CORRESPONDENCE

Readers are encouraged to write letters to the editor concerning articles that have been published in GASTROENTEROLOGY. Short, general comments are also considered, but use of the Correspondence section for publication of original data in preliminary form is not encouraged. Letters should be typewritten double-spaced and submitted in triplicate.

Insulin Resistance Influences Iron Metabolism and Hepatic Steatosis in Type II Diabetes

Dear Sir:

We read with interest the article by Mendler et al.[1] who described the association of hepatic iron overload, steatosis, and presence of one or more components of the insulin resistance syndrome. They suggested a role for insulin resistance in the development of hepatic iron overload, steatosis, and increased serum ferritin levels, although the mechanisms whereby insulin resistance would induce alterations in iron metabolism remain to be elucidated. Insulin resistance is very common among subjects with metabolic disorders, but its prevalence varies substantially among clinical conditions.[2] Type II diabetes is one of the metabolic conditions with the higher rate of insulin resistance and is frequently associated with increased serum ferritin levels.[2,3] We have evaluated iron indices in patients with type II diabetes before and after improvement of metabolic control to provide some insights into the relationship between iron and metabolic disorders. From 1989 to 1990, in our Department of Medicine, 10 outpatients with poorly controlled diabetes were enrolled to evaluate the incidence of hepatic steatosis and its reversibility after correction of the glycemic control. Results of this study have not been published. Chronic alcoholism, acute and chronic liver diseases, malignancies, and inflammatory and iron overload disorders were excluded. After baseline evaluation, patients received a strict diet regimen for 12 months in addition to antidiabetic therapy. The study schedule included monthly measurements of mean blood glucose, fructosamine, glycosylated hemoglobin, cholesterol, and triglyceride levels; assessment of uricemia and liver function tests for 12 months; and hepatic ultrasound examination and liver biopsy at baseline and after 12 months. Small aliquots of the serum samples were collected and stored at −80°. The study was approved by the Hospital's ethical committee. All patients gave their informed consent to the study. Four denied liver biopsy. We then retrospectively evaluated serum iron, transferrin, and ferritin levels of the patients on each sample and hepatic iron concentration (HIC) at baseline and at the end of study. HIC was determined by atomic absorption spectrophotometry (Perkin-Elmer S2380; Norwalk, CT) in deparaffinized specimens. Table 1 shows the main data of the patients at baseline and during the study. Significant improvement of glycemic control was obtained in each patient. At baseline, serum ferritin levels were significantly higher than those in 20 normal age-matched controls (223 ± 139 vs. 122 ± 66 μg/L; $P < 0.01$) and significantly decreased at the end of the study. Serum ferritin correlated with triglyceride at baseline ($r = 0.69$; $P = 0.026$) and with HIC only at the end of the study ($r = 0.87$; $P = 0.024$). Liver function test results were normal at baseline and did not change during the study. Hepatic steatosis, as defined by ultrasound examination and histology, was present in 80% of the patients at baseline and in 25% after 12 months; it was mild and often associated with very mild necroinflammatory activity. In 5 patients, HIC decreased after 12 months, but did not change in 1 patient. The results suggest the existence of a relationship between glucose metabolism, fatty liver, serum ferritin, and hepatic iron. Hyperinsulinemia, the main manifestation of insulin resistance, favors the accumulation of free fatty acid in the liver and increases the risk of steatosis.[4] High serum ferritin levels are common in patients with metabolic disorders and nonalcoholic steatosis or steatohepatitis, and some of them also have increased HIC.[4,5] In our patients, serum ferritin levels at baseline were increased to a degree disproportionate to liver iron stores because they significantly decreased after metabolic improvement when the expected correlation with HIC finally appeared. Also, the mild but significant decrease of HIC at the end of the study suggests a possible influence of diabetic-associated metabolic alterations on hepatocellular iron metabolism. The improvement of the glycemic control observed in the patients can be ascribed to increased insulin action in the liver and in the peripheral tissues. The concomitant improvement of steatosis and the decrease of serum ferritin and hepatic iron levels suggest that these alterations are distinct consequences of a common factor (probably insulin resistance in this case) and that they are at least partially reversible by the improvement of metabolic control. Hypertriglyceridemia is another condition typically associated with the insulin resistance syndrome,[2] and the correlation observed in our patients at baseline between serum ferritin and triglyceride further supports the hypothesis of a relationship between insulin resistance and alterations in iron metabolism.

Table 1. Iron and Metabolic Indices in 10 Patients With Poorly Controlled Type II Diabetes at Baseline and During Treatment

	Baseline	2nd month	6th month	12th month
Transferrin saturation (%)	29 ± 12	24 ± 9	24 ± 7	27 ± 12
Serum ferritin (μg/L)	223 ± 139	156 ± 102	140 ± 100	121 ± 101[b]
Mean blood glucose (mg/dL)	252 ± 53	157 ± 45	151 ± 27	157 ± 49[c]
Fructosamine (mmol/L)	350 ± 62	271 ± 43	273 ± 49	263 ± 100[c]
Glycosylated hemoglobin (%)	12.3 ± 2.5	9.1 ± 2.5	8.5 ± 2.1	8.6 ± 1.6[d]
HIC[a] (mg/100 mg)	109.2 ± 32			89.7 ± 45.6[e]

NOTE. Results are means ± SD.
[a]Measured in 6 patients.
[b]$P < 0.0001$, Kruskal–Wallis nonparametric analysis of variance (ANOVA) test.
[c]$P < 0.0001$ and [d]$P = 0.0011$, ANOVA.
[e]$P = 0.049$, paired t test.

including those that may be portable, light-weight, and available to a physician in an office setting, should have resolution capabilities of allowing all major findings to be seen. As the issues surrounding SSBE become clearer in the future, this instrumentation should indeed be able to allow its identification.

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A ''G Insertion'' at Nucleic Acids 481 of the *H. pylori fldA* Gene Is Not Associated With Gastric MALT Lymphoma

Dear Sir:

Chang et al.[1] recently showed that a 19-kilodalton *Helicobacter pylori* protein FldA was more commonly expressed in strains isolated from patients with gastric mucosa-associated lymphoid tissue lymphoma (MALToma) than in those isolated from patients with chronic gastritis. In addition, they disclosed a nucleotide G insertion at position 481 of the *H. pylori fldA* gene in 9 of 9 (100%) *H. pylori* strains associated with gastric MALToma but in only 6 of 17 (35.3%) strains isolated from patients with other gastric diseases.

The finding of Chang et al.,[1] commented on by Cover and Blaser,[2] is potentially significant, given its likely importance in elucidating the pathogenesis of *H. pylori* and its implications in the serological diagnosis of infection with high-risk strains of *H. pylori*. To further validate whether the "*fldA* G insertion variant" of *H. pylori* is associated with MALT lymphomagenesis, we studied biopsy specimens from *H. pylori*–positive subjects with and without MALToma.

Twenty-eight Italian cases of *H. pylori*–positive gastric MALToma (15 low grade and 13 high grade), 20 Italian cases of *H. pylori*–induced gastritis, and 24 English clinical strains of *H. pylori* isolated from patients with chronic gastritis were studied. DNA was extracted as described previously,[3,4] and the presence of *H. pylori* was confirmed by polymerase chain reaction (PCR) amplification of the urease A gene.[5] All the available *H. pylori fldA* gene sequences in Genebank database were retrieved, including those published by Chang et al.,[1] and a region of 140–base pair nucleotides of the *fldA* gene including the nucleotide 481, where a "G insertion" was reported, was selected for PCR. The antisense primer was designed with a mismatch to create an *Sca*I restriction digestion site spanning the nucleotide 481, so that the amplified PCR products would not be digested by *Sca*I should the *fldA* possess a G insertion at position 481, and vice versa (Figure 1). PCR was performed in duplicate on a thermal cycler (Phoenix, U.K.) using *Taq* polymerase (Life Technologies, U.K.) with a hot-start procedure at 94°C for 4 minutes followed by a touch-down program consisting of denaturing at 94°C for 45 seconds, annealing at 60–56°C (1 degree down each cycle) for 45 seconds, and extension at 72°C for 45 seconds, and then 40 cycles of denaturing at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds. A final extension step at 72°C for 10 minutes concluded the reaction. The amplified PCR products were purified (Life Technologies), and 5 μL of purified PCR product was mixed with 0.5 μg of pBR322 plasmid DNA (Promega), which contains an *Sca*I site and was used as an internal digestion control, and digested with *Sca*I (Promega) according to the manufacturer's instructions. Digests were then analyzed separately on 10% polyacrylamide gels and 0.5% agarose gels to examine the PCR products and plasmid DNA, respectively. In all experiments, pBR322 plasmid DNA showed complete digestion with *Sca*I, whereas none of the *fldA* PCR products

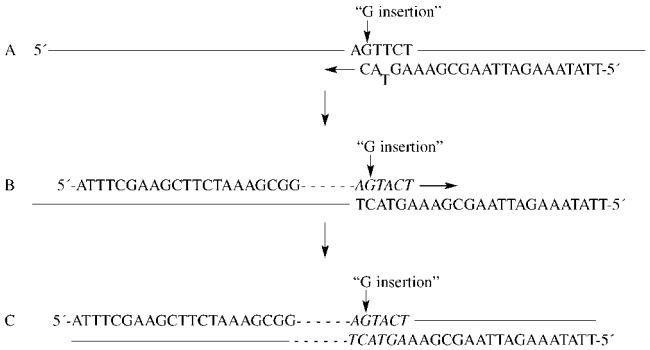

Figure 1. Detection of ''G insertion'' at nucleic acids 481 of the *H. pylori fldA* by PCR with a mismatched primer. (*A*) After separation of the original double-stranded DNA, the antisense primer with a single nucleotide substitution anneals to template and a complementary strand is synthesized with the mismatch primer incorporated at its 5′ end. (*B*) This strand then acts as a perfect template for synthesis of the sense strand DNA. (*C*) Both the newly synthesized strands are now perfect templates for subsequent rounds of amplification. Successful incorporation of the *Sca*I digestion site *AGTACT* was confirmed by sequence analysis of the representative PCR products.

displayed any digestion with *Sca*I, indicating the presence of the nucleotide G insertion at position 481 in all samples analyzed. To confirm the restriction digestion assay, PCR with a further downstream antisense primer (5′ GTTTGGTTGTCTATTTCTAGCA) was carried out in 5 cases of gastric MALToma and 16 *H. pylori* clinical strains, and the amplified products were sequenced using dRhodamine DNA polymerase on an ABI 373 automated sequencer (ABI). The G insertion at 481 was confirmed in all the cases examined, suggesting that this insertion is not associated with gastric MALToma in the sample series examined.

Our data do not support the recent report that a G insertion at nucleic acids 481 was seen more frequently in *H. pylori* strains associated with gastric MALToma than those associated with gastritis,[1] but are consistent with the published *fldA* sequences of three unrelated *H. pylori* strains, isolated from duodenal ulcer in United States (Genebank accession no. AE001439),[6] gastritis in United Kingdom (AE000511),[7] and an unknown source in United Kingdom (AF021093),[8] which all contain the G insertion at position 481. However, our sequence analysis did identify frequent nucleotide substitution, insertion, or deletion at other positions, especially at the region toward the 3′ end, as shown in the published *H. pylori fldA* sequences,[1,6–8] although none of these sequence variations have been found to specifically correlate with either gastritis or gastric MALToma. Frequent sequence variation of *fldA* is in agreement with the general understanding of the genetic diversity of *H. pylori*.[9] Whether one of the *fldA* variants is more cytotoxic than the other or is related to pathogenicity of *H. pylori* is unknown at present. Nevertheless, a G insertion at position 481 of *fldA* was conserved in each strain of *H. pylori* examined, suggesting that this represents the wild-type gene sequence rather than a polymorphism. Because strain-specific genetic diversity of *H. pylori*, such as the virulent factors *iceA* and *cagA* genes, has been shown to be geographically or population related,[10] it is possible that a G deletion at position 481 of *H. pylori fldA* exists in the Taiwanese population.

In addition, data from the referred study failed to show a direct association between the G insertion at nucleic acids 481 and expression of FldA protein or the presence of circulating antibodies directed against this bacterial molecule. The truncated recombinant FldA protein was used for testing the presence of circulating antibodies against FldA, but the test did not distinguish antibodies

against the truncated form from those recognizing the wild type of FldA. Because a deletion of 11 amino acids at the C-terminal of FldA caused by the "G insertion" at nucleic acids 481 would be unlikely to increase immunogenicity, the expression of FldA protein and the presence of its circulating antibodies in patients with gastric MALToma is probably not directly associated with this G insertion. However, it remains to be seen whether the expression of FldA protein or presence of circulating FldA antibodies is related to *H. pylori* strains associated with gastric MALToma in Western populations. (Supported by a grant from Cancer Research Campaign, U.K.)

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doi:10.1053/gg.2000.7696

Reply. We thank Liu et al. for their interest in our report on FldA protein and MALToma of the stomach.[1] Some of our answers have been addressed by Drs. Cover and Blaser in their excellent editorial.[2] Although our assay using recombinant fldA cannot differentiate truncated antibodies against the truncated form from those not truncated, our study revealed a significantly higher prevalence rate of these antibodies in patients with MALToma. Therefore, it is a bit early to assume that a short truncation at FldA will not increase its immunogenecity unless further evidence is available.

Because Fld antibodies are not perfect in terms of specificity and sensitivity,[1,2] to identify high-risk patients for MALToma by FldA alone will not succeed completely. Our report is just a small step; we hope follow-up studies, such as the expression of FldA in *H. pylori*, will help to clarify the pathogenesis of MALToma.[1,2]

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1. Chang CS, Chen LT, Yang JC, et al. Isolation of a *Helicobacter pylori* protein, FldA, associated with mucosa-associated lymphoid tissue lymphoma of the stomach. Gastroenterology 1999;117:82–88.
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Image of the Month Answer

Answer to the Image of the Month Question (page 820): The enteroscopic photographs show Dieulafoy's lesion. The artery projects above the mucosa, and there is a fresh coagulum. The patient's GI bleeding was controlled by endoscopic sclerotherapy using 1:10,000 3 mL epinephrine and then 2 mL ethanolamine oleate injection. During 12 months' follow-up, GI bleeding has not recurred.

Dieulafoy's lesion is a rare cause of massive GI bleeding, usually from the proximal stomach within 6 cm of the esophagogastric junction. The lesion may be encountered throughout the GI tract, however; the duodenal bulb and proximal jejunum are the next most common locations, followed by the proximal colon. The disorder presents most often in the elderly, but can be found in any age group; it is twice as common in men. The lesion possibly results from failure of transmural arterial ramifications to progress to smaller vessels, resulting in a muscular artery beneath the mucosa. Rupture of the artery can lead to severe bleeding, as seen in this case. Dieulafoy's lesion is best diagnosed by endoscopy during an acute bleeding episode. Often, multiple endoscopic procedures must be performed before the lesion can be identified. The mortality rate for elderly patients with this lesion has been high because of inability to localize the bleeding site. Various therapeutic endoscopic techniques, including injection therapy, heater probe, laser photocoagulation, and band ligation, have replaced surgery as the first-line approach to bleeding Dieulafoy's lesion.