'Leaning in' to Support Sex Differences in Basic Science and Clinical Research

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Sheryl Sandberg's new book *Lean In* (1)—along with *Nature*'s recent coverage (March seventh, 2013) on why women are less likely than men to pursue a career in science or reach the highest levels within their field—raise "big picture" questions about the gender gap in scientific and clinical professions. Yet it is also important that we recognize a "small picture" question related to sex in science: why an XX-XY knowledge gap persists in basic science, preclinical and even clinical research.

The focus of much of our work has been to raise awareness about the issue of sex-related chromosomal and hormonal influences on basic biology, drug development, and patient health. In 2010, we wrote an opinion paper, published in *Nature*, that called on the scientific community to ensure that male and female animals were included in the design of basic science discovery research (2). We further asked that the clinical community include a sufficient sample of males and females in their research trial design to evaluate whether sex alters the efficacy or adverse effects of tested drugs.

Since that paper was published, little has changed. As of 2011, there has been no significant difference in the percentage of women enrolled in nonsex specific studies (43% in 2004, 38% in 2009) (3). Outcome by sex is not reported in 64% of studies and sex-specific analysis remains low (3). A lack of analysis by sex extends to basic science as well. For example, a survey of more than 1200 neuroscience papers published in five top journals from June 2011 through May 2012 shows that studies using rodents included the sex of the animals in their analysis only 42% of the time (4). Of studies that did include the sex of subjects, females were studied only 24% of the time. The 2013 IOM Report, *Shorter Lives, Poorer Health"*, found that Americans may be wealthy compared to the rest of the world, but we are not particularly healthy compared to peer nations. Indeed the increase in female mortality raises a red flag and argues strongly that studying health risks and basic science through a sex and gender lens is critically important.

What are the reasons for this disparity? One possibility is the misguided presumption that beyond the reproductive system, sex differences are likely to be small and not meaningful. However, an important new discovery highlights the relevance of sex differences in neuroscience research: Huang and Woolley (2012) reported a sex-specific mechanism of synaptic modulation in the hippocampus, a brain region important in learning and memory, affective disorders, and epilepsy (5). This demonstrates that the basic mechanisms of neuromodulation can and do differ between males and females, even in brain regions unrelated to reproduction. Indeed published evidence from worms to humans confirms structural and functional sex differences throughout the brain (6). Determining how prevalent sex-specific or sex-biased mechanisms are in the brain will require that more basic scientists include both sexes in the design of their studies and that they consider sex differences in their data analysis.

Cardiovascular biology is another area in which sex-based research is critically important, yet journals in this field follow the general trend of publishing papers that do not specifically include or report sex differences (7, 8). These deficiencies persist despite numerous published

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Abbreviations:

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clinical studies demonstrating differences in the prevalence, presentation, and response to treatment in men and women with respect to cardiovascular disease. A glaring example of this male bias in basic research comes from the field of Dermatology where virtually all research on keratinocytes is done on cells from male foreskin, limiting our knowledge of this cell's function in response to estrogen or of other functions controlled by the chromosomal sex of the cell. There are also clear sex differences in the effects of many experimental treatments across diverse areas of preclinical research, such as obesity, sleep disorders, the microbiota and gastrointestinal (GI) diseases; (9–11) yet, male animals and cells continue to be the overwhelming experimental sex of choice for discovery research (4).

Since the *Nature* article was published we are heartened by the fact that several scientific societies, including The Endocrine Society and the Society for Neuroscience, now require a statement about the sex of animals used in studies published in their journals. This is an important step forward. Further progress is possible if investigators in other disciplines consider seriously the rationale for sex-selective studies and ask what they MIGHT learn if the other sex were also examined. The influences of Scientific societies, in concert with mandates by the National Institutes for Health (NIH) that females must be included in all NIH-funded clinical trials that are not examining a gender specific condition, might bend the arc of events to increase women in non-NIH funded studies as well.

Finally, we call on the NIH to ask basic scientists to indicate the sex of animals studied under the auspices of government-funded research. Just as with the clinical subjects, the investigator can include both sexes or explain why the study is restricted to one sex.

In 2010, the Canadian Institutes of Health added the following mandatory questions about sex and gender in their research funding applications:

Are sex (biological) considerations taken into account in this study: (Y/N)

Are gender (socio-cultural) considerations taken into account in this study? (Y/N)

If Yes, please describe how sex and/or gender considerations will be considered n in your research design.

If No, please explain why sex and/or gender are not applicable in your research design.

We call upon the NIH to follow the lead of our neighbors to the north and include similar language in future NIH grant opportunities. We also request that sex differences be reported even if they show no difference. We believe the American public who fund this research would find this an important step forward.

We must continue efforts to educate biomedical investigators throughout the world about sex differences related to health and disease beyond reproductive biology. While some progressive steps have been taken in the past 3 years, we need to continue to 'lean in' to this topic as a scientific community. We need to keep driving home the message that understanding how sex influences biology is a critical part of evaluating clinical outcomes. We should recognize that clinical trials will be less costly if we determine sex differences in preclinical testing—the earliest and least expensive stage of the discovery pathway—and that understanding sex-based biology will ultimately inform the development of more effective diagnostics, prevention strategies, and treatment interventions, making us all healthier. As we continue on the path towards personalized medicine, we must acknowledge the first differentiating characteristic within a patient population: male and female. We should use the twin motivators of 'it really matters' and 'I get something out of this' to encourage more scientists to consider this aspect of their work.

A critical part of the equation is to use policy changes, such as those implemented at the Endocrine Society and Society for Neuroscience, to catalyze these efforts. Legislators, scientists, policy makers, and the public must have clear reasons to support this line of research. We've argued above that there is strong scientific rationale for including sex in research and reporting – it is a physiological fact that differs between organisms that must be reported based on ordinary good lab practices. The next argument is intellectual: biological differences between men and women affect health, illness, and disease treatment across the lifespan and an improved understanding of these differences is necessary to improve the health of all people. The final argument is an economic one - that the study of sex-based basic biology provides a cost-effective way to increase the quality and reduce the costs of healthcare by learning earlier what drugs target different sectors of the population. Finally, it is possible that we can reduce adverse events in drug trials and approved drugs by learning who is more likely to benefit from treatment. Ambien is a good example of a drug that required different dosing in men and women – since the clinical trials were done only in men, women were harmed. Women require a smaller dosage of the drug in order for it to be effective, yet the drug was marketed at the 'male' dose, leading to harmful side-effects including erratic behavior in the morning after taking the medicine. This issue has now been rectified with the first ever 'gender specific labeling' of a drug, which is a model for the future (12). So, whether the argument is scientific, intellectual, economic, or just good medicine, sex-specific medicine matters to the health of all of us.

Finally, sex-based research will not only improve healthcare into the future, but will also send a message to rising young female scientists and the public in whose in-

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terest we work, that from early discovery research to the pinnacles of science leadership, women and their cells have an equal place at the table.

Let's all lean in and improve the research pipeline and reduce the gap in healthcare between men and women. This is an achievable goal.

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