Sex matters

In sickness and in health, men and women are clearly different

In July this year, the US National Institutes of Health (NIH) released preliminary results of a clinical trial investigating the effects of hormone replacement therapy (HRT) in post-menopausal women. The publication proved to be a bombshell, particularly as the NIH terminated the study and urged all participating women to stop taking the drugs. Just over 5 years into the 8.5-year study, the data collected by the NIH's Women's Health Initiative (WHI) showed that post-menopausal women taking combination HRT—estrogen and progesterone—had an increased risk of breast cancer, stroke and heart disease compared with those taking a placebo. Since numerous conditions, including heart disease, stroke and osteoporosis, manifest themselves post-menopause, it had always been assumed that estrogen is responsible for the maintenance of women's health; the NIH study overturned this long-held belief. 'Without any hard data it seemed that women taking HRT had fewer health problems,' said Marietta Anthony, Director of Women's Health Research in the Department of Pharmacology at Georgetown University Medical Center in Washington, DC. But even more broadly, the HRT study highlights the fact that, until recently, research into women's health—not just the effects of hormones in post-menopausal women—has been largely overlooked.

Women's health differences is a relatively new concept, which posits that there are biological differences between men and women that extend beyond their reproductive systems. Created in 1991, the WHI was the first and largest study of the chronic diseases that often affect women—cardiovascular disease, stroke, cancer, osteoporosis and depression—in the context of their overall health. Indeed, until the mid-1990s, most diseases were studied only, or primarily, in men, and the male model of a condition was the gold standard, in spite of centuries of circumstantial evidence that some diseases manifested themselves differently in each sex. It is not only the major threats to health that need to be considered, but also more subtle gender-specific effects on the outcome of any illness. Conclusions drawn from studies conducted primarily in men may not necessarily be applied to women, according to Florence Haseltine,

Senior Editor of the *Journal of Women's Health* and a founder of the Society of the Advancement of Women's Health Research. 'Science was never gender-neutral,' she commented. Women's health research has traditionally been equated with reproductive health; diseases that primarily affect women have not been taken seriously as an acceptable area of research, and since differences in disease manifestation are not generally recognised, appropriate treatment is too often delayed, said Haseltine.

Many studies conducted mostly within the past 5 years to specifically address gender-specific health issues have demonstrated that there are significant biological differences between the sexes. For example, heart disease strikes women later and harder than men; women who undergo either coronary bypass graft surgery or angioplasty fare worse than men of the same age and health status. Moreover, women have very different symptoms of heart attack, which makes diagnosis more difficult, especially given the still-widespread misconception that women have less heart disease than men. The male symptoms of a heart attack—crushing chest pain, jaw and shoulder pain—are still considered classical, although it has been shown that women's pain during a heart attack is very different, said Anthony.

Compared with men, women account for nine times more cases of lupus, 50 times more cases of thyroiditis, 15 times more cases of scleroderma during childbearing years, seven times more cases of Grave's disease (hyperthyroidism), three times more cases of migraine, and two times more cases of multiple sclerosis. They constitute 75% of autoimmune patients, 71% of rheumatoid arthritis cases and 90% of patients with Sjogren's syndrome. Conversely, men are twice as likely to suffer from Parkinson's disease and tend to be affected earlier in life. Men and women manifest very different patterns of addiction and feel pain differently. Women with high blood pressure are more likely to have a stroke than a heart attack, whereas heart attack is a more frequent outcome for men. The reason may be that women with high blood pressure experience a thickening around the left ventricle, whereas this chamber becomes enlarged in men. However, women tend to recover better from strokes, most likely because of greater brain plasticity

But more than merely cataloguing the differences between the sexes, the underlying causes need to be addressed. This was highlighted in the US Institute of Medicine's report in April 2001 entitled 'Exploring the Biological Contributions to Human Health; Does Sex Matter?' The study answered the question with a

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resounding 'yes'. It noted that, while research has focused on differences and similarities between the sexes at the societal and whole organism level, much less attention has been paid to the direct study of cellular and molecular differences. 'The next step is to move from the descriptive to the experimental and establish the conditions that must be in place to facilitate and encourage the scientific study of the mechanisms and origins of sex differences,' the authors of the study conclude.

Not surprisingly, studies investigating the molecular causes behind gender-specific outcomes have identified a prominent role for hormones, although the underlying mechanisms remain largely unclear. For instance, the effect of hormone fluctuations in migraine in women has been thoroughly studied. Also, progesterone is known to make gastric reflux more severe by relaxing the sphincter that holds stomach acids in place, which may occur more frequently during pregnancy when levels of this hormone are higher.

Also significant are the hormonal effects on the immune system. In an experimental mouse model of infectious myocarditis, Sally Huber and Barbara Pfaeffle of the University of Vermont in Burlington, VT, showed that sex hormones influence the type of immune reaction against coxsackie virus and the consequent severity of the disease. Male mice developed a predominately Th1-cell response to the virus with elevated immunoglobulin G2a, γ-interferon and IL-2, whereas female mice exposed to the same virus developed a Th2-cell response, with immunoglobulin G1 and elevated $CD4^+$ T-cells. Treating the females with testosterone and the males with estradiol before infection changed the Th-cell subset differentiation, suggesting that sex-associated hormones have an affect on $CD4^+$ lymphocytes.

Betty Diamond, Professor of Medicine, Microbiology and Immunology at the Albert Einstein College of Medicine in the Bronx, NY, has demonstrated another hormonal effect. She showed that excess estrogen in mice upregulates autoantibodies and causes non-autoimmune animals to develop a lupus-like disease by interfering with tolerance induction of naïve autoreactive B-cells. This in turn is associated with the increased expression of Bcl-2, an anti-apoptotic protein, which has been shown to be upregulated in a breast cancer cell line. Diamond noted that B-cells express estrogen receptors, so estrogen may exert a direct effect on these

cells, or the upregulation of Bcl-2 could be mediated through the interaction of the B-cell with another estrogen-responsive cell type. Overexpression of Bcl-2 is likely to contribute significantly to the autoimmune phenotype, she said.

Estrogen may also regulate other hormones affecting the immune response. George Chrousos, head of the Pediatric and Reproductive Endocrinology section at the NIH's National Institute of Child Health and Human Development, noted that estrogen stimulates the secretion of corticotropin-releasing hormone, which then governs the secretion of cortisol. He hypothesises that the higher ratio of autoimmune diseases in women is connected, at least in part, to these two stress hormones. In pregnancy, when cortisol levels are high, certain women are thus prone to developing lupus, whereas after pregnancy and in menopause, they may be more prone to multiple sclerosis and rheumatoid arthritis.

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In the realm of neuroscience, the influence of hormones is being examined in a range of diseases. Jill Goldstein of Harvard Medical School in Boston, MA, is interested in whether the clinical differences observed between schizophrenic men and women are related to known sex-based differences in brain structure and to the different brain abnormalities seen in men and women with the disease. Schizophrenia is thought to result from a complex mix of genetic, environmental and prenatal influences. Goldstein and colleagues hypothesised that the risk of developing the disease occurs about 6 months before birth—at the time when sex hormones exert their effect on the brain. Using magnetic resonance imaging, they compared the brains of 40 schizophrenic individuals with those of people without the disease and discovered that the characteristic brain abnormalities—mostly in the cortex—varied according to sex. Interestingly, the cortex is also different among healthy men and women. Goldstein believes that the same factors that influence normal sex differences may also cause schizophrenia. She noted that the differences in brain abnormalities may produce the dissimilar symptoms known to exist among male and female schizophrenic patients. Other studies have shown that female schizophrenic patients may have fewer cognitive problems, such as language processing, than males. Correspondingly, the cortex is involved in cognition, especially verbal processing, Goldstein noted.

Women and men also react differently to the same drugs. 'Of 10 drugs pulled off the shelves by the FDA [US Food and Drug Administration] in the past few years, 8 of those caused more severe adverse effects in women,' said Anthony. Many anti-arrhythmia drugs, tested primarily in men, actually cause life-threatening arrhythmias in women, whereas they decrease the chance of a male heart attack. Raymond Woosley, Vice President of the Arizona Health Sciences Center in Tucson, AZ, maintains a list of such drugs that can cause life-threatening arrhythmias or prolonged QT interval, the time the heart takes to rest between heartbeats, in women (www.torsades.org/druglist.cfm). The FDA also posts a list of about 40 drugs that may lengthen this interval in women (www.fda.gov/medwatch/safety.htm). Woosley's research has also shown that, in animal and tissue models, male hormones reduce the sensitivity of the cardiac potassium channel block, rendering the drugs more potent in women. 'We have also found that women have a

greater response to drugs that prolong the QT interval during the menstrual and ovulatory phases compared to the luteal phase,' he said. 'We also believe that there are likely to be other factors contributing to the difference but they have not yet been elucidated,' he added.

One such factor could be linked to the fact that male and female rats possess different forms of the liver enzymes responsible for metabolising drugs. Bernard Shapiro, Professor of Biochemistry at the University of Pennsylvania School of Veterinary Medicine in Philadelphia, PA, is now examining this trait in human liver cells. Humans have many isoforms of these enzymes, known as cytochrome P450s; he believes that men and women have different isoforms or different levels of the same isoform, which therefore metabolise drugs differently. Growth hormone determines which P450 is expressed; Shapiro and colleagues found that male and female rats express different levels of growth hormone in a 24-h cycle, and he believes this will also be true for humans.

There are signs that gender-specific health differences are being taken into consideration

The evidence for gender-specific health differences is increasing, not only due to the NIH's HRT study, and there are signs that these are being taken into consideration. Some drug companies have begun stratifying clinical trial subjects according to sex for pharmacogenomic purposes. GeneLogic (Gaithersburg, MD) recently launched its GeneExpress Women's Health DataSuite, with Marietta Anthony as its Scientific Director. The project gathers gene expression information from female human tissues representing key conditions, disorders and disease states, including osteoporosis, osteoarthritis, cancer, autoimmune diseases and heart disease, as well as in states of menopause, fertility and reproductive disorders. These profiles are combined with extensive patient clinical and pathology descriptions, medical history, medication history and other data. If it is understood how sex affects both disease states and drug action, it is hoped that drugs specifically tailored to men and women will be developed in the future, expects Anthony. This is clearly one area where women should wish for equality.

Vicki Brower

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