

High Prevalence of Small Intestinal Bacterial Overgrowth in Patients with Morbid Obesity: A Contributor to Severe Hepatic Steatosis

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Abstract

Background With the increasing prevalence of obesity, non-alcoholic fatty liver disease (NAFLD) has become a major cause of liver diseases. Small intestinal bacterial overgrowth (SIBO) could be related to NAFLD. Our aim was to determine the prevalence of SIBO and its relationship with liver lesions in morbidly obese patients.

Methods A glucose hydrogen (H₂) breath test (positive if fasting breath H₂ concentration >20 ppm and/or an increase of >10 ppm over baseline within the first 2 h) was performed in obese patients referred for bariatric surgery (body mass index [BMI] >40 kg/m² or >35 in association with comorbidities) and in healthy non-obese subjects. In obese patients, a surgical liver biopsy was performed.

Results One hundred and forty-six patients (129 women, age [mean±SE]: 40.7±11.4 years) were prospectively

included in the study. The mean BMI was 46.1±6.4 kg/m². A liver biopsy was available in 137 patients and a breath test in 136. The frequency of positive breath tests was higher in obese patients (24/136, 17.1%) than in healthy subjects (1/40, 2.5%; *P*=0.031). In the univariate analysis, SIBO was not associated with clinical variables, but tended to be associated with more frequent severe hepatic steatosis (26.3 vs. 10.3%, *P*=0.127), whereas the frequency of sinusoidal or portal fibrosis, lobular necrosis and non-alcoholic steatohepatitis (NASH) were not different. In the multivariate analysis, SIBO (*P*=0.005) and the presence of a metabolic syndrome (*P*=0.006) were independent factors of severe hepatic steatosis.

Conclusion In morbidly obese patients, bacterial overgrowth prevalence is higher than in healthy subjects and is associated with severe hepatic steatosis.

Keywords Morbid obesity · Bacterial overgrowth · Non-alcoholic fatty liver disease (NAFLD) · Steatosis

Abbreviations

BMI Body mass index
NAFLD Non-alcoholic fatty liver disease
NASH Non-alcoholic steatohepatitis
SIBO Small intestinal bacterial overgrowth

Introduction

Because of the increasing prevalence of obesity [1], non-alcoholic fatty liver disease (NAFLD) has become one of the major causes of liver diseases [2]. The spectrum of liver injury is broad, from steatosis and non-alcoholic steatohepatitis (NASH) through to cirrhosis, liver failure and

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hepatocellular carcinoma [3, 4], and almost all morbidly obese patients undergoing bariatric surgery have some liver lesions [5]. The prevalence of steatosis in these patients is very high, ranging from 85% to 98%, while the prevalence of NASH is highly variable, with an overall prevalence of 37% (24–98%) in a recent review [6]. Diabetes mellitus and insulin resistance are the conditions most frequently associated with NASH and hypertension with advanced hepatic fibrosis [6, 7].

Several data in the 1970s suggested that small intestinal bacterial overgrowth (SIBO) could play a role in NAFLD. Descriptions of fatty degeneration have been reported in morbidly obese patients with jejuno-ileal bypass [8, 9] and in small bowel diverticulosis [10], both conditions favouring bacterial overgrowth. Wigg et al. [11] and Sajjad et al. [12] showed that the prevalence of SIBO assessed by ^{14}C -D-xylose and lactulose or glucose breath tests was higher in patients with NASH compared with control subjects. However, in patients with morbid obesity, the prevalence of SIBO is unknown and its role in liver injuries has not been studied before. Our aim was, then, to determine the prevalence of SIBO in a prospective cohort of morbidly obese patients referred for bariatric surgery and to study the relationship between bacterial overgrowth and liver histology.

Patients and Methods

Study Design

This prospective study was part of an extensive preoperative and perioperative data collection on patients with morbid obesity referred to our institution to have adjustable gastric bands inserted laparoscopically or to undergo gastric bypass for weight reduction following an unsuccessful hypocaloric regimen. Morbid obesity was defined as body mass index (BMI) $\geq 40 \text{ kg/m}^2$ or $\geq 35 \text{ kg/m}^2$ in association with other comorbidities, such as sleep apnoea, diabetes mellitus or cardiovascular disease.

All obese patients systematically had a glucose hydrogen breath test before surgery to look for small intestinal bacterial overgrowth. A liver biopsy, taken surgically in the left lobe as a routine part of the operative procedure, was assessed for specific histological abnormalities. We looked for a relationship between SIBO and the presence of liver lesions.

We also performed a glucose breath test in a group of healthy non-obese ($18.5 \text{ kg/m}^2 < \text{BMI} < 25 \text{ kg/m}^2$) subjects matched for age and sex with the group of patients to compare the prevalence of positive breath test in the two groups.

Patients and Preoperative Assessment

Preoperative assessment included clinical and familial assessment, psychiatric assessment to detect chronic alcohol consumption and/or compulsive eating disorders, anthropometric measurements (weight, height, waist and hip circumferences) and laboratory tests. Increased waist-to-hip ratio (WHR), reflecting central obesity, was defined as ≥ 0.90 in men and ≥ 0.85 in women [13]. Laboratory tests included plasma aminotransferases (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) and gamma glutamyltransferase (GGT) activity (upper normal limits of 55, 45 and 50 IU/L, respectively). Other laboratory tests included iron studies, fasting lipid profile, fasting glucose profile, hepatitis B and C serologies, anti-smooth muscle antibodies, anti-liver–kidney microsome antibodies and anti-mitochondrial antibodies. Diagnosis of type 2 diabetes was based on the American Diabetes Association criteria [14]. Dyslipidaemia was defined as an increase in triglycerides or cholesterol or both, or if the patient was taking a hypolipaeamic agent. Hypertension was diagnosed if the patient had a past history of hypertension and was on antihypertensive medication or if the patient had a resting recumbent blood pressure greater than or equal to 140/90 mmHg on two repeated occasions. The NCEP-ATPIII definition was used to determine the presence or absence of the metabolic syndrome [15].

Patients were excluded from the study if they had a history of compulsive eating disorders, a daily consumption of alcohol greater than 20 g/day, evidence of hepatitis B or C, a positive serology for anti-smooth muscle antibodies or anti-liver–kidney microsome antibodies or anti-mitochondrial antibodies, any other specific liver disease, a severe associated pathology, previous bariatric surgery or were taking known hepatotoxic medication, such as amiodarone, diltiazem or corticosteroids.

Informed written consent was obtained from every patient and the study was conducted in accordance with the Helsinki Declaration.

Glucose Hydrogen (H_2) Breath Test

Subjects fasted overnight (12 h) and during the H_2 breath test. All subjects were asked to avoid eating food containing fibre during the previous evening, as this may cause prolonged excretion of H_2 . Cigarette smoking and exercise were not allowed for at least 2 h before and during the test. End-expiratory breath samples were obtained at 30-min intervals before and for 4 h after an oral load of 50 g of D-glucose, as described previously [16–18]. The results were expressed in parts per million (ppm). A positive result was defined as a fasting breath H_2 concentration above 20 ppm and or a breath H_2 concentration increase of more than 12

Table 1 Characteristics of the patients according to positive or negative breath tests

	Negative breath test (n=112)	Positive breath test (n=24)	P-value
Sex (% of female)	89.3	79.2	0.31
Age (years)*	40.9±11.9	41.3±9.3	0.85
BMI (kg/m ²)*	46.1±6.8	46.2±5.0	0.94
Waist circumference (cm)*	121.3±12.1	125.6±12.4	0.23
Diabetes mellitus (%)	20	18.8	1
Hypertension (%)	32	26.7	0.92
Dyslipidaemia (%)	18.9	12.5	0.8
Metabolic syndrome (%)	49.3	31.3	0.19
AST (IU/L)*	22.8±13.5	18.7±3.7	0.28
ALT (IU/L)*	44.4±17.8	36.7±6.8	0.26
GGT (IU/L)*	48.2±36.2	41.6±22.2	0.6

*mean±SE

% corresponds to the percentage of patients with the condition

ppm over baseline within the first 2 h of testing [19, 20]. H₂ concentrations of expiratory gas samples were measured using an electrochemical cell (Exhaled Hydrogen Monitor; GMI Medical Ltd., Renfrew, Scotland). The minimal detectable H₂ concentration was 1 ppm. Equivocal tests were considered as negative. Tests were not interpretable if antibiotics had been taken or a bowel washout was performed one month prior to testing.

Histological Assessment

All liver biopsies were fixed and examined using haematoxyline-eosin, Masson trichrome, silver reticulin and Perl's stain. Two pathologists, blinded to the patients' clinical condition and biochemical data, reviewed every biopsy. Steatosis was graded on a 0 to 3 semi-quantitative scale [21]: (0) no steatosis; (1) up to one third of the hepatocytes with steatosis; (2) from one to two thirds of the hepatocytes with steatosis; (3) more than two thirds of the hepatocytes with steatosis, defining severe steatosis. Liver fibrosis was assessed on a five-stage scale [21]: (0) no fibrosis; (1) perisinusoidal fibrosis without portal or periportal fibrosis; (2) perisinusoidal fibrosis with portal or periportal fibrosis; (3) perisinusoidal fibrosis with portal or periportal fibrosis with focal or extensive bridging fibrosis; (4) cirrhosis. Lobular necroinflammatory activity was defined as the presence of lobular polymorphonuclear leukocytes infiltrate associated with lobular necrosis. Non-specific inflammation was defined as sparse to mild focal inflammation without necrosis. The presence of ballooning degeneration and Mallory bodies was described.

Table 2 Histological findings according to positive or negative breath tests (n=127)

	Negative breath test (n=107)	Positive breath test (n=20)	P-value
Severe steatosis (>66%)*	10.3	26.3	0.13
Sinusoidal fibrosis*	39.3	55	0.19
Portal fibrosis*	24.3	25	1
Lobular necroinflammatory activity*	37.4	45	0.52
NASH*	23.4	30	0.73

*% of patients with the condition

Statistical Analysis

The results are expressed as mean±standard error (SE) and percentages. Comparisons of quantitative variables were made using Student's *t*-test. Categorical variables and proportions were tested by the χ^2 and Fisher's exact tests. The relationship between severe steatosis or NASH and various risk factors was studied using univariate comparison followed by multivariate analysis using binary logistic regression. Baseline variables that reached a univariate *P*-value ≤ 0.15 and variables found to be predictive in the literature were included in the multivariate analysis. A multivariate analysis was performed using either severe steatosis or NASH as the dependent variables. A *P*-value < 0.05 was considered as statistically significant. Analysis was performed with the SPSS software package (version 11.5; SPSS Inc., Chicago, IL).

Results

Characteristics of Patients

From October 2001 to October 2004, 146 consecutive patients with morbid obesity (129 F/17 M) who had a glucose H₂ breath test were included in the study. The mean age was 40.7±11.4 years (range: 17–72 years) and the mean BMI was 46.1±6.4 kg/m² (range: 35.4–73.3 kg/m²). Hypertension was present in 29.2%, diabetes mellitus in 19.8% and dyslipidaemia in 17.7% of the patients. A

Table 3 Univariate analysis of the predictive factors for severe steatosis (>66% of hepatocytes)

Variables	No severe steatosis (n=106)	Severe steatosis (n=19)	P-value
Age (years)*	39.5±11.7	45.4±8.6	0.037
Female sex (%)	89.6	84.2	0.77
BMI (kg/m ²)*	46.4±6.7	46.7±6.9	0.87
Hypertension (%)	27.8	25	0.95
Diabetes mellitus (%)	16.5	33	0.317
GGT (IU/L)*	49.0±36.9	40.7±6.6	0.66
ALT (IU/L)*#	43.1±16.0	55.6±25.2	0.066
Cholesterol (mmol/L)*	4.9±1.0	5.21±0.7	0.4
Triglyceride (mg/dL)*	114.5±51.6	159.6±115.4	0.03
Metabolic syndrome (%)	37.5	83.3	0.003

*mean±SE

ALT were available for 80 patients

% corresponds to the percentage of patients with the condition

metabolic syndrome was present in 46.3% of the patients. The mean waist size was 120.3±10.7 cm for females and 138.1±11.9 cm for males. The mean WHR was 0.86±0.09 for females and 1.01±0.05 for males. As liver tests were not routinely performed initially, pre-operative values for aminotransferases were only available for 80 patients. The mean liver enzyme levels were 44±17 IU/L for ALT, 23±15 IU/L for AST and 48±35 IU/L for GGT.

Glucose Hydrogen Breath Test

A glucose hydrogen breath test was performed in 40 healthy volunteers, which was positive in one case (2.5%). Of the initial 146 breath tests performed in obese patients, six were excluded because of antibiotic intake within the previous month. Of the remaining 140 breath tests, 24 were positive (17.1%), 112 were negative (80%) and four (2.9%) were equivocal because the increase of H₂ was not sufficient or not sustained. The frequency of positive breath tests was significantly higher in patients than in healthy subjects ($P=0.031$). The characteristics of the patients according to positive or negative breath tests are presented in Table 1. In the univariate analysis, no variable was associated with the presence of SIBO.

Histopathological Findings

Surgery was not performed in nine patients because of various contra-indications. A liver biopsy was available in 137 out of the 146 patients of the initial group.

Steatosis was present in 126 patients (92%) (grade 1 in 47.2%, grade 2 in 29.6% and grade 3 in 15.2%). According to the five-stage scale score of fibrosis, fibrosis was present

in 72 patients (52.6%) (grade 1 in 28.5%, grade 2 in 13.1% and grade 3 in 0.7%). Isolated portal fibrosis was present in 14 patients (10.2%), lobular necroinflammatory activity in 54 patients (39.4%) and NASH in 34 patients (24.8%). Five patients (3.7%) had normal histology.

The histopathological findings according to positive or negative breath tests are presented in Table 2. When SIBO was present, there was a small increase (from 7 to 16%) in the frequency of sinusoidal fibrosis, lobular necroinflammatory activity and NASH, but these increases never reached a level of statistical significance. The frequency of severe steatosis tended to be increased in patients with SIBO ($P=0.127$). Other factors associated with severe steatosis in the univariate analysis are presented in Table 3. Severe steatosis was associated with age, serum triglyceride level and the presence of a metabolic syndrome, with also a trend for higher ALT level ($P=0.066$). The multivariate analysis (binary logistic regression) for the presence of severe steatosis is presented in Table 4. The presence of a metabolic syndrome and SIBO were independent predictive factors for severe steatosis (the predictive value of ALT in the multivariate analysis could not be tested because of too much missing data).

In the univariate analysis, the presence of NASH was associated with higher waist circumference (127.5±15.4 cm vs. 120.7±11.0 cm, $P=0.037$) but not with age (41.6±11.4 years vs 39.7±11.4 years, $P=0.40$), sex (82.4% vs. 90.3% of women, $P=0.35$), BMI (47.2±6.7 kg/m² vs. 46.1±6.4 kg/m², $P=0.4$) or the presence of a metabolic syndrome (52.6% vs 41.9%, $P=0.4$). In the multivariate analysis (binary logistic regression), only waist circumference (OR 1.06. CI [1.001–1.116], $P=0.047$) was found to be predictive for the presence of NASH, whereas age, sex, BMI, the presence of metabolic syndrome and SIBO were not predictive.

Table 4 Multivariate analysis (binary logistic regression) for the presence of severe steatosis

Variable	Odds ratio	CI 95%	P-value
SIBO	27.5	2.7–276	0.005
Metabolic syndrome	51.2	3.1–834	0.006
Age	0.996	0.9–1.1	0.9

Discussion

In the present study, we found that bacterial overgrowth, defined by a positive glucose H₂ breath test, was more

frequent in morbidly obese patients compared to healthy subjects and was associated with severe steatosis.

The commonly accepted definition of SIBO is a total growth of $>10^5$ colony-forming units/ml of intestinal fluid. Because of the difficulties in obtaining cultures, different breath tests, including the glucose H₂ breath test, have been used. In our study, 17% of patients with morbid obesity had a positive glucose breath test; this was significantly higher than in healthy subjects. In the literature, the prevalence of the positive glucose breath test varies depending on the definition used for a positive breath test [22]. The prevalence of 2.5% of positive breath test found in our healthy subjects is similar to that reported before, varying from zero to less than 5% [20, 23–25]. We did not find any clinical or biochemical patient characteristic associated with SIBO. In the literature, older age has sometimes been associated with the increased prevalence of SIBO [25–27], but in our study, only four patients were greater than 60 years of age. Another known risk factor of SIBO is the use of proton pump inhibitors [27]. Because of elevated gastroesophageal reflux disease (GERD) prevalence in morbidly obese patients [28, 29], it could have been a confounding factor in our series, but proton pump inhibitors were stopped 7 days before the breath tests were performed. Bacterial overgrowth in morbidly obese patients could also be due to intestinal motility alterations, as described previously in humans or mice [30, 31]. Intestinal motility was not assessed in our patients, but one study performed in patients with severe obesity found motility disturbance in the fasting period with diminished Phase I, increased Phase II and more distal and less frequent occurrence of Phase III activity of the migrating motor complex [32].

A quarter of our patients had NASH and 92% had steatosis, which was severe in 15.2% of them. These findings are in accordance with the literature [5, 6]. In the presence of SIBO, the prevalence of portal fibrosis, sinusoidal fibrosis, lobular necroinflammatory activity and NASH was similar or slightly higher, but the difference never reached a level of statistical significance. However, there was a trend for an increased prevalence of severe steatosis from 10 to 26% of cases in the presence of SIBO, and SIBO was also found to be an independent predictive factor of severe steatosis. In the univariate analysis, other factors associated with severe steatosis were older age, serum triglyceride level and the presence of metabolic syndrome, but in the multivariate analysis, apart from SIBO, only the presence of a metabolic syndrome was found to be an independent predictive factor. Concerns about the variability of liver biopsy in NAFLD have been previously raised. However, in morbidly obese patients, excellent agreement for steatosis was seen in biopsy specimens obtained at surgery from the right and left lobes of the liver [33, 34]. Recently, Mathurin et al. [35] found

that, before bariatric surgery, severe steatosis was associated in the univariate analysis with ALT, GGT, fasting blood glucose, serum triglyceride levels and insulin resistance index, while in the multivariate analysis, only ALT level and insulin resistance index were independent predictive factors [35]. At the time of our study, insulin resistance testing was not routinely performed, but, despite no strict overlapping, a metabolic syndrome is significantly associated with insulin resistance [36].

The link between SIBO and steatosis is supported by a complete or partial reversal of steatosis after metronidazole treatment in patients with intestinal bypass [37] and in various rat models of SIBO [38, 39]. The mechanisms of liver lesions produced by bacterial overgrowth have been studied in rodent models. The peptidoglycan-polysaccharide, a bacterial cell wall polymer with potent inflammatory and immunoregulatory properties, seems to have a crucial role in promoting liver injuries. In a rat model of SIBO, treatment with mutanolysin, a highly specific muralytic enzyme, prevented the elevation of both plasma anti-peptidoglycan antibodies and tumour necrosis factor- α (TNF α) levels in Kupffer cells stimulated in vitro with peptidoglycan-polysaccharide [40]. The recent data of Cani et al. [41, 42] in mice also suggest that gut microbiota contribute to the pathophysiological regulation of endotoxaemia and may dysregulate the inflammatory tone, leading to insulin resistance and obesity. Moreover, in models of genetically obese rats and mice, there is an increased sensitivity to endotoxin hepatotoxicity caused by hepatic macrophage dysfunction [43]. In ob/ob mice, a model of NAFLD that develops intestinal bacterial overgrowth and overexpresses TNF α , treatment with probiotics or anti-TNF antibodies improves liver histology and reduces hepatic total fatty acid content [44] by reducing a TNF α kinase and the DNA binding of nuclear factor kappa B that promoted insulin resistance. These data and our results suggest that SIBO could play a role in the initial phase of steatohepatitis in the “two-hit” theory proposed by Day and James [45]. Severe steatosis constituting the “first hit,” the progression to more severe lesions, such as NASH, could be related to a “second hit” caused by oxidative stress/lipid peroxydation [46] and by factors promoting the expression of proinflammatory cytokines [47].

Conclusion

We found, in a population of morbidly obese patients, that bacterial overgrowth was more frequent than in healthy non-obese subjects and was associated with severe hepatic steatosis. Further studies are necessary to confirm our findings and to determine the mechanisms of such bacterial overgrowth. If our data are confirmed, this could open up a

new field of treatment with probiotics in order to limit the progression towards more severe liver lesions, such as non-alcoholic steatohepatitis (NASH) and cirrhosis.

References

- Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA*. 2006;295:1549–55.
- James OFW, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol*. 1998; 29:495–501.
- Fassio E, Alvarez E, Domínguez N, et al. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology*. 2004;40:820–6.
- Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology*. 2002;123:134–40.
- Hamois F, Msika S, Sabaté JM, et al. Prevalence and predictive factors of non-alcoholic steatohepatitis (NASH) in morbidly obese patients undergoing bariatric surgery. *Obes Surg*. 2006;16:183–8.
- Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. *J Hepatol*. 2006;45:600–6.
- Imagawa S, Yamaguchi Y, Ogawa K, et al. Interleukin-6 and tumor necrosis factor- α in patients with obstructive sleep apnea-hypopnea syndrome. *Respiration*. 2004;71:24–9.
- Bondar GF, Pisesky W. Complications of small intestinal short-circuiting for obesity. *Arch Surg*. 1967;94:707–16.
- Maxwell JG, Richards RC, Albo D Jr. Fatty degeneration of the liver after intestinal bypass for obesity. *Am J Surg*. 1968;116: 648–52.
- Nazim M, Stamp G, Hodgson HJF. Non-alcoholic steatohepatitis associated with small intestinal diverticulosis and bacterial overgrowth. *Hepatogastroenterology*. 1989;36:349–51.
- Wigg AJ, Roberts-Thomson IC, Dymock RB, et al. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor α in the pathogenesis of non-alcoholic steatohepatitis. *Gut*. 2001;48:206–11.
- Sajjad A, Mottershead M, Syn WK, et al. Ciprofloxacin suppresses bacterial overgrowth, increases fasting insulin but does not correct low acylated ghrelin concentration in non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2005;22:291–9.
- Chitturi S, Abeygunasekera S, Farrell GC, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology*. 2002;35:373–9.
- [No authors listed] Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 1997;20:1183–97.
- Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–97.
- Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology*. 1988;95:982–8.
- Flourie B, Turk J, Lemann M, et al. Breath hydrogen in bacterial overgrowth. *Gastroenterology*. 1989;96:1225.
- Attar A, Flourie B, Rambaud JC, et al. Antibiotic efficacy in small intestinal bacterial overgrowth-related chronic diarrhea: a cross-over, randomized trial. *Gastroenterology*. 1999;117:794–7.
- Chang C-S, Chen G-H, Lien H-C, et al. Small intestine dysmotility and bacterial overgrowth in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology*. 1998;28:1187–90.
- Lupascu A, Gabrielli M, Lauritano EC, et al. Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2005;22:1157–60.
- Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol*. 1999;94:2467–74.
- Bauer TM, Schwacha H, Steinbrückner B, et al. Diagnosis of small intestinal bacterial overgrowth in patients with cirrhosis of the liver: poor performance of the glucose breath hydrogen test. *J Hepatol*. 2000;33:382–6.
- Casafont MF, de las Heras Castaño G, Martín Ramos L, et al. Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci*. 1996;41:552–6.
- Yang C-Y, Chang C-S, Chen G-H. Small-intestinal bacterial overgrowth in patients with liver cirrhosis, diagnosed with glucose H₂ or CH₄ breath tests. *Scand J Gastroenterol*. 1998;33: 867–71.
- Mitsui T, Shimaoka K, Goto Y, et al. Small bowel bacterial overgrowth is not seen in healthy adults but is in disabled older adults. *Hepatogastroenterology*. 2006;53:82–5.
- Parlesak A, Klein B, Schecher K, et al. Prevalence of small bowel bacterial overgrowth and its association with nutrition intake in nonhospitalized older adults. *J Am Geriatr Soc*. 2003;51:768–73.
- Elphick DA, Chew TS, Higham SE, et al. Small bowel bacterial overgrowth in symptomatic older people: can it be diagnosed earlier? *Gerontology*. 2005;51:396–401.
- Suter M, Dorta G, Giusti V, et al. Gastro-esophageal reflux and esophageal motility disorders in morbidly obese patients. *Obes Surg*. 2004;14:959–66.
- Merrouche M, Sabaté JM, Jouet P, et al. Gastro-esophageal reflux and esophageal motility disorders in morbidly obese patients before and after bariatric surgery. *Obes Surg*. 2007;17:894–900.
- Quigley EM, Quera R. Small intestinal bacterial overgrowth: roles of antibiotics, prebiotics, and probiotics. *Gastroenterology*. 2006;130:S78–90.
- Nieuwenhuijs VB, Verheem A, van Duijvenbode-Beumer H, et al. The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats. *Ann Surg*. 1998;228:188–93.
- Pieramico O, Malfertheiner P, Nelson DK, et al. Interdigestive gastroduodenal motility and cycling of putative regulatory hormones in severe obesity. *Scand J Gastroenterol*. 1992;27:538–44.
- Larson SP, Bowers SP, Palekar NA, et al. Histopathologic variability between the right and left lobes of the liver in morbidly obese patients undergoing Roux-en-Y Bypass. *Clin Gastroenterol Hepatol*. 2007;5:1329–32.
- Merriman RB, Ferrell LD, Patti MG, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology*. 2006;44:874–80.
- Mathurin P, Gonzalez F, Kerdraon O, et al. The evolution of severe steatosis after bariatric surgery is related to insulin resistance. *Gastroenterology*. 2006;130:1617–24.
- Cheal KL, Abbasi F, Lamendola C, et al. Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. *Diabetes*. 2004;53: 1195–200.
- Drenick EJ, Fislis J, Johnson D. Hepatic steatosis after intestinal bypass—prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. *Gastroenterology*. 1982;82:535–48.

38. Lichtman SN, Sartor RB, Keku J, et al. Hepatic inflammation in rats with experimental small intestinal bacterial overgrowth. *Gastroenterology*. 1990;98:414–23.
39. Lichtman SN, Keku J, Schwab JH, et al. Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. *Gastroenterology*. 1991;100:513–9.
40. Lichtman SN, Okoruwa EE, Keku J, et al. Degradation of endogenous bacterial cell wall polymers by the muralytic enzyme mutanolysin prevents hepatobiliary injury in genetically susceptible rats with experimental intestinal bacterial overgrowth. *J Clin Invest*. 1992;90:1313–22.
41. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56:1761–72.
42. Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*. 2007;50:2374–83.
43. Yang SQ, Lin HZ, Lane MD, et al. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci U S A*. 1997;94:2557–62.
44. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology*. 2003;37:343–50.
45. Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology*. 1998;114:842–5.
46. Berson A, De Beco V, Letteron P, et al. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. *Gastroenterology*. 1998;114:764–74.
47. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest*. 2004;114:147–52.