Hypothesis: Women deposit more collagen after major abdominal surgery than men.

Design: A post hoc analysis of data obtained from 2 prospective, randomized, double-blind clinical trials.

Setting: University hospital general surgical service.

Patients: One hundred sixteen patients undergoing colon resection.

Main Outcome Measures: Protein and hydroxyproline (collagen) deposition during the first 7 postoperative days in expanded polytetrafluoroethylene implants positioned subcutaneously.

Results: On univariate analysis, men and women deposited comparable amounts of collagen (257 ± 120 vs 281 ± 117 ng/mm, respectively). When potential confounding factors were entered into a generalized mixed-effects model, only the interaction between age and sex was a significant factor ($P = .047$). Collagen deposition decreased with age in men, being 317 ± 133 ng/mm in men younger than 45 years, but only 238 ± 113 ng/mm in those older than 45 years ($P = .03$). In contrast, collagen deposition was virtually identical in women younger than 45 years (280 ± 133 ng/mm) and in those older than 45 years (281 ± 110 ng/mm). Only 3 of these women were receiving hormone replacement therapy.

Conclusions: Collagen deposition after surgery decreased significantly with age in men, while remaining unchanged in women. Younger men and women deposited similar amounts of collagen. Therefore, older men made less collagen after surgery than older women, perhaps explaining the consistent observation that wound dehiscence is twice as common in men than in women. Our results differ from previous studies conducted in healthy, nonsurgical volunteers, which showed that (1) young women made significantly more collagen than young men and (2) collagen deposition was reduced in postmenopausal women, but deposition returned to premenopausal values with hormone replacement therapy. Differences between our results and those reported previously likely stem from the populations studied. In particular, multiple perioperative factors decrease collagen deposition, which apparently obscures the differences observed previously in healthy, unstressed volunteers.

Arch Surg. 2000;135:71-74

I N ADEQUATE SCAR formation contributes to serious surgical complications, including wound dehiscence and postoperative hernias.1 A recent volunteer study concluded that young women heal significantly better than men (ie, deposit 85% more collagen in test wounds).2 That study simultaneously confirmed a previous finding in men that healing was worse in smokers.3 Ashcroft et al4 similarly demonstrated better healing in healthy premenopausal female volunteers than in postmenopausal volunteers. Improved healing was associated with a higher level of transforming growth factor β-1 in the wounds of young women. Hormone replacement therapy returned collagen deposition and growth factor concentration to premenopausal levels.

Young subjects tend to heal well, and the controlled nature of volunteer studies makes it relatively easy to identify physiological differences. Surgical patients, though, produce less collagen than healthy volunteers,5 in part because healing is impaired by factors including age, illness, malnutrition, medications, and physiological stress.6 Male sex has been shown to double the risk of wound dehiscence in numerous observational and retrospective studies.1 Male sex also increased the risk of wound infection in one study,7 but not in others.8,9 We therefore tested the hypothesis that women deposit more collagen than men after major abdominal surgery.

RESULTS

Sex, age, and weight were comparable in the patients participating in each study (Table 1).  Roughly half of the patients...
PATIENTS AND METHODS

With approval from an ethics committee and written consent, we studied 116 patients. All were aged between 18 and 80 years and were undergoing elective colorectal resection for cancer or inflammatory bowel disease. Patients having abdominal-peritoneal pull-through procedures were included, but not those scheduled for minor colon surgery such as polypectomy or isolated colostomy.

Patients were excluded when the surgeon did not anticipate primary wound closure. That is, patients were excluded when delayed primary closure techniques or healing by secondary intention was likely. Patients were also excluded when they had serious malnutrition (serum albumin <33 g/L, white blood cell count <2.5 × 10^9/L, or >20% weight loss), bowel obstruction, or reported a history of fever and/or infection. All patients were aggressively hydrated during and after surgery to minimize hypovolemia. All participants were given the opioid piritramid via a patient-controlled infusion in the postoperative period.

The study population consisted of a subset of patients, participating in 2 large outcome trials, in whom wound collagen deposition was measured. The first study evaluated the effects of maintaining intraoperative normothermia on the incidence of surgical wound infection.8 Exclusion criteria included use of steroids or other immunosuppressive drugs (including cancer chemotherapy) within 4 weeks of surgery. These patients were randomly assigned to normothermia (core temperature 36.6°C ± 0.5°C) or mild intraoperative hypothermia (core temperature 34.7°C ± 0.6°C). No postoperative thermal management was provided.

The second study evaluated the effects of 30% and 80% inspired oxygen (Grief et al, unpublished data, 1999). In each protocol were assigned to each randomized treatment (temperature or inspired oxygen concentration). The fraction of patients having colon or rectal surgery was also similar in the 2 study groups. Immunosuppressive drugs were excluded from the temperature trial and only a few patients in the oxygen study were receiving immunosuppressants.

Demographic and morphometric characteristics were comparable in the male and female patients, as were the type and duration of surgery. Univariate analysis similarly indicated that collagen and protein deposition, both measures of scar formation, were comparable in men and women (Table 2).

Potential confounding factors including age, sex, cigarette smoking, and study group were entered into a generalized mixed-effects model. Only the interaction between age and sex was a significant factor (P = .047); however, age alone was nearly statistically significant (P = .051). Collagen deposition decreased with age in men, being 317 ± 133 ng/mm in men younger than 45 years (n = 14), but only 238 ± 113 ng/mm in those older than 45 years (n = 46; P = .03). In contrast, collagen deposition was virtually identical in women younger than 45 years (n = 19; 280 ± 133 ng/mm) and in those older than 45 years (n = 37; 281 ± 110 ng/mm). Only 3 of 37 women older than 45 years (presumed to be postmenopausal) were receiving hormone replacement. On univariate analysis, collagen deposition did not differ significantly in men and women younger than 45 years (P = .42) or in those older than 45 years (P = .08). Neither smoking nor study group were significant predictive factors for collagen deposition.

Previous studies observed greater collagen deposition in healthy premenopausal women than in young men.2 Healthy postmenopausal women make less collagen than premenopausal women, a difference that disappears with hormone replacement therapy.3 In male mice, collagen deposition and the rate of healing decrease significantly with age.16 Our results in patients undergoing colon surgery contrast with previous results in that younger men and women deposited approximately equal amounts of collagen, while older women who were not receiving hormone replacement therapy deposited significantly more collagen than older men. This difference resulted from a decrease in collagen deposition with aging in men, whereas deposition remained largely unchanged in women. On univariate analysis, there was no difference between collagen deposition in men and women younger than 45 years (P = .42) or those older than 45 years

in this case, patients taking steroids or other immunosuppressive drugs (including cancer chemotherapy) were enrolled. Patients with asthma were included, but those with severe pulmonary disease were not. Severe pulmonary disease was defined by dyspnea at rest or a requirement for constant oxygen administration. The designated oxygen concentration was, in each case, maintained throughout surgery and for the first 2 postoperative hours. Core temperature was maintained near 36°C.

To evaluate collagen deposition, two 7-cm-long, 1-mm outer diameter, expanded polytetrafluoroethylene implants (Impra; International Polymer Engineering Inc, Tempe, Ariz) were inserted subcutaneously.10 The tubes were inserted several centimeters lateral to the incision on completion of surgery. On the seventh postoperative day, the tubes were removed for analysis.

Removed implants were assayed for protein11 and for hydroxyproline (a measure of collagen deposition) using high-performance liquid chromatography.32 Collagen ingrowth into such tubes is proportional to wound tensile strength in animal models15,16 and to subcutaneous oxygen tension in surgical patients.17 Since the hydroxyproline content of collagen is constant, the collagen content of the expanded polytetrafluoroethylene implants was calculated by multiplying the hydroxyproline content by 6.94.

Collagen deposition in men vs women was initially compared with unpaired, 2-tailed t tests. We also separately considered patients older than 45 years (because menopause is unlikely to occur earlier). Subsequently, the data were entered into a generalized nonlinear, mixed-effects model, taking into account age, sex, cigarette smoking, and study group. Factors having a univariate P <.25 were entered into the model, and retained in a stepwise elimination when P <.10. Results are presented as mean ± SD; P <.05 was considered statistically significant.

©2000 American Medical Association. All rights reserved.
(P = .08), although there was a trend toward increased collagen deposition in women older than 45 years. This difference was statistically significant using multivariate analysis, where potential confounding factors were included.

Factors known to impair collagen deposition include malnutrition,\textsuperscript{17} preoperative illness,\textsuperscript{18} hypovolemic,\textsuperscript{19} hypothermia,\textsuperscript{8} tissue hypoxia,\textsuperscript{9} and corticosteroids and other immunosuppressants.\textsuperscript{21,22} These factors are common in surgical patients; it is therefore hardly surprising that surgery reduces collagen deposition.\textsuperscript{5}

To the extent possible, we minimized potential confounding factors. All patients were aggressively hydrated. Severely malnourished patients were excluded from the study, although mild malnutrition is common in surgical patients. This is an important issue since collagen deposition is profoundly influenced by recent nutritional history and even a few days of preoperative calorie restriction may significantly reduce scar formation.\textsuperscript{18,23,24} Nonetheless, it seems most likely that our data contrast with previous reports because the study populations differed. Taken together, previous studies and our current report suggest that perioperative factors, including operative stress and underlying illness, overwhelm sex differences observed in healthy nonsurgical volunteers.

Interestingly, smoking status was not a significant predictor of collagen deposition. This result contrasts with previous studies in normal volunteers\textsuperscript{3,20} and older surgical studies.\textsuperscript{25-27} In contrast to normal volunteers who continued to smoke during the study, our patients were not permitted to smoke in their rooms (and most had limited mobility at least for the first few postoperative days). This is a critical factor because a major effect of smoking is an acute reduction in tissue oxygen partial pressure lasting roughly 1 hour.\textsuperscript{28} The older surgical studies may similarly differ in that patients in those days were frequently allowed to smoke in their rooms.

Expanded polytetrafluoroethylene implants as a measure of collagen deposition and scar formation have been validated in rats,\textsuperscript{31} where they predict ultimate wound tensile strength. They have been widely and successfully used in human volunteers\textsuperscript{3,20,28,32} and patients.\textsuperscript{3,19,23,24} The implants were placed at the time of surgery and removed after 7 days to reflect the early postoperative environment. Although high rates of collagen deposition are not found until 5 days after injury,\textsuperscript{31,32} collagen is measurable within wound implants by 24 to 48 hours after injury.\textsuperscript{33,34} Peak collagen deposition rates are found 7 to 14 days after injury.\textsuperscript{31} These early rates of collagen deposition reflect the early course of the wound as well as ultimate wound tensile strength, and thus are a useful surrogate measure for healing.

In the current study, we used the same type of implant as Jorgensen et al.\textsuperscript{7} (Ashcroft et al\textsuperscript{4} used wound biopsies.) However, the site of subcutaneous implantation differed. Jorgensen et al inserted the implants in the lateral upper arm,\textsuperscript{2} whereas we inserted them in the abdomen as in previous studies.\textsuperscript{35} This is unlikely to have affected the results; but to the extent that site is a confounding factor, implants only a few centimeters from the surgical incision may better reflect wound conditions than implants at a remote site.

An insufficient number of patients were enrolled in our study to correlate collagen deposition with wound dehiscence or hernia. However, it is well established that wound dehiscence is about twice as common in men than women. Similarly, men have a greater incidence of anastomotic leak.\textsuperscript{36} This observation is consistent with the relatively low level of postoperative collagen deposition (a predictor of low wound tensile strength) we observed in the older men.

### CONCLUSIONS

Collagen deposition after surgery decreased significantly with age in men, while remaining unchanged in women (who for the most part did not use hormone replacement therapy). Younger men and women deposited similar amounts of collagen. Older men therefore deposited less postoperative collagen than older women. These data are consistent with the observation that men have about twice the risk of wound dehiscence as women. Our results differ from previous studies conducted in healthy, nonsurgical volunteers, which showed that (1) young women made significantly more collagen than young men and (2) collagen deposition was reduced in postmenopausal women, but that deposition returned to premenopausal values with hormone replacement. Differences between our results and those reported previously likely stem from the populations studied. In particular, multiple perioperative factors decrease collagen deposition and apparently obscure sex differences observed in healthy, unstressed volunteers.

*This study was supported by grants GM49670, GM58273,
and GM27345 from the National Institutes of Health, Bethesda, Md; by the Joseph Drown Foundation, Los Angeles, Calif; the Fonds zur Förderung der wissenschaftlichen Forschung, Vienna, Austria; the Burgermeister Fond der Stat Wien, Vienna; and the Austrian National Bank Foundation, Vienna.

We appreciate the assistance of Alexander Schoellkopf, MS, and the statistical advice of Jessica Watson of the Biostatistical Consulting Unit at University of California, San Francisco.

Corresponding author: Daniel I. Sessler, MD, Department of Anesthesia and Perioperative Care, University of California, San Francisco, 374 Parnassus Ave, Third Floor, San Francisco, CA 94143-0648 (e-mail: sessler@anesthesia.ucsf.edu).

REFERENCES


©2000 American Medical Association. All rights reserved.