery is protective from high-fat diet-induced non-alcoholic fatty liver disease and hepatic endoplasmic reticulum stress

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Abstract

Aim—High-fat diets are known to contribute to the development of obesity and related co-morbidities including non-alcoholic fatty liver disease (NAFLD). The accumulation of hepatic lipid may increase endoplasmic reticulum (ER) stress and contribute to non-alcoholic steatohepatitis and metabolic disease. We hypothesized that bariatric surgery would counter the effects of a high-fat diet (HFD) on obesity-associated NAFLD.

Methods—Sixteen of 24 male Sprague Dawley rats were randomized to Sham ($N=8$) or Roux-en-Y gastric bypass (RYGB) surgery ($N=8$) and compared to Lean controls ($N=8$). Obese rats were maintained on a HFD throughout the study. Insulin resistance (HOMA-IR), and hepatic steatosis, triglyceride accumulation, ER stress and apoptosis were assessed at 90 days post-surgery.

Results—Despite eating a HFD for 90 days post-surgery, the RYGB group lost weight ($-20.7 \pm 6\%$, $P < 0.01$) and improved insulin sensitivity ($P < 0.05$) compared to Sham. These results occurred with no change in food intake between groups. Hepatic steatosis and ER stress, specifically glucose-regulated protein-78 (Grp78, $P < 0.001$), X-box binding protein-1 (XBP-1) and spliced XBP-1 ($P < 0.01$), and fibroblast growth factor 21 (FGF21) gene expression, were normalized in the RYGB group compared to both Sham and Lean controls. Significant TUNEL staining in liver sections from the Obese Sham group, indicative of accelerated cell death, was absent in the RYGB and Lean control groups. Additionally, fasting plasma glucagon like peptide-1 was increased in RYGB compared to Sham ($P < 0.02$).

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Conflicts of interest

The authors have no commercial associations that might represent a conflict of interest in relation to this research.

Conclusion—These data suggest that in obese rats, RYGB surgery protects the liver against HFD-induced fatty liver disease by attenuating ER stress and excess apoptosis.

Keywords

apoptosis; NAFLD; obesity; rats; RYGB

Long-term prospective and randomized control trials provide convincing evidence that Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy surgeries produce effective and durable weight loss and diabetes remission in obese patients (Sjostrom *et al.* 2004, Schauer *et al.* 2012, Courcoulas *et al.* 2014, Halperin *et al.* 2014). Bariatric surgery also improves the histopathological aspects of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), including steatosis, inflammation, ballooning degeneration and fibrosis (Mattar *et al.* 2005, He *et al.* 2013). However, while surgery is effective in over 75% of patients, revisional surgeries are needed for up to 20% of patients, and the reason for such a suboptimal outcome is unclear at this point (Aminian *et al.* 2015). There is the possibility that patients who need revisional surgery are not following recommendations regarding caloric and macronutrient intake. Recent studies suggest that there is little if any reduction in dietary fat intake in the months and years after surgery (Johnson *et al.* 2013, Moize *et al.* 2013). In addition, le Roux and colleagues found that changes in dietary fat preference and intake contributed to weight loss maintenance in rats and humans (le Roux *et al.* 2011). With these data in mind, we developed a study to examine the effect of bariatric surgery on weight loss and related liver conditions including NAFLD and NASH. We simulated the observations that patients do not reduce dietary fat intake after surgery by maintaining the animals on a high-fat diet for an extended period after the surgery.

The specific mechanism whereby bariatric surgery results in resolution of NAFLD and NASH has not been reconciled, but there is strong evidence that the effects are not due to weight loss alone (Rubino *et al.* 2004, Myronovych *et al.* 2014). One potential mechanism relates to obesity-induced stress on the endoplasmic reticulum (ER). The ER is a membrane-bound organelle responsible for folding and maturation of proteins during protein synthesis. An imbalance between assembly and folding leads to aberrant protein synthesis and triggers ER stress. Chronic exposure to nutrient overload in both obese mice and humans initiates the ER stress responses in various tissues including liver (Wei *et al.* 2008, Boden 2009). Accumulation of fat in the liver increases vulnerability to hepatic inflammation and injury that triggers oxidative and ER stress in the hepatocyte (Cnop *et al.* 2012, Verbeek *et al.* 2015, Yang *et al.* 2015). Moreover, hepatic ER stress is also associated with hepatic steatosis, and gene expression cloning studies show that inhibition of hepatic ER stress prevents steatosis (Li *et al.* 2011). Portions of the ER stress pathway control the expression of other metabolic hormones including fibroblast growth factor 21 (FGF21), a metabolic hormone that has emerged as a leading candidate in the regulation of lipid and glucose metabolism (Xu *et al.* 2009). FGF21 appears to play an important role in hepatic lipid metabolism by inducing hepatic fatty acid oxidation through the PPAR- α pathway (Inagaki *et al.* 2007). Chronic administration of recombinant FGF21 can reduce serum and hepatic triglyceride levels and reverse fatty liver disease in diet-induced obese mice (Xu *et al.* 2009). Furthermore, FGF21 has been shown to reduce ER stress-induced hepatic steatosis in mice via activation from the

IRE-1 α /XBP-1 arm of the ER stress pathway (Jiang *et al.* 2014). These data support the high therapeutic potential of FGF21 in obesity and chronic liver diseases.

We hypothesized that bariatric surgery would alter gut physiology and prevent expansion of HFD-induced hepatic steatosis in obese rats. To test this hypothesis, we performed RYGB surgery on obese rats, maintained the animals on a HFD and assessed clinical diagnostic criteria of NAFLD/NASH together with cellular changes in ER stress- and FGF21-related pathways and apoptosis. We had the expectation that bariatric surgery would reduce hepatic ER stress, apoptosis and insulin resistance and resolve the established hepatic steatosis and NAFLD.

Materials and methods

Animal care and surgery

The protocol was approved and performed in compliance with the Cleveland Clinic Institutional Animal Care and Use Committee (IACUC). Twenty-four adult male Sprague Dawley (SD) rats (Charles River Laboratories, Wilmington, MA, USA) were included in this investigation. Rats were received at 12 weeks of age and were housed in individual cages, kept at a constant temperature and ambient humidity in a 12-h light/dark cycle (Fig. 1). A total of 16 animals were provided an *ad libitum* high-fat diet (D12492, 60% fat, Research Diets, New Brunswick, NJ, USA) preoperatively to establish diet-induced obesity. This diet also produces hepatic steatosis, hyperglycaemia and insulin resistance. At age 24–25 weeks, the animals were randomly divided into: Sham surgery ($N=8$) or RYGB ($N=8$). An additional eight rats served as a Lean control group.

Animals undergoing surgery were fasted overnight (approx. 12 h). Ceftriaxone (75 mg kg⁻¹) was administered intramuscularly for prophylaxis; an isoflurane gas chamber was used for anaesthesia induction, which was switched to nose cone flow for maintenance of anaesthesia during the procedure. We used the gastric bypass model and bowel limb lengths as previously described (Gatmaitan *et al.* 2010) to achieve durable weight loss. The stomach and distal oesophagus were dissected free, and a 20% proximal gastric pouch was created. The jejunum was transected 30-cm distal to the ligament of Treitz, creating a 30-cm biliopancreatic limb. A 5-mm side-to-side gastrojejunostomy was made, and an 8-mm side-to-side jejunojejunostomy was made 10 cm below the gastrojejunostomy, creating a 10-cm alimentary limb. For the Sham operation, the terminal oesophagus and stomach were likewise exposed and dissected free. A 5-mm gastrotomy was made on the anterior surface of the gastric fundus and closed with interrupted 6-0 polyglactin sutures. The jejunum was also divided 30 cm below the ligament of Treitz and then reanastomosed in an end-to-end fashion. A liver biopsy was taken from each animal at the time of surgery. The rats were maintained on an *ad libitum* liquid diet with Boost (Nestle, Buffalo Grove, IL, USA) for up to 7 days after surgery. Thereafter, they were fed an *ad libitum* HFD. The control group was fed a normal chow diet once daily. Food intake was recorded daily. All rats were killed on post-operative day 90.

RNA extraction

RNA was extracted from the liver with RNeasy Mini kit (Qiagen, Valencia, CA, USA). Briefly, 10–20 mg of liver tissue was homogenized in buffer RLT using the FastPrep-24 tissue homogenizer (MP Biomedicals, Santa Ana, CA, USA). RNA was extracted, isolated and collected in 40 μ L nuclease free water. RNA concentration and purity was determined from absorbance at 230, 260 and 280 nm using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Isolated RNA was aliquoted and stored at -80°C until further analysis.

cDNA synthesis

One microgram of total RNA was reverse transcribed to cDNA (iScript cDNA synthesis kit, Biorad, Hercules, CA, USA) using a PX2 Thermal Cycler (Thermo Scientific). The volume of the reaction was set at 20 μ L, and synthesis was performed at 25°C for 5 min, 42°C for 30 min and 85°C for 5 min, respectively.

qRT-PCR primer pairs

Primer pairs for target genes were obtained from PrimerBank database (see Table 1). All primers were checked for specificity to the genes of interest by conducting a Blast analysis.

Semiquantitative RT-PCR analysis

Determination of relative mRNA expression was performed in duplicate on an MX3000P QPCR system (Agilent Technologies/Stratagene, La Jolla, CA, USA) using 10 ng of cDNA as the template and the Brilliant II SYBR Green QPCR Master Mix (Stratagene). The rat 18sRNA gene was used as an internal standard for sample normalization because it remains consistently expressed during fasting conditions (Yamada *et al.* 1997). The relative changes in mRNA abundance were calculated using the comparative DDCt method (Livak & Schmittgen 2001). Briefly, the threshold cycle (C_t) for 18sRNA was subtracted from the C_t for the gene of interest to adjust for variations in mRNA/ cDNA generation efficiency to generate the C_t value.

Hepatic steatosis and inflammation

Liver biopsy samples were obtained at the time of surgery, and then, the whole liver was harvested at 90 days after surgery. Samples were formalin-fixed and paraffin-embedded. Liver sections were de-paraffinized with xylene, rehydrated via a series of distilled water and graded ethanol solutions, sectioned and stained with haematoxylin and eosin. A pathologist (RKP) blinded to the animal groups assessed steatosis and inflammation using the Nonalcoholic Steatohepatitis Clinical Research Network criteria, as described previously (Kleiner *et al.* 2005). Total liver triglyceride accumulation was also measured using the Wako L-type TG kit (Wako Chemicals, Richmond VA, USA) following tissue extraction in 2 : 1 chloroform:methanol.

Immunohistochemistry

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) positive staining in paraffin-embedded liver sections was analysed using the In Situ Cell Death Detection Kit (Roche Applied Science).

Metabolic measures

Fasting plasma glucose, insulin and GLP-1 were determined pre-operatively and repeated 90 days post-operatively. On each occasion, 1.0 mL of blood was drawn from the femoral vein and placed into tubes containing aprotinin and dipeptidyl peptidase-4 inhibitor. Ketamine and local pain control was used during femoral access. The samples were centrifuged at 4 °C for 10 min at 2000 rpm, and the plasma was collected and stored at -80 °C for subsequent analyses. Glucose was measured using an Alphasnak blood glucose monitoring system (Abbott Laboratories, Abbot Park, IL, USA). GLP-1 was analysed using a commercially available rat gut hormone multiplex panel (Bio-Rad Laboratories). Insulin was analysed using a commercially available enzyme-linked immunosorbent assay kit for rat plasma (Millipore, Billerica, MA, USA).

Statistical analysis

The data are reported as mean $\pm$ the standard error. The statistical analysis of data from the different experimental groups was performed using either two-way analysis of variance followed by Bonferroni post-testing, or one-way analysis of variance followed by Tukey post-testing. Differences between groups were considered significant at $P < 0.05$.

Results

Metabolic and weight loss effects of diet and RYGB surgery

Baseline body weight was similar for all three groups (data not shown). The HFD successfully induced obesity and insulin resistance in the RYGB and Sham groups (Fig. 2a,c). Animals in the RYGB and Sham groups gained an average of 414.6 ± 21.1 grams and developed insulin resistance. At the completion of the study, the RYGB group lost 20% of their baseline weight, with significant weight loss differences between the groups ($P < 0.05$) (Fig. 2a). Overall food intake was not different between the Sham and RYGB groups throughout the study (Fig. 2b). Based on HOMA-IR calculations, the RYGB animals became significantly more insulin sensitive compared to the Sham group ($P < 0.03$) (Fig. 2c).

Hepatic steatosis and inflammation

During the 90-day post-surgery period, the HFD led to significant hepatic triglyceride accumulation in the Sham group (Fig. 3a), and this was accompanied by severe steatosis (Figs. 3b,c) and inflammation (Fig. 3d), all of which are typical clinical features of NAFLD/NASH. In contrast, the RYGB group showed remission of NAFLD as evidenced by a reduction in hepatic TG accumulation ($P < 0.0001$) (Fig. 3a), steatosis ($P < 0.001$) (Fig. 3b,c) and inflammation ($P < 0.001$) (Fig. 3d). The decrease in steatosis in the RYGB group

was notable and essentially normalized the liver when compared to the Sham control group (Fig. 3b,c).

Endoplasmic reticulum stress and apoptosis

ER stress is present in the liver of obese individuals with NAFLD (Gentile *et al.* 2011, Pagliassotti 2012). Knowing this, we searched for differences in the expression of genes in the ER stress-signalling pathway in the livers of these animals. The RYGB group had significantly reduced gene expression for two key ER stress sensors, IRE1 ($P < 0.001$) and PERK ($P < 0.001$) (Fig. 4a,b). There was also a trend towards a reduction in ATF6 ($P < 0.09$), and the chaperone protein grp78 ($P < 0.06$), when compared to the Sham group (Fig. 4c,d). Downstream of IRE1, we measured hepatic expression of XBP1 and XBP1s genes. There was a significant elevation of XBP-1 in both the Sham ($P < 0.001$) and the RYGB ($P < 0.001$) animals compared to the controls (Fig. 4e). There was a significant elevation of XBP-1s in the Sham group compared to the RYGB group ($P < 0.009$, Fig. 4f). Interestingly, FGF21, a gene that is regulated by XBP1s protein, was also up-regulated in Sham when compared to the control ($P < 0.0003$), but there was no difference with the RYGB group (Fig. 4g). Importantly, the circulating plasma level of FGF21 was significantly up-regulated in the Sham and down-regulated in the RYGB group (Fig. 6a). These data are consistent with FGF21 regulating the ER stress pathway in these animals. Downstream of PERK, we analysed ATF4 gene expression and its nuclear target CHOP. ATF4 expression was significantly elevated in the Sham group compared to the Lean control group (Fig. 4h). Expression of CHOP was significantly decreased in the RYGB ($P < 0.001$) group compared to Sham (Fig. 4i). ER stress is known to promote apoptosis (Kim *et al.* 2008), and therefore, we performed TUNEL staining on paraffin-embedded liver sections. The Sham group had significantly more TUNEL-positive nuclei ($P < 0.001$) compared to the Lean control group, and gastric bypass surgery virtually eliminated apoptosis as evidenced by the absence of TUNEL-positive staining in the liver of the RYGB group (Fig. 5a,b).

Gut peptide secretion

Gastric bypass surgery is known to stimulate changes in both fasting and postprandial gut peptide secretion (Gatmaitan *et al.* 2010, Kashyap *et al.* 2010). With this in mind, we analysed fasting plasma GLP-1 in all three groups and found that GLP-1 was significantly elevated in the RYGB animals compared to Sham ($P < 0.02$) (Fig. 6b).

Discussion

Herein, we investigated the effect of RYGB surgery on the progression of NAFLD in obese rats maintained on an obesogenic diet that is known to produce metabolic dysfunction in the liver. The main findings are that despite eating a HFD and RYGB surgery: (i) controlled and prevented hepatic lipid accumulation, (ii) attenuated hepatic ER stress-related pathways and (iii) maintained insulin sensitivity. These data demonstrate a beneficial effect of RYGB surgery on typical risk factors known to influence the development of NAFLD. The data presented here highlight the role of ER stress, and potentially apoptosis, in NAFLD and provide important insights into a novel mechanism that may be targeted to facilitate liver health.

We focused on the ER stress pathway because several studies suggest that ER stress is an early consequence of excessive nutrient intake that exacerbates NAFLD (Li *et al.* 2011). We show that RYGB surgery is protective from developing hepatic ER stress through pre-transcriptional regulation of key steps in the stress response pathway. ER stress begins with the recruitment of the chaperone protein grp78 to the lumen of the endoplasmic reticulum. RYGB surgery decreased grp78 gene expression suggesting that the stress response was blunted in this group. Further, we show a decrease in gene expression of the three ER stress sensors IRE-1, PERK and ATF-6 following surgery (Fig. 4a,b,c). It is known that activated IRE-1 signals downstream to splice XBP-1 into its active form XBP-1s, and the signal is propagated to the nucleus to increase the transcription of grp78 during ER stress (Schroder & Kaufman 2005). Other studies have shown that XBP-1s is at least partially responsible for *de novo* lipogenesis in mice (Lee *et al.* 2008). The decrease in XBP-1s gene expression in the RYGB group compared to Sham is consistent with decreased grp78 gene expression (Fig. 4d,f) and reduced hepatic lipid accumulation (Fig. 3a). In addition to splicing XBP-1, IRE-1 activation has other cell signalling roles including the activation of JNK and NF κ B via apoptosis signalling-regulating kinase 1 (Urano *et al.* 2000). Furthermore, IRE-1 has been linked to the activation of p38 mitogen-activated kinase (p38 MAPK) and extracellular-regulated kinase (ERK) (Hu *et al.* 2006). Taken together, these interactions suggest that IRE1 activation impacts cell survival through XBP-1, inflammation through NF κ B and insulin signalling through p38 MAPK. These interactions may explain the increased cell survival (Fig. 5a,b), decreased hepatic inflammation (Fig. 3d) and improved hepatic insulin sensitivity (Fig. 2b) in the liver of the RYGB animals. Activation of the second ER stress sensor PERK induces phosphorylation of eIF2 α and subsequent attenuation of most translation initiation (Wu *et al.* 2004). Interestingly, eIF2 α phosphorylation increases translation of the transcription factor ATF4. This transcription factor is responsible for the regulation of C/EBP homologous protein (CHOP), a proapoptotic protein that is responsible for initiating apoptosis in an overstressed cell (Oyadomari & Mori 2004). Aside from apoptosis, ATF4 may also play a role in hepatic lipid accumulation (Seo *et al.* 2009). These results suggest that PERK-mediated phosphorylation of eIF2 α may play a role in hepatic lipid accumulation, and possibly hepatocyte apoptosis. Our data support the view that RYGB surgery attenuates hepatic inflammation, lipid accumulation and cell death, by attenuating the PERK arm of the ER stress pathway at the transcriptional level. Further work is required to identify the transcription factors that are activated after surgery, and the mechanistic triggers that in turn regulate these transcription factors, and ultimately the ER process itself.

Uncontrolled ER stress and its subsequent downstream signalling often result in apoptosis. Hepatic apoptosis has been shown to play a large role in the progression of NAFLD and NASH (Feldstein *et al.* 2003). We assessed hepatic apoptosis in our animals through TUNEL staining and found that the RYGB group had significantly fewer TUNEL-positive nuclei and thus less apoptosis. The decreased occurrence of apoptosis in the RYGB group may be explained by the alleviation of ER stress in these animals as characterized by reduced expression in the ER stress-signalling pathway. The overall attenuation of ER stress and reduction of hepatic apoptosis may help to explain the improved histological appearance of the RYGB animals.

Recent studies suggest that the endocrine hormone FGF21 plays an important role in hepatic fatty acid oxidation, glucose metabolism and insulin sensitivity (Xu *et al.* 2009). In humans, high circulating levels of FGF21 have been found in obesity and NAFLD (Dushay *et al.* 2010). Furthermore, the IRE-1 α /XBP-1 arm of the ER stress pathway was recently shown to play a regulatory role in the expression of FGF21 in diet-induced obese mice (Jiang *et al.* 2014). This suggests that ER stress may initiate increased FGF21 expression as part of a carefully orchestrated strategy to combat hepatic steatosis. In the present study, we observed a significant decrease in circulating FGF21 levels in our RYGB group compared to the Sham controls (Fig. 6). This decrease in FGF21 was supported by the concurrent decrease in XBP-1s gene expression (Fig. 4f). In our study, the RYGB animals have reduced hepatic steatosis that leads to a reduced ER stress response and reduced FGF21 expression in the liver and plasma. Conversely, in Sham animals, hepatic steatosis was still present, and this was accompanied by up-regulation of the ER stress pathway and subsequently higher expression of FGF21 to counter lipid accumulation in the liver. Ongoing studies are under way to determine whether FGF21 is up-regulated in the immediate post-surgery period in patients and animal models. This may be the mechanism through which hepatic steatosis is attenuated, thus leading to an eventual decrease in FGF21 as the metabolic environment improves.

Multiple studies have now consistently shown an increase in both insulin and the GLP-1 response to nutrient ingestion following RYGB surgery (Kashyap *et al.* 2010). Importantly, GLP-1 is associated with enhanced pancreatic β -cell function via the attenuation of ER stress (Yusta *et al.* 2006). Based on the latter evidence in particular, there is the possibility that in the current study, some of the metabolic effects that were seen in the RYGB group may have been mediated through GLP-1 action in the liver. The presence of GLP-1 receptors on rat and human hepatocytes provides support for this view (Svegliati-Baroni *et al.* 2011). There is also evidence that GLP-1 has a direct effect on hepatocytes derived from NAFLD patients, and the resulting metabolic response includes activation of genes involved in beta-oxidation and insulin sensitivity (Svegliati-Baroni *et al.* 2011). Elevated beta-oxidation would help to explain the decrease in hepatic steatosis and inflammation, while a decrease in ER stress would explain the reduced occurrence of apoptosis. Further evaluation and validation of these effects are necessary.

The RYGB group lost a significant amount of weight following surgery even though food intake was similar for both groups. Animals in the RYGB group were able to maintain food intake by eating small amounts of food very frequently throughout the feeding period. These observations are consistent with many other studies that have reported that gastric bypass-induced weight loss is not associated with reduced food intake, but rather an increase in energy expenditure (Furnes *et al.* 2008, Bueter *et al.* 2010, Kodama *et al.* 2013).

A limitation of the present study is the absence of a control group pair-fed to the RYGB group. This would have allowed us to distinguish the effects of surgery from diet-induced caloric restriction and weight loss on improved liver health and metabolic function. Although we did not include a pair-fed group in this study, the absence of difference in food intake between the groups suggests that observed metabolic effects may be attributed to the surgery per se. A second limitation relates to the use of a rat model to assess NASH and

metabolic responses to bariatric surgery. There are other animal models used to study RYGB surgery including mice and pigs (Seyfried *et al.* 2011, Lindqvist *et al.* 2014). However, we are convinced that the rat model is appropriate because of similarities between the rat and human genomes with respect to the genes involved in multiple disease pathologies (Gibbs *et al.* 2004). In addition, diet-induced obesity in the Sprague Dawley rat is well documented and is a widely used model for obesity-related metabolic disease (Mercer & Archer 2005, Davidson *et al.* 2010, Gatmaitan *et al.* 2010). Finally, the RYGB surgery procedure used in this study was specifically developed to mimic the surgery that is now widely performed in humans with obesity (Meguid *et al.* 2004).

In conclusion, data from this study support the view that surgical re-routing of the gut facilitates important metabolic changes beyond the expected weight loss and enhanced insulin sensitivity. Importantly, protection from the cellular stress that is normally associated with a HFD suggests that ER stress and apoptotic pathways are potential mechanistic sites for the enduring effects of surgery on metabolic function. These data provide important insights that could lead to novel therapeutics against the progression of NAFLD to NASH in obesity. Further work is warranted to identify the specific molecular and cellular mechanisms so that these targets can be developed as effective therapies.

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Figure 1.
Schematic of the study design and timeline.

Figure 2.

RYGB surgery prevents HFD-induced weight gain and hepatic insulin resistance for up to 90 days after surgery. (a) Body weight 90 days after surgery. $*P < 0.05$ for the comparison between Obese Sham vs. Obese RYGB groups. (b) Average food intake for 90 days following surgery. (c) HOMA-IR was used as a measure of hepatic insulin resistance. $*P < 0.05$ for the comparison between Obese Sham vs. Obese RYGB groups.

Figure 3.

RYGB surgery prevents HFD-induced hepatic steatosis and inflammation for 90 days after surgery. (a) Chemical analysis of triglyceride content extracted from livers. **** $P < 0.0001$ for the comparison between Obese Sham, Obese RYGB and Lean controls. (b) Histopathological determination of hepatic steatosis based on per cent area of lipid accumulation. *** $P < 0.001$ for the comparison between Obese Sham, Obese RYGB and Lean controls. (c) H&E staining of representative liver sections. Liver biopsies were collected and fixed as described in Methods and then stained with haematoxylin and eosin. Black arrows are pointing to lipid accumulation in the liver of Obese Sham controls. (d) Inflammation assessed from H&E staining. ** $P < 0.01$ for the comparison between Obese Sham controls, Obese RYGB and Lean controls.

Figure 4.

Rats maintained on a HFD for 90 days after RYGB surgery are protected from ER stress.

Genes in the ER stress and FGF21 pathways were assessed by quantitative real-time PCR.

* $P < 0.05$, ** $P < 0.01$, **** $P < 0.001$, for the comparison between groups.

Figure 5.

Rats maintained on a HFD for 90 days after RYGB surgery are protected from excess apoptosis. Liver biopsies were collected and fixed as described in Methods and then probed with fluorescein-dUTP (Green), nuclei were counterstained with DAPI (Blue). (a) Histological comparison of TUNEL-stained livers. TUNEL staining is in green, and DAPI staining is in blue. (b) TUNEL-positive nuclei. *** $P < 0.001$ for the comparison between Obese Sham vs. Obese RYGB and Obese Sham vs. Lean controls.

Figure 6.

The surgical response to circulating metabolic hormones in rats fed a HFD. (a) The RYGB surgery-related increase in plasma GLP-1 is evident for up to 90 days despite feeding a HFD. (b) Plasma FGF21 is decreased following RYGB surgery. Plasma was collected as described in Methods. * $P < 0.05$ for the comparison between Obese Sham vs. Obese RYGB group.

Table 1

Primer list for quantitative real-time PCR analysis

Gene	Forward primer	Reverse primer	GenBank accession #	Amplicon size (nt)
18sRNA	GGA TCC ATT GGA GGG CAA GT	ACG AGC TTT TTA ACT GCA GCA A	NM_017008.4	110
GRP78	AGT AAG TTC ACT GTG GTG GC	GCG CTT GGC GTC GAA GAC	NM_013083.2	287
ATF6	GAT TTG ATG CCT TGG GAG TC	GGA CCG AGG AGA AGA GAC AG	NM_001107196.1	122
ATF4	TAT GGA TGG GTT GGT CAG TG	CTC ATC TGG CAT GGT TTC C	NM_024403.2	144
PERK	GAA GTC GAG AGG CGT CGG GG	GCC CGT CTC GCC GCT AGG AG	NM_031599.2	69
IRE1	CTG GTC GGA TGG GTG GCG TTC	CAG GGT CCT GGG TAA GGT CTC CGT	NM_001191926	159
CHOP	CTG GAA GCC TGG TAT GAG GAT	CAG GGT CAA GAG TAG TGA AGG T	NM_001109986.1	120
XBP-1	GGC GGC CCC CAA AGT GCT AC	CCC GGA ACC ATG AGC GGC AG	NM_001004210.2	74
XBP-1s	TTA CGA GAG AAA ACT CAT GGG C	GGG TCC AAC TTG TCC AGA ATG C	NM_001004210.2	288
FGF21	GTG TCA AAG CCT CTA GGT TTC TT	GGT ACA CAT TGT ATC CGT CCT	NM_130752.1	102

18sRNA – ribosomal RNA; GRP78– glucose-regulated protein, 78 kDa or heat-shock 70-kDa protein 5; ATF6 – activating transcription factor 6; ATF4 – activating transcription factor 4; PERK or Eif2ak3 – pancreatic EIF2-alpha kinase or eukaryotic translation initiation factor 2 alpha kinase 3; IRE1 or ERN1 – inositol-requiring enzyme 1 or endoplasmic reticulum to nucleus signalling 1 (Ern1); CHOP – C/EBP homologous protein; XBP-1 – X-box binding protein 1; XBP-1s – spliced form of X-box binding protein-1; FGF21 – fibroblast growth factor 21. (pga.mgh.harvard.edu/primerbank/).