Research Article

Weight Loss Induced by Bariatric Surgery Restricts Hepatic GDF15 Expression

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Introduction. Obesity and related nonalcoholic fatty liver disease (NAFLD) are an emerging health care issue that imposes substantial morbidity to individuals. Growth and differentiation factor 15 (GDF15) limits food intake, body weight, and energy balance by modulation of GDNF-family receptor α-like (GFRAL) signalling in the hindbrain. However, the regulation of GDF15 expression in obesity and NAFLD is incompletely understood. We sought to define the impact of weight loss achieved by laparoscopic adjustable gastric banding (LAGB) on hepatic and adipose GDF15 expression in a cohort of severely obese patients.

Methods. We analysed GDF15 expression of liver and subcutaneous adipose tissue before and 6 months after LAGB in severely obese patients undergoing LAGB by quantitative real-time PCR. To assess the role of inflammation on GDF15 expression, we analysed HepG2 hepatocytes stimulated with cytokines such as IL-1β, TNFα, IL-6, LPS, or cellular stressors such as tunicamycin.

Results. GDF15 expression was mostly confined to the liver compared to adipose tissue in severely obese patients. Weight loss induced by LAGB was associated with reduced hepatic (but not adipose tissue) expression of GDF15. Stimulation with IL-1β or tunicamycin induced hepatic GDF15 expression in hepatocytes. In line with this, hepatic GDF15 expression directly correlated with IL-1β expression and steatosis severity in NAFLD. These data demonstrated that amelioration of metabolic inflammation and weight loss reduced hepatic GDF15 expression.

Conclusion. Based on recent mechanistic findings, our data suggest that hepatic GDF15 may serve as a negative feedback mechanism to control energy balance in NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) and obesity are dramatically increasing worldwide. Systemic inflammation and tissue inflammation represent a critical driver of disease processes in obesity and its related disorders including NAFLD [1, 2]. We previously described that hepatic inflammation in NAFLD patients could be reversed by weight loss that was achieved by laparoscopic adjustable gastric banding (LAGB). LAGB not only improved metabolic dysregulation but also liver disease [3, 4].

Growth and differentiation factor 15 (GDF15), also known as MIC-1, is a member of the transforming growth factor β (TGF-β) family. Increased tumor-derived GDF15 concentrations mediated cachexia by modulation of food intake in mice [5]. In line with these findings, overexpression of GDF15 in mice reduced food intake and body weight while genetic deletion of GDF15 evoked obesity [6, 7], and administration of recombinant GDF15 ameliorated diet-induced obesity in mice [8]. Only recently, a mechanism of GDF15-controlled food intake and body mass has been revealed in a series of reports in Nature Medicine [9–11]. These studies demonstrated that GDNF-family receptor α-like (GFRAL) served as a receptor of GDF15 signalling in the hindbrain (i.e., area postrema and nucleus tractus solitarius) which was required for the metabolic effects of GDF15 [9–11]. Specifically, mice exposed to a high-fat diet exhibited decreased food intake and body weight when they were treated with recombinant GDF15 which was likely mediated by signalling in the brain [9]. In line with this
notion, intracerebroventricular GDF15 application in rats similarly resulted in reduced food intake [11]. Knockout models established that GDF15 signalling particularly protected against diet-induced obesity, while no phenotype was observed at baseline [9–11]. These data suggest that GDF15/GFRL signalling critically controls energy balance in a situation with unrestricted dietary access to high-caloric food. In line with this, GDF15-mediated GFRL signalling at the brainstem regulated food intake and energy expenditure in metabolic and toxic-induced stress [12]. As such, clear evidence accumulated that GDF15 allows limitation of food intake to control body weight under calorie-rich dietary conditions.

Based on these data, GDF15/GFRL signalling emerges as a promising target to treat obesity in the future. However, the regulation of GDF15 in obesity-related human disease processes is poorly understood. GDF15 is expressed in the liver [13], and patients with nonalcoholic steatohepatitis exhibited increased systemic GDF15 level when compared to healthy controls or patients without simple steatosis [14]. In this cohort of NAFLD patients, systemic GDF15 concentrations increased with hepatic fibrosis and correlated with liver stiffness measured by elastography [14]. Although GDF15 emerges as a critical driver of metabolism in diet-induced obesity, the impact of body weight on hepatic GDF15 expression remains unexplored. We tracked a cohort of 28 severely obese patients that underwent laparoscopic adjustable gastric banding (LAGB) and analysed the impact of 28 severely obese patients that underwent laparoscopic adjustable gastric banding (LAGB) and analysed the impact of weight loss on GDF15 expression in the liver and subcutaneous adipose tissue. We found that GDF15 expression was mostly confined to the liver and that weight loss induced by LAGB was associated with reduced hepatic (but not adipose tissue) expression of GDF15. Mediators of metabolic inflammation such as IL-1β and tunicamycin induced hepatic GDF15 expression in hepatocytes and IL-1β expression correlated with GDF15 expression in the liver of NAFLD patients. As such, weight loss and reduction in low-grade inflammation induced by LAGB in severely obese patients impact on hepatic GDF15 expression [3, 15]. In light of recent studies [9–12], our findings suggest that hepatic GDF15 may serve as a feedback mechanism to control energy balance in NAFLD.

2. Material and Methods

2.1. Study Design. Evaluation for LAGB was performed at the Department of Medicine, Innsbruck Medical University, Innsbruck, Austria. In this study, twenty-eight patients (21 females, 7 male) with a BMI of more than 35 kg/m² were included between 2003 and 2007 [4]. Patients with alcohol intake of more than 20 g per week, statin treatment, or other cause of chronic liver diseases (autoimmune or viral hepatitis, PBC, PSC, haemochromatosis, and Wilson’s disease) were excluded from the study. The protocol was approved by the ethics committee of the Medical University Innsbruck, and patients provided written informed consent before LAGB and sample collection. Liver and abdominal subcutaneous tissue specimens were taken intraoperatively at LAGB and per biopsy six months after LAGB along with blood samples from the fasting state. Clinical parameters were assessed, and blood and biopsy specimen were stored at −80°C. Patient characteristics are summarized in Table 1.

2.2. Quantification of Hepatic and Adipose mRNA Expression. Expression analysis was performed as previously reported [4]. Tissue samples were thawed and total RNA was extracted using TRIzol® Reagent (Invitrogen, Carlsbad, California). RNA was reverse transcribed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, Carlsbad, California). Quantitative real-time PCRs were performed with mesa green master mix (Eurogentec, Seraign, Belgium) on an Mx3000 qPCR Cycler (Stratagene, La Jolla, California). Expression was normalised to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following primer sequences were used: GAPDH, forward: GCC ACC AGC CGA GCC; reverse: CCC AAT TAC AAA TCC GT; and GDF15, forward: GAC CCT CAG AGT TGC ACT CC; and reverse: GCC TGG TTA GCA GGT CCT C.

2.3. Culture and Stimulation of Hep G2 Hepatocytes. Hep G2 human hepatocellular carcinoma cells were purchased from ATCC (HB-8065; Middlesex, UK) and cultured in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were stimulated with lipopolysaccharide (LPS 100 ng/ml; Invivogen, San Diego, California), recombinant human TNFa (50 ng/ml; Peprotech), rec. IL-1β (1 ng/ml, Peprotech, 200-01B), rec. IL-6 (10 ng/ml, Peprotech, 200-06), or tunicamycin (1 µg/ml, Sigma, T7765) for 24–48 hours overnight.

2.4. Histological Analysis of Hepatic Biopsies. Hepatic biopsies were formalin-fixed and paraffin-embedded and stained with hematoxylin and eosin. A blinded pathologist scored the severity of steatosis (0–4) as previously described [15].

| Table 1: Clinical characteristics of patients before and after LAGB. |
|----------------|----------------|----------------|
| N (female/male) | 28 (21/7) | 28 (21/7) |
| Age | 38 [19–66] | 38 [19–66] |
| BMI (kg/m²) | 43.01 ± 3.70 | 35.7 ± 4.53 | <0.001 |
| Weight loss (kg) | 21.90 ± 9.76 | — | — |
| % excessive weight loss | 39.57 ± 17.92 | — | — |
| Fasting glucose (mg/dl) | 103.02 ± 17.81 | 89.47 ± 9.17 | <0.001 |
| Insulin (U/l) | 20.85 ± 15.05 | 11.89 ± 7.87 | <0.001 |
| HOMA | 5.53 ± 4.54 | 2.71 ± 2.05 | <0.001 |
| AST (U/l) | 30.59 ± 12.93 | 25.44 ± 7.14 | <0.058 |
| ALT (U/l) | 36.45 ± 27.90 | 23.89 ± 12.30 | <0.05 |
| GGT (U/l) | 36.04 ± 24.57 | 25.64 ± 16.41 | <0.01 |
| AP (U/l) | 66.86 ± 17.87 | 66.00 ± 11.37 | <0.083 |
| CRP (mg/dl) | 1.01 ± 0.73 | 0.63 ± 0.35 | <0.05 |
| Leukocyte count (G/l) | 7.32 ± 1.88 | 6.48 ± 1.39 | <0.05 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; HOMA, homeostasis model assessment (calculated as Insulin (µU/ml) × glucose (mmol/L)/22.5); GGT, y-glutamyl transferase.
2.5. Statistical Analysis. Results are expressed mean ± standard error of the mean (SEM) or dot blot where appropriate. Statistical significance between two groups was determined by a two-tailed Student’s t-test, a Wilcoxon signed-rank test, or a two-way ANOVA where appropriate and considered significant at \( P < 0.05 \). Linear regression was analysed by GraphPad Prism version 6.0.

3. Results

3.1. LAGB Ameliorates Metabolic Inflammation. We hypothesised that weight loss induced by laparoscopic gastric banding impacted on GDF15 expression. We analysed hepatic and subcutaneous fat expression before and 6 months after laparoscopic gastric banding in severely obese patients in a longitudinal fashion [4]. Our cohort comprised 28 patients with an average age of 38 years who had lost 21.9 kg ± 9.76 kg 6 months after LAGB (Table 1) [4]. Weight loss was paralleled by reduced low grade systemic inflammation indicated by leukocyte counts and C-reactive protein (CRP). Furthermore, weight loss was associated with reduced hepatic injury indicated by a reduction in alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT). In line with this, metabolic inflammation improved after 6 months as demonstrated by an improved homeostasis model assessment (HOMA) index and reduced hepatic expression of inflammatory cytokines such as IL-1β and IL-6 (Table 1 and [3, 16, 17]).

3.2. LAGB-Induced Weight Loss in Obese Patients is Associated with Reduced Hepatic GDF15 Expression. We utilised this cohort to analyse the expression of GDF15 in liver and subcutaneous adipose tissue specimens before and 6 months after LAGB. Hepatic GDF15 expression was largely confined to the liver in obese patients before LAGB (Figure 1(a)). Six months after LAGB, hepatic GDF15 expression substantially decreased in all individuals while we observed no demonstrable effect in subcutaneous adipose tissue (Figures 1(b) and 1(c)).

3.3. Inflammation and Endoplasmic Reticulum Stress Induce GDF15 Expression. To understand the effect of weight loss on hepatic GDF15 expression, we utilised Hep G2 hepatocytes as a model system. As hepatic inflammation ameliorated 6 months after LAGB [3, 16, 17], we hypothesised that cytokines and cellular stress may induce the expression of GDF15. To address the impact of cytokines and cellular stress on GDF15 expression, we stimulated Hep G2 cells with IL-1β, TNFα, IL-6, LPS, and tunicamycin, the latter being an inducer of endoplasmic reticulum stress [18]. We noted that IL-1β, but not TNFα, IL-6 or LPS, induced the expression of GDF15 in hepatocytes (Figure 2(a), Supplementary Figure 1). Furthermore, endoplasmic reticulum stress induced by tunicamycin increased the expression of GDF15 in Hep G2 hepatocytes (Figure 2(b)). These data indicated that hepatic inflammation contributed to increased GDF15 expression in obese patients [14] which could be reversed by LAGB-induced weight loss.

3.4. GDF15 Expression Correlates with Hepatic Steatosis and IL-1β Expression in NAFLD. To assess a relationship between the regulation of GDF15 and metabolic inflammation in NAFLD, we correlated clinical parameters with GDF15 expression before LAGB. We did not note a correlation between hepatic GDF15 expression and BMI, HOMA, liver injury, systemic inflammation (C-reactive protein), or hepatic TNFα expression (Supplementary Figures 2(A)–2(E)). In contrast, we noted a direct correlation between hepatic GDF15 expression and steatosis assessed by histologic means (Figure 3(a)). Furthermore, hepatic expression of GDF15 correlated with IL-1β (Figure 3(b)). These data indicated a direct relationship between features of NAFLD and hepatic GDF15 expression.

4. Discussion

GDF15 limits food uptake and obesity in experimental models. However, the regulation in and impact on obesity and related diseases in humans is incompletely understood. A previous study demonstrated increased circulating GDF15 concentrations in advanced NAFLD [14]. We report that hepatic (but not adipose tissue) GDF15 expression decreased after LAGB-induced weight loss. In hepatocytes, GDF15 expression was promoted by IL-1β signalling and ER stress both of which have been implicated in the development of NAFLD [19, 20]. A previous study demonstrated that palmitic acid impacted on GDF15 expression particularly in Kupffer cells [14]. Collectively, these findings may explain why LAGB-induced weight loss was associated with reduced hepatic GDF15 expression as we previously noted reduced hepatic inflammation (i.e., IL-1β expression) and improved metabolic dysfunction consequent to bariatric surgery in this cohort [3,15–17].

Previous studies convincingly demonstrated that GDF15 shapes the susceptibility to developing obesity and that

![Figure 1](image1.png)

![Figure 2](image2.png)

![Figure 3](image3.png)
GDF15 treatment ameliorated diet-induced obesity [8–12]. These data provide the basis for a model in which hepatic GDF15 is strongly expressed in NAFLD [14] to limit food intake and diet-induced obesity. In line with this, a recent study demonstrated increased hepatic GDF15 expression in NASH animal models and humans which may protect against NAFLD [21]. GDF15 signalling may act locally (e.g., in the liver) or systemically which we cannot address in this study. Specifically, we were unable to provide systemic GDF15 level in our cohort due to lack of sample availability, and GFRAL was neither expressed in the liver (as previously demonstrated [10]) nor in adipose tissue of our cohort (data not shown). After LAGB-induced weight loss, which is in part mediated by restricted food uptake [22], a compensatory expression of GDF15 in the liver may be less pronounced. In line with this notion, GDF15 expression directly correlated with steatosis severity in our study, a critical feature of NAFLD which could be reverted by weight loss [17]. As such, GDF15 treatment may be beneficial in obese patients and after LAGB as many patients relapse [22]. In this context, a local inflammatory milieu (e.g., hepatic MIC-1 expression) may also impact on the regulation of body weight [5].

To further explore a therapeutic benefit of GDF15 in metabolic diseases and NAFLD, additional experimental studies are needed. It may be plausible that GDF15 mediated actions other than regulation of food intake control susceptibility to obesity and related disorders. For example, GDF15 may act anti-inflammatory by limiting neutrophilic inflammation as seen in myocardial infarction [23]. This observation appears important in NAFLD, as advanced stages are characterised by hepatic neutrophilic inflammation [24]. Moreover, GDF15 controls hepatic hepcidin expression and iron overload which may set the susceptibility to NAFLD [25, 26]. Interestingly, GDF15 serum level is a predictor of all-cause mortality which highlights the importance of GDF15 signalling in many disease processes [27].

In conclusion, our study demonstrated that weight loss induced by LAGB reduced hepatic GDF15 expression in patients with NAFLD which may be mediated by a reduction in low-grade inflammation [16]. Based on previous findings [6,9–12], these observations suggest that GDF15 expression in NAFLD [14] occurs in a compensatory manner and that targeting this pathway may ameliorate obesity and related disorders.

**Figure 2**: IL-1β and tunicamycin promote GDF15 expression in hepatocytes. (a, b) GDF15 expression in Hep G2 hepatocytes over the course of 48 hours stimulation with interleukin 1b (a) or the endoplasmic reticulum stressor tunicamycin (b) determined by qPCR and normalised to GAPDH. Data from 3 independent experiments are shown. *P < 0.05.

**Figure 3**: Correlation of hepatic GDF15 expression with steatosis and inflammation. (a, b) Hepatic GDF15 mRNA expressions correlated with histologically quantified steatosis (a) and IL-1β expression (b). Respective R values and level of significance are shown in each panel. Each dot represents individual patient before or after LAGB.
Abbreviations

ALT: Alanine aminotransferase  
BMI: Body mass index  
GDF15: Growth differentiation factor 15  
GFT: c-Glutamyl transferase  
HOMA: Homeostasis model assessment  
LAGB: Laparoscopic gastric banding  
NAFLD: Nonalcoholic fatty liver disease.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

(i) GDF15 is strongly expressed in the liver compared to adipose tissue in obesity. (ii) Weight loss induced by LAGB is associated with reduced hepatic GDF15 expression in obese patients. (iii) Inflammatory signals such as IL-1β or unresolved endoplasmic reticulum stress induce GDF15 expression in hepatocytes. (iv) Hepatic GDF15 expression directly correlates with features of human NAFLD i.e., IL-1β expression and steatosis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

T.E.A. designed and analysed all experiments. F.G., L.M., C. G., and B.E. helped preparing the manuscript and performed experimentations. A.R.M. and H.T. developed and coordinated the project and prepared the manuscript.

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Supplementary Materials

Supplementary Figure 2: correlation of hepatic GDF15 expression with clinical features. (A–E) Hepatic GDF15 mRNA expressions did not correlate with body mass index (BMI) (A), homeostasis model assessment (HOMA) index (B), liver injury (C), systemic inflammation (D), and hepatic TNFα expression (E). Respective R values and level of significance are shown in each panel. Each dot represents an individual patient before or after LAGB. (Supplementary Materials)

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