SELECTED SUMMARIES

Henry J. Binder, M.D.
Selected Summaries Editor
Yale University School of Medicine
New Haven, Connecticut

MANAGEMENT OF GERD IN THE MILLENNIUM: IS THERE ROOM FOR ENDOSCOPY?

Filipi CJ, Lehman GA, Rothstein RI, Raijman I, Stiegmann GV, Waring JP, Hunter JG, Gostout CJ, Edmundowicz SA, Dunne DP, Watson PA, Cornet DA (Creighton University, Omaha, Nebraska; Indiana University, Indianapolis, Indiana; Dartmouth Medical Center, Lebanon, New Hampshire; Hermann Hospital, Houston, Texas; University of Colorado, Denver, Colorado; Emory University, Atlanta, Georgia; Mayo Clinic, Rochester, Minnesota; and Graduate Hospital, Philadelphia, Pennsylvania). Transoral, flexible endoscopic suturing for treatment of GERD: a multicenter trial. Gastrointest Endosc 2001;53: 416–422.

The ABCDEs of major therapeutic endoscopic advances during the last quarter century include: (A) ablation of tumors, (B) banding of blood vessels, (C) cautery of bleeding vessels or Barrett's mucosa, (D) dilatation of strictures and decompression of luminal obstruction with tubes and stents, and (E) excision of tumors, extraction of stones and foreign bodies, and endoscopic ostomies (PEG and PEJ). "Fundoplication" through the endoscope (the first step for the endoscopists in the surgical arena) may be added to this list. Swain et al. from the Royal London Hospital, London, England, should be congratulated for their pioneering work in the design of tools for endoscopic surgery and the development of techniques for endoluminal surgery to treat gastroesophageal reflux.

Endoluminal surgical instruments include: (1) an endoscopic sewing machine to place a suture through a fold of tissue, (2) endoscopic knot tying techniques and knot pushing instruments to place a secure knot, (3) an endoscopic suture cutting device to cut the threads after placing the knots (Gastrointest Endosc 1986;32:36–38, 1994;40:722–729, 1996;44:667–674), and the development of techniques for endoluminal surgery to treat gastroesophageal reflux (Gastrointest Endosc 1996;44:133–143). Filipi et al. conducted the first United States multicenter trial to evaluate the safety and efficacy (decrease the heartburn severity score by 50% in addition to a reduction in the use of acid suppressive therapy and prokinetic agents to less than 4 doses per month) of this new technique in the management of gastroesophageal reflux disease (GERD).

Patients with GERD were enrolled for the study in 8 centers. Inclusion criteria were 3 or more heartburn episodes per week while not taking medication, dependency on antisecretory medicine, documented acid reflux by pH monitoring, and grade 0 to 2 esophagitis on the modified Savory–Miller scale. Exclusion criteria were dysphagia, grade 3 or 4 esophagitis, obesity (body mass index of >40 kg/m^2), hiatal hernia greater than 2 cm in length, and GERD refractory to proton pump inhibitors. Patients underwent manometry, endoscopy, 24-hour pH monitoring, and symptom severity scoring before and 6 months after the procedure. The patients were randomized to 1 of the 2 plication configurations ("linear" vs. "circumferential"). Minimums of 2 plications were placed for each procedure. Endoscopic plication involves the following steps: (1) placement of an oroesophageal overtube, (2) advancement of the Bard Interventional Endoscopic Suturing System device through the overtube and distal to the squamocolumnar junction, (3) suction of a fold of tissue into the cavity of the sewing capsule and placement of a stitch and withdrawal of the suturing system and the tilt-tag attached to the suture, (4) reloading the same suture attached to the metal tilt-tag into the sewing machine's hollow needle, and reinsertion of the suturing system to the location of the previous stitch, rotation of the endoscope, and placement of the next stitch adjacent to the first, (5) withdrawal of the suturing system followed by traction on both suture ends until the redundant loop has been eliminated, (6) tying a half hitch outside the patient and passing it with a knot pusher (a minimum of 5 half hitches) to secure plication, and (7) introduction of a suture cutter to cut the suture strands above the knot. (Each plication takes at least 8 passes with different instruments to place 1 plication). The procedure is repeated to complete the second and third plication.

Sixty-four patients with GERD were enrolled in the study (mean age, 46.4 years; M:F, 7:3; mean weight, 193 lb). Heartburn was present daily or 3–5 episodes per week in 98% of patients, moderate to severe regurgitation was reported in 61% of patients, and 86% of patients were taking proton pump blockers, 39% H_2 receptor antagonists, 20% antacids, and 19% prokinetic agents.

All the physicians performing the procedure underwent training with animals (4–15 procedures per physician) before participation in the trial. Patients were randomized either to a linear configuration (52%) or a circumferential configuration (48%) of procedures. The following types of sedation were used: conscious sedation (69%), monitored anesthesia (14%), and general anesthesia (17%). The mean time for the procedure was 68 minutes. The intended suture location was accomplished for 81% of the plications. Forty-six patients received 2 plications and 18 received 3 plications. The procedure was completed successfully in 60 patients in a single session (93%); 4 patients required a second session on a different day (technical difficulties prevented adequate number of plications in 3 patients and hypoxia occurred during the procedure in 1 patient). Eleven patients (17%) required a repeat procedure after suboptimal results with the first procedure. In total, 15 patients (23%) required 2 sessions. Fifty-one patients completed the 6-month follow-up; 13 patients (20%) were excluded (unwillingness to complete the study, poor results, and moving to a different city).

There was a significant improvement in the GERD symptom score at 6 months: heartburn severity (23 to 9.5), frequency (2.75 to 1.31), and regurgitation (1.81 to 0.61) ($P < 0.0001$ for each). Quality of life assessment (by SF-36 questionnaire) showed improvement in bodily pain and social functioning. Sixty-two percent of patients were taking less than 4 doses of medication per month at 6 months, compared with the number of patients on treatment with the following: PPIs, 86%; H_2RA, 39%; antacids, 20%; and prokinetic agents, 19% at baseline. There was a significant improvement in percent of upright time (11.5 to 9.7; $P < 0.005$), total pH was less than or equal to 4 (9.63 to 8.50; $P < 0.01$), and there was a decrease in the total number of reflux episodes (158 to 117; $P < 0.0002$). There was no difference in esophageal body or lower esophageal sphincter (LES) pressure measurements. The mean grade of esophagitis did not change significantly (0.72 at base line to 0.59 at 6 months). Degradation of plication folds was noted in those patients experiencing treatment failure. There were no procedure-related deaths. There was 1 suture perforation that resolved with intravenous antibiotics and hospitalization. Other complications include pharyngitis, chest pain, abdominal pain, vomiting, hypoxia, and mucosal tear. Sewing capsule malfunction accounting for problems was minimal.

Comment. Review of the extensive animal experimental work is crucial to the understanding of the above report. Paul Swain's group has been instrumental in the development of the following endoluminal surgical techniques: (1) endoscopic gastroplasty, accomplished by suturing the anterior and posterior walls of the stomach to create a neoesophagus along the lesser curve; (2) fundoplication, created by invaginating the esophagus and fixing it to the stomach 2 cm distal to the cardioesophageal junction; and (3) anterior gastropexy, performed by fixing the anterior wall of the stomach to the rectus sheath by a technique similar to that used in the placement of PEG tubes. All animals survived the operation (no mortality), and there were no perforations, bleeding, and serious postoperative infections. Postmortem studies did not show any serosal inflammatory reaction or adhesions at the operation site. The median duration of the procedure was 30 minutes for the gastroplasty, 40 minutes for the fundoplication, and 35 minutes for the anterior gastropexy. The stitches were seen at endoscopy performed at a median of 6 weeks, and in 2 animals repeat endoscopy at 357 and 345 days after the original operation showed that all the stitches were still identifiable. There was an increase in the lower esophageal pressure (preoperative median, 4.6 mm Hg; postoperative median, 13.33 mm Hg; $P = 0.008$) and cardiac yield pressures (preoperative median, 10 mm Hg; postoperative median, 19 mm Hg; $P = 0.007$) after the gastroplasty. The lower esophageal sphincter pressure and endoscopic yield pressure decreased with time, but still the pressures were maintained well above the preoperative pressures (Gastrointest Endosc 1996;44:133–143).

Martinez-Serna et al. (Gastrointest Endosc 2000;52:663–670) evaluated a different suture arrangement: 3 bulking sutures either in a linear arrangement on the lesser curvature of the stomach just below the gastroesophageal junction (GEJ) (group I) or in a circumferential arrangement just distal to the GEJ (group II) in baboons. The intraabdominal length of esophagus increased in both the groups (13.7 mm to 17.9 mm; $P = 0.004$ in group I and 14.5 mm to 19.7 mm, $P = 0.004$ in group II), the LES pressure increased only in group I (5.39 mm Hg to 7.64 mm Hg; $P = 0.008$), and the total LES length increased in group II (20.1 mm to 26.8 mm; $P = 0.02$). Suture retention noted at 6 months varied between 12% and 50% in group I and between 33% and 90% in group II. The yield pressure and yield volume did not differ significantly from those measured in control animals. Pathologic examination showed that 3 sutures penetrated to the submucosa, 18 penetrated to the superficial muscularis, and 2 penetrated to the deep muscularis layer. There was no transmural penetration of the sutures. Chronic inflammation of the proximal esophagus was observed in all the animals. Mild to moderate esophageal hyperplasia was noted in about half of the animals. Interestingly, animals with the most fibrosis had a greater change in the abdominal length of the LES compared with those with less fibrosis, although this value did not reach significance ($P < 0.08$).

Endoscopic suturing is the new kid on the block. Before accepting this new kid, one should put this to the same strict scrutiny of scientific investigation as we do for any new therapy before embracing it and offering it to our patients with GERD. The 2 most important questions that need to be answered during the preliminary evaluation of any new device or technique are the following: (1) is the device and the technique safe? and (2) does this benefit the patient? Has it been done by the investigators? Regarding the issue of safety of the device, in this multicenter trial of 64 patients, there were no procedure-related deaths. There was 1 suture perforation that resolved with intravenous antibiotics and hospitalization. This was caused by excessive suture tension, and this problem could be avoided with training. Sewing capsule malfunction accounting for problems was minimal. Other complications (pharyngitis, chest pain, abdominal pain, vomiting, hypoxia, and mucosal tear) were caused by a combination of prolonged procedure requiring repeated intubations (mean of 68 minutes) and use of an overtube. These could be improved by considering general anesthesia or monitored anesthesia to make the procedure more tolerable, thereby avoiding retching and minimizing complications related to the overtube placement. One could envision avoiding the placement of an overtube if the current endoscopic suturing (many pass technique) could be simplified to a single pass technique. A novel suture-anchor device was shown to reduce the number of esophageal intubations in animal experimental study (Gastrointest Endosc 2001;53:AB3435).

The second question is the success of placement of plications and the "wear and tear" of the plications. This procedure definitely requires patience (8 steps per plication). Successful plication was possible in over 80% (range, 50%–75%) of patients. The best endoscopists in the country did these procedures after training (4–15 animals per physician) organized by the manufacturer. Like any other new procedure, with time these results could be improved. "Wear and tear" of the plications occurred in 11 patients between 49 and 405 days after the original procedure and required a repeat procedure. Does this plication improve the symptoms of reflux and pH and manometric readings and heal the esophagitis? There was no sham arm in the study for comparison and the subjects were used as their own controls. Symptoms of GERD improved. Because of the study design (patients with severe erosive esophagitis, GERD refractory to proton pump inhibitors, hiatal hernia greater than 2 cm, and obesity were excluded), we do not know how this technique will pan out in patients with severe reflux refractory to PPIs. Erosive esophagitis did not improve at 6 months after antireflux procedure, and this brings the issue of the effects of suboptimal control of reflux in patients with severe disease and those with Barrett's esophagus. Prospective long-term studies are needed to assess the durability of the endoscopic fundoplication and define its role in the management of patients with GERD.

At about the same time, 2 other new kids came to the arena of GERD management: endoscopic radiofrequency energy delivery to the gastroesophageal junction (Gastrointest Endosc 2001;53:407–415) and endoscopic implantation of Plexiglas microspheres (Gastrointest Endosc 2001;53:423–426). Since the presentation of all 3 antireflux endoluminal surgeries at the 2000 Digestive Disease Week plenary session, a lot of enthusiasm was ignited to develop new methods to augment the anti-reflux barrier (Gastrointest Endosc 2001;53:AB132, 2001;53:AB74). All these new kids need to be put to the same vigorous investigation and let the time allow these techniques to define their survival. Even if these new techniques pan out in the long run, would they find a niche, especially with the price wars among the pharmaceuticals trying to make the proton pump blockers dirt-cheap? How do they compare with the gold standard—the surgical fundoplication?

Filipi and his colleagues should be congratulated for their collaborative efforts to document the safety and efficacy of endoluminal surgery in the management of GERD—the first step, a successful venture. The next decade will be interesting for all of us involved in the management of GERD to watch the exciting developments.

GOTTUMUKKALA S. RAJU, M.D., D.M., MRCP (UK)

Reply. Dr. Raju's comments concerning the new technologies for treatment of gastroesophageal reflux disease are appropriate. Sham studies, long-term follow-up, and comparative analysis of the new treatment modalities with standard forms of treatment are in order. Physicians need to remain cautious but also willing to enter the intraluminal antireflux procedural milieu. But one should examine the capabilities and reasons for proceeding.

The details of the procedure cannot all be transmitted by written reports or video recordings. Therefore, direct observation of procedures is important. Most physicians are flattered by requests to observe their technique. Do not hesitate to participate in training courses and discuss your involvement with respected but noncompetitive colleagues. Also understand that endoscopic suturing requires a team approach. Qualified personnel that can consistently be present for all procedures are necessary at first. There is a steep learning curve. Finally, the patient volume must be sufficient or the peak of the curve will never be reached. As a laparoscopic surgeon, I can attest to the great pleasure of observing the benefits of quick and successful patient recovery.

CHARLES FILIPI, M.D.

IS THERE A PLACE FOR PLACEBOS?

Hrobjartsson A, Gotzsche PC (Department of Medical Philosophy and Clinical Theory, University of Copenhagen, Panum Institute, and the Nordic Cochrane Centre, Rigshospitalet, Copenhagen, Denmark). Is the placebo powerless? N Engl J Med 2001;344:1594–1602.

Noting that placebos are believed to improve clinical outcomes, Hrobjartsson and Gotzsche systematically reviewed the medical literature in an effort to determine what effect such an intervention really has. They viewed a placebo as a treatment that is similar to the study therapy but without any known specific activity. The placebo intervention could be a pharmacologic agent (e.g., a tablet containing presumably inert substances), a physical intervention (e.g., a manipulation), or a psychological one (e.g., a conversation).

To assess the efficacy of placebo therapy, these investigators sought randomized controlled trials (RCTs) that included both a placebo arm and a no treatment arm. They were able to identify 114 such RCTs, and they then combined the data from these studies in a series of meta-analyses.

RCTs can provide data in either continuous or categorical (binary) manners. In the former, the outcome can vary over a wide range (e.g., blood pressure or scales of pain scores). In the latter, the data are presented as events that did, or did not, occur (e.g., blood pressure above or below a certain level or pain that is either present or absent). Meta-analysis cannot combine these 2 types of data together. Hence, Hrobjartsson and Gotzsche separately calculated the relative risk (RR) of an unwanted outcome in 32 RCTs (including 3795 patients) that collected binary outcome data and the standardized mean difference (SMD) in the 82 RCTs (including 4730 patients) reporting continuous outcome data. This latter number represented the difference between the mean values for an unwanted outcome in the 2 groups (placebo minus untreated) divided by the pooled standard deviation.

Thus, any RR less than 1.0 represented a favorable effect of the placebo, and any 95% confidence interval (CI) that did not overlap 1.0 was interpreted as showing a significant effect. Likewise, any SMD less than 0% represented a favorable effect of the placebo and any 95% CI not overlapping 0 was considered to be significant. The investigators also separately looked at outcomes that were subjective and that were objective.

The overall RR for the binary outcomes was 0.95 (95% CI, 0.88–1.02). The respective RRs (95% CIs) for the subjective and objective ones were 0.95 (0.86–1.05) and 0.91 (0.80–1.04). In other words, the placebo therapy was not shown to have any significant effect on a binary outcome. On the other hand, placebo therapy did have a demonstrable effect with regard to continuous outcomes (SMD, −0.28; 95% CI, −0.38 to −0.19), especially

for subjective ones (SMD, −0.36; 95% CI, −0.47 to −0.25). No significant effect was observed on objective continuous outcomes (SMD, −0.12; 95% CI, −0.27 to +0.03).

Whenever there were at least 3 combinable RCTs assessing a particular clinical problem, the investigators also undertook a meta-analysis. They identified 3 RCTs providing binary data regarding nausea, 6 regarding smoking, and 3 regarding depression; the RRs in all were close to unity and the 95% CIs overlapped 1.0. Relatively small numbers of RCTs (3–7 for each category) provided continuous data for obesity, asthma, hypertension, insomnia, and anxiety; again, no significant effect of placebo was identified. On the other hand, 27 RCTs assessed pain, and the SMD (−0.27) was significant (95% CI, −0.40 to −0.15).

Hrobjartsson and Gotzsche concluded that placebos do not have powerful clinical effects. In fact, even the effects that they did demonstrate translated into relatively small gains. (For example, the difference in pain score was equivalent to a 6.5-mm reduction on a 100-mm visual-analogue scale.) They stated that the use of a placebo outside of a controlled trial could not be recommended.

Comment. In the 1950s, uncontrolled reports suggested that cardiac ischemic pain could be substantially alleviated by ligating the internal mammary artery. (Note: This is not the same as the Vineberg procedure, in which the internal mammary artery is implanted into the myocardium.) Two small RCTs compared internal mammary ligation to sham surgery (N Engl J Med 1959;160:1115–1118, Am J Cardiol 1960;5:483–486). In both studies, all of the patients were sent to the operating room, where an incision was made in the chest. At that point, the patient was randomly allocated to having the procedure or to having the incision closed without any ligation. Neither the patient nor the staff caring for him or her knew whether or not an actual ligation was performed. When the data were analyzed, pain relief occurred in 60%–70% of those receiving the ligation and in 70%–100% of the recipients of the sham surgery.

I am fond of quoting these trials as examples of the placebo effect. (I have assumed that cutting the skin would otherwise be ineffective treatment for cardiac ischemic pain.) It is hard to believe that such a high rate of spontaneous improvement in pain is consistent with the natural history of chronic angina. This point is mentioned to differentiate the "placebo" effect from the effect that can be expected simply from the natural history of a disease. As an example of the latter, it is not surprising that peptic ulcers can come and go over time even without any specific intervention. When pharmacologic trials have compared active drugs to "placebos," the role of the placebo is to provide insight into a natural history that is not confounded by observer bias, not to produce nonpharmacologic ulcer healing (although this possibility cannot be excluded by such study designs).

Hence, my concept of the placebo effect is not that it actually alters the natural history of a disease process, but rather that it interferes in some beneficial way with the patient's perception of some of the symptoms, particularly those we refer to as subjective. (This may be a different perspective than that of Hrobjartsson and Gotzsche, who may have been asking if the placebo actually altered natural history.) As such, I was not surprised by the observation that the placebo could not be shown to be more useful than no treatment in altering objective evidence of disease (whether it is measured in a binary or continuous manner). What was more surprising to me was that placebo therapy did not have a consistent effect on subjective outcomes. After all, why should it matter whether those outcomes were measured with a scale or with a category?

As is true for most systematic reviews emanating from the Cochrane collaboration, the bulk of the discussion focused on potential shortcomings of the study, not on citing selected literature to support the authors' conclusions. Hrobjartsson and Gotzsche commented on several biases. Obviously, the participants in the no treatment group knew that they were receiving no treatment, whereas the placebo recipients may have thought that they were receiving active therapy. Hrobjartsson and Gotzsche hypothesized that, if this resulted in the placebo recipients reporting better outcomes simply to please the individual study investigators, a reporting bias would have occurred with regard to subjective outcomes (thus accounting for the effect that they did observe). ("Placebo" is Latin for "I will please.") Of course, if the patients really perceived what they were reporting (rather than just trying to satisfy the investigator), this is my perspective of the placebo effect.

Hrobjartsson and Gotzsche also wondered if the patients in the no treatment arm were more likely to seek alternative treatment outside of the trial, thus masking the effect of the placebo. They discounted this possibility because they were unable to detect any difference in effect between those trials in which some type of standard therapy was also provided to all of the study participants and those in which none was offered. Hrobjartsson and Gotzsche reasoned that out-of-study treatment would be more prevalent if the patient was not receiving any treatment at all. Hence, this bias would not have as frequently masked a placebo effect in trials that also provided standard therapy. However, they did acknowledge that there was virtually no information about concomitant therapy in the original reports.

The placebo effect was seen more commonly in small trials than in large ones. This was not because the larger trials were of higher quality. Another explanation for this observation could be publication bias. However, because it was not the primary purpose of the RCTs to assess the effect of placebo per se, Hrobjartsson and Gotzsche believed that it was unlikely that other trials were not submitted for publication because such an effect was not observed.

As might have been inferred from many of the numbers cited above, the power of some of the analyses to see a difference was low (type II error). Hrobjartsson and Gotzsche also noted that although they did attempt to do sensitivity analyses to tease out potential confounding issues, the pooling of heterogeneous RCTs might have obscured an effect in an undefined subgroup.

Hrobjartsson and Gotzsche claimed that they were unable to assess the effect of the patient-provider relationship, a phenomenon that they considered separate from the placebo effect. From my perspective, that interaction is part of the placebo effect.

Because there was a lack of blinding between the 2 groups, one might predict that the various biases so introduced should have produced differences. Hence, even if the placebo had no true effect, those biases should have tended to create a difference. The fact that it was hard to find differences despite these biases suggested to Hrobjartsson and Gotzsche that no such effect does exist.

It may not be true that the lack of blinding would have tended to create a difference. First of all, one such potential bias, discussed earlier (no treatment group seeking alternative therapy), would tend to obscure a difference. Furthermore, these RCTs were all designed to include both a placebo group and an untreated one. Because this was not done in general to assess the role of placebo per se, why did those investigators choose to create more work for themselves? Is it possible that the investigators who designed these trials tended to be partic-

ularly skeptical, and that this bias acted to obscure differences? (After all, those investigators were also unblinded.)

In any event, the observations of Hrobjartsson and Gotzsche again remind us that much of what we take for granted in medicine may not be true, or at least not as true as we would like to think. Although I still believe that there is such a thing as a placebo effect (as I have described it), it may very well be small in most cases (the situation for internal mammary artery ligation notwithstanding). On the other hand, because it comes at essentially no cost if we are going to prescribe any particular intervention anyway, the issue may be moot. On the other hand, I would agree with these Danish investigators that, outside of clinical trials, we should not be ordering interventions solely to achieve a placebo effect.

RONALD L. KORETZ, M.D.

HEREDITARY HEMOCHROMATOSIS AND CANCER RISK: MORE FUEL TO THE FIRE?

Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, Losco A, Fargion S (Dipartimento di Medicina Interna, Cattedra di Gastroenterologia, and Unita di Epidemiologia, Universita di Milano, Ospedale Maggiore IRCCS, Milano, Italy). Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron related chronic liver disease. Hepatology 2001;33: 647–651.

Fracanzani et al. studied 230 consecutive patients diagnosed with hereditary hemochromatosis (HH) presenting to an outpatient liver clinic over a 20-year period, along with an equal number of control patients with non-iron-related chronic liver disease. The diagnosis of HH was based on laboratory tests, liver biopsy specimens, and, in 200 cases, mutations in *HFE,* the hemochromatosis gene. The control group consisted of 230 patients with chronic liver disease caused by hepatitis C (38%), alcohol (21%), hepatitis C and alcohol combined (22%), hepatitis B (9%), and a small number of other causes. Each HH patient was then matched individually to a control patient based on sex, age, duration of follow-up (±5 years), and the severity of liver disease. In the final analysis, there was a slight trend towards longer follow-up in the HH group (79.9 months compared with 76.9 months). One discrepancy between the groups was a nonsignificant trend towards a stronger family history of cancer in the non-iron-related group (27% vs. 22%).

Cancer surveillance in both groups involved ultrasonography every 6 months, α-fetoprotein testing every 3 months, and a detailed yearly history and physical examination. Those patients in whom these screening tests revealed concerning signs or symptoms were subjected to further diagnostic evaluations. All malignancies were histologically confirmed.

Hepatocellular cancer (HCC) developed only in patients with cirrhosis from both groups. Forty-nine of 134 (36%) of the HH patients developed HCC compared with only 29 of 134 (21%) of controls (relative risk, 1.8; 95% confidence interval [CI], 1.1–2.9). Interestingly, the average age at diagnosis of HCC was 58.5 in the HH group compared with 53.5 in the control group. With regards to extrahepatic malignancy, there was a trend towards an increased risk of nonhepatic cancer in those with HH compared with the control population (20/230 HH patients [9%] compared with 11/230 control patients [5%]; 95% CI, 0.8–4.0). The nonhepatic cancers that developed in both the control and HH patients included colorectal, lung, prostate, tongue, and bladder.

Comment. HH is the most common inherited disease in persons of Northern European descent. Over time, inappropriately increased absorption of iron from the gastrointestinal tract leads to iron deposition in the liver, pancreas, heart, joints, anterior pituitary, and skin. One of the most feared complications of untreated HH is the development of hepatic cancer. Indeed, primary liver cancer (predominately HCC) has been determined to account for 20% to as many as 45% of deaths in patients with HH (Hepatology 1992;15:655–659). The increased risk of HCC in patients with HH compared with the general population has been well documented. Hsing et al. followed a population-based cohort of Danish men discharged from the hospital with a diagnosis of HH and found that the risk of hepatic cancer was almost 100 times greater than the expected rates within the Danish population (Int J Cancer 1995;60:160–162). Niederau et al. prospectively analyzed the cause of death among 163 patients with documented HH and found that the risk of hepatic cancer was 219 times greater than in a normal population (N Engl J Med 1985;313: 1256–1262). A follow-up analysis by this group showed that liver cancer could develop even in patients who had undergone iron depletion, with an average time interval between depletion and cancer of 9.4 years (Gastroenterology 1996;110:1107–1119). In this study, all 21 cases of hepatic cancer developed in cirrhotic livers, a typical finding in patients with HH. However, there are now 13 case reports in the literature of patients with noncirrhotic HH who developed HCC.

Although an increased rate of HCC in patients with HH is not in debate, controversy surrounds the rate of nonhepatic cancers in HH. Ammann et al. were among the first to suggest an increased incidence of extrahepatic cancer in patients with HH. In a study of 36 consecutive HH patients followed for an average of 8 years, the investigators observed a total of 6 extrahepatic cancers, 4 of which were lung cancer (Scand J Gastroenterol 1980;15:733–736). Hsing et al. found an increased rate of esophageal cancer (2 cases), melanoma (2 cases), and all nonhepatic cancers (13 cases) compared with the general population, although the sample size was small, and the average follow-up was only 4.1 years after enrollment (Int J Cancer 1995;60:160–162). Tiniakos et al. studied 71 patients with HH who enrolled in venesection therapy, and they noted an 8.4% rate of nonhepatic cancer development over an average of 7 years of follow-up. The total number of cases of nonhepatic cancer in this study was only 6, however, and no claim was made as to statistical significance (Appl Pathol 1988;6:128–138). Nelson et al. provide supporting evidence by demonstrating a statistically significant increased risk of colorectal and stomach cancer in patients heterozygous for HH, but their conclusions are limited by the fact that their primary endpoint included many different types of cancer, some of which showed no increased risk of occurrence compared with controls (Cancer 1995; 76:875–879). By contrast, Bradbear et al. followed 208 patients with HH and found no increase of extrahepatic malignancies compared with expected values from cancer registry incidence data (J Natl Cancer Inst 1985;75:81–84).

The current study by Fracanzani et al. adds fuel to the fire with regards to iron playing a causative role in hepatic and nonhepatic

cancers. The study is a survival analysis, adjusted for multiple factors known to predispose to carcinogenesis. There was an adequate number of cancers found to allow for an adjustment for rates of hepatitis C, alcohol, and family history. Unlike many previous studies, which often use national cancer databases as controls, Fracanzani et al. wisely compare their HH patients with a population of chronic liver disease patients without HH, eliminating much of the referral and selection bias that plagues earlier studies.

The investigators state that patients with HH are at high risk of liver cancer as well as other malignancies. Their study conclusively proves an increased risk of hepatic cancer compared with the control population, but the "increased risk" of other malignancies does not reach statistical significance (95% CI, 0.8–4). Their multivariate analysis groups the hepatic and nonhepatic cancers together, leading to a statistically significant result that does not hold true when the extrahepatic cancers are considered alone.

HH patients in this study developed 20 extrahepatic cancers, compared with 9 in the control group. However, the breakdown of the site of these cancers shows increased numbers within sites such as the colon, lung, and bladder, which are organs that are not known to accumulate excess iron in HH. Thus, to ascribe the increased incidence of cancer in these HH patients, one would have to formulate a carcinogenic hypothesis in which iron excess at remote sites leads to distant cancer development.

The potential causative role of iron in HCC and nonhepatic cancer is intriguing. Iron has long been suggested to be a biocarcinogen. Deugnier et al. showed an increase in mouse tumor cell growth with iron supplementation, and an inhibition with iron removal (J Hepatol 1998;28:21–25). One argument implicating iron's causative role derives from the discovery that patients with HCC have a small but significantly increased hepatic iron index compared with normal individuals (Hepatology 1995;22:446–450). Another argument derives from the discovery of increased iron stores on magnetic resonance imaging studies of livers with hepatocellular carcinoma compared with controls (Radiology 1999;212:235–240).

The putative mechanisms of iron-related carcinogenesis involve iron's role in oxidative injury and in cell growth. Iron has been shown to cause lipid peroxidation and oxidant stress in patients with HH undergoing liver biopsy evaluation. This finding is supported by a growing number of studies showing increased lipid peroxidation products in the liver of iron-overloaded animals. The formation of sufficient reactive oxygen species leads to an impairment of basic immune defenses such as antigen-specific immune responses, cytotoxic T-cell proliferation and function, and enhancement of suppressor T-cell activity. These alterations in T-cell function may lead to impaired immune surveillance against cancer.

One argument against iron's role as a carcinogen has been the tendency for cancers to arise after iron depletion, a phenomenon once again demonstrated in this study. This fact should not discount the potential role of iron. However, the carcinogenic effects of other toxins have been shown to occur as many as 20 years after exposure. Indeed, it could be hypothesized that the generation of free radicals, lysosomal and DNA damage, or other mechanisms are irreversibly underway before iron depletion, and that the eventual toxin removal does not stop what is already an inevitable process.

The story of iron and carcinogenesis is an unfinished one, with many questions still left to be answered. The laboratory evidence supporting iron's role as a carcinogen has yet to be conclusively shown in clinical studies. Nonetheless, Fracanzani et al. succeed in drawing attention to the importance of prompt iron depletion in patients diagnosed with HH.

MARK A. MALLORY, M.D.
KRIS V. KOWDLEY, M.D.

Reply. Mallory and Kowdley report that the results of our study unequivocally indicate an increased risk of liver cancer in patients with HH compared with matched control patients, but they are skeptic on the increased risk of extrahepatic malignancies. Although we agree that the CI reported (0.8–4) does not reach statistical significance, nevertheless, we retain that a relative risk of 1.8 indicates a definite trend towards an increased risk.

Interestingly, after the publication of this article, we showed that the polymorphisms of tumor necrosis factor (TNF)-α may affect the expression and severity of liver damage of patients with HH (Blood 2001;97:3707–3712). A significantly lower prevalence of 1 of the 2 polymorphisms studied, the 238, was found in patients with HH than in controls. A lower prevalence of cirrhosis was observed in patients with TNF-α polymorphisms than in those without it (4 of 15 [27%] vs. 28 of 49 [57%]; $P = 0.07$). In nonhomozygotes for the C282Y mutation, severe liver siderosis was less prevalent in patients with the 308 polymorphisms than in those without it ($P = 0.05$) and alanine aminotransferase values were significantly lower in patients with TNF-α polymorphisms ($P = 0.008$ and $P = 0.045$, respectively, in homozygotes and nonhomozygotes for the C282Y mutation). Non-HFE genetic factors could interfere with the risk of developing HCC, and only a subset of patients with HH could be at increased risk of malignancies. Large cooperative clinical studies are needed to assess this complex issue.

A further fuel to the fire comes from another recent study by our group. We studied the prevalence of HFE gene mutations and the interaction between these mutations and known exogenous risk factors in 81 male patients with hepatocellular carcinoma occurring in cirrhosis (Blood Cells Mol Dis 2001;27:505–511). None of the patients had a phenotype compatible with homozygous hereditary hemochromatosis. The analysis was performed by using the case-only approach specifically designed to estimate departure from multiplicative risk ratios under the assumption of independence between genotype and environment exposure. An increased prevalence of the C282Y mutation was observed in patients with hepatocellular carcinoma than in normal controls (8.6% vs. 1.6%; $P < 0.03$). At univariate analysis, iron overload was significantly associated with both HFE mutations ($P < 0.0001$), whereas ongoing hepatitis B virus infection was associated with the C282Y mutation ($P < 0.05$). By multivariate analysis, a trend for increased risk of being positive for hepatitis virus markers (odds ratio, 2.9; 95% CI, 0.9–9.5) was observed in patients heterozygous for HFE mutations. These data suggest an involvement of iron in carcinogenesis even in heterozygotes for HFE mutations. These subjects, once exposed to risk factors, could have an increased risk of developing cirrhosis and later liver cancer than people without the mutation exposed to the same risk factors. In conclusion, the interaction between iron and cancer seems progressively more complex with increasing fuel added to the fire!

A. L. FRACANZANI
D. CONTE
M. FRAQUELLI
E. TAIOLI
M. MATTIOLI
A. LOSCO
B. S. FARGION

DIET AND DISEASE: THE "PHYTE" OVER INTESTINAL CHOLESTEROL

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH (Department of Molecular Genetics and McDermott Center for Human Growth and Development and Howard Hughes Medical Institute and Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas; Tularik, Inc., San Francisco, CA; Department of Pediatrics, Johns Hopkins University, Baltimore, MD). Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771–1775.

Sitosterolemia is an autosomal recessive disease first described by Bhattacharyya and Connor in 1974 (J Clin Invest 1974;53:1033–1043). The original description reported 2 sisters with extensive tendinous xanthomas and marked phytosterolemia. Afflicted individuals have significantly elevated plasma levels of phytosterols (campesterol, stigmasterol, and β-sitosterol) and are hypercholesterolemic, primarily during childhood. Studies have shown that these patients hyperabsorb all sterols (cholesterol, in addition to structurally similar phytosterols) and poorly secrete phytosterols into bile (Arterioscler Thromb Vasc Biol 1991;11:1287–1294). In addition to xanthomas, these patients exhibit accelerated atherosclerosis, leading in many cases to premature death caused by deposition of cholesterol and phytosterols in coronary arteries (J Lipid Res 1992;33:945–955). To date, some 45 cases of sitosterolemia have been reported worldwide (Curr Opin Lipidol 2001;12:141–149). More cases likely exist but are probably misdiagnosed as other forms of hyperlipidemias. Treatment is directed towards reducing intestinal absorption of cholesterol and phytosterols with alterations in diet, bile acid–binding resins, or ileal bypass surgery. A molecular understanding of sitosterolemia may lead to a better means of reducing cholesterol absorption, both for sitosterolemic patients and for the typical American suffering from diet-induced hypercholesterolemia.

Patel et al. previously mapped the sitosterolemia locus to chromosome 2p21 (J Clin Invest 1998;102:1041–1044). Based on this positional information, Berge et al. used a combination of sophisticated molecular cloning techniques to determine that sitosterolemia results from mutations in either 1 of 2 newly identified ABC (ATP-Binding Cassette) transporter genes, *ABCG5* and *ABCG8.* First, they used DNA microarrays to search for messenger RNAs (mRNAs) in mouse liver and intestine that were induced by an agonist of the nuclear receptor LXR (Liver X Receptor), a gene regulator recently reported to reduce the intestinal absorption of cholesterol (Science 2000;289:1524–1529). This resulted in an identification of an expressed sequence tag (EST) transcript that resembled known ABC transporter genes in *Drosophila* (*brown, scarlet,* and *white*). A human homolog of *white, ABCG1,* had been previously implicated as a cholesterol efflux protein in macrophages (J Biol Chem 2000;275:14700–14707). Hence, Berge et al. reasoned that this EST might represent the mouse version of the gene mutated in sitosterolemia. A full-length complementary DNA (cDNA) corresponding to the EST was cloned, and GenBank used it to identify a human homolog. The full-length human cDNA was then sequenced and named *ABCG5* according to current nomenclature.

Berge et al. detected a mutation in *ABCG5* in only 1 of 9 unrelated sitosterolemic patients, suggesting that there might be another gene located within 2p21 that is part of the sitosterolemia locus. Thus, they embarked on a complicated, but ultimately rewarding, search that revealed a second ABC transporter, also homologous to the *Drosophila white* gene, immediately adjacent to *ABCG5* on chromosome 2p21. They named this second gene *ABCG8.* Alignment of the amino acid sequences revealed 28% sequence identity between these 2 ABC transporters. The 2 genes are arrayed in a head-to-head configuration separated by only 374 nucleotides. The entire sitosterolemia locus on chromosome 2 spans ~56 kb (~28 kb per gene), each gene containing 13 exons, 6 membrane-spanning domains, and an adenosine triphosphate (ATP) signature motif at the N-terminus. Importantly, each transporter is structurally considered a "half-transporter." A multiple tissue Northern blot performed by Berge et al. revealed that *ABCG5* and *ABCG8* are expressed at highest levels in human liver and intestine.

The remaining 8 patients studied had mutations in *ABCG8.* Three were caused by nonsense mutations in both alleles. Two were heterozygotes and 1 was homozygous for a missense mutation in *ABCG8.* Two more patients were found to have a nonsense mutation in a single allele of *ABCG8.* In summary, Berge et al. identified 7 different mutations in 9 patients. None of these reported mutations were detected in 100 alleles from healthy controls, and no patient had mutations in both genes.

To test their hypothesis that together *ABCG5* and *ABCG8* provide a barrier to cholesterol accumulation, Berge et al. next studied the expression of these transporters in mice fed a high cholesterol diet. These animals showed a 2-fold increase in the level of *Abcg5* mRNA in the intestine and greater than a 3-fold increase of *Abcg8* mRNA in the liver. These data support the clinical findings that the sitosterolemia gene must have selective intestinal and hepatic expression (J Lipid Res 1992;33:945–955). Interestingly, these cholesterol-fed mice did not have significantly elevated plasma cholesterol levels. Berge et al. suggest that LXR plays a role in this cholesterol homeostasis because LXR is a nuclear receptor activated by oxysterols, hydroxylated derivatives of cholesterol. In mice, LXR agonists increase both *Abcg5* and *Abcg8* mRNA expression in liver and intestine (primarily jejunum). Taken together, Berge et al. suggest that the up-regulation of *Abcg5* and *Abcg8* mRNA expression in the scenario of cholesterol feeding may be occurring via induction of LXR, and that this is a normal adaptive response to a hypercholesterolemic diet.

Comment. Berge et al. have conducted groundbreaking research that has led not only to the understanding of the genetic basis of this rare disease, but also provides key insights that may lead to new models of the absorption and metabolism of both phytosterols and cholesterol. Given the tissue expression of this pair of ABC transporters, up-regulation by a cholesterol-enriched diet, and proposed structure, it appears likely that

both intestinal uptake and hepatic export of sterols is regulated via this pair of newly discovered ATP-binding cassette, half-transporter proteins. Berge et al. propose that these 2 genes are obligate heterodimers. Moreover, their close proximity and orientation on chromosome 2 suggest that they might use a single bidirectional promoter and common elements may regulate their coordinated expression.

These investigators suggest that, in the intestine, these 2 ABC transporters limit sterol absorption by re-excreting sterols that have entered enterocytes. They propose that these same transporters are resident in the canalicular membrane of hepatocytes and likely participate in the secretion of sterols into bile. Another group, Lee et al., has named these proteins "sterolin-1" and "sterolin-2," corresponding to the gene products of *ABCG5* and *ABCG8,* respectively (Curr Opin Lipidol 2001;12:141–149). Lee et al. propose 2 similar models of *ABCG5* and *ABCG8* function.

However attractive these physiologic proposals may be, they still require experimental confirmation. If possible, tissue specimens from patients with *ABCG5* and *ABCG8* mutations should be examined to determine if either cholesterol or phytosterol accumulate in various organs. Previous investigations have established that patients with sitosterolemia accumulate elevated levels of sitosterol and campesterol in plasma, erythrocytes, cardiac muscle, lung, liver, and aorta (J Lipid Res 1985;26:1126–1133). Interestingly, these same patients also accumulate high levels of the 5α derivatives of phytosterols (phytostanols) in these tissues in comparison to controls. Whether these stanol derivatives play a key role in the regulation of these ABC genes is yet another very intriguing question. Recently, Brown and Goldstein postulated that cholesterol accumulation in sitosterolemic livers suppresses the low-density lipoprotein receptor (LDLR) which, in turn, leads to increased serum levels of LDL (Science 2001;292:1310–1312). However, the cholesterol levels in the livers of sitosterolemia patients are normal, or even lower than healthy controls (J Lipid Res 1985;26:1126–1133). Moreover, Salen et al. previously reported that sitosterolemic livers expressed an increased number of LDLRs in comparison to controls (J Lipid Res 1992;33:945–955). Clearly, these newly identified ABC transporters now need to be reconstituted into various experimental cell systems so that the true effects of sterols and stanols on these genes can be further clarified.

Berge et al. propose that these 2 half-transporters work in concert as sterol exporter proteins, but do not provide support with functional studies. Moreover, it is not known which cells express these genes, nor their cellular location. Although it is likely that they are expressed on the apical membrane of enterocytes and hepatocytes, these important questions await the development of *ABCG5*- and *ABCG8*-specific antibodies. The currently held models of *ABCG5* and *ABCG8* function may very well be altered after immunohistochemical studies. Such was the case for another member of this gene family, *ABCA1,* which functions as a cholesterol-efflux protein and is mutated in Tangier disease (J Lipid Res 2000;41:433–441). There is some recent controversy about the initially proposed localization of the *ABCA1* protein in the plasma membrane of epithelial cells (Arterioscl Throm Vasc Biol 2001;21:378–385).

It would be of significant interest to know the noncholesterol sterol and stanol composition of the cholesterol-enriched diet fed to these mice. In normal individuals, phytosterols seem to block cholesterol absorption via intraluminal competition for uptake (Am J Clin Nutr 2000;71:908–913), at a molecular level, or both. Indeed, many studies conducted on human subjects have shown that a diet high in phytosterols is accompanied by significant reductions in total and LDL cholesterol levels in the blood (Am J Med 1999;107:588–594). Patients with sitosterolemia do have a higher concentration of LDL in comparison to controls, but these LDLs are unique in that they are enriched with phytosterols (J Lipid Res 1992;33:945–955). In contrast, high-density lipoprotein (HDL) levels in sitosterolemic patients are normal or reduced. A 1997 study showed that only HDL was a successful vehicle for sterol transport into bile (J Clin Invest 1997; 99:380–384). Therefore, the preferential incorporation of phytosterols into LDL rather than HDL may partly explain the low phytosterol content in bile from sitosterolemic individuals.

Although Berge et al. make great strides in unraveling the genetic basis of sitosterolemia, their mutational analyses still leave some questions unanswered. Specifically, with molecular investigations limited to the coding regions of the 2 transporter genes, there remains some genotype/phenotype disparity in several patients. Five of 9 patients had mutations detected in the coding region of only 1 allele of either transporter. With an autosomal recessive disorder, several possibilities should be considered. The most likely is that the allele with a normal coding region has a significant mutation in a regulatory, or intronic, region that would not have been detected by only searching exons for mutations. Another perhaps more intriguing possibility is that there is another related half-transporter that remains to be discovered. Several of these issues will be resolved when the patients' tissue samples are analyzed for transporter gene expression, when their *ABCG5* and *ABCG8* genes are explored for noncoding region mutations, when there is characterization of potential new transporter genes, and finally when transport studies are performed to characterize mutated transporter gene function.

In conclusion, the recent discovery of new ABC transporters *ABCG5* and *ABCG8* in the intestine and liver has markedly broadened our knowledge of sterol metabolism and has provided a genetic cause for the rare autosomal recessive disease sitosterolemia, and perhaps other hyperlipidemias. In addition, these studies remind us of the important role of the intestine in sterol metabolism. The typical daily Western diet consists of 250–500 mg of cholesterol and an additional 200–400 mg of noncholesterol sterols, of which phytosterols comprise the majority. Whereas the average individual absorbs ~50% of dietary cholesterol, normally less than 5% of phytosterols are retained (J Clin Invest 1970; 49:952–967). In the current climate of "nutri-ceuticals," soy, one of the primary sources of dietary phytosterols, is emerging as advertised treatments for a variety of disorders. Moreover, commercially manufactured foods (i.e., margarine and salad dressing) that substitute phytosterols for cholesterol are entering the American market, being touted as "cholesterol-lowering" dietary options. What brings together these dietary medicaments and this study on sterol metabolism is the common and clinically relevant link of potentially altering the intestinal absorption of cholesterol. It is important to note that the majority of people with hypercholesterolemia do not have genetic mutations in cholesterol metabolic pathways, but rather they seem to have an increased susceptibility to cholesterol in the diet. This is where studies that identify the means of cholesterol absorption, like Berge et al., may open the door for novel therapeutic manipulations of intestinal cholesterol absorption for the benefit of millions of people in the US alone. Perhaps new directions towards exploiting the use of certain phytosterols to lower cholesterol absorption may have real therapeutic benefit. Thus, the novel concept of a luminal "phyte" between phytosterols and cholesterol for intestinal absorption may prove beneficial for the typical cholesterol-enriched American diet, which could ultimately have profound ramifications in the battle against the number one cause of death in the United States, coronary artery disease.

BETH A. CARTER, M.D.
SAUL J. KARPEN, M.D., Ph.D.