

Modeling Women's Health with Nonhuman Primates and Other Animals

Jay R. Kaplan

Interest in the health problems of women as distinct from those of men is not new (Pinn 2003). The Popular Health Movement of the 1830s and -40s (which targeted health advice to women as the caretakers of their families), the creation of women's hospitals after the Civil War (which provided a venue for female physicians and nurses to train and practice), and the birth control and maternal and child health movements of the early 1900s all represent major efforts to increase awareness of health concerns specific to women (Weisman 1998). Even so, research investigating diseases and conditions unique to women and evaluating sex differences in the manifestation of common diseases was slow until the very latter part of the 20th century. As late as 1980, for example, the Food and Drug Administration routinely discouraged the inclusion of women in drug trials (Kinney et al. 1981). This exclusionary atmosphere extended also to large clinical trials funded by the National Institutes of Health (NIH¹), including the Physicians Health Study (Hennekens and Eberlein 1985) and the Multiple Risk Factor Intervention Trial ("MR. FIT" 1982), and is documented in numerous publications (e.g., NIH 1999; Wize- mann and Pardue 2000).

Change, at least with respect to government policy, began in the mid-1980s with publication of a US Public Health Service Task Force report concluding that women's health care had been compromised by the failure to conduct research specifically on women's health issues (USPHS 1985). As a result, the NIH issued a policy encouraging the inclusion of women in research and the assessment of sex differences in research outcomes. However, real transformation of research practices did not begin until an investigation instigated by the Congressional Caucus for Women's Health revealed poor compliance with the new NIH policy

(USGAO 1990). In response to this report and the resulting public and Congressional furor, the NIH established the Office of Research on Women's Health and initiated a further change in policy that culminated in a new law prescribing the inclusion of women and minorities as subjects in clinical research (PL 103-43 1993). Other NIH actions at this time included the establishment of the Women's Health Initiative (WHI¹) in 1991. This 15-yr effort encompasses a controlled clinical trial evaluating effects of hormone replacement therapy, a large observational study assessing predictors of disease, and community-based approaches to developing healthful behavior. The WHI is perhaps the most extensive manifestation of a mandate to address common causes of death, disability, and impaired quality of life in postmenopausal women.

Recently, the Institute of Medicine released a report (*Exploring the Biological Contributions to Human Health: Does Sex Matter?*) that provides an historical perspective on women's health issues, reviews the current state of science in this area, and makes recommendations for future research (Wizemann and Pardue 2001). Notably, the summary statement calls for increased development and utilization of animal models in the study of sex differences in disease. This recommendation emphasizes nonhuman primates, recognizing that these species probably have the greatest potential for advancing such investigations. In light of this document, it is perhaps appropriate that this issue of *ILAR Journal* is devoted to the use of animal models in the study of women's health, and, furthermore, that the major focus is on nonhuman primates. It will be seen from the included articles that these animals, especially monkeys of the genus *Macaca*, have played a pivotal role in extending understanding of both normal reproductive function and pathological processes affecting women.

Researchers first made systematic use of macaque monkeys to elucidate the reproductive biology of women. Much of this research was organized around study of the menstrual cycle, a phenomenon shared uniquely by women and the Old World anthropoid primates. The first article in the issue (Kaplan and Manuck 2004) provides a brief summary of this work and then describes a phenomenon (functional hypothalamic anovulation syndrome) in which environmental factors (e.g., psychological "stress," caloric restriction, and exercise) cause women to transition from normal men-

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¹Abbreviations used in this article: CEE, conjugated equine estrogens; CHD, coronary heart disease; GnRH, gonadotropin-releasing hormone; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; MR. FIT, Multiple Risk Factor Intervention Trial; NIH, National Institutes of Health; PCOS, polycystic ovarian syndrome; WHI, Women's Health Initiative.

strual cyclicity to a state of relative ovarian dysfunction and estrogen deficiency. The authors hypothesize that this condition not only reduces fertility, but also increases vulnerability of premenopausal women to early acceleration of atherosclerosis and osteoporosis. Importantly, stress, exercise, and caloric restriction readily induce ovarian dysfunction in rhesus and cynomolgus macaques (*Macaca mulatta*, *Macaca fascicularis*). As in women, a return to normal reproductive function accompanies removal of the initiating stimuli. Of particular interest are data showing that stress-induced estrogen deficiency (secondary to subordinate social status) accelerates coronary artery atherosclerosis in premenopausal monkeys, an outcome that predicts postmenopausal disease in the same animals and that can be prevented by exposure to exogenous estrogen. Bone density is similarly reduced among subordinate, estrogen-deficient females. These data imply that efforts to reduce the postmenopausal disease burden should begin premenopausally. Finally, the authors speculate that functional reproductive deficits in female primates represent an adaptive complex allowing individuals to maximize lifetime reproductive success by foregoing reproduction in suboptimal circumstances.

Diseases unique to women do not necessarily result in mortality but nonetheless may cause significant mental distress or physical discomfort. Ovulatory infertility and endometriosis—two such conditions—are reviewed in the next two articles of the volume. In the first, Abbott and colleagues (2004) suggest that infertility is a substantial problem for 10 to 20% of married couples in the United States, with ovulatory dysfunction accounting for the greatest impairment among female partners. Nonprimates have limited usefulness in studies of this phenomenon owing to reproductive dissimilarities with women. Even nonhuman primates vary in suitability, with Old World monkeys more appropriate for modeling ovulatory dysfunction and New World monkeys more amenable to studies requiring strict control over the timing of ovulation. Importantly, studies of Old World monkeys revealed that alterations in the pulsatile release of gonadotropin-releasing hormone (GnRH¹) underlie anovulatory infertility. After reviewing the use of nonhuman primates to explore contributions of caloric restriction, strenuous exercise, and stress to ovulatory dysfunction, the authors focus on the significant differences in the neuroendocrine mechanisms mediating hypogonadic anovulation in Old versus New World monkeys. Among the most significant contributions in this area has been the ability to develop and test therapies that desensitize the pituitary to GnRH, thus reversing hormone-dependent diseases, allowing assisted reproduction to occur more smoothly, and restoring natural ovulation. Finally, Abbott and coauthors also review the role of primates in elucidating the origins of polycystic ovarian syndrome (PCOS¹), one of the most common causes of secondary amenorrhea and an epidemiologically significant cause of menstrual irregularity and thus infertility. The observation that androgen excess during monkey fetal life leads to a syndrome that mimics PCOS is

particularly noteworthy. Such individuals preferentially accumulate abdominal fat and thus are also vulnerable to defects in carbohydrate metabolism, including increased insulin resistance. These observations provide the basis for investigations that could significantly advance our understanding of the development and sequelae of PCOS, which, in addition to reduced fertility, also increases the risk of diabetes and cardiovascular disease.

Endometriosis is a gynecological condition characterized by the presence of endometrial tissue outside the uterus. Although usually considered benign, the condition is often manifested by reduced fertility as well as painful menstrual periods and intercourse. Furthermore, although numerous treatments reduce the pain associated with endometriosis, few effectively reverse the infertility. According to Story and Kennedy (2004), knowledge in this area has been hampered by an incomplete understanding of the syndrome, a problem stemming from the difficulty of studying the disease in women. Owing to their small size and convenience of use, rodents are frequent subjects in this research. However, these animals do not develop spontaneous disease and thus must be stimulated with either human or autologous uterine tissue, producing lesions quite dissimilar from those seen in women. In contrast, nonhuman primates (e.g., macaques and baboons) develop endometriosis naturally. The spontaneous lesions developed by these primates are morphologically identical to those seen in humans and occur at similar locations. The prevalence among colony-dwelling individuals is about 30%, a value increased by estrogen implant and hysterectomy. Data also indicate the presence of genetic susceptibility and reveal an inverse association between lesion severity and degree of infertility. Finally, the authors review the use of primate models in evaluating novel diagnostic tools (magnetic resonance imaging) and therapies (tumor necrosis factor binding protein 1 and progesterone receptor modulators and antagonists). The major drawback of nonhuman primate models is that although they develop disease spontaneously, most lesions are minimal. For this reason, investigators generally must evaluate lesions induced by either cervical ligation (to cause retrograde menstruation) or intraperitoneal injection of endometrial tissue.

The initial articles in this volume review health and disease in premenopausal women. However, postmenopausal individuals bear the greatest health burden. There are currently 34 million women in this category, comprising 12% of the entire US population and increasing rapidly. Williams and Suparto (2004) consider the contributions of nonhuman primates to our current understanding of coronary heart disease (CHD¹), the largest cause of death in this group. This topic is especially timely in view of the outcome of the randomized trial arm of the WHI, which suggested that women in their mid-60s with an intact uterus gain no cardiovascular benefits from treatment with conjugated equine estrogens (CEE¹) and medroxyprogesterone acetate (MPA¹). This finding was contrary to expectations based on positive outcomes from observational (cohort) studies and

experiments performed with animal models. Williams and Suparto consider three possible causes for this discrepancy: (1) the so-called “healthy woman” phenomenon (individuals choosing to take hormone replacement therapy [HRT¹] or prescribed HRT by their physicians are initially healthier, better educated, and take better care of themselves than age-matched counterparts); (2) the WHI intervention occurred too late, because these women probably had pre-existing atherosclerosis; and (3) MPA may have antagonized any beneficial effects of CEE in the WHI. Studies with nonhuman primates are randomized and thus not confounded by pre-existing disparities in the health of treated and untreated individuals. Furthermore, such studies shed light on the possibility that the timing or components of hormone replacement may account for unanticipated findings of the WHI. The authors emphasize that most studies using nonhuman primates to model postmenopausal women initiate an atherogenic diet and hormone treatment simultaneously upon ovariectomy (surgical menopause). This research design evaluates the ability of hormone treatment to inhibit the *development* of atherosclerosis, which it does effectively. Similarly, hormone treatment reduces lipid accumulation in the artery wall of such monkeys and acts to improve endothelial function (the endothelium, which is the inner lining of the artery, controls the movement of substances in and out of the artery wall and mediates arterial responses to changes in flow demand). In contrast to the situation modeled in early monkey studies, current evidence suggests that the majority of postmenopausal women have some degree of pre-existing atherosclerosis. Notably, two recent monkey studies indicate that pre-existing atherosclerosis diminishes or eliminates the atheroprotection associated with hormone replacement. The investigations using monkeys also suggest that MPA, in contrast to other progestins, may antagonize the beneficial effects of estrogen treatment. Williams and Suparto end their article by urging investigators using animal models to go beyond measuring plaque size to assessing indices of plaque stability and risk of rupture to ensure that experimental outcomes will be indicative of clinical risk.

Stroke is the third leading cause of death in women, after coronary disease and all forms of cancer. Murphy and colleagues (2004) review the use of animals to study this devastating disease. As with CHD, women enjoy an age-related epidemiological advantage relative to men. This pattern of protection suggests that female reproductive hormones play a role in delaying the onset of stroke. Although numerous animal models of stroke exist, including nonhuman primates, research on sex differences has been performed exclusively in rodents. In such studies, premenopausal females sustain smaller injuries than males after ischemic insults and have greater survival times or reduced mortality in response to spontaneous or induced stroke. Furthermore, the protection against stroke damage characteristic of females increases concomitantly with increases in endogenous estrogen. Importantly, this sex difference is abolished by ovariectomy. Overall, in both rodents and

people, the stroke burden is lower in reproductively intact females than in ovariectomized (surgically postmenopausal) individuals or males. Studies in rodents also show that estrogen administration or replacement is neuroprotective after an ischemic insult. In contrast, human trials assessing stroke either as a primary or a secondary endpoint have indicated that there is increased risk associated with estrogen treatment. However, estrogen can influence both the vessels and nerves of the central nervous system, suggesting that both kinds of sites must be monitored to evaluate estrogen’s “global” effects on the brain. At present, the conditions under which estrogen is harmful versus inhibitory with respect to stroke risk are not fully understood. The authors suggest that additional progress in understanding the natural history of sex differences in stroke requires nonhuman primate models, owing to their greater degree of encephalization compared with rodents.

Bruns and Kemnitz (2004) next review the use of nonhuman primates in the study of insulin resistance and diabetes. Insulin resistance, the first step in a cascade of physiological changes that culminates in type 2 (“adult onset”) diabetes, often emerges in relation to obesity and a sedentary lifestyle, and it greatly increases the risk of cardiovascular disease. Although insulin resistance and type 2 diabetes affect both men and women, adverse outcomes are more prominent among women. Notably, diabetes has been studied intensively in monkeys. However, there has not been systematic assessment of body composition and patterns of fat distribution during the juvenile period, the time during which vulnerability to adult onset diabetes is probably first established. The authors also suggest that despite the many similarities between women and female monkeys in growth and development, monkeys (unlike women) do not display sex differences in the pattern of fat deposition. This distinction is important and helpful, because it implies that sex differences in insulin resistance in monkeys must be due to factors other than pattern of fat deposition. Importantly, premenopausal monkeys are more insulin sensitive than males, suggesting that ovarian hormones contribute to glucoregulation. Supporting this view is the observation that insulin sensitivity is greater in the follicular phase of the menstrual cycle, when estradiol is rising, than in the luteal phase, when it is reduced. In continuing their review, Kemnitz and Bruns indicate that many monkey studies are probably flawed by their use of relatively young, ovariectomized animals to model menopause, which generally co-occurs with aging in women. Nonetheless, data from monkeys consistently show that unopposed estrogen improves insulin sensitivity, an effect that is antagonized by the addition of a progestin or by treatment with compounds like tibolone (which has combined estrogenic, progestogenic, and androgenic characteristics). In view of the substantial health costs—especially to women—represented by insulin resistance and diabetes, the authors urge an increase in the scope of hormone-related diabetes research using nonhuman primates.

Osteoporosis, a condition characterized by low energy

fractures arising from low bone mass and altered microarchitecture, is another prominent cause of morbidity among postmenopausal women. Jerome (2004) reviews the use of nonhuman primates in the study of this disease entity. Non-human primates are the most suitable models for osteoporosis, not only because they resemble women anatomically and reproductively, but also because they similarly remodel bone in response to aging and pharmacological treatment. With respect to skeletal biology, Jerome suggests that female macaques reach peak bone mass by about 9 to 11 yr of age (approximately equivalent to a 30-yr-old woman) and that losses in bone mass occur with further aging. These observations imply that the age of monkeys used in experiments affects the ability of researchers to translate study outcomes to women. If animals are too young, for example, they will be acquiring bone and thus fail to closely model conditions in peri- and postmenopausal women. Nonetheless, almost all studies to date indicate that adult female monkeys resemble women in the profound adverse effects induced by estrogen depletion. Furthermore, natural menopause in macaques, as in women, is associated with decreased bone mass and increased bone turnover. Similar changes are induced by ovariectomy, provided the animals have reached peak bone mass and consume a diet relatively low in calcium. Interestingly, bone loss in monkeys is relatively lower than that which occurs in ovariectomized rats. Nonetheless, the monkey pattern of bone loss—a sigmoid-shaped decline—and its magnitude closely resemble events in women, except that the period of loss is compressed into a 1- or 2-yr period compared with approximately 6 yr of loss observed in women passing through the menopausal transition. Monkey studies also demonstrate that estrogen replacement effectively reduces bone loss. Jerome argues that monkeys will be used increasingly in future bone studies as researchers and pharmaceutical companies attempt to develop alternatives to estrogen/progestin replacement therapy, which is being refused by many women on the basis of the WHI outcome. Future monkey studies are likely to be increasingly efficient, as researchers employ chemical castration with a GnRH agonist to induce reversible estrogen deficiency. This manipulation produces alterations in bone turnover consistent with those seen after surgical ovariectomy and allows the same animals to be used in numerous studies.

Cancer of the reproductive tract (endometrium, ovarian surface, and cervix) is a major source of morbidity and mortality in women. Worldwide, for example, cervical cancer is second only to breast cancer as the most common type of cancer affecting women. In reviewing the epidemiology of the reproductive tract cancers, Cline (2004) identifies obesity, insulin resistance, hyperandrogenemia, PCOS, and anovulation as major premenopausal risk factors. Furthermore, estrogen promotes cancers of the uterus, which is the rationale for combining estrogen with a progestin in postmenopausal replacement therapy. Old World monkeys are among the most appropriate models for studying cancers of the reproductive tract because they have a similar endome-

trial and menstrual physiology. However, relatively few spontaneous neoplasms have been described in monkeys, an outcome that probably reflects the relatively small populations subjected to scrutiny. However, endometrial hyperplasia is readily induced in macaques by unopposed estrogen, and it can be used as a precancer index of risk. Importantly, precancerous increases in cellular proliferation, regardless of cause, can be recognized by using appropriately stained tissue. In general, hormone treatments associated with increased cancer risk in women reveal increased endometrial proliferation in monkeys, whereas those that are protective in women generally reduce proliferation in monkeys. In addition to monkeys, rodents are frequently used to study reproductive tract cancer, especially in complex experiments requiring large numbers of individuals. Rats are often used in such studies because they exhibit a relatively high incidence of hormone-dependent uterine neoplasms, while genetically altered (“knockout”) mice permit mechanistic studies that are impossible to conduct in primates. Other models in current use include Chinese hamsters and rabbits, which develop spontaneous uterine carcinomas. In contrast to the foregoing animals, domestic species (cats, dogs, bovines) are not widely used to model reproductive tract cancers. The review ends with a call for development of additional and more sophisticated models that would allow study of either spontaneous or induced carcinomas that more closely resemble those of women.

The issue articles to this point emphasize physical conditions affecting pre- and postmenopausal women. In the next article, Shively and Bethea (2004) focus on the links between estrogen activity in the brain and alterations in mood, cognition, and memory. The authors argue that understanding effects of cyclic hormone exposure and its cessation on the central nervous system could aid the development of strategies capable of ameliorating cognitive decline and mood disorders, both of which are more prominent in postmenopausal women than age-matched men. Current evidence in this area is mixed. Most recently, the WHI showed that CEE plus MPA adversely affects cognition and quality of life. Shively and Bethea suggest that the interpretation of current human studies is limited by the small number of hormone treatments that have been tested. The authors argue that additional testing in animal models could clarify whether there are situations in which particular hormones or hormone combinations might exert beneficial central nervous system effects. Not surprisingly, large brain size, high intelligence, and a reproductive physiology similar to that of women make Old World monkeys among the most salient models for evaluating hormonal influences on emotional states and cognitive abilities. The authors review studies in monkeys showing that aged, cycling females perform better on cognitive tests than similarly aged animals that no longer cycle. These and other initial data suggest that estrogen has beneficial cognitive effects. However, the paucity of such studies means that little is known about the underlying mechanisms or how such effects might translate to competency in everyday life. The authors then review

their own important work in developing an animal model of depression, the mood disorder that is most prominent among postmenopausal women. Shively and Bethea show that social subordination substantially increases the vulnerability of group-housed macaque females to depression. Among hormone treatments, oral contraceptives appear to have persistent adverse effects on factors that could affect mood. In contrast, studies of ovariectomized monkeys reveal that CEE and soy protein with isoflavones are potentially mood elevating. Finally, the authors review their investigations and evaluate the influence of gonadal hormones on the brain serotonergic system of macaques, a research area of particular relevance to mood disorders. The authors end by urging an increase in the number and scope of investigations that target hormonal influences on neural systems.

The issue articles cited above describe the health problems experienced by women and highlight the role of animal models, especially primates, in advancing understanding of these conditions. In the next section, Appt (2004) reviews the health effects of soy, used increasingly by postmenopausal women as an alternative to hormone therapy. Several observations support the view that soy might in fact promote health. First, populations that habitually consume soy generally have a lower incidence of chronic disease (especially heart disease, breast cancer, prostate cancer, and osteoporosis) than populations that do not use soy. Moreover, when individuals move from soy- to non-soy-consuming regions, they take on the disease characteristics of the host populations. Finally, soy contains substances (isoflavones) that are thought to activate estrogen receptors and are therefore sometimes termed "phytoestrogens." In turn, many scientists believe that phytoestrogens account for the majority of soy's beneficial effects. Appt next reviews the cardiovascular effects of soy in female macaques. These effects include beneficial alterations in both lipid- and non-lipid-related indices of cardiovascular risk, with outcomes that may vary in relation to gonadal hormones and extent of pre-existing atherosclerosis. Soy has also been studied in monkeys in relation to risk of breast cancer and osteoporosis. Importantly, soy inhibits adverse increases in mammary and breast proliferation observed in monkeys exposed to estrogen, suggesting that it may protect against the development of breast cancer. However, investigations to date indicate that soy does not prevent the increases in bone turnover or losses in bone density that typically result from estrogen deficiency. These negative findings should temper some of the enthusiastic claims sometimes made on behalf of soy products. Appt provocatively concludes her section by hypothesizing that in the future, soy may be used in combination with existing hormonal compounds to create an ideal postmenopausal treatment regimen.

Investigators employing animal models in research or seeking to understand the significance of this investigational strategy cannot help but benefit from the views of those physicians who must interpret experimental findings and,

ultimately, decide what is relevant to patient care. For this reason, the final article of the issue (Archer 2004) is a clinical perspective by a recognized expert in menopausal medicine, David Archer. In this clinical perspective, Dr. Archer judges each of the contributions with an eye toward identifying the ways in which they enlighten the provision of healthcare to women. He concludes that the greatest contribution of animal models, especially nonhuman primates, is that they offer the ability to conduct controlled experiments that would be logistically or ethically proscribed in women. According to Dr. Archer, such studies have advanced the cause of women's health by providing the incremental increases in knowledge upon which evidence-based medicine has come to depend.

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