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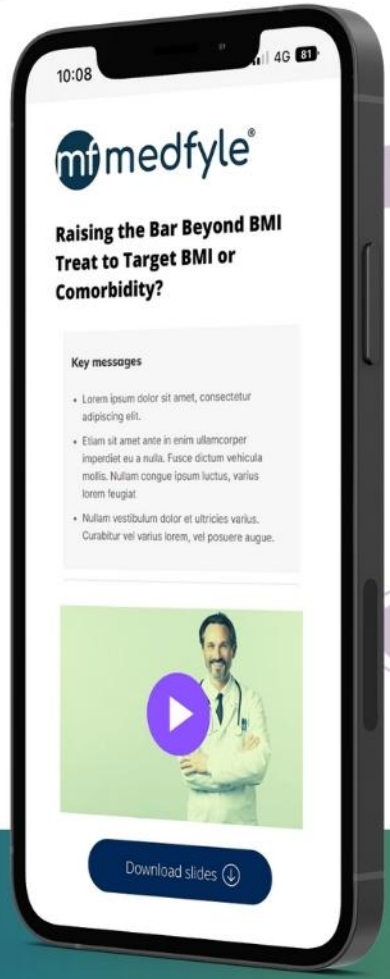

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Original Article
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Obesity

Liver Fat Reduction After Gastric Banding and Associations with Changes in Insulin Sensitivity and β-Cell Function

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Objective: The aim of this study was to examine the relationship between changes in liver fat and changes in insulin sensitivity and β-cell function 2 years after gastric banding surgery.

Methods: Data included 23 adults with the surgery who had prediabetes or type 2 diabetes for less than 1 year and BMI 30 to 40 kg/m^2 at baseline. Body adiposity measures including liver fat content (LFC), insulin sensitivity (M/I), and β-cell responses (acute, steady-state, and arginine-stimulated maximum C-peptide) were assessed at baseline and 2 years after surgery. Regression models were used to assess associations adjusted for age and sex.

Results: Two years after surgery, all measures of body adiposity, LFC, fasting and 2-hour glucose, and hemoglobin A1c significantly decreased; M/I significantly increased; and β-cell responses adjusted for M/I did not change significantly. Among adiposity measures, reduction in LFC had the strongest association with M/I increase (r = –0.61, P = 0.003). Among β-cell measures, change in LFC was associated with change in acute C-peptide response to arginine at maximal glycemic potentiation adjusted for M/I (r = 0.66, P = 0.007). Significant reductions in glycemic measures and increase in M/I were observed in individuals with LFC loss >2.5%.

Conclusions: Reduction in LFC after gastric banding surgery appears to be an important factor associated with long-term improvements in insulin sensitivity and glycemic profiles in adults with obesity and prediabetes or early type 2 diabetes.

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Study Importance

What is already known?

- The importance of excess liver fat in the pathogenesis of type 2 diabetes is increasingly recognized, yet limited studies have assessed effects of changes in liver fat on changes in insulin sensitivity and β-cell function, the two major determinants of type 2 diabetes.

What does this study add?

- Weight loss induced by gastric banding over 2 years resulted in significant reduction in body adiposity, increase in insulin sensitivity, and reduction in glycemic measures.
- Among adiposity measures, reduction in liver fat was the strongest correlate with improvement in insulin sensitivity and the only adiposity measure significantly associated with change in one of the three β-cell response measures derived from the hyperglycemic clamp.
- Significant reductions in glycemic measures and increase in insulin sensitivity were observed in individuals with liver fat loss >2.5%.

Introduction

Progressive loss of pancreatic β-cell function on a background of chronic insulin resistance is characteristic of the pathogenesis of type 2 diabetes (T2D) (1,2). Obesity is an important factor in this process. Indeed, observational studies have shown that weight gain over time is one of the most important factors contributing to worsening insulin

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resistance and falling β-cell function (3). Interventions that target obesity, whether through lifestyle, bariatric surgery, or medications, have produced the greatest relative and absolute reductions in incidence of T2D in people at high risk for the disease (4,5).

Obesity is associated with fat accumulation in the liver (6,7). Nonalcoholic fatty liver disease is an important entity that underlies the metabolic abnormalities associated with obesity (7). Furthermore, the importance of excess liver fat in the pathogenesis of T2D has been increasingly recognized (8). However, studies relating liver fat content (LFC) to insulin sensitivity and β-cell function remain limited. In cross-sectional studies, liver fat had a fairly strong and significant positive association with insulin resistance in adults (9-11) and adolescents with obesity (12), whereas the association with β-cell function has been inconsistent: some studies show no association (9,10), whereas others show association with early defects in β-cell function (11,12). In longitudinal studies, a recent mechanistic study in adults with T2D who participated in a low-calorie diet intervention to lose weight showed that T2D remission requires a decrease in liver fat (8). Two studies have assessed the longitudinal impact of bariatric surgery on liver fat in relation to changes in insulin sensitivity and β-cell function over time (13,14). Both studies included women with obesity and without diabetes, and follow-up time was limited to 3 months (13) or 12 months after gastric banding surgery (14). Insulin sensitivity and β-cell function were measured only by fasting blood samples but not dynamic measures. The 3-month study showed significant reduction in LFC, and the reduction in LFC was associated with improvement in insulin sensitivity measured by fasting samples (13). However, these results were not observed in the 12-month study (14).

The goal of the present report is to examine longitudinal associations between changes in liver fat induced by gastric banding surgery and changes in insulin sensitivity and β-cell function over 2 years of follow-up. Liver fat was measured by magnetic resonance imaging (MRI). Insulin sensitivity and β-cell function were measured by hyperglycemic clamps. We hypothesized that weight loss would lead to liver fat loss over 2 years, and reduction in liver fat would be important in improving insulin sensitivity and stabilizing or improving of β-cell function and glycemic measures.

How might these results change the direction of research or the focus of clinical practice?

- Reduction in liver fat appears important in improving insulin sensitivity and glycemic profiles in adults with obesity and prediabetes or early type 2 diabetes. This finding may direct future research toward understanding how liver fat impacts glucose regulation.

Methods

Data source

Data in this report were from participants who received gastric banding surgery as part of the BetaFat study of the Restoring Insulin Secretion (RISE) Consortium. The BetaFat study was a single-center mechanistic trial to test the impact of sustained weight loss on β-cell preservation over 2 years using gastric banding or metformin treatment (ClinicalTrials.gov identifier NCT01763346). Details of the BetaFat study have been described previously (15-17). The study was approved by the Institutional Review Boards at the University of Southern California and Kaiser Permanente Southern California. Written informed consent was obtained from each participant, consistent with the Helsinki Declaration and guidelines of the participating Institutional Review Boards. Twenty-three out of the thirty-six participants who received gastric banding surgery completed baseline and 2-year assessments of LFC and hyperglycemic clamps. Eligibility for the BetaFat study were as follows: adults (age 21-65 years) with moderate obesity (BMI 30-40 kg/m^2) and either impaired glucose tolerance (IGT) or mild T2D (fasting glucose >90 mg/dL, 2-hour glucose ≥140 mg/dL on 75-g oral glucose tolerance test [OGTT], hemoglobin A1c [HbA1c] ≤7.0%). For participants with diabetes, known diabetes duration had to be less than 1 year with no history of antidiabetic medication use except during pregnancy. Hyperglycemic clamps, OGTT, dual-energy x-ray absorptiometry (DXA), and abdominal MRI were performed at baseline and 2 years following laparoscopic gastric banding surgery.

Body anthropometrics and DXA. Weight was measured on a calibrated digital scale. Height was measured with a stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared (2). Waist circumference was measured midpoint between the iliac crest, and the lowest rib and hip circumference was measured at the top of the femur (hip); both were measured with the participant standing upright. DXA was used to measure total and fractional (percentage) body fat and trunk fat.

MRI. MRI was used to measure the proportion of fat in liver, expressed as LFC, as well as visceral fat volume. The validity of MRI to quantify liver fat has been established in previous studies (18,19). Approximately fifty 5-mm axial slices were obtained from the top of the liver to the pelvis using a series of 10- to 15-second breath holds on a GE Signa EXCITE HDxt 3.0T MR scanner (GE Healthcare, Waukesha, Wisconsin). Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL) was used to delineate visceral fat depots and quantify organ fat (20,21).

Image postprocessing for fat analysis and quantification was performed using Synapse 3D (Fujifilm, Stamford, Connecticut). Fat fraction maps were used as the base images for segmentation. For each scan, three volumes were manually segmented: total body volume (excluded noisy outer body voxels), abdominal cavity volume, and whole liver volume. Visceral fat volume was created from the abdominal cavity volume. A ≥50% fat fraction threshold was applied on a per-voxel basis to all fat volumes to remove lean tissue. The visceral fat volume was further manually segmented to remove intramuscular and digestive tract fat. The liver volume was eroded by 2 pixels to generate a region of interest completely within the body of the liver. The average fat fraction within liver volume was reported as LFC.

Hyperglycemic clamps. Details of the hyperglycemic clamp have been published (14,16). Briefly, a two-step hyperglycemic clamp (11.1 mmol/L, then >25 mmol/L plus arginine) was performed at baseline and 24 months as described previously (15,17). The hyperglycemic clamp was used to simultaneously quantify insulin sensitivity (calculated as M/I, defined subsequently) and three β-cell response measures: (1) acute (first-phase) C-peptide response to glucose (ACPRg; mean incremental C-peptide above baseline for the first 10 minutes after the glucose bolus), (2) steady state (at plasma glucose ~200 mg/dL) C-peptide concentration (SSCP), and (3) acute C-peptide response to arginine at maximal glycemic potentiation (>450 mg/dL; ACPRmax). M/I was calculated from clamps as the mean glucose infusion rate (M) at the steady-state glucose of ~200 mg/dL divided by the corresponding steady-state plasma insulin concentration (I).

Glucose tolerance and glycemia. Three-hour 75-gm OGTTs with samples collected at fasting and 10, 20, 30, 60, 90, 120, 150, and 180 minutes after glucose ingestion were performed on separate days from clamps. HbA1c was measured at each visit.

Assays. Laboratory assessments were performed in a central laboratory at the University of Washington (15-17). Glucose was measured using the glucose hexokinase method on a Roche c501 autoanalyzer (Roche Diagnostics Inc., Indianapolis, Indiana). C-peptide and insulin were measured by a two-site immunoenzymometric assay performed on the TOSOH 2000 autoanalyzer (TOSOH Biosciences, South San Francisco, California). Interassay coefficients of variation (CVs) on quality control samples with low, medium, medium-high, and high concentrations were ≤2.0% for glucose, ≤4.3% for C-peptide, and ≤3.5% for insulin. HbA1c was measured on a TOSOH G8 analyzer, under Level 1 National Glycohemoglobin Standardization Program (NGSP) certification. The interassay CVs as measured on quality control samples with low and high HbA1c levels were 1.9% and 1.0%, respectively (15-17).

Data analysis

Measures at baseline and 2 years and changes between these time points in anthropometrics, glycemia, insulin sensitivity (M/I), and β-cell responses are presented as mean (SD) for normally distributed variables and medians (interquartile ranges) for non-normally distributed variables. Significance of changes in these measures over time were tested by paired *t* test or Wilcoxon signed rank test where appropriate. Significance for changes in β-cell responses over time after adjusting for insulin sensitivity were assessed using regression models with random intercept and repeated measure over time.

Linear regression models were used to assess the correlations of LFC with other anthropometric measures (weight, BMI, waist circumference, hip circumference, total and percentage body and trunk fat, and visceral fat volume) at baseline and for changes during follow-up, adjusted for age and sex to control for potential confounding. Similarly, linear regression analyses were used to assess the associations between LFC and other body anthropometrics and insulin sensitivity (M/I) and β-cell responses (ACPRg, SSCP, ACPRmax, fasting C-peptide) at baseline and for changes during follow-up, adjusted for age and sex. We also calculated the ratio of fasting C-peptide to fasting insulin as a surrogate index of insulin clearance. For β-cell responses and insulin clearance, we further adjusted for insulin sensitivity. LFC, insulin sensitivity, and measures of β-cell responses and insulin clearance were log-transformed to normalize the data distribution prior to data analysis. To make the regression coefficients directly comparable across different measures with different measurement units, standardized regression coefficients are reported. The standardized regression coefficients are scale-independent and represent change per SD in the dependent variable associated with change per SD in the independent variable. SAS version 9.4 (SAS Institute Inc., Cary, North Carolina) was used for data analysis. All statistical tests were two-sided.

Results

Cohort characteristics

Of the 23 participants included in this report, 14 had IGT, and 9 had mild T2D at baseline. The mean (SD) age and BMI were 48.2 (9.5) years and 35.3 (3.3) kg/m^2, respectively (Table 1). The median LFC (interquartile range) was 8.0% (6.2%-15.0%), ranging from 2.9% to 31.8%. A total of 22 (96%) of the participants had LFC >3.0%, a threshold that has been used to identify people with steatosis and metabolic syndrome using the IDEAL method (22,23). During follow-up, body weight, BMI, waist and hip circumferences, total and percentage body and trunk fat, and LFC all significantly decreased ($P < 0.001$). The mean weight change was −10.2 kg and ranged from −38.9 kg to 1.6 kg; 96% of the participants lost weight. The mean percentage weight change from baseline was −10.3% and ranged from −35.4% to 1.7%. The median LFC change from baseline was −2.5% and ranged from −20.7% to 5.8%; 78% of the participants had a reduction in LFC. However, among the 22 participants who had LFC >3.0% at baseline, only two improved their LFC to ≤3.0% at 2 years.

During the 2 years of follow-up (Table 1), fasting and 2-hour glucose and HbA1c were significantly decreased ($P < 0.04$). Insulin sensitivity (M/I) increased significantly ($P = 0.004$), whereas fasting C-peptide ($P < 0.0001$), fasting insulin ($P < 0.0001$), and SSCP ($P = 0.0008$) decreased significantly; ACPRg did not change significantly; ACPRmax also decreased but the change was of borderline statistical significance ($P = 0.08$). Insulin clearance increased significantly ($P = 0.0004$). After adjusting for baseline values and change in M/I, fasting C-peptide ($P < 0.0001$) and fasting insulin ($P = 0.0003$) remained significantly decreased from baseline, and insulin clearance remained significantly increased ($P = 0.017$). After the same adjustments, ACPRg showed a trend toward increasing ($P = 0.07$), SSCP showed a trend toward decreasing ($P = 0.08$), and ACPRmax showed no significant change ($P = 0.43$).

Correlations of liver fat with other measures of body anthropometrics at baseline and during follow-up

At baseline, LFC had no significant correlation with other anthropometrics except for percentage trunk fat by DXA ($r = 0.43$, $P = 0.05$) (Table 2). In contrast, changes in LFC during follow-up were highly correlated with changes in weight ($r = 0.66$, $P = 0.001$), BMI ($r = 0.64$, $P = 0.002$), and total and percentage body and trunk fat by DXA ($r \geq 0.61$, $P \leq 0.004$ for each). Changes in LFC were not significantly correlated with changes in waist or hip circumference or visceral fat volume.

Associations of liver fat with insulin sensitivity and β-cell responses

At baseline, LFC was significantly associated with fasting C-peptide levels without adjusting for M/I ($r = 0.46$, $P < 0.05$), whereas associations between LFC and M/I or measures of β-cell response failed to reach significance when adjusting for M/I (Table 3). Among other measures of anthropometrics, the only significant association was the well-known negative association between BMI and M/I ($r = -0.47$, $P < 0.05$).

For changes during follow-up, change in LFC was negatively associated with change in M/I ($r = -0.61$, $P = 0.003$, Figure 1A) and positively associated with change in ACPRmax ($r = 0.50$, $P = 0.01$, Figure 1B) and fasting C-peptide ($r = 0.57$, $P = 0.007$, Figure 1C). Correlations between LFC and ACPRmax or fasting C-peptide became stronger after adjusting for change in M/I ($r = 0.66$, $P = 0.007$ for ACPRmax, and $r = 0.74$, $P = 0.005$ for fasting C-peptide) (Table 3). Similar patterns of association were observed between changes in other anthropometric measures (weight, BMI, total and percentage body and trunk fat) and

TABLE 1 Baseline and follow-up characteristics

	Baseline	Year 2	Change
Demographics			
Age (y)	48.2 ± 9.5		
Female (%)	17 (73.9%)		
Race/ethnicity			
White	7 (30.4%)		
Black	5 (21.7%)		
Hispanic	11 (47.8%)		
Anthropometrics			
Weight (kg)	98.8 ± 13.6	88.8 ± 14.7	−10.2 ± 8.0*
BMI (kg/m^2)	35.3 ± 3.3	31.6 ± 3.5	−3.7 ± 2.9*
Waist circumference (cm)	104.7 ± 8.1	99.4 ± 9.5	−5.4 ± 6.6*
Hip circumference (cm)	119.5 ± 8.0	112.7 ± 7.7	−6.8 ± 6.7*
Total body fat (kg)	42.8 ± 7.5	35.7 ± 8.0	−7.1 ± 5.9*
Total trunk fat (kg)	23.5 ± 5.3	19.0 ± 5.5	−4.5 ± 3.7*
Percentage body fat (%)	43.1 ± 5.5	40.0 ± 6.1	−3.2 ± 3.6*
Percentage trunk fat (%)	45.0 ± 5.7	40.8 ± 7.2	−4.2 ± 4.7*
Liver fat content (%)	8.0 (6.2, 15.0)	5.2 (3.8, 8.8)	−2.5 (−7.8, −0.3)*
Visceral fat volume (L)	3.5 ± 1.6	2.9 ± 1.8	−0.6 ± 0.6*
Glucose			
Fasting (mmol/L)	6.2 ± 0.89	5.8 ± 1.18	−0.4 ± 0.8**
2-hour (mmol/L)	10.5 ± 2.7	9.5 ± 3.1	−1.0 ± 2.2**
HbA1c (mmol/mol)	40.3 ± 4.8	38.8 ± 6.4	−1.5 ± 3.2**
Hyperglycemic clamp			
M/I (10^{-5} mmol/kg/min per pmol/L)	3.09 (1.93, 5.31)	5.68 (2.61, 7.34)	1.61 (−0.14, 4.03)***
ACPRg (nmol/L)	0.55 (0.16, 1.13)	0.75 (0.36, 1.09)	0.04 (−0.14, 0.48)
SSCP (nmol/L)	3.90 (3.18, 4.80)	3.22 (2.94, 4.35)	−0.60 (−0.90, −0.11)***
ACPRmax (nmol/L)	6.15 (3.52, 9.07)	4.36 (3.00, 7.15)	−0.55 (−2.03, 0.40)
Fasting C-peptide (nmol/L)	1.32 (0.97, 1.51)	0.94 (0.74, 1.16)	−0.26 (−0.43, −0.15)*
Fasting insulin (nmol/L)	126.1 (96.6, 168.2)	66.0 (46.3, 95.1)	−51.1 (−75.4, −18.3)*
Fasting C-peptide/fasting insulin × 100	1.09 (0.81, 1.13)	1.15 (1.15, 1.63)	0.29 (0.04, 0.52)*

Data presented as mean ± SD, *N* (%), or median (25th, 75th percentiles).
ACPRg = acute C-peptide release above fasting, defined as mean incremental plasma concentration above baseline during the first 10 minutes after the glucose injection at the initiation of the glucose clamp; ACPRmax = acute C-peptide response to arginine maximal glucose concentration, defined as mean incremental C-peptide response during the 5 minutes after the arginine injection; M/I = insulin sensitivity, defined as mean glucose infusion rate divided by mean insulin concentration at steady-state hyperglycemia (100, 110, and 120 minutes during glucose clamp); SSCP = steady-state C-peptide, defined as mean of C-peptide concentrations at steady state (100, 110, and 120 minutes during glucose clamp).
*$P < 0.001$ by one-sample *t* test or Wilcoxon signed rank test where appropriate.
**$0.01 < P < 0.05$ by one-sample *t* test or Wilcoxon signed rank test where appropriate.
***$0.001 \leq P \leq 0.01$ by one-sample *t* test or Wilcoxon signed rank test where appropriate.

changes in M/I (negative associations) and fasting C-peptide (positive associations) but not for change in ACPRmax (no significant associations) (Table 3). To assess whether the associations between change in LFC and changes in M/I and β-cell responses were because of change in weight, we further adjusted for weight change in the models. Adjusting for weight change reduced the association between change in LFC and change in M/I from r of −0.61 to −0.48, and it became borderline significant ($P = 0.06$), but it did not affect the association between change in LFC and change in fasting C-peptide (r changed from 0.57 to 0.58, $P = 0.047$). By contrast, adjustment for weight change increased the association between change in LFC and change in ACPRmax adjusted for M/I (r changed from 0.66 to 0.76, $P = 0.009$). Changes in waist circumference, hip circumference, and visceral fat volume were not significantly associated with changes in M/I or β-cell responses. None of the changes in body anthropometrics were associated with changes in ACPRg, SSCP, or fasting insulin clearance (Table 3).

When the study cohort was divided at the median of change in LFC (i.e., −2.5%), reduced fasting ($P = 0.0005$) and 2-hour ($P = 0.02$) glucose and HbA1c ($P < 0.0001$) and increased M/I ($P = 0.004$) were limited to the 12 participants who lost at least 2.5% of LFC (Figure 2). Although SSCP ($P = 0.006$) and ACPRmax ($P = 0.013$) decreased significantly in the subgroup with relatively large loss of LFC, the significance disappeared after adjusting for change in M/I ($P = 0.59$ for SSCP and $P = 0.10$ for ACPRmax). Adjusting for change in weight loss did not explain the decrease in fasting ($P = 0.024$) and HbA1c ($P = 0.0003$)

TABLE 2 Correlations of LFC with other measures of body anthropometrics at baseline (cross-sectional) and with changes during follow-up (longitudinal)

	Baseline (cross-sectional)		Changes (longitudinal)	
LFC vs.	*r**	*P*	*r**	*P*
Weight	−0.11	0.64	0.66	0.001
BMI	0.09	0.70	0.64	0.002
Waist circumference	0.12	0.61	0.40	0.08
Hip circumference	−0.05	0.83	0.28	0.22
Total body fat	0.11	0.62	0.68	0.0007
Total trunk fat	0.26	0.25	0.70	0.0005
% Body fat	0.33	0.15	0.63	0.002
% Trunk fat	0.43	0.05	0.61	0.004
Visceral fat volume	0.21	0.37	−0.12	0.61

LFC = liver fat content.
*Values shown are correlation coefficients after adjusting for age and sex.

and increase in M/I ($P = 0.027$) in this subgroup. No significant changes in glycemia, M/I, or β-cell response measures were observed in the 11 participants with relatively small loss of LFC.

Discussion

We found significant reductions in fat in all measured depots, including the liver, 2 years after laparoscopic gastric banding in participants affected by overweight and obesity with IGT or recently diagnosed T2D. Simultaneously, insulin sensitivity increased, and fasting and steady-state C-peptide levels and glucose concentrations fell, consistent with β-cell unloading and improved glucose metabolism. Reduction in LFC was the strongest correlate with improvement in insulin sensitivity. Reduction in body weight may have contributed as well, because adjusting for weight attenuated the association between change in LFC and change in insulin sensitivity. Reduction in LFC was also associated with a reduction in circulating levels of C-peptide at both fasting and acute response to arginine at maximal glycemic potentiation both before and after adjusting for improvement in insulin sensitivity. This finding suggests that the association of change in liver fat with change in insulin secretion is not simply changing compensation for changing insulin resistance, rather, there may be direct effects of changing liver fat to mediate same directional change in insulin secretion, as has been shown during fat feeding in animal models (24,25). Of note, reductions in C-peptide following weight loss occurred in the face of improved glucose levels, indicating that the reductions did not reflect deterioration of β-cell function. Finally, significant reductions in glycemia and improvements in insulin sensitivity and β-cell unloading measured by ACPRmax were limited to the subset of participants with reductions in LFC greater than the median for the cohort, and the significant improvement in fasting, A1c, and M/I were independent of weight loss. Our findings suggest that changes in liver fat are fundamentally important to improvement in insulin sensitivity and glycemic control associated with gastric banding over a 2-year period in people with IGT or recently diagnosed T2D.

To our knowledge, this is the first relatively long-term study aimed at understanding the role of liver fat and multiple other adiposity measures on changes in insulin sensitivity, β-cell responses, and glycemic measures after weight loss induced by gastric banding surgery. Although many measures of adiposity and fat distribution improved following surgery, we found that changes in liver fat had the strongest association with changes in insulin sensitivity. Further, change in liver fat was the only measure of body adiposity that was significantly associated with changes in one of the three β-cell response measures derived from hyperglycemic clamp. The association was direct and became stronger after adjusting for change in insulin sensitivity and weight. The biological mechanisms behind this direct association remain to be studied. However, the fact that it occurred in the face of reduced glucose levels supports β-cell unloading rather than β-cell deterioration as the cause. Of note, our results should be interpreted in light of the fact that most study participants had mild to moderate fatty liver based on the level of LFC.

Interestingly, cross-sectionally at baseline, LFC had little association with all other measures of body adiposity. In contrast, change in LFC over time was significantly associated with changes in weight loss and body fat loss. However, change in LFC was not associated with changes in waist or hip circumferences or visceral fat volume. Cross-sectionally at baseline, LFC was not associated with insulin sensitivity or with β-cell responses, except for a positive association with fasting C-peptide. This result is contrary to those from prior cross-sectional studies in which liver fat had a fairly strong and significant positive association with insulin resistance in adults (9-11). Differences among the study cohorts could explain the differing results. Our study cohort included individuals with a BMI of 30 to 40 kg/m^2 at baseline with IGT or early T2D, whereas the other cohorts included individuals with normal glucose tolerance without restriction of BMI minimum or maximum. The narrow range of BMI, hyperglycemia, and LFC at baseline in our study may have limited our ability to detect important associations. Gastric banding induced a wide range of individual changes in body fat content and distribution, providing greater opportunity to find and examine associations. Of note, the significant associations between change in liver fat and changes in weight, BMI and total or trunk fat are expected and consistent with previous reports (26).

Although the effect of weight loss induced by gastric banding on markers of insulin sensitivity and β-cell function in individuals with severe obesity has been shown more than a decade ago (27), few and limited longitudinal studies have directly assessed changes in liver fat relative to changes in insulin sensitivity and β-cells responses. A very low-calorie diet in 11 individuals with T2D for 8 weeks showed that liver fat reduction was accompanied with the normalization of hepatic insulin sensitivity and β-cell function (28). Two earlier longitudinal gastric banding studies included 29 women affected by obesity and without diabetes who were assessed 3 months after surgery (13) or 18 women affected by obesity and without diabetes who were assessed 12 months after surgery (14). Insulin resistance was estimated from the homeostatic model assessment of insulin resistance (HOMA-IR) in both studies. In the former study, gastric banding surgery led to a significant reduction in LFC, and the change was significantly associated with reduction in HOMA-IR and decrease in fasting insulin (13). In the latter, LFC did not change significantly, and there was no association between change in LFC and change in either HOMA-IR or fasting insulin (14). In one study in patients affected by obesity and without diabetes, insulin sensitivity and β-cell function were assessed in response to mixed meal testing or banding and Roux-en-Y gastric bypass (RYGB)

TABLE 3 Associations between LFC and other measures of body anthropometrics with insulin sensitivity and β-cell responses at baseline and for changes during follow-up

		Not adjusting for M/I					Adjusting for M/I				
	M/I	ACPRg	SSCP	ACPRmax	Fasting C-peptide	Fasting IC	ACPRg	SSCP	ACPRmax	Fasting C-peptide	Fasting IC
Cross-sectional correlations at baseline											
LFC	−0.14	−0.24	0.10	−0.04	**0.46***	−0.19	−0.29	0.01	−0.06	0.42	−0.14
Weight	−0.32	0.27	0.31	0.03	−0.18	0.15	0.18	0.11	−0.004	−0.32	0.28
BMI	**−0.47***	0.37	0.44	0.25	−0.10	0.06	0.29	0.17	0.27	−0.36	0.29
Waist circumference	−0.43	0.05	0.26	0.01	0.34	−0.32	−0.10	−0.03	−0.04	0.21	−0.21
Hip circumference	−0.01	−0.07	−0.04	−0.16	−0.13	0.02	−0.07	−0.05	−0.16	−0.14	0.02
Total body fat	−0.32	0.18	0.27	0.02	−0.05	0.11	0.09	0.06	−0.01	−0.20	0.25
Total trunk fat	−0.35	0.09	0.27	−0.03	0.16	−0.06	−0.03	0.04	−0.07	0.03	0.07
Percentage body fat	−0.42	0.18	0.32	0.05	0.07	0.12	0.05	0.05	0.01	−0.09	0.29
Percentage trunk fat	−0.44	0.04	0.28	−0.02	0.36	−0.18	−0.12	−0.01	−0.08	0.22	−0.05
Visceral fat volume	−0.13	−0.30	0.02	−0.40	0.37	−0.24	−0.35	−0.07	−0.41	0.32	−0.20
Longitudinal correlations for changes (follow-up – baseline)											
LFC	**−0.61****	0.02	0.24	**0.50****	**0.57****	−0.16	−0.004	0.17	**0.66****	**0.74****	−0.03
Weight	**−0.51***	0.07	0.33	0.20	**0.50***	−0.17	0.08	0.30	0.18	**0.56***	−0.07
BMI	**−0.44***	0.05	0.29	0.28	**0.44***	−0.05	0.05	0.24	0.28	**0.46***	0.07
Waist circumference	−0.46	−0.15	0.09	0.17	0.43	−0.19	−0.20	−0.02	0.13	0.43	−0.10
Hip circumference	−0.37	0.14	0.11	0.31	0.18	−0.02	0.15	0.03	0.29	0.14	0.08
Total body fat	**−0.51***	0.11	0.37	0.25	**0.49***	−0.08	0.12	0.32	0.23	**0.52***	0.05
Total trunk fat	**−0.54***	0.06	0.35	0.27	0.45	−0.04	0.06	0.29	0.25	0.46	0.12
Percentage body fat	−0.36	0.11	0.35	0.20	**0.58***	−0.23	0.11	0.29	0.16	**0.57***	−0.16
Percentage trunk fat	−0.36	−0.02	0.28	0.15	**0.59***	−0.31	−0.03	0.21	0.11	**0.58***	−0.24
Visceral fat volume	0.17	−0.01	0.11	−0.12	0.33	−0.16	0.0002	0.15	−0.10	0.36	−0.21

Data given as standardized regression coefficients (beta) adjusted for age and sex; bold are statistically significant.
ACPRg = acute C-peptide response to glucose, defined as mean incremental plasma concentration above baseline during the first 10 minutes after the glucose injection used to initiate the hyperglycemic clamp; ACPRmax = acute C-peptide response to arginine at the maximal glucose concentration, defined as mean incremental C-peptide response during the 5 minutes after the arginine injection; fasting IC = fasting insulin clearance, defined as ratio of fasting C-peptide over fasting insulin; LFC = liver fat content; M/I = insulin sensitivity, defined as mean glucose infusion rate divided by mean insulin concentration at steady-state hyperglycemia (100, 110, and 120 minutes during glucose clamp); SSCP = steady-state C-peptide, defined as mean of C-peptide concentrations at steady-state hyperglycemia (100, 110, and 120 minutes during glucose clamp).
*$0.01 < P < 0.05$.
**$P \leq 0.01$.

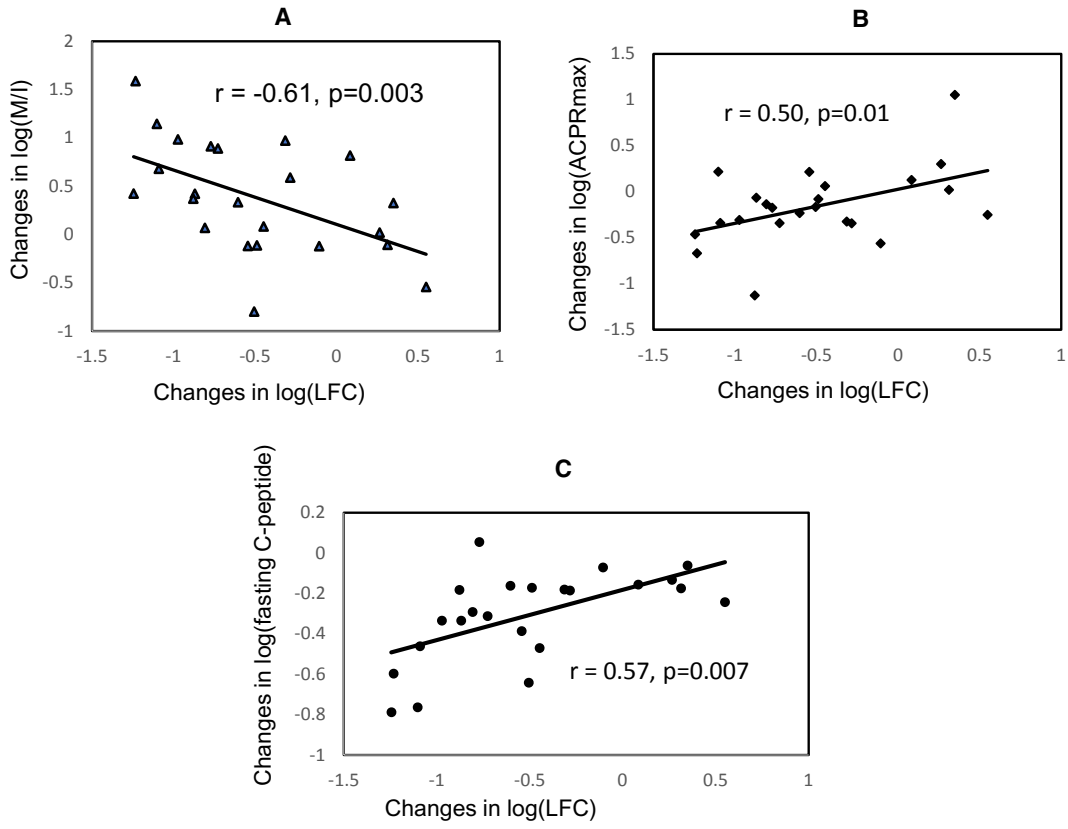

Figure 1 Relationships between changes in log of LFC and changes in (A) log of insulin sensitivity (M/I), (B) log of ACPRmax, and (C) log of fasting C-peptide 2 years after gastric banding surgery. ACPRmax, acute C-peptide response to arginine at maximal glycemic potentiation; LFC, liver fat content.

surgery. After achieving >20% weight loss via either approach (22 weeks for gastric banding and 16 weeks for RYGB), RYGB led to rapid delivery of ingested glucose and dynamic insulin secretion and early postprandial increases in plasma glucose and insulin. However, the two surgery groups had similar, modest improvements in β-cell function and large improvements in muscle and hepatic insulin sensitivity with an approximate 60% decrease in liver fat (29). The authors did not examine the relationship between changes in liver fat and changes in insulin sensitivity and β-cell function. Our study expands on this finding by extending the duration of treatment to 2 years and the use of hyperglycemic clamps to measure insulin sensitivity and β-cell function contemporaneously.

Although decreases in liver fat and dysglycemia following multiple types of bariatric surgery are well documented, this is often attributed to improvements in multitissue insulin sensitivity secondary to weight loss. Immonen et. al. (30,31) found that 6 months following RYGB surgery, patients with obesity, with or without diabetes, had decreased uptake of fatty acids to the liver, likely due to decreased fat mass and improved adipose insulin sensitivity. Within the liver, glucose uptake was improved, and excess endogenous glucose release was nearly normalized. Similar findings were observed in patients with extreme obesity 12 months after gastric bypass surgery, and adipose insulin sensitivity improved by 47% (32). We found that decreases in liver fat were most closely associated with improvements in whole body insulin sensitivity, confirming the strength of this relationship 2 years after gastric banding surgery. Future studies should include assessments of tissue-specific fatness, including skeletal muscle and pancreas and their relationship with changes in insulin sensitivity and β-cell function, and extend the follow-up time to more than 2 years after surgery.

This study has several unique strengths. First, the significant and sustained weight loss induced by gastric banding allowed us to examine longitudinal relationships between changes in hepatic fat and other anthropometrics and changes in insulin sensitivity and β-cell function over time. Second, the methods we used to assess β-cell function are state-of-the-art, allowing contemporaneous evaluation of multiple aspects of secretory function in relation to directly measured insulin sensitivity. Finally, the moderate obesity that we chose to study is common among people with IGT or early T2D and may be better suited to gastric banding than more intensive bariatric approaches that induce much greater weight loss.

Important weaknesses of this report include the fact that only a subset of BetaFat participants had MRI scans at follow-up. The relatively small number of participants limited power to detect small changes and associations. Nonetheless, we were able to detect notable changes in LFC and its association with changes in insulin resistance and β-cell responses. We did not measure pancreatic fat content thus precluding us to assess its role as demonstrated in the recent study (8). Our results

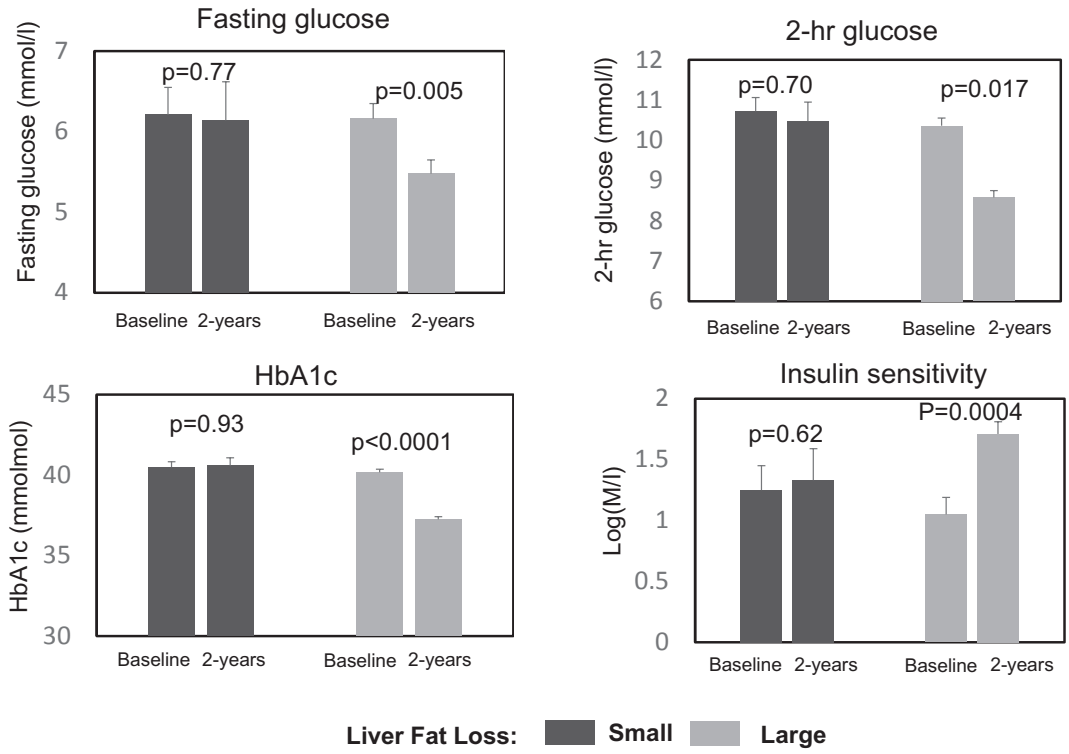

Figure 2 Mean (SE) of fasting glucose, 2-hour glucose from OGTT, HbA1c, and log of insulin sensitivity (M/I) at baseline and 2 years after gastric banding surgery stratified by the median change in LFC from baseline (–2.5%). Small = LFC loss less than or equal to 2.5% from baseline (n = 11); Large = LFC loss from baseline greater than 2.5% (n = 12). P value tests for within-group differences between baseline and 2 years. HbA1c, hemoglobin A1c; LFC, liver fat content; OGTT, oral glucose tolerance test.

should be interpreted in the context that individuals included in this study had narrow range of BMI and limited to IGT and T2D diagnosed less than 1 year at baseline, therefore the results may not be generalizable to a broader population. In addition, although the longitudinal design allowed us to assess changes and associations among them, the study design precluded us to make causal inferences.

In summary, in adults with mild to moderate obesity and IGT or recently diagnosed mild T2D, weight loss induced by gastric banding over 2 years resulted in a significant reduction in liver fat, which appeared to be an important factor associated with improved insulin sensitivity and reduced glucose levels.O

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Author contributions: AHX and TAB designed and implemented the study, researched data, and wrote the first draft of the manuscript. AHX, MPM, ET, DHH, NK, KSN, and TAB conducted the study. AHX performed data analysis. All of the listed authors edited the manuscript, as did members of the **RISE Steering Committee:** Kristen J. Nadeau, Tamara S. Hannon, Sharon L. Edelstein, Ellen W. Leschek, Philip S. Zeitler, Kieren J. Mather, Barbara Linder, and Steven E. Kahn.

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