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Gastric Bypass Resolves Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) in Low-BMI Patients

A Prospective Cohort Study

Adrian T. Billeter, MD, PhD, Katharina M. Scheurlen, MD,* Barbara Israel, BSc,* Beate K. Straub, MD,† Peter Schirmacher, MD,† Stefan Kopf, MD,‡§ Peter P. Nawroth, MD,‡§‖ and Beat P. Müller-Stich, MD*✉*

Objective: Metabolic dysfunction-associated fatty liver disease (MAFLD) reflects the multifactorial pathogenesis of fatty liver disease in metabolically sick patients. The effects of metabolic surgery on MAFLD have not been investigated. This study assesses the impact of Roux-en-Y gastric bypass (RYGB) on MAFLD in a prototypical cohort outside the guidelines for obesity surgery.

Methods: Twenty patients were enrolled in this prospective, single-arm trial investigating the effects of RYGB on advanced metabolic disease (DRKS00004605). Inclusion criteria were an insulin-dependent type 2 diabetes, body mass index of 25 to 35 kg/m^2, glucagon-stimulated C-peptide of >1.5 ng/mL, glycated hemoglobin $>7\%$, and age 18 to 70 years. A RYGB with intraoperative liver biopsies and follow-up liver biopsies 3 years later was performed. Steatohepatitis was assessed by expert liver pathologists. Data were analyzed using the Wilcoxon rank sum test and a P value <0.05 was defined as significant.

Results: MAFLD completely resolved in all patients 3 years after RYGB while fibrosis improved as well. Fifty-five percent were off insulin therapy with a significant reduction in glycated hemoglobin ($8.45 \pm 0.27\%$ to $7.09 \pm 0.26\%$, $P = 0.0014$). RYGB reduced systemic and hepatic nitrotyrosine levels likely through upregulation of NRF1 and its dependent antioxidative and mitochondrial genes. In addition, central metabolic regulators such as SIRT1 and FOXO1 were upregulated while de novo lipogenesis was reduced and β-oxidation was improved in line with an improvement of insulin resistance. Lastly, gastrointestinal hormones and adipokines secretion were changed favorably.

Conclusions: RYGB is a promising therapy for MAFLD even in low-body mass index patients with insulin-treated type 2 diabetes with complete histologic resolution. RYGB restores the oxidative balance, adipose tissue function, and gastrointestinal hormones.

From the *Department of General, Visceral, and Transplantation Surgery, University of Heidelberg, Heidelberg, Germany; †Institute of Pathology, University of Heidelberg, Heidelberg, Germany; ‡Department of Internal Medicine I and Clinical Chemistry, University of Heidelberg, Heidelberg, Germany; §German Center for Diabetes Research (DZD), Heidelberg, Germany; and ‖Joint Heidelberg-IDC Translational Diabetes Program, Helmholtz-Zentrum, München, Germany.

✉beat.mueller@med.uni-heidelberg.de.

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Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are rapidly increasing worldwide and comprise a major challenge due to the risk of progression to liver cirrhosis or development of hepatocellular carcinomas.[1] NAFLD/NASH are associated with many metabolic disease including type 2 diabetes (T2D), dyslipidemia, and obesity.[2] Patients with NAFLD/NASH have a high risk for cardiovascular disease further highlighting the multiorgan involvement of NAFLD/NASH.[1] Lastly, NASH is increasingly observed in patients without obesity albeit with similar liver-related complications challenging the role of obesity in this disease.[3] Due to these complex interaction with other metabolic diseases, metabolic dysfunction-associated fatty liver disease (MAFLD) was recently described to reflect the multifactorial pathogenesis of fatty liver disease in metabolically sick patients.[4]

The progression from steatosis to steatohepatitis/fibrosis and the role of diabetes remain poorly understood. The recent observations of lean NASH and the concept of MAFLD as liver injury combined with metabolic disease independent of severe obesity strongly suggest that overweight/obesity may be less important than initially thought. Albeit many mechanistic and therapeutic studies have been performed in mouse models, there is little mechanistic data on the changes during reversal of MAFLD/NASH in humans.[5] Metabolic surgery has repeatedly been shown to improve NASH and to improve fibrosis in severely obese patients in observational studies.[6–8] However, all studies reporting histological data on NASH-resolution after bariatric surgery report on cohorts with a mean preoperative body mass index (BMI) of at least 45 kg/m^2 and all had inclusion criteria of a BMI >35 kg/m^2.[8] Hence, it is unclear whether metabolic surgery is an effective treatment for NASH/MAFLD in low-BMI patients outside the current guidelines and a BMI <35 kg/m^2. Thus, the main aim of this trial was to investigate whether metabolic surgery is an effective therapy for MAFLD in low-BMI patients as has been shown for T2D.[9]

To our knowledge, no study has investigated the effects of metabolic surgery in patients with MAFLD independent of criteria for obesity surgery. The aim of this study was to investigate the impact of Roux-en-Y gastric bypass surgery (RYGB) on histologically proven MAFLD in overweight patients with a BMI <35 kg/m^2 and insulin-treated T2D.

METHODS

Study Design and Participants

Since safety of RYGB in patients with low preoperative BMI was not clear yet, this study was designed as a prospective, single-arm trial investigating the effects of RYGB on advanced metabolic disease in patients outside the criteria for bariatric-metabolic surgery. It was registered at the German clinical study registry (DRKS00004605), approved by the local institutional review board (IRB, S-078/2010) and performed in accordance with the declaration of Helsinki. Twenty consecutive patients (10 male and 10 female patients) underwent RYGB from 11/2010 to 08/2012. Inclusion criteria were: insulin-treated T2D with at least three months of insulin therapy, a BMI of 25 to 35 kg/m^2, preserved pancreatic β-cell function (glucagon-stimulated C-peptide of > 1.5 ng/mL), glycated hemoglobin (HbA1c) > 7%, and age 18 to 70 years. Known liver disease or intraoperative finding of liver cirrhosis were exclusion criteria. All patients had histologically proven liver injury combined with an insulin-treated T2D fulfilling the criteria for MAFLD (Supplementary Table 1, http://links.lww.com/SLA/E98). A laparoscopic RYGB with a 75 cm biliopancreatic and a 150 cm alimentary limb was performed. Deep liver wedge biopsies (Seg. 2) as well as visceral (omentum majus) and subcutaneous adipose tissue (trocar sites) biopsies were taken intraoperatively. All patients received a ketogenic (protein-rich, low caloric) diet for 2 weeks before RYGB.

Patients were followed-up after 3, 6, 12, 24, and 36 months. No patient had signs of increased alcohol consumption, history of viral hepatitis, history of liver-related complications, and all patients had normal liver function. After 3 years, percutaneous repeat liver (core needle biopsies) and subcutaneous adipose tissue biopsies were taken in 10 patients after an amendment to the study protocol and approval by the local IRB as well as informed consent of the patients. The histology of the subcutaneous adipose tissue and changes in systemic inflammation, postoperative care, and clinical outcomes up to 2 years of follow-up have previously been reported.[10–14] Detailed methods are provided in the supplements (Supplemental Digital Contents 1 and 2, http://links.lww.com/SLA/E97 and http://links.lww.com/SLA/E98).

RESULTS

Effects on BMI and Glycemic Control

Mean BMI dropped from a preoperative stable weight before perioperative ketogenic diet of 32.8 ± 0.5 kg/m^2 (10 patients with BMI 33–35 kg/m²) to 24.5 ± 0.7 kg/m^2 after 36 months ($P < 0.0001$, Fig. 1A). Fasting glucose and HbA1c also improved significantly (fasting glucose: 201.4 ± 15.7 to 124.5 ± 8.6 mg/dL, $P = 0.001$; HbA1c: 8.45 ± 0.27% (69.1 ± 2.8 mmol/L) to 7.09 ± 0.26% (53.9 ± 2.8 mmol/L), $P = 0.0014$; Figs. 1B, C). Insulin use decreased significantly (81.6 ± 11.9 to 12.4 ± 4.8 IE/d; $P < 0.0001$, Fig. 1D) while metformin use remained unchanged. After 36 months, 55% of patients were off insulin therapy and 1 patient achieved diabetes remission according to the 2021 consensus criteria.[15] No gliflozins (SLGT2i), Glucagon-like peptide 1 receptor agonists or dipeptidyl peptidase-4 inhibitors were used during the study period since they were not yet available at the time of recruitment.

Improvement in Liver Function Tests and Liver Histology

ALT-levels decreased after RYGB (36.8 ±3.2–24.5 ± 3.2U/l, $P = 0.038$, Fig. 1E) while AST remained unchanged (data not shown). NAS decreased from 4 ± 0.33 to 1.1 ± 0.23 after 36 months ($P = 0.0002$, Fig. 1F) with steatosis, ballooning, and inflammation all improving significantly (Figs. 1G–I). Liver fibrosis improved after RYGB (1.8 ± 0.2 to 1.2 ± 0.2; $P = 0.0197$, Fig. 1J). None of the patients had progression of fibrosis and all had an improvement of the NAS resulting in a 100% success of RYGB regarding histological outcomes according to the NASH clinical research network (CRN). Exemplary histological images of 2 patients are shown in Fig. 1K. Also, there was no association of improvement of liver histology (decrease in NAS) with preoperative BMI ($r = 0.0002$; $P = 0.968$), with decrease in HbA1c ($r = -0.24$; $P = 0.521$), or homeostatic model assessment insulin resistance (HOMA-IR) ($r = 0.203$; $P = 0.601$).

Changes in Systemic and Hepatic Oxidative Stress and Hepatic Oxidative Defense Genes

Oxidative stress is a hallmark of NASH.[16] Serum nitrotyrosin (190 ± 60–31.5 ± 14 nM; Fig. 2A) and nitrotyrosin modifications in the liver (Fig. 2B) both significantly decreased 36 months after RYGB. Since nuclear respiratory factor 1 (NRF1) and nuclear factor erythroid 2-related factor 2 (NRF2) both have been shown to regulate oxidative defense genes in the liver,[17,18] their expression was assessed. NRF1 was significantly upregulated while NRF2-expression remained unchanged (Figs. 2C, D). NRF1 targets superoxiddismutase 2 (SOD2) and Glyoxalase 1 (GLO1) were significantly upregulated after RYGB (Figs. 2E, F) while the NRF2 targets Heme Oxygenase-1 (HO-1), Glutamate-Cysteine Ligase (GCLC), and NAD(P)H dehydrogenase (quinone 1) (NQO1) remained unchanged (data not shown).

The NRF1 mitochondrial targets MtND3 (complex 1), UQCRC1 (complex 3), and mtCOX1 (complex 4) were all strongly upregulated following RYGB (Figs. 2G–I). Mitochondrial fusion and fission proteins, MFN1 and FIS1, were both also increased following RYGB (Figs. 2J, K). These data collectively suggest that RYGB increases NRF1 expression which upregulates its target of the oxidative defense and mitochondria while NRF2 and its targets were not affected. Furthermore, the increase in MFN1 and FIS1 indicates a profound change in mitochondrial function. These changes are associated with a reduction in systemic and hepatic oxidative-nitrosative damage.

Changes in Hepatic β-Oxidation and Denovo Lipogenesis

The genes of the β-oxidation (ACADM and ACSL1) were both upregulated after RYGB (Figs. 2L, M). SCD1 was also upregulated while ACACA, the rate limiting step in the fatty acid synthesis, remained unchanged (Figs. 2N, O).

Expression of Central Metabolic Regulators in the Liver

Sirtuin-1 (SIRT1), a central liver metabolism regulator that improves liver steatosis,[19] was significantly upregulated following RYGB (Fig. 3A). FOXO1, which plays a complex and poorly understood role in glycemic control and antioxidative defense, was similarly upregulated (Fig. 3B). However, the peroxisome proliferator-activated receptors (PPAR) α and γ both showed a clear decrease although results were not statistically significant (Fig. 3C, D; $P = 0.06$). Thus, the beneficial metabolic effects of RYGB appear to be independent of the PPARs despite the upregulation of the metabolic master switch SIRT1.

Changes in the Gut-Liver Axis

Fibroblast growth factor (FGF)-19 and FGF-21 are significantly increased after RYGB (Figs. 3E, F). Although fibroblast growth factor receptor (FGFR)1 is low expressed in human liver, it was significantly upregulated after RYGB while the expression of FGFR4 remained unchanged (Fig. 3G;

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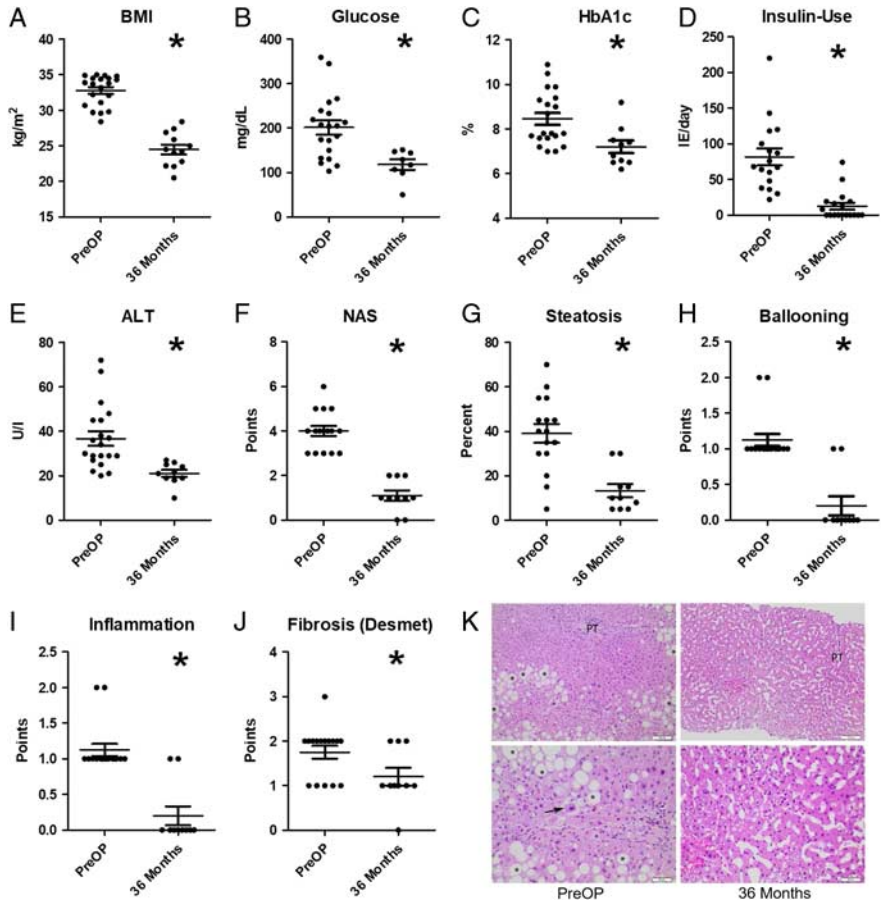

FIGURE 1. Change in BMI, glycemic control, and liver histology. Change in BMI (A), fasting glucose (B), HbA1c (C), and daily insulin use (D) preoperatively and 36 months after RYGB. Change in liver function tests and histology are shown for ALT (E) as well as NAS (F) and the histological components of the NAS [liver steatosis (G), hepatocyte ballooning (H), and portal inflammation (I)] after RYGB. Change in liver fibrosis assessed by the Desmet-score (J) with representative images of two patients before and 36 months after RYGB (K). Data are shown as mean ± SEM; $*P < 0.05$.

Supplemental Fig. 1A, Supplemental Digital Content 3, http://links.lww.com/SLA/E269). Furthermore, the two well-defined FGF-19 target genes, CYP7A1 and CYP8B1 (Figs. 3I, J), were both upregulated despite increased FGF-19 levels indicating that FGFR4 signaling in the liver seems not to play a relevant role in restoration of liver function after RYGB in humans.

While FGF-21 serum levels were significantly upregulated after RYGB (Fig. 3F), hepatic FGF-21 mRNA expression was barely not significantly increased ($P = 0.07$, Supplemental Fig. 1B, Supplemental Digital Content 3, http://links.lww.com/SLA/E269). These observations are in line with experimental findings that SIRT1, which was upregulated after RYGB (Fig. 3A), stimulates FGF-21 expression.[19] Lastly, serum total bile acids were also increased after RYGB (Fig. 3H). The increase in serum bile acids is also in line with the increased CYP7A1 and CYP8B1 expression (Figs. 3I, J). However, the expression of the receptors for bile acids, farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5), in the liver were unchanged (Supplemental Figs. 1C, D, Supplemental Digital Content 3, http://links.lww.com/SLA/E269). While the expected changes in FGF-19/-21 and bile acids after RYGB were observed, only the expression of FGFR1 was increased after RYGB suggesting that FGFR1 may play a central role in mediating the effects of RYGB on the liver.

In line with the changes in the gut-liver axis, CYP7A1 and CYP8B1 which both comprise the rate limiting steps in the bilirubin synthesis but are also essential for cholesterol secretion into the bile, were both upregulated following RYGB (Figs. 3I, J). Since FOXO1 is a regulator of the glucose metabolism, the insulin resistance was investigated. The overall insulin resistance (HOMA-IR) significantly improved from 16.12 ± 2.48 to 4.25 ± 0.6 ($P = 0.0002$, Fig. 3K) in line with the improved glycemic control and reduced insulin use. The upregulation of glycogen synthase 2 (GS2) indicates a reduced hepatic insulin resistance too (Fig. 3L). These changes indicate an improvement of the hepatic insulin resistance on the molecular level with improved β-oxidation, reduced lipogenesis, and reduced hepatic insulin resistance.

Effects on Lipid and Adipose Tissue Metabolism

In line with improved systemic and hepatic insulin sensitivity and reduced lipogenesis, serum triglycerides and serum free fatty acids were significantly reduced after RYGB (Supplemental Figs. 2A, B, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). Low-density lipoprotein remained unchanged while high-density lipoprotein significantly increased (Supplemental Figs. 2C, D, Supplemental Digital Content 4, http://links.

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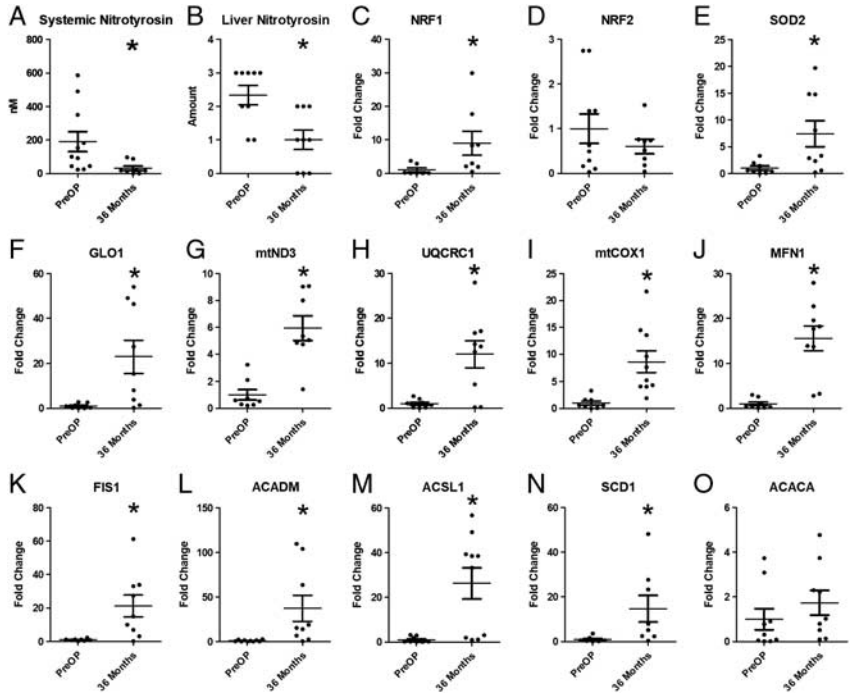

FIGURE 2. Reduction in systemic and hepatic oxidative stress through upregulation of NRF1 and its target antioxidative defenses, mitochondrial biogenesis, β-oxidation, and fatty acid synthesis. Reduction in systemic and liver oxidative stress (A) & (B) 36 months after RYGB. Changes in NRF1 (C) and NRF2 (D) and NRF1 targets SOD2 (E), GLO1 (F). Upregulation of genes of the mitochondrial respiratory chain: (G), UQCRC1 (H), and 4 (I). MFN1 as a marker for mitochondrial fusion (J) and FIS1 and a marker for mitochondrial fission (K) are both upregulated 36 months after RYGB. Changes in β-oxidation [ACADM (L), ACSL1 (M)] and fatty acid synthesis [SCD1 (N), ACACA (O)]. Data are shown as mean ± SEM; $*P < 0.05$.

lww.com/SLA/E270). The decrease in leptin and increase in adiponectin are in line with improved adipose tissue function (Supplemental Figs. 2E, F, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). The increase in adiponectin is also in line with experimental data showing a direct effect of FGF-21 on adiponectin secretion from the adipose tissue.[20] To further investigate whether the receptors of FGF-21 and FGF-19 are differentially expressed in the adipose tissue, the expression of FGFR1 and FGFR4 was determined. FGFR1 remained unaffected while FGFR4 was significantly reduced after RYGB (Supplemental Figs. 2G, H, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). FXR expression was not detectable in adipose tissue while TGR5 had a very low expression which remained unchanged (data not shown). PPARα was unexpectedly downregulated while PPARγ remained unchanged (Supplemental Figs. 2I, J, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). Uncoupling protein 1 (UCP1) is very low expressed and was not affected by RYGB (Supplemental Fig. 2K, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). UCP2, however, was significantly upregulated after RYGB in the subcutaneous white adipose tissue (Supplemental Fig. 2L, Supplemental Digital Content 4, http://links.lww.com/SLA/E270). These findings indicate a profound change in adipose tissue function after RYGB in a favorable manner. However, the contribution of the different hormones (ie, FGF-21, FGF-19, bile acids) and through which signaling cascades these hormones act, requires further studies. Also, whether adult human adipose tissue can be brightened as suggested by UCP2 upregulation requires more in-depth investigations.

Complications and Safety

There was one perioperative surgical complication in this cohort, an obstruction of the jejuno-jejunostomy which required reoperation. No other perioperative complications occurred. One patient had an acute cholecystitis 3 years after RYGB requiring an emergency cholecystectomy. Two patients developed tumor disease, one died from a cholangiocarcinoma within 2 years after RYGB and the other patient developed a small bowl neuroendocrine tumor which resulted in an obstruction of the small bowel 3 years after RYGB. He also developed lymph node metastases 4 years after resection of the small bowl neuroendocrine tumor which were again resected. Currently, he is without any sign of tumor disease. Until the 3-year follow-up, no patient developed any cardiovascular events or malnutrition. No relevant vitamin or micronutrient deficiencies occurred, which was published elsewhere.[13]

DISCUSSION

This is the first study investigating the effects of metabolic surgery on liver injury in low-BMI patients. Thus far, the cohorts reporting histological data on NASH-resolution after metabolic surgery included cohorts with a mean BMI >45 kg/m² and all patients were operated within the standard guidelines for bariatric-metabolic surgery. This cohort was operated outside these guidelines with a BMI <35 kg/m² as an inclusion criterion (and

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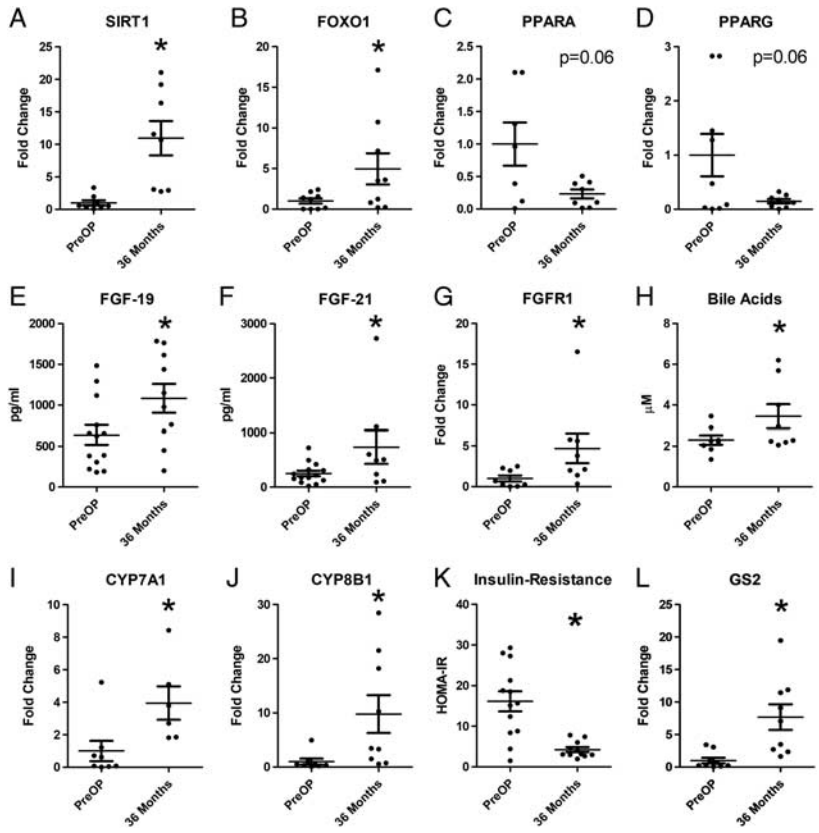

FIGURE 3. Change in hepatic transcription factors, gut-liver axis, bile acid synthesis, and insulin resistance. Expression of SIRT1 (A), FOXO1 (B), PPARα (C), and PPARγ (D) in the liver before and 36 months after RYGB. Change in serum FGF-19 (E), serum FGF-21 (F), and hepatic FGFR1 (G), 36 months after RYGB. Increase in serum total bile acids after RYGB (H) as well as changes in the rate limiting steps of the bile acid synthesis [CYP7A1 (I), CYP8B1 (J)]. Improvement in insulin resistance determined by the systemic HOMA-IR (K) and hepatic GS2 (L). Data are shown as mean ± SEM; $*P < 0.05$.

therefore a mean preoperative BMI-difference of >10 kg/m² compared with all other published studies) and focused specifically on metabolically sick patients (insulin-treated T2D) with histologically proven liver injury (steatohepatitis with fibrosis), which therefore are prototypical for MAFLD. The complete histological resolution of MAFLD and regression of fibrosis 36 months after RYGB indicates that metabolic surgery seems to be an effective treatment option for MAFLD and likely NASH in low-BMI patients outside the traditional indications of bariatric-metabolic surgery. Furthermore, the finding that liver injury resolved completely despite a persistent T2D demonstrates that the effects of metabolic surgery on metabolic complications are independent of the glycemic control. This finding is in line with findings for diabetic nephropathy and neuropathy.[10,14,21] The molecular analysis in these patients suggest that the reduction in oxidative stress and the recovery of the defense systems after RYGB are potential mechanisms by which metabolic surgery improves organ damage independent of glycemic control.

The complete resolution of MAFLD was associated with a plethora of changes in the liver metabolism, gut-liver axis and adipose tissue (Supplementary Fig. 3, Supplemental Digital Content 5, http://links.lww.com/SLA/E271). As the main mechanism, RYGB seems to restore the oxidative balance in the liver by reducing oxidative stress and beneficial changes in genes related to mitochondrial function and antioxidative defense. Thus, many of the mechanistic studies from animal models could be evaluated in human MAFLD-resolution. A key finding of this study is the reduction in oxidative stress, both systemically and in the liver (Figs. 2A, B) which is in line with prior studies both in humans and mice.[16,22–24] This study adds that the oxidative defense is improved after RYGB and during MAFLD-resolution in an NRF1 dependent manner while NRF2 and its targets were not affected. These observations are in line with studies demonstrating distinct actions of NRF1 and NRF2 regarding oxidative defense.[25] Furthermore, the role of NRF1 in MAFLD-resolution is in line with NASH-progression in liver-specific NRF1 knock-out mice.[18] Additionally, NRF1 may also mitigate cholesterol-induced ER-stress and cholesterol accumulation in hepatocytes.[26] This newly described role of NRF1 may also contribute to the improved cholesterol metabolism after RYGB. In line with an improved cholesterol clearance from hepatocytes is also the upregulation of CYP7A1 and CYP8B1 which produce bile acid from cholesterol and thus secret it into the bile.

NRF1 is also a key activator of mitochondria biogenesis.[27] In line with the NRF1 upregulation, all examined genes of the mitochondrial respiratory chain were upregulated after RYGB. These changes are in line with experimental data in diet-induced NASH models in mice.[28] In addition, Verbeek et al[28] showed that mitochondrial function was improved by RYGB in mice. To further evaluate the changes in mitochondrial structure, MFN1 and FIS1

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were investigated. Surprisingly, both genes were upregulated suggesting a complex modulation of mitochondria architecture that requires further investigation. In skeletal muscle, exercise has been shown to induce MFN1 resulting in improved respiratory capacity while mitochondrial fission with reduction in MFN1 and increase in FIS1 was associated with insulin resistance and reduced oxygen respiratory capacity.[29] Overall, our observations demonstrate that mitochondrial function undergoes a significant change during MAFLD-resolution. The findings of this study indicate that the increased oxygen leak and mitochondrial dysfunction in human NASH[16] may be reversed by RYGB. Further studies are needed to investigate the exact changes in mitochondrial structure and function during MAFLD-resolution.

Also, hepatic metabolism and insulin resistance is normalized as indicated by the HOMA-IR, GS2, and increased β-oxidation (Fig. 3). SCD1, the rate limiting step of the lipogenesis pathway and an enzyme that has been shown to reduce obesity when blocked and is therefore a target for obesity therapy,[30] was surprisingly upregulated after RYGB. Several studies have demonstrated the beneficial effects of SCD1 deficiency/knowdown.[31] However, SCD1 plays also an essential in the lipid metabolism since it is the enzyme that provides monounsaturated fatty acids[32] which are essentially in many biological processes including signaling cascades and membrane formation. In contrast to the beneficial effects of SCD1 deficiency/knowdown, a recent study demonstrated that SCD1 deficiency increases ER-stress through a mTORC1 pathway.[33] On the basis of our findings with a reduction in cellular stress after RYGB, this hypothesis that SCD1 plays an important role during MAFLD-resolution by limiting ER-stress fits well in the overall hypothesis.

Several regulators of hepatic metabolism including NRF1, SIRT1, and FOXO1 were upregulated after RYGB while NRF2, PPARα, and PPARγ were not. This study confirms the experimental data that SIRT1 plays an essential role in NASH/MAFLD-resolution[19,34] and it may be upregulated by RYGB. Further examinations if and by which mechanisms SIRT1 is upregulated after RYGB are needed. Importantly, our results strongly suggest that MAFLD-resolution after RYGB are independent of PPARα and PPARγ. This is in line with clinical trials showing that PPAR-stimulation does not achieve a profound improvement of NASH despite some features of NASH being resolved.[35] Lastly, the role of FOXO1 in NASH-resolution deserves further investigation. FOXO1 plays a complex role in liver metabolism which is currently not fully understood.[36] SIRT1 is an inducer of FOXO1 in some tissues[37] and liver-specific deletion of FOXO1 results in NAFLD/NASH.[38] The upregulation of FOXO1 during MAFLD-resolution after RYGB supports a relevant role of FOXO1 during MAFLD-resolution. Fitting to our results, PGC1α, a major metabolic regulator, can directly bind FOXO1 mRNA counteracting gluconeogenetic actions by glucagon.[39]

The role of gastrointestinal hormones was also investigated. While the expected changes after RGYB such as increased FGF-19, FGF-21, and serum bile acids were observed, only hepatic FGFR1 was upregulated while the main FGFR in the liver, FGFR4, and the bile acids receptors FXR and TGR5 were not altered. Although the beneficial impact of FGF-19 on the liver has been shown in mice and humans,[40] the signaling pathway through which FGF-19 acts in human NASH-resolution needs further investigation. We did not observe the typical FGF-19 action, the suppression of CPY7A1 and CYP8B1 through FGFR4,[41] indicating that FGF-19 signaling through FGFR4 may not play a major role during MAFLD-resolution in humans. This finding is in line with experimental data that FGFR4 signaling does not influence hepatic glucose metabolism.[42] Further studies using FGF-19 analogs will shed more light on the exact role and involved pathway of FGF-19. Alternatively, the liver metabolism may be affected through upregulation of FGFR1 after RYGB by FGF-21, or even FGF-19, which is not observed under normal circumstances.[43]

Lastly, the effects of RYGB on adipose tissue function were investigated. We have shown previously that RYGB improves adipose tissue and systemic inflammation.[11] This investigation adds that adipose tissue function is improved by RYGB. Triglycerides, free fatty acids, and leptin were decreased while adiponectin increased. Adiponectin is likely increased through FGF-21, similar to recombinant FGF-21 in humans.[44] Experimental data from mice also showed that adiponectin mediates the effects of FGF-21.[20] The downregulation of PPARα is in line with other studies in human adipose tissue after RYGB[45] indicating that animal studies of PPARα actions in adipose tissue may lead to different results than in humans. Lastly, the UCP2 upregulation indicates a sustained increased energy expenditure after RYGB.[45]

This study has several limitations. Due to the limited availability bv of liver tissue and patient samples, especially of the follow-up biopsies, no additional in-depth analyses were possible, for example the analysis of nitric oxide synthase isoforms, stability of FXR and its interaction with FGF-19 or dipeptidyl peptidase-4 expression. Due to the rarity of these patients that were operated outside the guidelines for metabolic and bariatric surgery, we had no access to more tissue for further analyses within this study. However, these and other points will be investigated in future trials. Furthermore, due to the loss of follow-up of some patients, there are concerns of selection bias. However, there were no signs that the patients that did not complete the 36 months follow-up were different from the patients with follow-up biopsy. Also, the preoperative ketogenic diet may have improved the liver injury although all patients had steatosis and NASH at the time of surgery despite this intervention. Despite these limitations, the findings presented here are in line with the results of experimental data in mice as well as human studies. Nonetheless, further studies are necessary to understand the mechanisms by which RYGB improves liver injury and the interactions of peripheral tissues and the gut. This study provides an overview of the manifold changes in various tissues following RYGB in metabolically sick patients with MAFLD. Lastly, it remains unclear what the relevance of weight loss on the metabolic improvement is. Since all patients lost a significant amount of weight, the role of weight loss cannot be fully elucidated in this cohort. However, the correlation analysis strongly indicate that preoperative BMI and improvement of glycemic control do not seem to play a major role. Furthermore, despite most patients not achieving a diabetes remission, the liver injury resolved completely. Therefore, this study further supports the movement that BMI-independent indications for metabolic surgery should be established. Future trials should be designed to compare metabolic surgery with other treatment options such as GLP-1 analogs/SLGT2i and to investigate the main contributing factors for MAFLD and NASH-resolution. This GLP-1 analog/SLGT2i naïve cohort allows to estimate the effects of RYGB alone without any drugs interfering with liver histology.

CONCLUSION

RYGB is an effective treatment completely resolving MAFLD without progression of fibrosis in low-BMI patients outside the current criteria for bariatric-metabolic surgery and it should be considered as a treatment option in such patients. RYGB induces a profound change in liver and adipose tissue metabolism resulting in complete resolution of MAFLD. A regulatory axis consisting of SIRT1, FOXO1, and NRF1 results in upregulation of

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oxidative defense genes, improvement of mitochondrial function and normalization of hepatic glucose and lipid metabolism restoring the oxidative balance. Systemically, the increased FGF-21 expression improves adipose tissue function including increased energy expenditure while adiponectin may further enhance the beneficial metabolic effects in the liver.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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DISCUSSANTS

Nicoló De Manzini (Trieste, Italy)

I would like to congratulate you and your colleagues for this interesting work, which implies that the role of surgery in the resolution of Metabolic Disfunction Associated Fatty Liver Disease (MAFLD). This is a prospective, single-arm observational study on a relatively limited cohort of patients, describing a multitude of phenomena observed, and not necessarily associated with the RYGBP.

It has been quite difficult to understand the relationships between the different metabolic pathways you have analyzed, mostly because they are difficult to interrelate; many questions arise regarding these relationships. However, my questions essentially focus on surgical aspects:

First, considering gut-liver axis, how can you explain the stability of the farnesoid X receptor despite the operation, compared to the upregulation of FGF-19 and FGF-21?

Second, between the multitude of data, has a portal tension gradient been studied?

Third, for this group of patients, "out of common guidelines", could you specify the mortality and morbidity rates?

Fourth, considering the inclusion criteria, could you specify how many patients had a BMI that was lower than 33, as the main issue of this paper could be an extended indication for RYGBP?

Finally, what considerations can a bariatric surgeon retrieve from your interesting work to apply to their clinical practice?

Response from Adrian Billeter (Heidelberg, Germany)

Thank you very much for these questions. First, regarding the wide array of analyses we did, this was the very purpose of this study because there is much basic science data on single pathways or single proteins and their effects on healthy and fatty liver, but there is no study investigating the changes during resolution of NASH/MAFLD in humans. Using these patients with NASH/MAFLD-resolution, we can test some of these basic science driven hypotheses in actual patients. While some of the data is conflicting, we generated a few new hypotheses, and now, these must be investigated again. Among these questions is the role of the farnesoid X receptor pathway, which was not affected, in contrast to some experimental data. The effects of FGF-19 and FGF-21 can be explained by the various FGF-receptors and are well in line with experimental data.

Second, regarding the portal tension gradient, we did not study that in this trial because none of the patients had signs of cirrhosis or portal hypertension, which was an exclusion criterion for operating these patients in this study. However, we do study patients with portal hypertension in another trial.

Third, regarding the surgical risk, it is comparable with the complication rates of obesity surgery in "normal" obese patients. There is no difference regarding perioperative or long-term complications. We also did a follow-up study, which was published a couple of years ago, reporting the nutritional outcomes. These patients have the same rate of vitamin deficiencies as patients with morbid obesity after a gastric bypass. They do not have an increased risk of protein-deficiency or vitamin deficiency, provided that they take the same vitamin supplementation that every bariatric patient must take.

With regard to your fourth question, ten patients had a BMI below 33 kg/m², and their outcomes were identical to the ones with a BMI >33 kg/m². However, it is very important to consider that the mean BMI of this cohort was at least 12 BMI-points lower than the average BMI of all other surgical cohorts investigating the effect of metabolic surgery on NASH/MAFLD. In fact, this cohort has a comparable or even lower BMI than the cohorts treated with new drugs for NASH. Therefore, we believe that this study clearly demonstrated the potential value of metabolic surgery in the treatment of NASH in patients with low-BMI, and future studies should compare surgery versus medical treatment in these patients. Metabolic surgery should be considered as a treatment option for NASH/MAFLD in patients with a BMI of 30 kg/m² or higher.

François Pattou (Lille, France)

Congratulations on this very nice paper. My question regards the clinical implications of your choice of gastric bypass versus sleeve gastrectomy. In our paper, which you cited at the beginning, we showed clear directions by surgery to prevent cirrhosis. Of course, these patients are difficult, and the risk is very high. Gastric bypass is a good option, in terms of efficacy, but it's a demanding operation, which forbids any follow-up of the gastric remnant with endoscopy. My real question regards weight loss. In previous studies, weight loss appears to be the real driver. You show that you have weight-independent factors that may justify a gastric bypass. However, what is your reason for the choice of operation with an apparently similar weight loss outcome?

Response from Adrian Billeter (Heidelberg, Germany)

This is an excellent point. We do not have data comparing sleeve gastrectomy with gastric bypass. There are some studies that looked at this, but the patient number was very low. Therefore, the biggest evidence for the effects of metabolic surgery on NASH/MAFLD is currently for gastric bypass. As you pointed out, we do have less weight loss than the other trials, due to the low starting BMI of the patients in this cohort. However, all these patients lost all their excessive weight, which represents very significant weight loss. To really investigate the role of weight loss and weight loss-independent effects, these data must be compared with the results of more obese patients after an adjustment for weight loss to see if there is a weight-independent effect. In our cohort, there was no association between weight loss and improved liver histology. However, this cohort is, perhaps, too small to make any firm associations.

Gastric bypass was chosen in this cohort based on the available data at the time of study inception that RYGB is the operation with the stronger metabolic effect. Since the aim of this study was to treat patients with advanced metabolic disease and low-BMI, gastric bypass was the obvious choice. However, I do agree that sleeve gastrectomy should also be evaluated in future studies.

Martin K. Angele (Munich, Germany)

Thank you very much for this nice paper. I just have one question: In patients transplanted for NASH, do you apply gastric bypass surgery in those patients prior to or after transplantation, in order to avoid those detrimental effects of obesity to the new liver?

Response from Adrian Billeter (Heidelberg, Germany)

We perform bariatric surgery in transplant candidates. This is a trial we are doing as a prospective single-arm study in patients with liver cirrhosis and portal hypertension. However, in these patients, we use a sleeve gastrectomy because the surgical risk of sleeve gastrectomy is lower; it is easier to manage complications and you have access to the biliary tree, if needed.

Michael Olausson (Göteborg, Sweden)

Out of your 20 patients, how many developed alcohol-dependency?

Response from Adrian Billeter (Heidelberg, Germany)

None of our patients developed alcohol-dependency in this cohort.

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