ORIGINAL ARTICLE

Increased Gastric Cytokine Production After Roux-en-Y Gastric Bypass for Morbid Obesity

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Hypothesis: Mucosal cytokines may be involved in the process of gastric bacterial contamination that may occur after Roux-en-Y bypass for morbid obesity in both gastric chambers, with inflammation and gastritis mostly in the excluded stomach.

Design: A prospective observational study in a homogeneous population with nonspecific complaints.

Setting: Outpatient clinic of a large, public, academic hospital.

Patients: Subjects (n=37; 26 [70.3%] female; mean±SD age, 42.4±9.9 years) seen a mean±SD of 7.3±1.4 years after Roux-en-Y gastric bypass and nonoperated on morbidly obese control subjects (n=10; 7 [70%] female; mean±SD age, 44.0±8.9 years).

Intervention: Enteroscopy was performed to collect samples for cytokine assays and bacteriologic studies.

Main Outcome Measures: Concentrations of tumor necrosis factor α and transforming growth factor β in the gastric mucosa of both chambers in patients undergoing Roux-en-Y gastric bypass and correlation with bacterial overgrowth and *Helicobacter pylori* infection.

Results: High microbial counts ($>10^5$ colony-forming units per milliliter) were detected in 22 (59.5%) and 7 (18.9%) of the 37 samples from the functional pouch and excluded reservoir, respectively; and *H pylori* investigation was positive in 6 of 37 samples (16.2%). The tumor necrosis factor α concentration (mean±SD, 2.1±1.9 pg/g of protein) and the transforming growth factor β concentration (mean±SD, 24.2±12.8 pg/g of protein) in the excluded stomach, but not in the proximal pouch, were elevated with regard to the corpus or antrum of controls, and correlation with bacterial overgrowth and with *H pylori* infection was demonstrated.

Conclusion: Overexpression of tumor necrosis factor α and transforming growth factor β occurred in the distal stomach, positive cytokine correlation with microbial invasion by *H pylori* and nonspecific germs was seen, and further studies addressing phenotypic and genotypic changes of gastric mucosa are recommended.

Arch Surg. 2007;142(10):962-968

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CYTOKINES ARE MARKERS OF cell functional or inflammatory activation and may be expressed in response to sepsis, ischemia, necrosis, regeneration, and other events. Abnormal cytokine production by gut mucosa is well established in Crohn disease, ulcerative colitis, and other intestinal conditions, including human immunodeficiency virus infection and food allergy.[1-5] Some gastric cytokines also have been the focus of attention, mostly within the context of *Helicobacter pylori* infection and gastric cancer[6,7] but also of stress and peptic ulcer.[8]

On the other hand, visceral fat cytokines and adipokines are commonly elevated within the context of morbid obesity, and repercussions concerning insulin resistance, nonalcoholic fatty liver disease, and cardiovascular complications have been highlighted for some time.[9] This is a phenomenon originating from the fat compartment, and although there is systemic and probably hepatic impact, there is no evidence that the gastrointestinal tract is involved.

Reports concerning gastric cytokine production in bariatric populations, or more specifically in patients after Roux-en-Y gastric bypass (RYGB), have not been found in the literature.

Evidence of bacterial and fungal colonization of the proximal gastric pouch and the excluded stomach has been registered, along with occasional positive test results for *H pylori* infection,[10] in the late postoperative period. In these patients, a study of local production of 2 cytokines

was undertaken, aiming to correlate such findings with microbial test results and with the clinical and biochemical profile of that population.

METHODS

From August 2, 2004, to August 1, 2005, patients who previously underwent open-banded RYGB for morbid obesity were enrolled in this protocol.

Inclusion criteria were nonspecific upper gastrointestinal complaints, such as nausea, vomiting, or intolerance to certain foods under investigation at the Endoscopy Service; absence of *H pylori* or confirmed eradication by the time of bariatric intervention; and more than 48 months after RYGB.

Exclusion criteria were critical illness, reversal of the bariatric procedure, upper gastrointestinal obstruction or history of bacterial overgrowth, use of antibiotics or antacids, tube feeding, gastrostomy or jejunostomy, inflammatory bowel disease, gastrointestinal bleeding, systemic or gastrointestinal infection, human immunodeficiency virus infection or AIDS, use of immune-modulating drugs, and refusal to participate in the study.

Among 42 recruited subjects, 5 were excluded because of technical impossibility to reach the excluded stomach. The remaining 37 subjects (26 female [70.3%]; mean±SD age, 42.4±9.9 years [range, 21-66 years]) are the focus of this study.

Ten symptom-free morbidly obese adults, screened for future bariatric operation but not yet registered at the Surgical Service, were examined as the control population for general biochemical test results and cytokine production in the intact stomach.

All patients were seen at the Endoscopy Service of Sao Paulo University Medical School, and written informed consent was obtained. This protocol was approved by the Ethical Committee of Hospital das Clinicas.

EXPERIMENTAL DESIGN AND CLINICAL AND LABORATORY DATA

This was a prospective clinical study; nevertheless, part of the studied variables (date of operation and perioperative findings pertinent to inclusion and exclusion criteria) was retrieved from the clinical chart or hospital records.

The clinical questionnaire included the attainment of a general medical history and a survey of clinical records, including operations and hospitalizations, immunologic conditions, and medical prescriptions; nutritional assessment (weight, height, and body mass index); biochemical and hematological tests (complete blood cell count, including hemoglobin level, white blood cell count, and levels of neutrophils and lymphocytes; and levels of serum albumin, total cholesterol, and triglycerides); and an endoscopic survey.

The double-balloon enteroscope (Fujinon EN-450P5/20; Fuji Photo Optical Co, Ltd, Omiya, Japan) (diameter, 8.5 mm; and length, 200 cm) was used together with the soft overtube (TS-12140; Fuji Photo Optical Co, Ltd). Sedation was done with midazolam maleate, fentanyl, and propofol if necessary, and supplementary oxygen was offered via nasal catheter. By the push-pull double-balloon technique, the enteroscope was inserted orally and advanced to the excluded stomach, thus performing full retrograde gastroscopy[11] (**Figure 1**).[12]

PATIENT BIOPSY SPECIMENS

Mucosal biopsy specimens were collected from the corpus of the excluded stomach, and subsequently from the corpus of the functional gastric pouch, for cytokine and *H pylori* investigations. Gastric fluid was also collected for microbiological studies. In controls, specimens were removed from the gastric corpus and antrum and all materials were kept on melting ice until transferred to the laboratory.

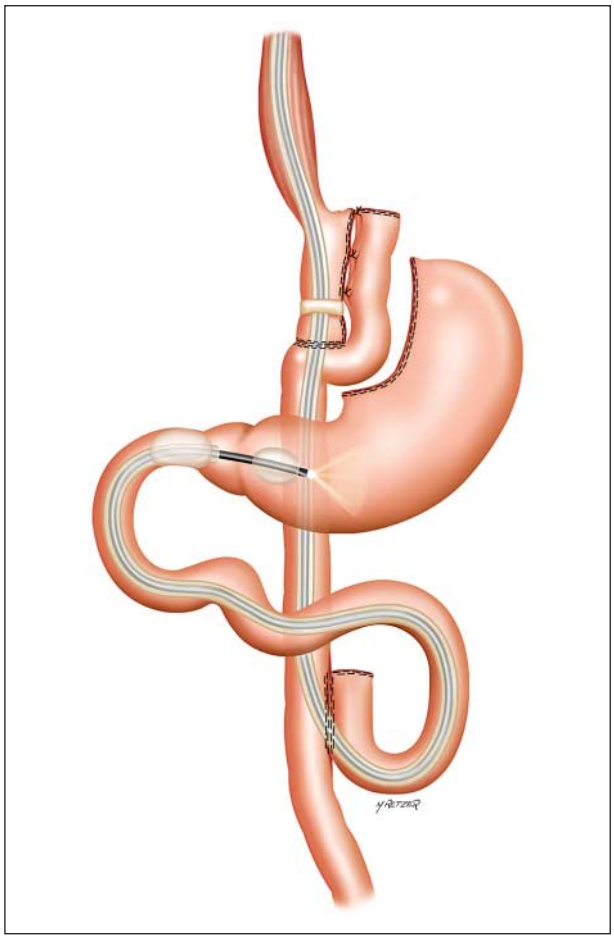

Figure 1. Enteroscopic access to the distal stomach after Roux-en-Y gastric bypass. From Kuga et al.[12]

The endoscope was sterilized per the manufacturer's instructions. All biopsy specimens were collected via the biopsy channel using a new silicone-protected device for each maneuver.

MICROBIAL EXAMINATION, *H PYLORI* INVESTIGATION, AND CYTOKINE ASSAY

Quantitative determination of viable mesophile aerobic bacteria in the stomach, namely, those that grow at body temperature, was done by the serial dilution agar plating procedure. The same method was used for mesophile anaerobes, by adding an anaerobiosis-generating device (BBL GasPack Plus; Becton Dickinson, Franklin Lakes, New Jersey).

For fungi and yeasts, serial (1.0 and 0.1 mL, respectively) deep inoculation in a combination of acidified agar, dextrose, and potato plates was done, and incubation proceeded for 5 days (at 25°C). The number of colonies was computed by colony counter, and results were expressed as colony-forming units per milliliter.[13] *Helicobacter pylori* infection was diagnosed in histological specimens by Giemsa staining.

Tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) concentrations were measured by enzyme-linked immunosorbent assay. The TNF-α and TGF-β protein

Table 1. General and Nutritional Features[a]

Variable	Patients	Controls	*P* Value
Age, y	42.4 ± 9.9	44.0 ± 8.9	.39
Female sex[b]	26/37 (70.3)	7/10 (70.0)	.86
BMI			
Preoperative	53.5 ± 10.6	51.6 ± 5.0	.35
Current	32.6 ± 7.8	51.6 ± 5.0	<.001
Follow-up, y	7.3 ± 1.4	NA	NA

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); NA, data not applicable.
[a] Data are given as mean ± SD unless otherwise indicated.
[b] Data are given as number/total (percentage) of each group.

Table 2. Biochemical Profile of the Population[a]

Variable	Patients		Control Subjects
	Preoperative Data	Current Data	
Total cholesterol level, mg/dL	196.7 ± 30.5	181.8 ± 27.4[b]	180.7 ± 13.4[c]
Triglyceride level, mg/dL	140.6 ± 57.2	91.5 ± 33.4[b]	159.1 ± 59.5[c]
Hemoglobin level, g/dL	13.2 ± 0.9	12.3 ± 1.5[b]	11.4 ± 3.6
White blood cell count, $\times 10^3$/mL	7.5 ± 1.7	6.1 ± 1.7[b]	7.4 ± 1.9
Neutrophils, $\times 10^3$/mL	4.5 ± 0.6	3.5 ± 0.5[b]	4.3 ± 0.7
Lymphocytes, $\times 10^3$/mL	2.2 ± 0.5	2.3 ± 0.5	2.4 ± 0.6
Serum albumin level, g/dL	4.2 ± 0.4	4.2 ± 0.2	4.3 ± 0.1

SI conversion factors: To convert total cholesterol to millimoles per liter, multiply by 0.0259; to convert triglycerides to millimoles per liter, multiply by 0.0113.
[a] Data are given as mean ± SD.
[b] $P < .05$ compared with preoperative results in the same group.
[c] $P < .05$ compared with preoperative results in the different group.

levels were calculated by commercial kits (Quantikine HS Human TNF-α and TGF-β immunoassay kits, respectively; R&D Systems, Minneapolis, Minnesota) modified for tissue extraction.[14]

ASSESSMENT OF COVARIATES AND COLLECTION OF CLINICAL DATA

Imaging procedures, biopsies, and additional tests for diseases or conditions mentioned in the exclusion criteria were not performed. The identification of such variables was based on the clinical questionnaire, with emphasis on the diagnosis made by a physician or information available in the medical record and on findings of suggestive symptoms and disease-specific drug prescriptions.

STATISTICAL ANALYSES

All data were reviewed and analyzed by the Biostatistical Team of the Department of Gastroenterology. Values are presented as mean ± SD. Parametric (*t* test) and nonparametric (Kruskal-Wallis and Mann-Whitney tests) methods were applied as appropriate. Linear regression analysis (Pearson product moment correlation) correlating cytokine assays and clinical and microbiological findings was also done. $P < .05$ was considered significant. The protocol was approved by the institutional review board of Hospital das Clinicas, and informed consent was signed by all subjects.

RESULTS

General and demographic findings of the population can be seen in **Table 1**. This was a comparatively homogeneous group of patients who underwent a standard procedure by the same surgical team; they were reassessed between 4 and 11 years after the intervention, when substantial weight loss had occurred. Nonoperated on controls were similar, except with regard to current body weight, which was still in the superobesity range, different from subjects who underwent RYGB.

Initially abnormal biochemical test results clearly benefited by the operation, although complete normalization was not present because weight regain tended to occur in the late postoperative period (**Table 2**). Serum albumin level and lymphocyte count were adequate and remained stable, indicating good nutritional status. Hemoglobin level diminished somewhat, but most remarkably, white blood cell count and neutrophil level decreased after the operation, possibly as a consequence of less systemic inflammation following weight loss, resulting in a reduced fat content of the adipocytes.[15]

Both gastric compartments, but not the stomach, of controls often displayed high microbial counts ($>10^5$ colony-forming units per milliliter). Proportions were 59.5% (22 of 37) and 18.9% (7 of 37) of the samples from the functional pouch and excluded reservoir, respectively. In addition, *H pylori* investigation was positive in 6 of the 37 patients (16.2%) who underwent RYGB and in 4 of the 10 nonoperated on controls (40%).

Concentrations of TNF-α and TGF-β in both gastric chambers of patients are as follows: distal chamber, 2.1 ± 1.9 and 24.2 ± 12.8 pg/g of protein, respectively; and proximal chamber, 0.1 ± 0.7 and 12.5 ± 9.3 pg/g of protein, respectively. Results (TNF-α and TGF-β) in the corpus and antrum of controls are as follows: corpus, 1.3 ± 1.2 and 10.0 ± 6.3 pg/g of protein, respectively; and antrum, 1.3 ± 1.5 and 15.6 ± 17.5 pg/g of protein, respectively. Values in the excluded (distal) chamber were elevated when compared with those in the functional pouch and with those of nonoperated on obese subjects (**Figure 2** and **Figure 3**).

Differences in absolute concentrations of TGF-β and TNF-α in the presence or absence of ordinary microbes did not reach statistical significance, neither in the excluded stomach (26.3 ± 18.7 and 1.2 ± 1.1 pg/g of protein, respectively, vs 19.4 ± 3.4 and 1.1 ± 0.8 pg/g of protein, respectively; $P = .37$ and .42, respectively) nor in the proximal stomach (12.0 ± 6.4 and 0.09 ± 0.6 pg/g of protein, respectively, vs 15.1 ± 6.3 and 0.1 ± 0.8 pg/g of protein, respectively; $P = .18$ and .16, respectively).

The same occurred, for both cytokines, when *H pylori* infection was considered in the excluded stomach (20.8 ± 15.1 and 1.1 ± 0.6 pg/g of protein, respectively, vs 26.5 ± 11.8 and 1.2 ± 0.9 pg/g of protein, respectively; $P = .24$ and .51, respectively) or in the proximal stomach (12.2 ± 6.5 and 0.1 ± 0.6 pg/g of protein, respectively, vs

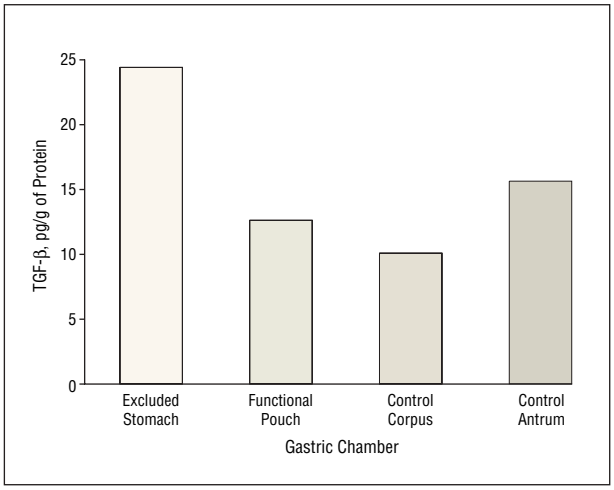

Figure 2. Production of transforming growth factor β (TGF-β) in operated on subjects and in the corpus and antrum of control subjects. $P<.05$ for value in the excluded chamber vs other measured values.

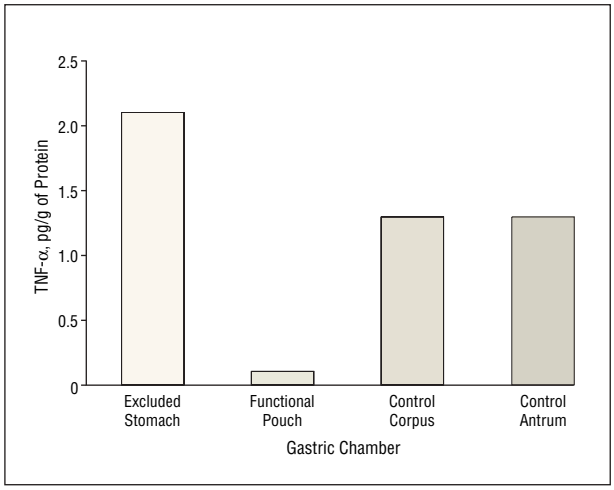

Figure 3. Production of tumor necrosis factor α (TNF-α) in operated on subjects and in the corpus and antrum of control subjects. $P<.05$ for value in the excluded chamber vs other measured values.

12.9±8.4 and 0.07±0.2 pg/g of protein, respectively; $P=.33$ and .63, respectively).

Linear regression analysis aimed to correlate cytokine concentrations in the 2 gastric chambers and general features of the patients, such as body mass index, sex, and hemoglobin concentration (**Table 3**). The purpose was to unveil possible systemic influences on gastric inflammatory activity. Intercorrelation between different cytokines and gastric segments, such as TNF-α concentration in the excluded stomach and TGF-β concentration in both parts of the stomach, was searched as well, and significance was achieved.

Correlations in healthy subjects, either between cytokines themselves, assessed at the corpus and antrum, or between cytokines and selected systemic measurements, were also depicted. Synergy was shown for release of the 2 cytokines in homologous regions of the control stomach (Table 3).

The Pearson product moment correlation was further applied regarding cytokine concentrations and degree of colonization of the 2 stomach parts. Such analysis yielded noteworthy correlations between conventional microbial contamination and TGF-β in the distal pouch, whereas for *H pylori* correlation, contamination was more widespread (**Table 4**).

COMMENT

The principal cytokine source in gastrointestinal mucosa is probably not visceral tissue but infiltrated macrophages and other mononuclear cells.[16] That does not diminish the diagnostic and prognostic importance of these biomarkers for prediction of local inflammatory, degenerative, and even neoplastic diseases.[17]

Tumor necrosis factor α is one of the central elements of the T helper cell 1 (Th1) lymphocyte response, and its local and systemic injuring potential has been repeatedly demonstrated in connection with obesity, infections, allergy, arthritis, and immune disorders.[1-8] Within the gastrointestinal system, Crohn disease, ulcerative colitis, and human immunodeficiency virus–associated diarrhea have been widely viewed as cytokine-mediated morbidity, if not truly TNF-α diseases.[1,2,4,5] Directly relevant to the current protocol is the connection between local Th1 profile, which includes augmented TNF-α output, and *H pylori*–triggered gastric ulcerations.[15-18] In simple gastritis, the connection has been identified as well, albeit in combination with Th2 cytokine secretion.[19]

The importance of TGF-β for wound healing in general has been recognized for a long time, and recent investigations indicate that it may be appropriate for clinical outcome of gastric ulcerations as well. Patients with cured gastric ulcers display abundant expression of TGF-β and its receptors, whereas lack of these tends to predict poor healing.[20-22] Bacterial invasion by ordinary aerobes, anaerobes, and fungi has not received as much emphasis as *H pylori* infection concerning gastric cytokine activation, but it might also be damaging to mucosa via release of Th1 cytokines, which provide an interface with gastric exocrine and endocrine secretions.[23]

The current protocol identified, for the first time to our knowledge, in patients who underwent a bariatric intervention an aberrant pattern of these important biological mediators in the excluded and the proximal stomach, associated with the presence of *H pylori* and conventional bacterial overgrowth.

The Th1 cytokine synthesis during *H pylori* infection is usually conspicuous, and correlation between abnormal gastric TNF-α results and the presence of that pathogen was expected, as demonstrated for children[24] and adults.[25] Overexpression of TGF-β was an original finding, and probably mirrors the defensive response of injured mucosa.[20-22] Table 4 supports the hypothesis that gastric colonization by conventional bacteria and fungi was not devoid of effect on the production of those cytokines in the distal stomach. Changes in cytokine output with and without *H pylori* and nonspecific colonization could not be shown, although the results of the Pearson product moment correlation were significant.

When those variables were compared with demographic and biochemical results, further links became evi-

Table 3. General Cytokine Correlations

Cytokine	Systemic Measurement	*r* Value[a]	*P* Value
	Data for Operated on Subjects		
TNF-α			
Excluded stomach	Hemoglobin level	–0.34	.03
	TGF-β (excluded stomach)	0.60	<.001
	TGF-β (proximal stomach)	0.43	.08
Proximal stomach	Triglycerides level	–0.33	.03
TGF-β			
Excluded stomach	BMI	0.40	.01
	Sex	0.35	.02
	TGF-β (proximal stomach)	0.39	.01
Proximal stomach	Hemoglobin level	–0.45	.005
	Data for Control Subjects		
TNF-α			
Corpus	Hemoglobin level	–0.67	.04
	Serum albumin level	0.62	.05
	TGF-β (corpus)	0.72	.02
Antrum	TGF-β (antrum)	0.96	<.001
TGF-β (corpus)	Hemoglobin level	–0.61	.05

Abbreviations: BMI, body mass index; TGF, transforming growth factor; TNF, tumor necrosis factor.
[a] Determined by Pearson product moment correlation.

Table 4. Cytokine Correlations With Bacterial Colonization and *Helicobacter pylori* Infection

Cytokine	Type of Colonization	*r* Value[a]	*P* Value
	Data for Operated on Subjects		
TNF-α			
Distal stomach	*H pylori*	0.51	<.001
Proximal stomach	*H pylori*	0.48	.001
TGF-β			
Distal stomach	Aerobes (proximal stomach)	0.42	.008
	Anaerobes (proximal stomach)	0.33	.03
	Fungi (proximal stomach)	0.92	<.001
	Yeasts (proximal stomach)	0.66	<.001
Proximal stomach	*H pylori*	0.76	<.001
	Data for Control Subjects (in the Antrum)		
TNF-α	*H pylori*	0.37	.22
TGF-β	*H pylori*	0.41	.16

Abbreviations: See Table 3.
[a] Determined by Pearson product moment correlation.

dent, notably regarding sex,[26] age, and markers of nutritional status, such as albumin levels and body mass index.[27] For obscure reasons, correlation with hemoglobin and triglycerides levels was negative. Intercorrelation between the local cytokines in different gastric locations was another finding, similar to findings in the reports of others.[24,28]

Although TGF-β is related to Th3 cytokines and the formation of T-regulatory cells, not to Th1 processes, it may be released by macrophages analogously to TNF-α, as part of the aggressive-defensive immune cascade in some settings, thus justifying the association.[15,29,30]

Taken together, these results are consistent with cytokine-mediated chronic gastric inflammation after RYGB. For some time, researchers have witnessed gastritis of variable extension and severity in patients undergoing RYGB who were endoscopically examined in the late postoperative period, in particular in the excluded stomach,[10,11] which is coherent with the current observations.

The rationale of this outcome, namely, mucosal cytokine overexpression basically promoted by opportunistic or incidental bacterial invasion, was not supported by standard clinical experience, because most patients exhibit favorable quality of life and a symptomless long-term clinical course after bariatric gastric bypass. Thus, this study originated from our questions about the meaning of late postoperative gastritis and occasional suspicion of gastric microbial invasion. These led to the hypothesis of a blind-loop syndrome with or without *H pylori* infection, despite the strongly held belief that few such findings should be expected in either the biliopancreatic limb or the functional gastric pouch.

Evaluation of gastric acid output is part of another ongoing protocol. In this sense, results are not available

herein; however, initial findings suggest high pH in both gastric chambers.

The differential behavior of the proximal and distal stomach, given the fact that both were affected by aberrant microbiological stimuli, reinforces the suspicion that some other factors could be involved in the stronger inflammatory response of the second chamber. Indeed, within the context of *H pylori* infection, Billroth I vs II architecture influences cytokine generation, with some mediators less expressed after Billroth I anastomosis.[17]

In clinical and experimental studies with the same pathogen, inflammatory molecules tended to exhibit lower concentrations in the fundus and upper corpus than in more distal regions of the stomach.[17,31] Biliary reflux also has been incriminated in augmented gastric cytokine generation.[17]

The excluded stomach after RYGB is prone to biliary reflux, as perceived in clinical experience,[10,11] whereas the functional chamber contains exclusively high corpus and some fundus-type mucosa, which could explain the differences in cytokine release.

The lack of absolute increase in mucosal cytokines in all subgroups, and the confirmation of only indirect influence on cytokine output by *H pylori* infection and bacterial overgrowth, as evidenced during Pearson product moment correlation regression analysis, is probably not contradictory. Information about TGF-β distribution during gastric bacterial aggression is extremely scarce; however, in those protocols dealing with TNF-α, which are less rare, release was not always homogeneous or predictable, with other markers occasionally displaying better correlation.[17-19,22-25]

Moreover, experimental investigations suggest that after many years of gastric colonization, as was probably the case in this bariatric population, not all cytokines are equally expressed; therefore, exhaustion of responses for some mediators should not be surprising,[31] thus preventing a more obvious link between microbial aggression and cytokine profile.

Additional studies are necessary to elucidate the prevalence and clinical course of such an immune-inflammatory pattern. The participation of these and other cytokines and biomarkers in intracellular pathways potentially promoting phenotypic and genotypic changes deserves attention, not missing messenger RNA expression and signal-transducing intermediates of cell proliferation and differentiation.[19-24,28-30]

Gastric bypass for morbid obesity is not listed as a cancer risk factor, and the reported long-term profile is safe. Just a few cases of postoperative gastric malignancies have been recorded, after 3 to 29 years, without any specific histologic pattern or anatomical location. Principal concern relates to diagnostic difficulties with the excluded stomach, justifying careful preoperative investigation.[31-36]

Nevertheless, therapeutic options should not be overlooked, because *H pylori* infection and bacterial overgrowth are amenable to appropriate antibiotics, yet biliary reflux could represent a greater challenge. Chemoprevention of *H pylori* infections as a means of aborting future cancer has been proposed for some time,[37] and although relatively low postoperative infection rates were registered in this series, eradication is obligatory. A similar consensus about primary deleterious effects of standard bacterial overgrowth on gastric mucosa is still lacking, and certainly this condition cannot be rated as a class 1 carcinogen[23,38]; nevertheless, long-term monitoring seems warranted.

In conclusion, overexpression of TNF-α and TGF-β occurred in the distal stomach of the population undergoing RYGB, regression analysis showed positive cytokine correlations with microbial invasion by *H pylori* and nonspecific germs, and further studies addressing the role of these and other cytokines in phenotypic and genotypic changes of gastric mucosa are recommended.

Accepted for Publication: April 6, 2007.
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Author Contributions: Dr Faintuch had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Faintuch. *Acquisition of data:* Faintuch, Ishida, Jacabi, Ribeiro, Kuga, Sakai, H. V. Barbeiro, D. F. Barbeiro, Soriano, and Cecconello. *Analysis and interpretation of data:* Faintuch, Jacabi, Ribeiro, H. V. Barbeiro, D. F. Barbeiro, Soriano, and Cecconello. *Drafting of the manuscript:* Faintuch. *Critical revision of the manuscript for important intellectual content:* Faintuch, Ishida, Jacabi, Ribeiro, Kuga, Sakai, H. V. Barbeiro, D. F. Barbeiro, Soriano, and Cecconello. *Obtained funding:* Faintuch. *Administrative, technical, and material support:* Faintuch, Ishida, Jacabi, Ribeiro, Kuga, Sakai, H. V. Barbeiro, D. F. Barbeiro, Soriano, and Cecconello. *Study supervision:* Faintuch.
Financial Disclosure: None reported.
Additional Contributions: The Obesity Surgery Service of Hospital das Clinicas valuably contributed during the study period; and Mitsunori Matsuda, MD, PhD, Paulo E. Pinto Jr, MD, PhD, Bruno Zilberstein, MD, PhD, and Arthur B. Garrido Jr, MD, PhD, valuably contributed to the study.

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