CARDIOVASCULAR RESEARCH

Estrogen: Key Player in Heart Disease Among Women

Female vulnerability in affairs of the heart has long been the domain of poets. But now cardiologists are getting involved. And while the physicians' story may be less romantic than the poets', it could be more practical. They are trying to unravel the complex workings of a disease that is not only killing 480,000 U.S. women each year but is striking them down in a pattern that suggests its modus operandi differs in key respects between men and women.

Statistics show that slightly more than one of every two women in the United States will die from cardiovascular illnesses. Since 1984, the number of female deaths due to heart disease has inched above the number of male deaths, according to the American Heart Association (AHA). And although the overall death rate does not differ greatly between genders, experts say the incidence pattern does—the disease typically develops 10 to 15 years later in women, with a woman's risk rising exponentially after menopause. Researchers believe that this distinctive pattern suggests that estrogen, which is produced in women's bodies at relatively high levels before menopause, offers some protection against heart disease.

Many women aren't familiar with these data, however. Most postmenopausal women, for example, don't know that heart disease is their number one killer, experts say. By and large, women are much more concerned about breast cancer, even though only 4% will actually develop that disease during their lifetimes, according to the National Center for Health Statistics.

Some advocates for women's health lay responsibility for such ignorance at the door the medical profession. "Heart disease in women was either trivialized or ignored for years," says cardiologist Bernadine Healy, senior policy adviser for the Page Center of the Cleveland Clinic Foundation and former director of the National Institutes of Health (NIH). "When I began studying cardiovascular disease, it was all about women taking care of their husbands' hearts."

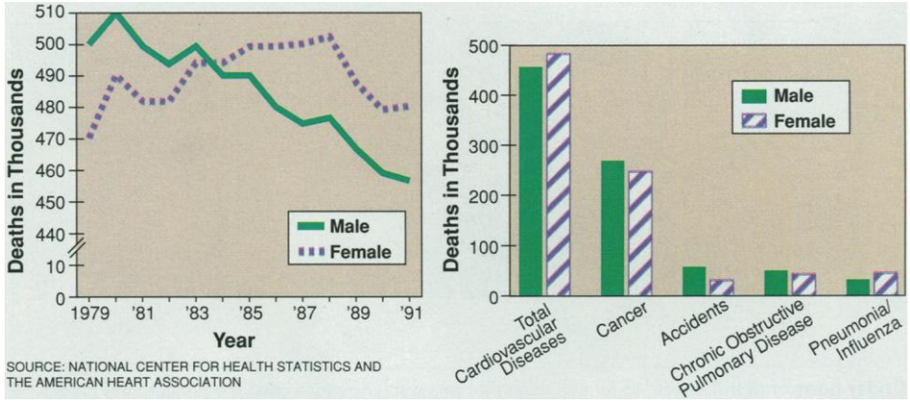
Healy points out that, amazingly enough, the first estrogen supplementation trials were limited to male participants—researchers were studying whether boosting estrogen levels in male heart attack patients might protect against a second attack. During her tenure as director of NIH, Healy argued that the paucity of women in previous studies has lead to a substantial knowledge gap when it comes to cardiovascular disease. "The only way we are ever going to address that gap," Healy says, "is with a consistent, sustained, and relentless commitment."

Now that commitment is taking shape—thanks in part to Healy's efforts. A large research effort has been launched to study gender-based differences in cardiovascular disease. The prime focus is on finding out what mechanisms protect women from heart disease during their childbearing years and leave them vulnerable after menopause. Healy's main contribution to the effort took shape in 1993, with the 10-year, $628 million Women's Health Initiative (WHI). To date, this huge trial has recruited 10,000 of the expected 163,000 postmenopausal women between the ages of 50 and 79. The goal is to analyze the effects of hormone replacement, diet, and other lifestyle factors on the prevention of illnesses such as heart disease and stroke.

This new focus on cardiovascular disease in women has been widely welcomed, but the WHI itself has stirred controversy. The study has met with opposition from researchers who argue that its funds would be better spent on smaller projects with less risk and more flexibility that have already begun (*Science*, 5 November 1993, p. 838). Some critics also contend that even if WHI data support what other trials have already shown—that estrogen can cut the risk of heart disease by as much as 50%—the WHI, because of its study design, will not be able to pinpoint the mechanism behind the reduction.

Long before the WHI produces its first results, however, many investigators are trying to piece together a biologically plausible mechanism for estrogen's apparent cardioprotective effect. Based on clinical observations, researchers have already proposed eight or more potential pathways by which the hormone might protect against heart disease. At least four of these potential mechanisms now have basic research evidence to support clinical findings. And of these, perhaps the most widely studied is the effect of estrogen on levels of blood lipoproteins, fatty molecules known to be correlated with the risk of heart disease.

A 3-year, multicenter trial begun in 1987 by the National Heart, Lung, and Blood Institute (NHLBI), called the Postmenopausal Estrogen/Progestin Intervention Trial (PEPI), showed that orally administered estrogen, alone or in combination with proges-



Different strokes. U.S. cardiovascular disease rates have been falling sharply for men; less so for women *(left)*. Heart disease is the greatest killer of women, as shown by data for 1991 *(right)*.

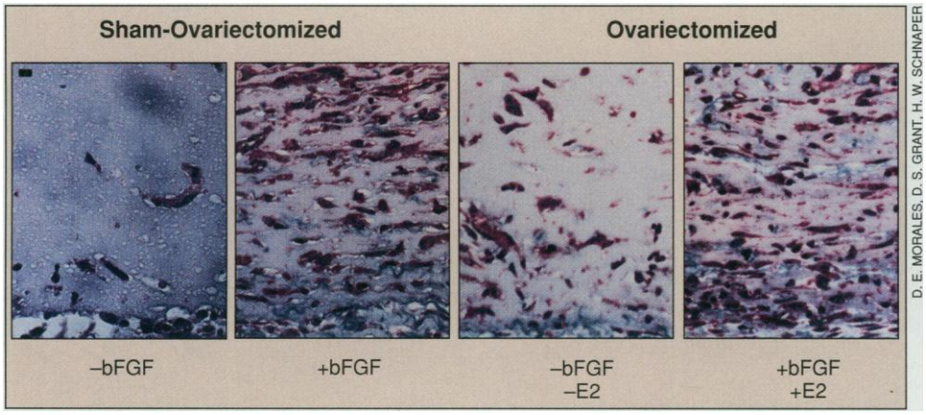
terone, increased levels of high-density lipoprotein (HDL) cholesterol in a group of 875 postmenopausal women. HDL (sometimes dubbed "the good cholesterol") is associated with decreased risk of heart attacks. At the same time, estrogen lowered the participants' levels of low-density lipoprotein (LDL, sometimes called the "bad cholesterol").

"What some people don't realize is how important a risk factor HDL cholesterol is in women," says epidemiologist Trudy Bush, one of the co-directors of PEPI. "For women who have low HDL levels, there is a sevenfold increase in risk on the basis of this one lipoprotein.". A correlation between low LDL levels and increased heart-disease risk also holds in males. But PEPI has pinpointed an important difference between men and women in their response to LDL: While high levels of LDL correlate with high risk of heart disease in males, Bush says, that marker does not appear to be as important in determining a woman's risk.

"PEPI is helping us sort out what the issues are," says Irma Mebane-Sims, an epidemiologist at the NHLBI and a PEPI co-director. "The study will be important in directing further research." PEPI, whose first results were published in January in the *Journal of the American Medical Association*, is the first and

largest trial of its kind completed to date. In contrast to observational studies, PEPI was a double-blinded, randomized clinical trial. In the earlier studies, women chose which form of treatment they would get before entering the trial. "Healthier women are more likely to take estrogens," says Bush. "And therefore you have a protective effect not because the women are using estrogens but because they are healthier."

"PEPI goes a long way toward giving us real data when making statements about preventive measures," says Mebane-Sims. But she adds that there are a "a host of questions yet to be answered." Some of these questions are about the biochemical details of



Under hormonal influence. In female mice that retain their ovaries *(two left panels)*, the growth factor bFGF is enough to cause blood cells to aggregate on a small piece of gel. In mice without ovaries *(two right panels)*, estrogen is required to get that effect.

estrogen's apparent alterations of blood lipid chemistry. Other studies have shown that estrogen directly inhibits LDL accumulation and increases the lipoprotein's metabolism in arterial walls—but those results are far from conclusive.

Even if researchers can decipher how estrogen affects lipid levels, that would explain only part of the hormone's cardiovascular effect: The PEPI study itself indicated that alteration in lipid chemistry accounts for only about 25% to 50% of the observed decrease in heart disease risk. "Therefore, many investigators are trying to understand what else estrogen does," says Geoffrey Tofler, director of the Institute for the Prevention of Cardiovascular Disease at Deaconess Hospital and Harvard Medical School in Boston. Some clues to the additional effects come from another PEPI finding: Estrogen lowered levels of a blood-clotting factor called fibrinogen. Lower fibrinogen levels might in turn reduce blood clotting, which, by causing blockages in blood vessels, carries the risk of heart attacks.

Work by other investigators, including Tofler and his colleagues at Harvard Medical School, provides support for an anti-clotting mechanism for estrogen. Tofler compared 293 premenopausal women and 82 postmenopausal women taking estrogen with 374 postmenopausal women not taking hormones and 682 men. Participants with "high estrogen status" had significantly lower blood levels of plasminogen activator inhibitor, an enzyme that blocks the action of the natural clot dissolver, tissue plasminogen activator. But neither this study nor related ones by other workers has elucidated the mechanism for this action. "We don't know the mechanism by which estrogen affects clotting factors," Tofler concedes. "But it is an active area of interest."

Another active area of interest, being pursued by Mark Nelson, a pharmacologist and physiologist at the University of Vermont College of Medicine, is the hypothesis that estrogen causes blood vessels to relax by interacting with potassium channels on the membranes of blood vessel cells. "Our laboratory had been studying ion channels that control blood-vessel function," Nelson recalls. "I sort of backed into estrogen because I was interested in coronary arteries and NIH had an initiative for investigators to look at estrogen's role in heart disease."

Nelson found that when arteries taken from male and female rats were subjected to elevated pressure, the vessels from females constricted less than those from males under the same pressure conditions. What's more, the effect appears to be governed by the female reproductive system: The blood vessels of female rats whose ovaries were removed constricted just as much as male arteries did. When timed-release estrogen pellets were implanted into the ovariectomized females, those rats' arteries responded in the normal female pattern. "Physiological estrogen dilates arteries in females in response to pressure," Nelson sums up. "That means that women are probably better prepared to deal with" blood flow cutoff in certain vessels.

To help determine how estrogen relaxes arterial walls, Nelson began looking into a connection to nitric oxide, which is a potent dilator of blood vessels. Other labs had already shown that nitric oxide activates smooth muscle cells lying underneath the endothelial layer that lines artery walls. Nelson combined the existing nitric oxide data with his own earlier discoveries (*Science*, 14 July 1989, p. 177; and 24 April 1992, p. 532) and proposed a mechanism: Endothelial cells, triggered by estrogen, release nitric oxide, which travels to underlying smooth muscle cells and sets in motion a biochemical cascade that ends in the opening of potassium channels and vessel relaxation. "The overall mechanism makes sense, and it may contribute to the effect of estrogen on the heart," said Nelson, whose co-workers presented results in February at the AHA meeting in Salt Lake City.

Even if Nelson's hypothesis is correct, his cascade is probably not the only way that estrogen interacts with blood vessels. A different role for estrogen has been proposed by William Schnaper, associate professor of pediatrics at Northwestern University. Schnaper recently published a report in *Circulation* demonstrating that estrogen enhances angiogenesis—the formation and growth of new blood vessels.

Researchers believe angiogenesis occurs through a pathway involving digestion of the outer arterial wall by enzymes, migration of cells from inside the artery into the resulting space, and formation of new blood vessels as cells line up and join side by side in the form of a tiny tube. That process could be of value for heart disease prevention in two ways, Schnaper says. First, more blood vessels decrease vascular resistance, lowering blood pressure. Second, the movement of endothelial cells that characterizes angiogenesis might help repair the inner lining of injured arteries. "The same events that form new vessels may help and protect already existing vessels," Schnaper contends.

Schnaper has tested the effect of estrogen on various steps of the angiogenesis pathway, using cultured human endothelial cells derived from umbilical veins. He found that the cell proliferation, adhesion, and formation of capillary-like tubular networks is greater in cell culture when cells are treated with estrogen than it is when the cells are untreated or given testosterone or progesterone.

Schnaper has also explored these effects in intact animals. He chose two groups of mice—one with ovaries removed, one without—and implanted in both groups tiny segments of gel designed to simulate the arterial basement membranes through which angiogenesis occurs. Later the gel was removed for staining and microscopic observation. The result: The mice with ovaries grew new vessels at a higher rate.

The next phase in the search for gender-based differences in cardiovascular disease could center on the genes. Although genes

that specifically affect gender differences in heart-disease risk have not yet been found, several groups are looking for them as a part of a broader investigation of genetic influences on cardiovascular disease. If defects in such gender-specific genes are found, functional copies of the genes might ultimately be administered via one of a number of forms of gene therapy for heart disease that are currently under development.

Former NIH Director Healy says she is encouraged by the blossoming of all these studies into gender-specific mechanisms of heart disease—a development she believes was stimulated by the WHI project she initiated at NIH. "Let's face it, the way to get scientists to move into a certain area is to fund that area," Healy says. "And the WHI brought money to the table and attention to the issue."

But Healy isn't by any means satisfied. She calls the studies now in progress "beginnings." "But in the case of heart disease, she says, "we still have a long way to go."

–Trisha Gura

Trisha Gura is a reporter at the Chicago Tribune.

IMMUNOLOGY

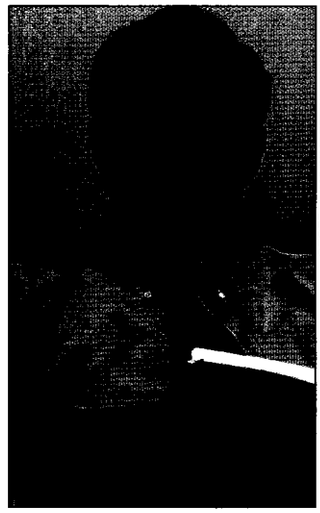
Zeroing In on How Hormones Affect the Immune System

Inject an immunized male cockroach with a honeybee's venom, and chances are that cockroach will bite the dust. Do the same thing to a female cockroach, on the other hand, and she will almost certainly recover. That stark difference in outcomes, immunologists say, illustrates a basic disparity between the male and female immune systems, a difference that extends all the way from cockroaches to humans: Females are immunologically stronger than males. "In the eyes of God or biology or what have you, it is just very important to have women," quips Norman Talal, an immunologist at the University of Texas Health Science Center in San Antonio. "And so they are hyperprotected.

Adds Noel Rose, an immunologist at Johns Hopkins University: "It's a well-documented fact: Women are simply more immunologically talented than men." Yet such talent is a sword with two sharp edges. While a woman may be less susceptible to infections, she is far more likely to contract an autoimmune disease, such as systemic lupus erythematosus or multiple sclerosis—diseases in which the immune system turns against its own. Indeed, nine out of 10 lupus sufferers are women; overall, researchers estimate that 75% or more of autoimmune disease patients are women.

In seeking explanations and cures for these disorders, scientists have begun unraveling the mysteries of the female immune system itself. "By showing us what goes wrong, they give us a marvelous window on the functioning of a normal immune system," explains Joan Merrill, a protein chemist in immunologist Robert G. Lahita's lab at St. Luke's Roosevelt Hospital in New York, where several autoimmune disease studies are under way. Through that window, researchers like Merrill have come to see "that a woman's sex hormones and gonads play a central role in regulating her immune system and vice versa," she says. These studies have demonstrated that a woman's reproductive system and immune responses are so tightly interwoven that one researcher terms it "a feedback system"—but one that varies according to a woman's age, what point she is at in her monthly cycle, and whether or not she is pregnant.

Although the idea that hormones affect the immune response has been around since the late 19th century, only now are researchers beginning to closely scrutinize the cyclic nature of the female immune system. "It is an area that has been tremendously underinvestigated," says Charles R. Wira, a reproductive immunologist at Dartmouth's Medical School. Investigations into this area, which are now picking up speed, are vital to understanding women's health, because they have implications for everything from drug testing to the timing of vaccinations to treating certain kinds of infertility problems to administering chemotherapy to finding treatments—and cures—for many autoimmune diseases.



GEOFF HANSEN/DARTMOUTH COLLEGE

Lack of study. Charles Wira says the female immune system has been "tremendously underinvestigated."

The basic observation that women's immune responses are stronger than those of men has been confirmed by a host of studies. It's true in both main branches of the immune system: the cell-mediated (which controls the killer T cells) and the humoral (which controls the antibodies produced by B cells). For example: Female mice will reject a foreign skin graft faster than males will; male mice and guinea pigs that have had gonadectomies show increased resistance to infections; and in both human and animal models, females have higher circulating levels of the major immunoglobulins (IgG, IgM, and IgA). As early as age six, the levels of IgM in human females exceed those of males of comparable age.

At the heart of the female cycle—and the yin-yang of male and female immune responsiveness—lies that key biological difference: reproduction. "Women have to make the offspring to carry on the species," says Charles J. Grossman, an immunoendocrinologist at Cincinnati's Veterans Administration Medical Center. "So it's not surprising that they have a better [immune] system." To become pregnant in the first place, he adds, a woman must be healthy, which may explain why her immune responses are stronger than a man's. And once pregnant, she must "overcome the stresses of a long gestation; then after the child is born, she has to face the stresses of nursing and protecting that child. A man doesn't have to do any of these things."

Indeed, while a woman's estrogens keep her immune response "revved up," as Grossman puts it, a man's androgens tend to suppress his immune system. The male's system is also subject to little variation after puberty, while the woman's spikes up at puberty, is depressed during pregnancy, returns to its previous high level after pregnancy, and is lowered again at the onset of menopause.

Yet, paradoxically, the mother's revved-up immune system places the fetus at risk, because it is in a sense a foreign body and therefore liable to be attacked. And that, Grossman and others say, is where the cycling sex hormones come into play. "Studies have shown that prior to ovulation, the sex hormones up-regulate the immune system," says Grossman. Estrogen, he suggests, acts to increase secretions of both prolactin and growth hormones, which, in turn, increase the production of T and B immune cells—thus giving the woman an added boost in fighting off viruses or bacteria.

But in the second half of a woman's cycle, after ovulation, the level of estrogen drops, while progesterone is increased, regulating her immune response. "The immune system