



Concomitant PPAR α and FXR Activation as a Putative Mechanism of NASH Improvement after Gastric Bypass Surgery: a GEO Datasets Analysis

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Abstract

Background Compared to non-surgical weight loss (Diet), weight loss after Roux-en-Y gastric bypass (RYGB) results in greater rates of non-alcoholic steatohepatitis (NASH) resolution. Changes in bile acid physiology and farnesoid X receptor (FXR) signaling are suspected mediators of postoperative NASH improvement. Recent experimental evidence suggests that upregulation of hepatic peroxisome proliferator-activated receptor α (PPAR α) activity might also impact NASH improvement. As FXR partly regulates PPAR α , we compared resolution of NASH and changes in hepatic PPAR α and FXR gene expression following Diet and RYGB.

Methods We searched the Gene Expression Omnibus database to identify human studies with liver biopsies containing genomic data and histologic NASH features, at baseline and after Diet or RYGB. Microarray data were extracted for PPAR α and FXR gene expression analyses using GEOquery R package v.2.42.0.

Results We identified one study (GSE83452) where patients underwent either Diet ($n = 29$) or RYGB ($n = 25$). NASH prevalence was similar at baseline (Diet 76% versus RYGB 60%, $P = \text{ns}$). After 1 year, NASH resolved in 93.3% of RYGB but only in 27.3% of Diet ($P < 0.001$). Hepatic PPAR α and FXR gene expression increased only after RYGB ($P < 0.001$). These changes were also found when analyzing only patients that resolved NASH ($P < 0.01$), and patients without NASH at baseline and follow-up ($P < 0.05$).

Conclusions Compared to Diet, RYGB results in greater NASH resolution with concurrent upregulation of hepatic PPAR α and FXR. Our findings point to concurrent PPAR α and FXR activation, triggered by RYGB, as a potential mechanism to improve NASH.

Keywords Bariatric surgery · Gastric bypass · NASH · NAFLD · Bile acids · FXR · PPAR α

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Introduction

Severe obesity is a potent risk factor for progression of non-alcoholic fatty liver disease (NAFLD) to its clinically aggressive form of non-alcoholic steatohepatitis (NASH).¹ While weight loss through lifestyle modification is the staple treatment option for NAFLD and NASH, Roux-en-Y gastric bypass (RYGB) has shown the most promising effects in patients with severe obesity (body mass index, BMI > 35 kg/m²) and NAFLD.² The mechanisms behind the postoperative reversal of NAFLD and NASH are not yet fully understood,³ but they are thought to include effects that are both dependent on and independent of weight and fat mass loss, including restoration of insulin responsiveness and sensitivity as well as improvements in liver fat oxidative metabolism.² Given the projected

increases in the prevalence of severe obesity and NAFLD over coming years,⁴ identification of the specific pathways through which RYGB reverses NAFLD and NASH could improve current understanding of these diseases' pathologies and translate to new therapies.

Among the multiple neurohormonal and gastrointestinal signaling factors elicited by RYGB, bile acids have emerged as suspected mediators of many postsurgical metabolic benefits,^{5, 6} and alterations in bile acids physiology after RYGB appear at least partly independent of weight loss and may also foster fat mass loss.⁷ Findings of sustained modifications in bile acid concentrations up to 5 years after RYGB⁸ further suggest a possible contributory role to long-term effects of surgery. The modifications in bile acid pool size and composition have been shown to alter bile acid signaling through the nuclear farnesoid X receptor (FXR) and Takeda G protein-coupled receptor (TGR-5).⁹ Several studies have demonstrated an increase in serum BA levels after RYGB, especially chenodeoxycholic acid, which is a specific FXR agonist.^{10,11} Rodent models of RYGB and other bariatric surgeries demonstrate such mechanistic links between bile acids and their FXR and TGR-5 receptors to the postoperative restoration of insulin sensitivity, glucose and lipid homeostasis, and NAFLD improvement.^{12,13} FXR is highly expressed in hepatocytes and is centrally involved in balancing hepatic fatty acid and cholesterol synthesis and secretion.⁹ Specifically, FXR activates the small heterodimer partner (SHP), which subsequently inhibits sterol regulatory element-binding protein (SREBP-1c) to downregulate de novo lipogenesis.¹⁴ Despite intense interest in FXR as a putative therapeutic target for NASH, we are unaware of any human study that has documented changes in hepatic FXR gene expression after RYGB.

Acting in conjunction with FXR, other nuclear receptors in the liver also serve important functions in regulating lipid metabolism. The peroxisome proliferator-activated receptor α (PPAR α) is part of a subfamily of nuclear receptors and is highly expressed in liver, where it stimulates the transcription of genes involved in mitochondrial fatty acid uptake and β -oxidation.¹⁵ Of note, PPAR α is the molecular target for the fibrate class of drugs that lower plasma triglycerides and elevate plasma high-density lipoprotein levels.¹⁶ In humans, hepatic PPAR α gene expression negatively correlates with whole-body insulin resistance and NASH severity¹⁷ and has been suggested as a therapeutic target for NASH.¹⁷

Additionally, a bile acid-mediated cross-talk between FXR and PPAR α activation has been previously described,¹⁸ perhaps through FXR binding to a PPAR α receptor element.¹⁹ The limited available experimental evidence also suggests that concurrent activation of PPAR α and FXR may lead to synergistic improvements in NAFLD.²⁰ Taken together, it is plausible that RYGB simultaneously upregulates hepatic PPAR α and FXR via overlapping pathways and that this will coincide with favorable changes in NASH-related liver histology. Our aim was to compare hepatic PPAR α and FXR expression and

NASH resolution in human patients submitted to either Diet alone or RYGB, using the publicly available Gene Expression Omnibus (GEO) database.

Material and Methods

Gene Expression Data

Gene expression data were explored using the publicly available GEO datasets.²¹ The GEO is a public repository that archives and freely distributes comprehensive sets of microarray, next-generation sequencing, and other forms of high-throughput functional genomic data submitted by the scientific community.²² GEO contains raw data that facilitates the unambiguous interpretation of the index data and potential verification of conclusions of peer-reviewed publications. Many journals require accession numbers for microarray or sequence data before acceptance of a paper for publication, and reviewers and editors may need access to genomic data during the review process. Thus, data is usually deposited in GEO before a manuscript describing the data is sent to a journal for review, and GEO records may remain private until a manuscript quoting the GEO accession number is made available to the public. All data submitted to GEO is initially curated by GEO personnel before being accepted to be displayed on the GEO records. After publication, GEO data can be searched and re-used after the index publication to provide evidence of specific gene expression to support hypotheses, test material for algorithm development, and aggregate and analyze data in ways not anticipated by the original authors.¹⁹ The database is built and maintained by the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine, located on the campus of the National Institutes of Health in Bethesda, MD, USA.

Search terms were “liver” AND “bariatric” and filtered for “human” to identify eligible microarray datasets from studies performed in adult patients who had paired liver biopsies before and after bariatric surgery or non-surgical caloric restriction (Diet). Only studies that described histological features of NAFLD or NASH were considered. Additionally, we only included in our analyses the patients that we could identify liver biopsies and genomic data at both baseline and follow-up. Pre-processed gene expression data were obtained from GEO using GEOquery R package v. 2.42.0. Gene expression was analyzed on the \log_2 scale and log-fold changes (\log_2FC) in gene expression were calculated.

Statistical Analysis

Categorical variables were compared using Fisher's exact test, and numerical variables were compared using the paired-samples *T* test or Mann-Whitney *U* test. Statistical significance

was considered to be $P \leq 0.05$. Statistical analyses were performed using SPSS v 24 for Windows (SPSS Inc., Chicago, IL).

Results

Our search revealed two studies in which liver biopsies were performed before and after bariatric surgery. Only the study that reported hepatic gene expression changes after both RYGB and Diet alone (GSE83452) was included in this analysis. In that study, Lefebvre et al.²³ presented hepatic transcriptomic data in a cohort of patients with liver biopsies before and 1 year after undergoing either Diet or RYGB. Patients in the Diet group underwent a weight management program with emphasis on lifestyle modification and without prescription of specific anti-obesity drugs. The authors defined NASH according to the combined presence of steatosis, ballooning, and lobular inflammation.²⁴ Liver biopsy was performed percutaneously (16G Menghini) or perioperatively (14G Tru-Cut). Patients were excluded in case of significant alcohol consumption (> 20 g/day), history of bariatric surgery, diagnosis of another liver disease, or pre-existing diabetes.

We selected 25 patients submitted to RYGB and 29 patients submitted to Diet alone that we could identify liver biopsies and genomic data at both baseline and follow-up. Of note, the authors of GSE83452 only reported microarray data and did not use other methods, such as PCR or Western blotting, as they judged it was technically unfeasible due to the limited size of the spare biopsy sample that was mostly entirely used for RNA extraction. Other additional data available in the GEO public dataset for these patients were sex and age.

Table 1 depicts patient demographic data made available by Lefebvre et al.,²³ as well as NASH status at baseline and 1-year follow-up. Patients that underwent RYGB were younger (RYGB 38.9 ± 13.5 years versus Diet alone 47.7 ± 14.4 years, $P = 0.03$). Both groups were comprised of 68% females ($P = 0.94$). There was no difference in the baseline prevalence of NASH between groups (RYGB 60% versus Diet alone 78%, $P = 0.25$). In direct contrast, NASH resolution at the 1-year reassessment was significantly higher after RYGB compared to Diet alone [NASH Resolution: RYGB 14/15 (93.3%) versus Diet alone 6/22 (27.3%), $P < 0.001$].

Table 1 Demographic data and NASH prevalence in patients with paired liver biopsies at baseline and 1-year follow-up, as available in the GEO dataset (GSE83452)

	RYGB ($n = 25$)	Diet alone ($n = 29$)	<i>P</i> value
Age (mean \pm SD)	38.9 ± 13.5	47.7 ± 14.4	0.03
Female sex ($n, \%$)	17 (68%)	20 (68.9%)	0.94
NASH at baseline ($n, \%$)	15 (60%)	22 (76%)	0.25
NASH at 1-year follow-up ($n, \%$)	1 (4%)	17 (58.6%) ^a	<0.001

^a 1 patient with de novo NASH

SD, standard deviation; RYGB, Roux-en-Y gastric bypass; NASH, non-alcoholic steatohepatitis

Dataset GSE83452 does not provide individual weight and weight loss data. However, weight loss results from that cohort of patients are reported in another study by the same group,¹⁷ where mean BMI unit lost was greater for bariatric surgery subjects after 1 year (11.9 versus 3.7 in diet group, $P < 0.05$).

Hepatic PPAR α and FXR Gene Expression

Table 2 shows the PPAR α and FXR gene expression on the \log_2 scale. Figure 1 shows the \log_{FC} in PPAR α and Fig. 2 shows \log_{FC} in FXR expression after 1 year. Only RYGB patients had a significant increase in both PPAR α and FXR gene expression after 1 year (PPAR α and FXR, $P < 0.001$). In addition, when analyzing only patients that resolved NASH after 1 year (RYGB, $n = 14$; Diet alone, $n = 6$), only RYGB patients had a significant increase in both PPAR α and FXR gene expression (PPAR α , $P < 0.001$; FXR, $P = 0.003$). To attempt examine the effects of RYGB relative to Diet alone, independently of NASH improvement, we conducted a separate analysis in the subgroup of patients who were free of NASH at both baseline and follow-up time points (RYGB, $n = 10$; Diet alone, $n = 6$). Similar to the whole-group analysis, only RYGB patients had a significant increase in both PPAR α and FXR gene expression after 1 year (PPAR α , $P = 0.001$; FXR, $P < 0.001$).

Discussion

Our secondary analysis of the publicly available dataset GSE83452 demonstrated that RYGB but not non-surgical Diet increased both PPAR α and FXR gene expression in the liver. These transcriptional changes coincided with remarkably greater rates of NASH resolution with RYGB (93%) than Diet alone (27%), rates which are consistent with prior reports of NASH improvement 1 year after RYGB or non-surgical weight loss.^{25,26} Although several experimental and clinical studies have shown changes in bile acid pool after bariatric surgery,²⁷ to our knowledge, only non-human experimental studies have demonstrated changes in bile acid receptor FXR in liver.²⁸ Thus, we suspect that this is the first research work that documents changes in gene expression of the bile acid receptor FXR in the human liver after RYGB. Also, while

Table 2 PPAR α and FXR gene expression on log₂ scale (mean \pm SD) at baseline and 1 year after RYGB and Diet alone.

	All patients			Diet alone (<i>n</i> = 29)		
	RYGB (<i>n</i> = 25)			Baseline	Follow-up	<i>P</i> value
PPAR α (log ₂)	7.385 \pm 0.301	7.934 \pm 0.261	< 0.001	7.735 \pm 0.249	7.724 \pm 0.202	0.81
FXR (log ₂)	7.981 \pm 0.204	8.276 \pm 0.128	< 0.001	8.027 \pm 0.225	8.029 \pm 0.224	0.96
	Patients with NASH resolution			Diet alone (<i>n</i> = 6)		
	RYGB (<i>n</i> = 14)			Baseline	Follow-up	<i>P</i> value
PPAR α (log ₂)	7.281 \pm 0.254	7.925 \pm 0.298	< 0.001	7.684 \pm 0.234	7.751 \pm 0.149	0.32
FXR (log ₂)	8.084 \pm 0.165	8.286 \pm 0.150	0.003	8.041 \pm 0.276	8.077 \pm 0.220	0.687
	Patients without NASH			Diet alone (<i>n</i> = 6)		
	RYGB (<i>n</i> = 10)			Baseline	Follow-up	<i>P</i> value
PPAR α (log ₂)	7.496 \pm 0.324	7.999 \pm 0.136	0.001	7.673 \pm 0.232	7.823 \pm 0.215	0.27
FXR (log ₂)	7.817 \pm 0.146	8.262 \pm 0.103	< 0.001	8.019 \pm 0.327	7.991 \pm 0.266	0.6

SD, standard deviation; RYGB, Roux-en-Y gastric bypass; NASH, non-alcoholic steatohepatitis

aiming to eliminate the influence of NASH improvement on the changes in gene expression, we excluded the NASH patients and compared PPAR α and FXR gene expression only in patients without NASH. Even in that subset of patients, RYGB group had a statistically significant increase in both PPAR α and FXR gene expression in the liver that was not observed after Diet alone. While the RYGB group lost significantly more weight than the diet group, we believe that greater weight loss per se is not the main driver behind the significant increase in both PPAR α and FXR gene expression. This is because others have already demonstrated that non-surgical calorie restriction does not increase bile acid concentration in serum, while RYGB does.⁷ Thus, we believe that altered bile acid nuclear receptor signaling through FXR and PPAR α might be the mechanism triggered specifically by RYGB, a finding that was independent of the presence of NASH in our analyses.

We hypothesized that RYGB could lead to changes in nuclear receptor expression and regulation in the liver. PPAR α is the main regulator of lipid metabolism in the liver during fasting state, but it works integrated to a network of nuclear receptors, in a nutrient-sensitizing fashion, differentiating fed from fasting state.^{29,30} On the other hand, FXR activation by bile acids exerts its effect in liver lipid metabolism mainly by its downstream pathways, but also in coordination with PPAR α activation.³¹ Besides the ability of FXR to directly activate PPAR α gene by binding to a receptor element,¹⁹ SHP also interacts physically with PPAR α gene, acting as a coactivator of gene transcription.³² Experimentally, HepG2 cells treated with bile acids exhibited increased expression of carnitine palmitoyltransferase 1 (CPT-1), a key enzyme for lipid oxidation that is under control of PPAR α .³⁰ Finally, there is also experimental evidence suggesting that simultaneous PPAR α and FXR activation may have a synergic effect in CPT-1 expression and NAFLD/NASH enhancement.^{19,33}

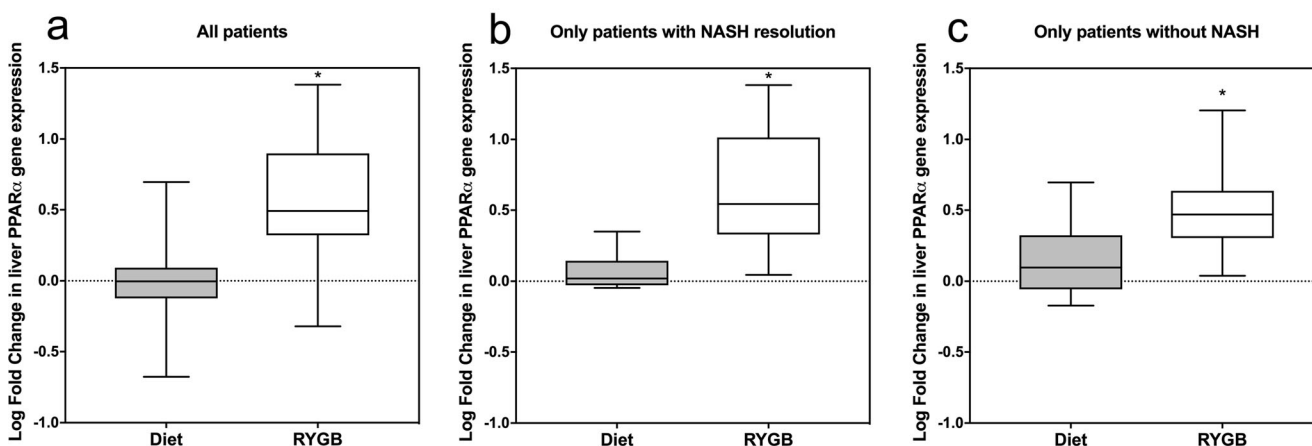


Fig. 1 Median logFC in liver PPAR α gene expression 1 year after Diet alone or RYGB. **a** Analysis of all patients. **b** Subset analysis of patients that resolved NASH after 1 year. **c** Subset analysis only with

patients without NASH at baseline and follow-up time points. Asterisk indicates only logFC from RYGB patients were statistically significant

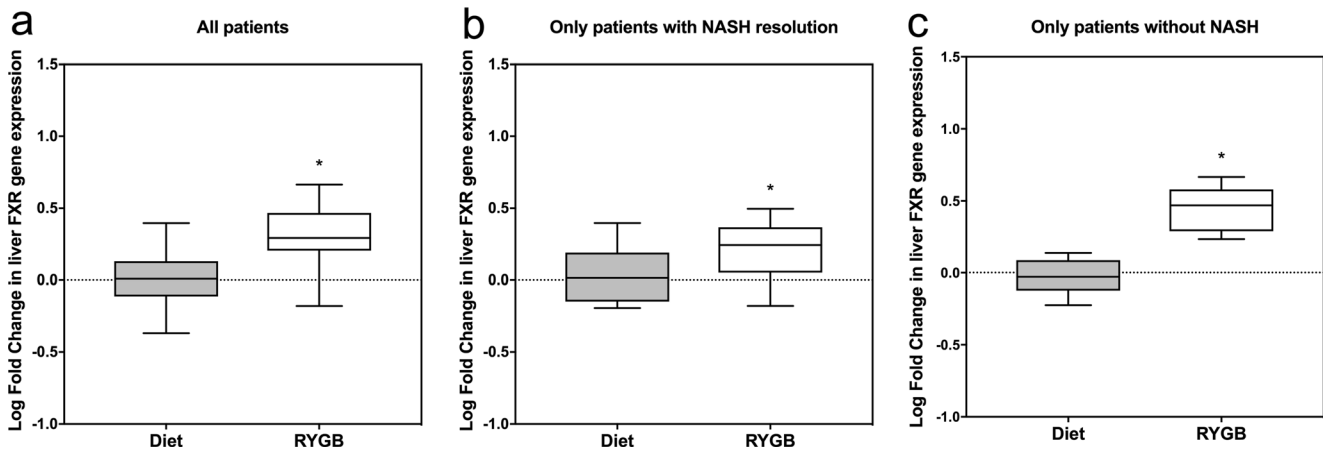


Fig. 2 Median $\log_{2}FC$ in liver FXR gene expression 1 year after Diet alone or RYGB. **a** Analysis of all patients. **b** Subset analysis of patients that resolved NASH after 1 year. **c** Subset analysis only with

patients without NASH at baseline and follow-up time points. Asterisk indicates only $\log_{2}FC$ from RYGB patients were statistically significant

Alongside FXR, PPAR α has a crucial role in hepatic lipid metabolism that is demonstrated by excess triglyceride accumulation in the liver of PPAR α -null mice that are starved or fed a high-fat diet.³⁴ PPAR α acts as a transcription factor during the fasting state, under free fatty acid activation, inducing hepatic fatty acid oxidation and ketogenesis.¹⁶ Downregulation of liver PPAR α seems to play a central role in NAFLD, triggering liver steatosis in obesity-induced oxidative stress and insulin resistance.³⁵ Experimentally, diet-induced obese mice treated with the PPAR α agonist improved hepatic steatosis accompanied by enhancement of the hepatocyte ultrastructure favoring β -oxidation and decrease in gene expression associated with hepatic de novo lipogenesis.³⁶ Regarding NASH pathophysiology, PPAR α downregulation enhances the DNA binding capacity of proinflammatory factors such as NF- κ B in the liver of obese patients, favoring the progression to steatohepatitis³⁵ and its activation was capable of reducing liver inflammation and fibrosis in studies with rodents.^{37,38} In humans, PPAR α expression in liver was demonstrated to be negatively correlated with NASH severity, visceral adiposity, and insulin resistance.¹⁷

The relationship between the various techniques of bariatric surgery and changes in bile acid pool, FXR, and PPAR α expression remains to be elucidated. Two studies from the same group in Belgium have brought initial light to this field.^{17,23} In the study by Francque et al.,¹⁷ 85 patients admitted for weight loss treatment with suspected NAFLD were submitted to either bariatric surgery ($n = 35$) or a weight management program ($n = 50$). After 1 year, mean BMI unit lost was greater for bariatric surgery subjects (11.9 versus 3.7 in diet group, $P < 0.05$), metabolic improvements were also significantly greater, and NASH resolution was observed in 100% of patients in bariatric surgery group versus only 32.3% in weight management program group ($P < 0.001$). Histological improvement in NASH subjects was associated with an increase in expression of PPAR α and its target genes. While the changes in PPAR α gene expression were significant in both bariatric and non-bariatric groups, it was

significantly greater after bariatric surgery, and the authors acknowledge that the greater effect on PPAR α expression with surgery may be related to a larger improvement in weight and other metabolic parameters and could not be attributed to a specific or independent metabolic effect of bariatric surgery.¹⁷ The study by Lefebvre et al.²³ (the origin for GSE83452) carried an interspecies whole-genome analysis and found a molecular signature for NASH + fibrosis. That molecular signature involved the activation of dermatopontin gene, which is under PPAR α regulation by a transrepressive mechanism, suggesting PPAR α as a putative therapeutic target for NASH. Additionally, the authors demonstrated that 1 year after RYGB, NASH improvement was accompanied by an increase in liver PPAR α expression and repression of dermatopontin gene expression. These two publications^{17,23} studied a similar cohort of patients and brought groundbreaking information about the role of PPAR α in NAFLD/NASH pathophysiology and resolution. Also, both studies support the contention that bariatric surgery may activate PPAR α through some intrinsic mechanism. However, design and analyses of these studies were directed toward understanding NAFLD/NASH pathophysiology and did not attempt to dissect further the mechanisms by which RYGB could have triggered those changes. It is important to underscore that our current analyses of the public GEO dataset GSE83452 were carried only in the patients that we could identify as having paired baseline and follow-up liver biopsies and genomic data, and that we compared gene expression data on the \log_{2} scale. These methodological differences to the studies of Francque et al.¹⁷ and Lefebvre et al.²³ likely have led to some differences in the results we obtained, specifically that we did not find a statistically significant increase in liver PPAR α gene expression 1 year after Diet, as Francque et al.¹⁷ reported.

From a broader perspective, it is very likely that additional nuclear receptor pathways may overlap or functionally interact with the PPAR α and FXR pathways. Considering that nuclear receptors work in an integrated way, our results point to a

possible effect of RYGB in modulating nuclear receptor function, probably triggered by changes in circulating bile acid pool. The understanding of these mechanisms may help us identify targets for new therapies, which could extend those benefits beyond the population with severe obesity. Despite the scientific advances in NAFLD/NASH pathophysiology, weight loss remains the main available treatment for NASH,^{25,39} and nuclear receptor modulators are under thorough investigation.⁴⁰ Special attention has been directed to a phase III trial studying the obeticholic acid, which is an FXR agonist.⁴¹ Thus, together with the previous experimental data presented, our results reinforce the idea that multi-target nuclear receptor modulators might be a suitable strategy to treat NASH.

While we were able to obtain, analyze, and present the data that study our hypothesis, our study has methodological limitations, mostly inherent in working with publicly available databases. We did not have access to important demographic information for the individual patients selected for genomic analyses such as baseline weight and weight loss, and other metabolic parameters, as well the whole histologic features of the biopsies. Also, we could not study whether the increase in both PPAR α and FXR gene expression in liver was a cause of the greater weight loss after RYGB relative to Diet alone, or if greater weight loss itself promotes changes in bile acid physiology and gene expression in liver. However, past findings that bile acids decrease after non-surgical weight loss and increase with RYGB^{7,42} seem to suggest that the disparities in postoperative bile acid profiles after RYGB and consequent PPAR α and FXR gene expression in liver are likely attributed to the respective anatomic rearrangements of the intestines and the resultant effects on bile acid enterohepatic circulation and/or reuptake, rather than differences in weight loss outcomes. Lastly, while the changes reported are of a relatively modest effect size, our dataset was sufficiently powered to detect those small effect sizes, indicating that the changes, albeit small, are consistent between the subgroups. Given these limitations, we conclude that compared to Diet, weight loss with RYGB results in greater NASH resolution with concurrent upregulation of hepatic PPAR α and FXR. Our findings point to concurrent PPAR α and FXR activation as a potential mechanism to improve NASH, triggered by RYGB. Prospective and controlled studies should be implemented to elucidate better the role of different bariatric surgery techniques in regulating energy metabolism, fat mass loss, impact on the many bile acid species, and activation of nuclear receptors not only in the liver but also in other tissues.

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Author Contributions Mazzini: conception, analysis, drafting, and final approval. Khoraki: GEO search and final approval. Dozmorov: GEO query and extraction, statistical analyses, drafting, and final approval. Browning: design, interpretation of data, drafting, and final approval.

Wijesinghe: design, interpretation of data, drafting, and final approval. Wolfe: study design, statistical analyses, and final approval. Gurski: interpretation of data, revising critically, and final approval. Campos: study conception, design, interpretation of data, drafting, revision, and final approval.

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