

Nonalcoholic Fatty Liver Disease: Improvement in Liver Histological Analysis With Weight Loss

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The effect of significant weight loss on nonalcoholic fatty liver disease remains unclear. In this case series of 36 selected obese patients, we examined the effect of weight loss on nonalcoholic fatty liver disease, including nonalcoholic steatohepatitis (NASH) and hepatic fibrosis. These 36 patients (11 males, 25 females) had paired liver biopsies, the first at the time of laparoscopic adjustable gastric band placement and the second after weight loss. Second biopsies were obtained from two groups: those requiring a subsequent laparoscopic procedure (n = 19) and those with index biopsy score of 2 or greater for zone 3-centric hepatic fibrosis (n = 17). All biopsies were scored, blinded to the patient's identity and clinical condition, for individual histological features and for NASH stage and grade. Initial biopsies demonstrated NASH in 23 patients and steatosis in 12 patients. Repeat biopsies were taken at 25.6 ± 10 months (range, 9–51 months) after band placement. Mean weight loss was 34.0 ± 17 kg, and percentage of excess weight loss was $52 \pm 17\%$. There were major improvements in lobular steatosis, necroinflammatory changes, and fibrosis at the second biopsy ($P < .001$ for all). Portal abnormalities remained unchanged. Only four of the repeat biopsies fulfilled the criteria for NASH. There were 18 patients with an initial fibrosis score of 2 or more compared with 3 patients at follow-up ($P < .001$). Those with the metabolic syndrome (n = 23) had more extensive changes before surgery and greater improvement with weight loss. **In conclusion**, weight loss after surgery provides major improvement or resolution of obesity and metabolic syndrome-associated abnormal liver histological features in severely obese subjects. (HEPATOLOGY 2004;39:1647–1654.)

The more progressive forms of nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and associated hepatic fibrosis, are strongly associated with obesity and metabolic syndrome.¹ As a result of the rising prevalence of obesity, liver disease may become the most common liver disorder in developed countries.² On the basis that obesity is a principal factor in the pathogenesis of NAFLD, it would be expected that weight loss should be therapeutic.

The effect of weight loss on NAFLD remains unclear. Several small studies have examined the effect of diet-induced weight loss on NAFLD and have demonstrated an improvement in liver enzyme levels and steatosis, but variability in its effect on other histological features.^{3–6} Bariatric surgery produces significant weight loss, but the additional effects of malabsorption and diversion of gut contents have confounded the effect of weight loss after these procedures. This was particularly evident with jejunioileal bypass, in which global malabsorption and isolation of a significant segment of gut from the enteric contents set up the possibility of immune and metabolic changes leading to progressive liver damage and liver failure in some cases.^{7,8} Currently used bariatric surgical procedures, such as biliopancreatic diversion and long limb Roux-en-Y gastric bypass, that provide significant diversion and malabsorption remain a concern.^{9–11}

Laparoscopic adjustable gastric banding (LAGB) provides weight loss without malabsorption because it acts by inducing satiety and by restricting the amount of food taken. Its restriction can be adjusted at any time after placement, providing satiety without undue obstruction,

Abbreviations: NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; LAGB, laparoscopic adjustable gastric banding; HbA1c, glycosylated hemoglobin A1c; ALT, alanine aminotransferase.

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and it allows for controlled weight loss. This form of bariatric surgery has proved to be safe and effective.¹² LAGB thus provides a potential model for observing the effect of controlled weight loss without major alteration to the structure and function of the gastrointestinal tract.

The aim of the present study was to compare the histological features of NAFLD in selected severely obese subjects before and after weight loss induced by LAGB. In addition, we examined the relationship between changes in features of liver histology and changes in the clinical and biochemical features of obesity, with weight loss.

Materials and Methods

Patients and Preoperative Assessment. Selection and preoperative assessment of patients for Lap-Band (Inamed Health Corp, Santa Barbara, CA) surgery (LAGB) and inclusion in the study of NAFLD has been described previously.¹ Briefly, patients with a body mass index of more than 35 kg/m² who had significant medical, physical, or psychosocial disabilities are considered for entry into the program. All patients undergo extensive preoperative assessment that includes a careful assessment of alcohol consumption, anthropometric measurements, and laboratory tests. Laboratory tests include liver function tests, fasting lipid profile, fasting plasma glucose, fasting insulin, C-peptide, glycosylated hemoglobin A1c (HbA1c) and hepatitis B and C serological analysis. Investigations were performed to exclude hemochromatosis, α_1 -antitrypsin deficiency, Wilson disease, or autoimmune liver disease if indicated on the liver biopsy. Patients were excluded from the NAFLD study if they had a history of alcoholism, consumed more than 200 g of alcohol per week, had evidence of hepatitis B or C, were taking known hepatotoxic medications, or had a history of or finding consistent with another specific liver disease. At the time of the second biopsy, the clinical assessment and anthropometric and biochemical measures were repeated.

All index liver biopsies were taken at the time of laparoscopic surgery to place an adjustable gastric band during the period from January 1999 until June 2002, and a total of 197 unselected biopsies were obtained. Follow-up biopsies were obtained either at the second laparoscopic procedure or as a percutaneous biopsy. There were 19 patients (9.6%) who had a second laparoscopic procedure and consented to a liver biopsy at that time. Fifteen of these had revisional surgery for prolapse or slippage of the band. Two were obtained at the time of laparoscopic band explantation for intolerance of the band restriction, and two at laparoscopic cholecystectomy. All eligible patients having a laparoscopic procedure agreed to the follow-up

biopsy. The remaining 17 patients agreed to have percutaneous liver biopsies because of concern regarding their index biopsy that showed NASH and centrilobular fibrosis of stage 2 or more. These 17 patients were from a group of 23 (11.7%) selected for rebiopsy on clinical grounds, because the effect of weight loss on the more advanced forms of NAFLD, especially fibrosis, is unclear. Five declined the opportunity of biopsy, and one patient had emigrated and could not be contacted.

All index liver biopsies and intraoperative follow-up biopsies were performed percutaneously under laparoscopic view as a routine part of the operative procedure as previously described.¹ Nonoperative specimens were obtained percutaneously with the assistance of ultrasound guidance. Biopsy samples were obtained using a 14-gauge 200-mm Temno Biopsy needle (Allegiance; Health Care Corp, McGraw Park, IL). Adequacy of the biopsy was assessed macroscopically, and an additional core was taken if a specimen of less than 8 mm in length was obtained. All biopsies contained at least eight portal tracts. Informed written consent was obtained from all patients at the time of the index biopsy as part of an approved prospective study previously published.¹ Informed written consent also was obtained from all subjects before the second biopsy, and the study was conducted conforming to the ethical guidelines of the Helsinki Declaration.

Diagnosis of type 2 diabetes was based on the American Diabetes Association criteria.¹³ A diagnosis of metabolic syndrome was based on Adult Treatment Panel III criteria that is the presence of three or more of the following five conditions¹⁴: (1) waist circumference more than 102 cm in men and greater than 88 cm in women; (2) fasting serum triglyceride concentration of 1.7 mmol/L or more; (3) high-density lipoprotein cholesterol level of less than 1.04 mmol/L in men and less than 1.29 mmol/L in women; (4) fasting plasma glucose of 6.1 mmol/L or more; (5) blood pressure of 130 mmHg/85 mmHg or more.

Insulin sensitivity was estimated using the homeostatic model assessment method.^{15,16} Preoperative excess weight was calculated as baseline weight (kg) less ideal weight (kg) as measured by the Metropolitan Life tables.¹⁷ Percentage of excess weight loss was calculated by dividing the weight change between paired biopsies by the excess weight before surgery, multiplied by 100.

Histological Assessment. All liver biopsy specimens were stained with hematoxylin and eosin, silver reticulin, Masson trichrome, and Sirius red for collagen¹⁸ and Perls stain and ubiquitin, an immunostain demonstrating Mallory bodies.¹⁹ All sections were number coded such that the pathologist and observer performing the histological

Table 1. Criteria Used for Histological Scoring

Steatosis	
0	<5% of parenchyma involved
1	5% to 25% of lobular parenchyma involved
2	25% to 50% of lobular parenchyma involved
3	50% to 75% of lobular parenchyma involved
4	>75% of lobular parenchyma involved
Cellular injury*	
Mallory bodies	
0	No Mallory bodies
1	Fewer than two in 10 to 20× fields
2	More than two in 10 to 20× fields
Ballooning degeneration	
0	Nil
1	Limited to zone 3 and affecting <50% of lobules
2	More extensive changes
Lobular inflammation	
0	No inflammation
1	Sparse zone 3 inflammation (less than one focus per lobule)
2	Mild focal zone 3 inflammation (1 to 2 foci per lobule)
3	Notable zone 3 inflammation (3 to 4 foci per lobule)
4	Severe zone 3 inflammation (>4 foci per lobule)
Portal inflammation extent	
0	No portal inflammation
1	Portal inflammation of less than 25% of portal tracts
2	Portal inflammation between 25% and 50% of tracts
3	Portal inflammation between 50% and 75% of tracts
4	Portal inflammation of greater than 75% tracts
Portal inflammation intensity	
	In addition, any portal inflammation was graded as nil, mild, moderate, or severe (0-3)
Fibrosis	
0	Normal connective tissue
1	Perivenular and pericellular fibrosis limited to zone
2	Perivenular and pericellular fibrosis confined to zone 2 and 3
3	Bridging or extensive fibrosis with architectural distortion; no obvious cirrhosis
4	Cirrhosis
Portal fibrosis (scored independently from overall fibrosis score above)	
0	No portal inflammation
1	Portal fibrosis of less than 25% of portal tracts
2	Portal fibrosis of between 25% and 50% of tracts
3	Portal fibrosis of between 50% and 75% of tracts
4	Portal fibrosis of greater than 75% tracts

*There are two scores for cellular injury, (1) Mallory bodies and (2) ballooning degeneration, that were scored independently.

scoring were blinded to the patient identity, clinical condition, biochemical data and whether it was a biopsy from before weight loss or after weight loss. The 72 sets of biopsies slides were examined in a random order.

Histological Scoring. One pathologist (PSB) and a second observer (JBD) scored all biopsy material in a standard manner. Individual histologic features listed in Table 1 were observed and scored separately. These features were a modification of our previous scoring method, with the view of additionally scoring cellular injury and portal features in a manner similar to that of the National Institutes of Health group and that recently reported by others.^{1,20,21} This is very similar to the recently presented histologic scoring system for NAFLD and NASH.²² Additional features such as lipogranulomas and other granulomas were noted but not scored. Finally, all were graded and staged for NASH according to the system proposed at

the American Association for the Study of Liver Diseases single topic conference in September 2002.²³ These criteria are a modification of those of Brunt et al.²⁴ and are summarized in Table 2.

LAGB Surgery. LAGB surgery involves the placement of an adjustable silicone gastric band just below the gastroesophageal junction. The band has an inflatable inner balloon that can be adjusted by adding or removing normal saline via a small subcutaneous access port attached to the anterior rectus sheath. In this way, the band restriction can be altered to induce satiety in a controlled fashion. In a recent report from our group of 700 consecutive patients who underwent LAGB, there were no deaths, and perioperative complications occurred in 1.2% of patients.²⁵ Late problems with slippage or prolapse of the stomach through the band were common (up to 30%) in early reports, but recent modifications of technique

Table 2. Summary of Grading and Staging for NASH as Proposed by the American Association for the Study of Liver Diseases Single Topic Conference^{23,24}

Grade	
Grade 1, mild	Steatosis in 33% to 66% of lobules, occasional ballooning degeneration in zone 3, mild lobular inflammation with or without mild portal inflammation
Grade 2, moderate	Steatosis, ballooning present in zone 3, lobular inflammation with polymorphs in association with ballooned hepatocytes, pericellular fibrosis, or both, with or without mild chronic inflammation; none, mild, to moderate portal inflammation
Grade 3, severe	Steatosis: usually >66%, Marked ballooning especially zone 3, scattered lobular acute and chronic inflammation, plus mild to moderate portal inflammation (not marked)
Stage	
1	Perivenular and pericellular fibrosis limited to zone 3
2	Stage 1 plus focal or extensive portal fibrosis
3	Bridging fibrosis, focal or extensive
4	Cirrhosis with or without residual perisinusoidal fibrosis

have reduced the incidence of this complication markedly. The procedure typically involves an overnight hospital stay and has the attributes of adjustability and reversibility. During a follow-up of up to 6 years, weight loss of between 50% and 60% of excess weight was achieved.²⁵

Statistical Analysis. Continuous demographic and anthropometric variables were expressed as mean \pm SD change with between biopsies assessed using the paired Student *t* test. Histologic scoring and features were treated as ordinal categorical variables. Any change in scoring between index and second biopsies was assessed using the nonparametric Wilcoxon signed ranks test. All laboratory measures were expressed as median \pm interquartile range, and changes were assessed using the Wilcoxon signed ranks test. Correlation between ordinal or continuous variables was performed using Spearman-rho coefficients. The chi-square method (Fisher exact test) was used to test the significance of differences between proportions and categorical variables. Multivariate analysis was tested using binary logistic regression (forward and backward), and some β -coefficients have been shown. SPSS statistical software (SPSS Inc., Chicago, IL)²⁶ was used for statistical analysis. A *P* value of less than 0.05 was considered statistically significant.

Results

There were 36 patients with paired biopsies. This represents 18.3% of the 197 unselected patients from whom liver biopsy samples were obtained during the defined period. Patient characteristics are shown in Table 3. Paired biopsies were assessed in 36 (11 male and 25 female) patients. The second biopsy was obtained 25.6 ± 11 months (range, 9–51 months) after surgery. The mean weight loss at this time was 34.0 ± 17.0 kg (range, 8–68.5 kg). Other clinical, demographic, and weight loss data, including the percentage of excess weight lost, are shown in Table 3. Index biopsies had a mean of $13.8 \pm$

4.8 portal tracts (range, 8–30 portal tracts), and second biopsies had a mean of 15.5 ± 5.1 portal tracts (range, 8–28 portal tracts). Weight loss was accompanied by significant favorable changes in anthropometric measures, including waist, hip, and neck circumferences and waist-to-hip ratio (Table 3). There were also significant decreases in both systolic and diastolic blood pressure. There were major improvements in the biochemical markers of metabolic syndrome, lower fasting plasma glucose, HbA1c, insulin levels, and improved insulin sensitivity, along with higher fasting high-density lipoprotein cholesterol and lower triglyceride concentrations. Twenty-three of the 36 subjects (64%) fulfilled the Adult Treatment Panel III criteria for metabolic syndrome¹⁴ before surgery, and only 7 (19%) fulfilled these criteria at follow-up. All enzyme concentrations in the panel of liver function tests fell significantly with weight loss, and there was a significant rise in the aspartate aminotransferase-to-alanine aminotransferase (ALT) ratio (Table 3).

Scores for steatosis, lobular inflammation, centrilobular fibrosis, Mallory bodies, and ballooning degeneration all improved significantly with weight loss. There were no significant changes in any of the scores for portal fibrosis or inflammation (Table 4). There were no histological features that changed unfavorably with weight loss.

NASH Grade and Stage. All specimens were scored for NASH grade and stage using the American Association for the Study of Liver Diseases proposed method (Table 5).²³ There were 24 patients with a score of at least 1 for grade or stage before surgery, compared with 9 such patients at follow-up (*P* < .001).

We classified subjects as having NASH if their biopsy scored at least 1 for both grade and stage. There were 23 subjects with NASH in their index biopsy, 20 (87%) of whom had metabolic syndrome. By contrast, only four of the follow-up biopsies demonstrated NASH (*P* < .001); in two, the second biopsy had an improved combined grade and stage score, and in two, there was no change.

Table 3. Changes in Anthropometric Measurements and Biochemistry Between the Patients First and Second Liver Biopsy (n = 36)

	Pre Weight Loss (Mean, Median, %)	With Weight Loss (Mean, Median, %)	P Value
Number	36	36	
Age (years)	43 ± 10.3		
% in group male, %	31	31	
Diabetic %	39	8*	0.005
Hypertensive %	50	19*	0.006
Metabolic syndrome %	64	19*	<0.001
BMI (kg/m ²)	47 ± 10.6	34.0 ± 5.5	<0.001
Weight (kg)	134.8 ± 26	99.8 ± 17.2	<0.001
Percentage of excess weight lost		51.6 ± 17.3	
Waist (cm)	132.1 ± 17.4	108.3 ± 15.4	<0.001
W:H ratio	0.93 ± 0.10	0.87 ± 0.97	0.028
Neck (cm)	43.6 ± 4.8	39.4 ± 3.2	<0.001
Systolic blood pressure (mm Hg)	139.5 ± 19.4	128.7 ± 16.2	0.016
Diastolic blood pressure (mm Hg)	87.8 ± 12.3	81.6 ± 10.9	0.032
Fasting P glucose (mmol/l)	6.3 ± 2.2	5.0 ± 0.8	<0.001
Hb A1c (%)	6.2 ± 2.0	5.3 ± 0.9	<0.001
F P insulin (mu/l)	22.4 ± 16.7	10.6 ± 8.3	<0.001
C peptide (pmol/ml)	1.39 ± 0.50	0.96 ± 0.53	<0.001
Insulin sensitivity HOMA% _S	28.0 ± 12	56.0 ± 33.0	<0.001
Total cholesterol (mmol/l)	5.7 ± 1.55	5.4 ± 1.1	0.044
Fasting triglycerides (mmol/l)	2.1 ± 1.1	1.2 ± 0.7	<0.001
HDL-C (mmol/l)	1.20 ± 0.38	1.35 ± 0.46	0.012
LDL-C (mmol/l)	3.7 ± 1.5	3.4 ± 1.2	0.28
AST (IU/l)	27.0 ± 33.0	17.0 ± 8.0	<0.001
ALT (IU/l)	43.0 ± 33.0	21.0 ± 15.0	<0.001
AST/ALT ratio	0.77 ± 0.39	0.93 ± 0.54	0.012
Gamma-glutamyl transpeptidase (IU/l)	38.0 ± 36.6	18.0 ± 9.0	<0.001
Alkaline phosphatase (IU/L)	105.5 ± 27.0	62.0 ± 25.0	<0.001

For all laboratory measures: Median (interquartile range), *P* value Wilcoxon signed ranks test.

For continuous demographics and anthropometric : Mean (SD), *P* value paired T-test.

Proportions: Percentage, *P* value chi-square.

*Indicates the percentage of those fulfilling the criteria for these conditions at follow-up. The difference indicates those that are in remission from the condition.

Thus, in this series, 82% of subjects had resolution or remission of NASH with weight loss, 9% demonstrated improvement, and 9% were unchanged. The four in whom NASH was unresolved included a male who was the only case of established cirrhosis, a male with long-standing insulin-dependent type-2 diabetes who attained a normal body mass index but still required insulin, and two patients who had only lost 16% and 39% of excess weight at the time of the second biopsy.

The changes in fibrosis or NASH stage are perhaps one of the more significant features of this analysis. The one patient with established cirrhosis had no change in stage score, but necroinflammatory changes had reduced. However, of the 10 patients with stage 3 fibrosis in their index biopsy, 7 regressed to stage 0, 1 to stage 1, and 1 to stage 2, but 1 remained at stage 3 at follow-up. Of the 23 patients with any zone 3 fibrosis found in the index biopsy, 16 patients (70%) had no fibrosis at follow-up.

The median changes in NASH grade and NASH stage between paired biopsies fell by a score of 1.0. We there-

fore looked at those patients who had more than this median change. There were 17 patients with a decrease in grade of 2 or more and 16 patients with a decrease in stage of 2 or more. We looked for factors associated with weight loss and metabolic syndrome that may be associated with these greater-than-median decreases. Measures of weight loss were not significantly different between those grouped by above or below median decreases in grade or stage scores. Patients with greater than median decreases were more likely to have features of metabolic syndrome.

The preoperative measures that were associated with a decrease of 2 or more of NASH grade were higher: fasting plasma glucose (*P* = .013), HbA1c (*P* = .007), and ALT (*P* = .025) concentrations and higher waist-to-hip ratio (*P* = .023). These 17 patients experienced greater decreases in fasting plasma glucose (*P* = .022), HbA1c (*P* = .048), ALT (*P* = .041), and aspartate aminotransferase (*P* = .031) concentrations than the remainder with weight loss. Patients in this group were far more likely to have metabolic syndrome (*P* = .004) or type 2 diabetes (*P* = .037) before surgery.

Table 4. Histologic Scores for the 36-Paired Biopsies Reported Blinded to the Patient's Identity, Clinical Features and Timing

Feature*	Scores					P Value
	0	1	2	3	4	
Steatosis						
A	1	3	6	12	14	
B	21	9	2	3	1	<0.001
Lobular inflammation						
A	12	5	8	8	3	
B	25	8	3	0	0	<0.001
Fibrosis						
A	13	5	7	10	1	
B	29	4	1	1	1	<0.001
Mallory bodies						
A	15	9	12			
B	34	1	1			<0.001
Ballooning degeneration						
A	10	12	14			
B	27	9	0			<0.001
Portal inflammation (extent)						
A	1	18	4	5	8	
B	2	13	8	10	3	0.9
Portal inflammation (intensity)						
A	1	28	8	1		1.0
B	2	24	9	1		
Portal fibrosis						
A	13	12	4	4	3	0.34
B	11	10	5	5	5	

*A = Pre-weight loss or index biopsy and B = follow-up biopsy. P values calculated using Wilcoxon signed rank test.

The preoperative measures that were associated with a decrease of 2 or greater NASH stages were higher fasting plasma glucose ($P = .012$), HbA1c ($P = .003$), ALT ($P = .004$) and aspartate aminotransferase ($P = .012$) concentrations, and higher waist circumference ($P = .033$) and waist-to-hip ratio ($P = .022$). These 16 patients experienced greater decreases in fasting plasma glucose ($P = .004$), HbA1c ($P = .022$), ALT ($P = .009$), and alkaline phosphatase ($P = .008$) concentrations, and a significantly greater decrease in waist circumference than the

Table 5. American Association for the Study of Liver Diseases Proposed Scoring for the Grade and Stage of NASH

	Scores					P Value*
	0	1	2	3	4	
Grade						
A†	12	5	6	13		
B‡	30	4	2			<0.001
Stage						
A†	13	3	9	10	1	
B‡	29	2	2	2	1	<0.001

*Calculated using the Wilcoxon signed rank test.

†Before weight loss or index biopsy.

‡Follow-up biopsy.

Table 6. Correlation Between the Metabolic Syndrome Ordinal Score Before Weight Loss and Features in Index Biopsy and the Change in Histological Scores Between Biopsies

	Metabolic Syndrome 1, 2, or 3	
	Correlation Index Biopsy Score	Correlation with Change in Score Between Biopsies
Steatosis	0.56†	-0.17
Lobular inflammation	0.56†	-0.52†
Fibrosis	0.38*	-0.40*
Mallory bodies	0.41*	-0.52†
Ballooning degeneration	0.48†	-0.33*
Portal inflammation	0.18	-0.11
Intensity of portal inflammation	0.17	0.07
Portal fibrosis	0.30	-0.16
NASH grade	52†	-0.53†
NASH stage	0.37*	-0.38*

NOTE. Metabolic syndrome ordinal score 1 = 0-2 of 5 features (n = 13), 2 = 3 of 5 features (n = 7), and 3 = 4 or 5 of 5 features (n = 16).

*Spearman correlation was significant at the 0.05 level (two-tailed).

†Spearman correlation was significant at the 0.01 level (two-tailed).

remainder with weight loss. Patients in this group were far more likely to have metabolic syndrome ($P = .008$) or type 2 diabetes ($P = .017$) before surgery.

Binary logistic regression revealed that the presence of metabolic syndrome was the predominant preoperative predictor for a change in 2 or more in NASH grades and stages with B coefficients for change in grade of 10.3 (95% CI, 1.8-58; pseudo $R^2 = .22$; $P = .008$) and B coefficients for change in stage of 13.7 (95% CI, 1.4-130; pseudo $R^2 = .23$; $P = .02$), respectively. After controlling for metabolic syndrome, no other factors were significant.

Metabolic Syndrome. Patients were scored by the number of Adult Treatment Panel III-defined features of metabolic syndrome that they had before surgery. There were three patients with 1 feature, 10 patients with 2 features, 7 patients with 3 features, 12 patients with 4 features, and 4 patients with all 5 features. They were put into three ordinal categories: 13 patients scoring <3, therefore, without metabolic syndrome; 7 patients scoring 3 features; and 16 patients with 4 or 5 features. Spearman correlation coefficients for the relationship between metabolic syndrome score and index biopsy histologic feature score, and the change in score between biopsies, are shown in Table 6. Features more strongly associated with metabolic syndrome improved more with weight loss.

Liver Function Tests. The median \pm interquartile range liver function panel enzyme concentrations at the time of liver biopsies are shown in Table 3. Using the upper laboratory reference range as the cutoff, 17 patients had a raised

ALT (>34 IU/L) at the time of the index biopsy and 2 patients at follow-up; aspartate aminotransferase (>40 IU/L) measurements were 11 and 2, respectively; γ glutamyl transpeptidase (>65 IU/L) measurements were 9 and 4, respectively; and alkaline phosphatase (>120 IU/L) measurements were 4 and 1, respectively. There was a significant positive correlation between the change in NASH grade and change in all enzymes of the liver function panel (not shown).

Other Histological Features. Small numbers of lipogranulomas were observed in some of the biopsy specimens, but none showed extensive changes. There was no significant relationship between the presence of lipogranulomas and other histological features or clinical and biochemical measures of metabolic syndrome. There was no significant change in the number of specimens showing lipogranulomas with weight loss (not shown).

Discussion

In this study, we demonstrated that the key features of NAFLD and NASH improve or resolve dramatically with weight loss. These key features include zone 3 features of steatosis, necroinflammatory change, and fibrosis. Greater improvement is seen in those selected subjects who had been diagnosed with metabolic syndrome before surgery. In addition, individual histological abnormalities that correlated with metabolic syndrome improved significantly more with weight loss in those with metabolic syndrome (see Table 6). The histological features of NASH resolved or remitted in 82% of patients. These findings support the hypothesis that, in these selected obese subjects, obesity and metabolic syndrome are causally associated with the liver condition observed. Our findings also assist in validation of which specific histological features are associated with obesity- and metabolic syndrome-associated NASH.²²

Our results demonstrate major improvement in necroinflammatory activity and fibrosis with weight loss, findings that have not always been reported consistently.²³ The method chosen to achieve weight loss may be important. Studies involving diet, lifestyle, and exercise interventions often are shorter-term studies and usually do not achieve such substantial sustained weight loss.²⁷ Weight loss programs involving low- or very low-calorie diets typically are followed by some weight gain, and biopsies taken during such a phase may not truly represent the effects of weight loss. At a mean duration of 25 months after surgery, most of our patients not only have lost substantial weight but also are relatively weight stable.

We have not found an increase in portal or lobular inflammation with the gradual weight loss that follows LAGB surgery, findings that have been described with

often more rapid weight loss after a very low-calorie diet⁴ and gastroplasty,²⁸ respectively. It is possible that the very rapid weight loss after gastroplasty, with an increase in visceral free fatty acid release, produces a temporary increase in inflammatory changes.

Assessment of the biopsy specimens also may hold some clues. Few studies have reported a wide range of features, and rarely are individual features scored independently. Steatosis may be such an overwhelming feature in some of the specimens obtained before weight loss that a careful assessment of necroinflammatory and portal tract changes may be overlooked. A dramatic reduction in steatosis may enhance or unmask the view of inflammatory cells or portal changes.

Neuschwander-Tetri and Caldwell²⁹ carefully explored the histologic changes induced by the use of the intracellular insulin-sensitizing medication, Rosiglitazone, on patients with NASH. They describe significant improvements in zone 3 histologic features, including necroinflammatory changes and fibrosis. These reported changes are entirely consistent with our findings, and the persistence of mild portal inflammation is a common feature. This suggests that these portal changes may not be associated directly with metabolic syndrome or insulin resistance. It is interesting that weight loss and improved insulin sensitivity without weight loss have similar effects on liver histological features, suggesting a common mechanism.

LAGB surgery has several features that allow us to look relatively cleanly at the effect of significant weight loss on NASH. Diversionary surgical weight procedures with significant malabsorption may lead to nutritional deficiencies and alterations of intestinal flora, potentially confounding this effect. A recent report follows up patients after biliopancreatic diversion and describes a generally positive benefit with weight loss, but some subjects had increased fibrosis that was related to lower albumin levels and uncontrolled diarrhea.¹¹

Metabolic syndrome clearly is a hepatotoxic condition and, along with alcohol and hepatitis C, is one of the three most common hepatotoxic conditions in developed countries. There is growing evidence that metabolic syndrome acts in conjunction with the other common hepatotoxic conditions, producing a synergistic effect.³⁰⁻³⁴ The association between metabolic syndrome and steatohepatitis has important clinical implications, and perhaps the nonalcoholic part of NASH and NAFLD is not ideal, because it implies a diagnosis of exclusion and may divert focus from recognizing and treating problems of obesity and metabolic syndrome. From a public health perspective, prevention of metabolic syndrome-associated steatohepatitis should be related integrally with the prevention

of obesity and its metabolic consequences. For those with established obesity and metabolic syndrome-associated steatohepatitis, weight reduction should be a central component of therapy.

In conclusion, we have demonstrated major improvement in the biochemical and histological features of liver disease associated with obesity and metabolic syndrome with weight loss after LAGB surgery. Those with metabolic syndrome have greater initial histological abnormalities and greater improvement with weight loss.

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