Sex and basic science. A Title IX position

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No person in the United States shall, on the basis of sex, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any education program or activity receiving federal financial assistance.

Section 901(a) of the Title IX of the Education Amendments of 1972 [44 Fed. Reg. 71,413, 71,423 (1979); http://www.ed.gov/about/offices/list/ocr/docs/9interp.html]

POINT (KS AND JGV): On January 17, 2014, Representative Nita M. Lowey (17th District, New York) and Representative Rosa DeLauro (3rd District, Connecticut) wrote to Dr. Francis S. Collins, Director of the National Institutes of Health (NIH), to express their concerns over the fact that basic science is still primarily conducted in male animal models of disease and that this bias from the outset has contributed to the costly waste of drugs being removed from the market due to unexpected adverse consequences in women. These two congresswomen have a historic interest in ensuring NIH-funded biomedical research benefits both men and women in a balanced, equitable manner, and they were instrumental in enacting the NIH Revitalization Act in 1993 that greatly improved the balance in clinical research by requiring the inclusion of women in NIH-funded phase III clinical trials. Four months later, on May 14, 2014, Janine A. Clayton, Director of the Office of Research on Women’s Health at the NIH, and Dr. Collins announced in the journal Nature that NIH plans to address the “over-reliance on male animals and cells in preclinical research” and to “ensure that the health of the United States is being served” by “requiring sex and gender inclusion in preclinical research” (2).

To build on that important announcement, we contend that adopting a Title IX concept in funding basic science research would be the most effective way NIH could achieve an appropriately balanced portfolio of biomedical research utilizing male and female cells and animals. Here, the Title IX analogy does not refer to career opportunities for men and women investigators. Rather, we are using the Title IX concept to advocate for balance in biomedical research between male and female animal models of disease to ensure that both men and women will receive equivalent benefits from federally funded basic science.

COUNTERPOINT (GLCY AND WKS): NIH proposes to start requiring applicants “to report their plans for the balance of male and female cells and animals in preclinical studies in all future applications, unless sex-specific inclusion is unwarranted, based on rigorously defined exceptions” (2). Will this increase costs?

POINT (KS AND JGV): Some investigators are concerned that including both sexes in preclinical studies would quintuple animal costs and investigator time because in addition to males, the study would now require females at each stage of the estrous cycle; however, the assumption that variability in females is intrinsically greater than in males due to the estrous cycle is unfounded. A meta-analysis of 293 articles covering molecular, morphological, physiological, and behavioral traits revealed that variability was not significantly greater in females than males for any endpoint and, in fact, was substantially greater in males for several traits (25). Thus animal costs need not quintuple; however, each experiment in which both sexes were studied would certainly increase animal budgets. In contrast, a Title IX approach would not require investigators to increase project animal costs or investigator time. Title IX did not dictate that half the football team had to be women. What Title IX did do was to require federally funded institutions to provide girls and women similar amounts of resources as for boys and men in aggregate, not within a sport. Similarly, the Title IX concept for biomedical research would mean an individual investigator would not be required to study both sexes in their experimental models; however, it would be the responsibility of NIH to ensure basic research in male and female animal models proceeds in a balanced manner in aggregate throughout the 27 NIH Institutes and Centers.

One of the most important advantages of this approach is that many experimental models of disease show a sex difference in the severity of injury. Take recent studies on immune contributions to hypertension as an example. Guzik et al. (7) showed that male T cells play a prohypertensive role in a male T cell-deficient mouse (Rag1−/−); however, we found that male T cells do not elevate blood pressure if the sex of the Rag1−/− mouse is female (24). Furthermore, we found that female T cells do not increase blood pressure in the male Rag1−/− mouse (12). In other words, whereas male T cells elevated blood pressure in the male but not the female Rag1−/− mouse, female T cells did not elevate blood pressure in either sex. These two studies illustrate that this model of hypertension is not useful for studying mechanisms of T cell susceptibility to hypertension in the female but would be valuable for investigating mechanisms of resistance. The converse example is the NZBWF1 mouse, which is a commonly used female mouse model of systemic lupus erythematosus (lupus). Unlike the females, which exhibit hypertension by 36 wk of age (26), the male animals do not exhibit pathology until the end of their life span (17). Thus this lupus model is not optimal for studying mechanisms of susceptibility to lupus in the male but would be useful for investigating resistance to lupus. The implication of these studies is that male-female differences in mechanisms of susceptibility to hypertension could impact response to therapeutic strategies for treating hypertension (35).

Since most men and women will develop hypertension before they die (29), basic research in hypertension is warranted in both sexes; however, some models of hypertension have no counterpart in the opposite sex. For example, postmenopausal hypertension and hypertension associated with premature ovarian failure is often modeled by ovariectomy, ovarian follicle depletion, or aging in the female (5, 9, 14). Thus single sex studies like these are warranted as it would not...
be possible to include male animals in these female-specific models of hypertension.

Hypertension is not the only condition in which male models of disease are not ideal for investigating disease susceptibility in females. Whereas women are susceptible to chronic kidney disease, in many of the experimental models, female animals develop little renal pathology (4, 28). Thus another advantage of the Title IX approach to achieving a male-female balance in basic biomedical research is the likely development of new experimental models that represent the understudied sex throughout diverse fields of preclinical research.

COUNTERPOINT (GLCY AND WKS): Are you saying that regardless of merit, 50% of all grants funded should study female animals and/or cells and 50% male animals and/or cells? While most would agree that drug trial-based (clinical) research should examine sex differences in responsiveness to potential therapeutics, this assumes that all NIH-funded work focuses solely on therapeutic development. Would discovery of new peptides or receptors, or work matching endogenous ligands to orphan receptors (41, 42), then not get funded? Should a Title IX approach apply to anything other than therapeutic development? Certainly, studying sex differences in basic physiological and pathophysiological processes is important, but initial descriptions of those basic principles may not require, in the early stages, the analysis of sex differences. Studies following up on those foundational discoveries designed to elucidate the physiological (and potential therapeutic) relevance, to be sure, should address the issue of potential differences in responses or actions based on sex.

POINT (KS AND JGV): Given that we are just beginning to understand how the sex of cells impacts basic cellular processes including cell fate and metabolism (6), it is imperative that we study new peptides, ligands, and receptors within the context of both male and female cells and animals to ensure the translation of basic mechanisms into drug development is not sex biased from the outset, since molecular and cellular signaling pathways provide the basis for discovering biomarkers and targets for novel therapeutic interventions of the future (30). Searching for new peptides or receptors and factors that regulate them solely in male cells and animals could result in missing potential biomarkers and drug targets that are differentially expressed and/or regulated in females. Mandating an absence of sex bias only once new drugs have reached the late stage of clinical trials is akin to closing the barn door once the horse has already bolted. So yes, the NIH should strive toward achieving a 50–50 balance in funding basic science research in experimental models using male and female cells and animals though not necessarily study by study. As to merit, there is no inherent reason why basic science research on one sex would be more or less meritorious than on the other sex.

COUNTERPOINT (GLCY AND WKS): Will this latest mandate from NIH spur other advocacy groups to similarly demand special attention in the review process? Clearly, age-dependent differences in physiological and pathophysiological organ and tissue responsiveness have been established (20, 33). Will the American Aging Association be the next to demand special consideration, in which case grant proposals would be required to include young, middle-aged, and aged subpopulations in all studies? Would you then advocate for age equality as well? If the Title IX approach were to be established, they would have a valid argument for similar consideration. What other advocacy group would step up to the plate next?

POINT (KS AND JGV): We are not advocating for each basic science study to contain equal numbers of males and females and neither should investigators be required to study all ages in their model of disease. The National Institute of Aging was established to promote age-related research and the National Institute of Child Health and Human Development focuses on the young, so there are already venues for the American Aging Association and child interest groups to lobby for research. Similarly, disease-specific advocacy groups have individual NIH Institutes and Centers to respond to their concerns. One’s sex, on the other hand, transcends virtually every aspect of research related to human health and thus should be incorporated into the missions of all NIH institutes and centers. Furthermore, because one’s sex is a fundamental biological factor that affects all aspects of disease including incidence, age of onset, symptoms, diagnostic criteria, response to treatment, and outcomes (18), it uniquely elevates it to the level of requiring specific safeguards to ensure that the health of the United States is in fact being best served by both as well as clinical research. What is vital is that NIH ensure there is a balance between females and males in basic science research utilizing cells and animals in aggregate and that the proportion of male and female models used to investigate specific diseases reflect disease prevalence in the general population. The NIH is already charged with balancing research across disciplines and fields at the macrocosm and microcosm levels throughout its institutes and centers and thus has the ability to also balance representation of male and female experimental models.

Although one’s sex is a fundamental biological factor and regulation, integration, and homeostasis in health and in disease are equally pertinent to both males and females, out of 678 papers published since 2012 in The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology (AJP-RIC), which publishes “original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations” (http://ajpregu.physiology.org/about), 18% did not indicate the sex of the species (Table 1). About half of studies on human volunteers included both sexes, though many of these studies did not analyze the data by sex. Of the human studies conducted in only one sex, 79% were conducted in men whereas only 21% were conducted in women. When studies conducted in only one sex were considered across all species including humans, 77% were conducted in males and only 23% were conducted in females. Furthermore, of all the studies that included both sexes (24%), many did not analyze the data by sex. This underrepresentation of published studies in females is not unique to this journal as a historical survey of 10 biological fields revealed a similar underrepresentation of studies published on female cells and animal models (1). Thus this is clearly a long-standing systemic problem.

COUNTERPOINT (GLCY AND WKS): Journals only publish what they receive for review and what passes the peer-review process. It is dangerous to imply that the scientific journals are responsible for any “sex bias” in basic science. As an extension of your proposed “funding Title IX,” would Society-based journals then have to fall in line and publish
equal numbers of female and male studies, and then by extension, a comparison across the lifespan? Are you suggesting that NIH should “follow” a journal’s progress if this Title IX approach is initiated and that NIH would dictate what is and what is not published? Will NIH then become the new “Big Brother”? Don’t forget that not all work published in scientific journals is funded from US Government sources.

**POINT (KS AND JGV):** Although journals reveal the striking disparity in basic science research between males and females, they should not be held responsible for the lack of balance between the sexes in basic biomedical research unless reviewer bias entered into the scientific review process. Journals could, however, facilitate the goal of achieving balance by providing forums for education and discourse as to the impact of one’s sex in a multitude of cellular and physiological processes. On the other hand, NIH should bear the responsibility for ensuring that both men and women are not denied the benefits of biomedical research due to underrepresentation of one sex in experimental models of disease.

While NIH should not dictate to journals what to publish, the NIH could use journals to evaluate their progress on initiatives that are designed to improve the balance of the underrepresented sex in various disciplines across the NIH biomedical research portfolio. The NIH already tracks publications resulting from government funds as part of grant progress reporting to the agency by Principal Investigators. Thus NIH could utilize these data to track the proportion of single-sex studies that are male-only versus female-only and of the studies that included both sexes, they could track the percentage of these that analyzed the impact of one’s sex in a statistically meaningful manner. The NIH should obtain this information not just for clinical research but also for basic science research as a tangible metric (as in Table 1).

**COUNTERPOINT (GLCY AND WKS):** Journals can do a better job requiring authors, like those in the 18% category above, to specify the sex of their animal models and all the American Physiological Society journals now request the sex of the animal be described (18). However, for a journal like *AJP-RIC* that has the mandate to publish comparative studies (27), some designations would be hard to establish, e.g., when studying zebrafish larvae (32) or *Caenorhabditis elegans* (37). What would they categorize the sex of the amphibian horned beetle (38) or Pacific white shrimp (8) to be? Would comparative studies of lower species to elucidate basic physiological principles (3, 11, 19, 22, 31) net merit funding?

**POINT (KS AND JGV):** Of course, comparative studies of nonmammalian species do indeed merit funding. These comparisons have made major contributions to our understanding of physiology and have led to new treatments for disease. For example, the discovery in 1984, of a vasoactive intestinal peptide-secreting-like peptide in Gila monster venom (10) led to the development of exenatide (36), a peptide analog of glucagon-like peptide-1 that has been developed and approved for the treatment of Type 2 diabetes mellitus (21). Furthermore, a Title IX approach would only apply to mammalian species and cells. However, given that one’s sex is a fundamental biological factor that affects physiology and pathophysiology, if the sex of the organism, regardless of species, was not considered, authors should at least acknowledge that point in their study when they discuss other experimental caveats. Note that studying sex determination and gonad differentiation in nonmammalian species including zebrafish (15) has advanced our basic understanding of these core physiological processes, and the first report of males of any animal species exhibiting endogenous reproductive cycles was done using shrimp (23).

Comparing the sexes in basic research is conceptually quite similar to the field of comparative physiology, which investigates novel mechanisms used by animals from different taxa and diverse environments to understand basic physiological processes. Similarly, sex difference research is an important area of study, and in fact, comparing the sexes has led to novel findings including how an imbalance of sex hormones can exacerbate diabetic renal disease (39) and the discovery of gonad-independent sex chromosome effects on hypertension (13) and autoimmune disease (34) as well as sex chromosome-independent effects of gonadal hormones on enzyme activity (16). Just as comparative physiology is one approach to understanding physiology, sex difference research investigating the impact of gonadal hormones and sex chromosomes on a biological parameter is one approach but not the only approach to understanding biology. We also need to conduct single-sex studies in models of disease for the reasons discussed above.

**COUNTERPOINT (GLCY AND WKS):** Somewhere lost in the excitement over the Clayton and Collins article in *Nature* is an equally pressing issue, that of equal career opportunities for women in science. NIH may be able to monitor and eventually mandate equality in terms of sex-dependent differences in all funded grants, but one must ask if they have led the way in advocating for equality in terms of hiring and salaries for women scientists? Until those inequalities are effectively addressed, sex bias for most will mean the reality of lower pay for women scientists? Until those inequalities are effectively addressed, sex bias for most will mean the reality of lower pay for women and a steeper climb up the career ladder. What better way to eradicate sex bias in research than to give women an equal place at the table?

**POINT (KS AND JGV):** The role of NIH in improving equity in career opportunities for women scientists is an important topic; however, that is a separate discussion for another time and we would not want this subject to be conflated with our use of the Title IX analogy as a mechanism for ensuring

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**Table 1. Distribution by sex of animal models described in manuscripts published in the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology from January 1, 2012 through May 15, 2014**

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of Manuscripts</th>
<th>Females Only</th>
<th>Males Only</th>
<th>Both Sexes</th>
<th>Sex Not Indicated</th>
<th>Females Only</th>
<th>Males Only</th>
<th>Both Sexes</th>
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<td>01/2012–12/2012</td>
<td>328</td>
<td>39</td>
<td>156</td>
<td>83</td>
<td>50</td>
<td>4</td>
<td>16</td>
<td>16</td>
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<tr>
<td>01/2013–12/2013</td>
<td>271</td>
<td>36</td>
<td>103</td>
<td>66</td>
<td>66</td>
<td>4</td>
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<tr>
<td>01/2014–05/2014</td>
<td>79</td>
<td>15</td>
<td>39</td>
<td>16</td>
<td>9</td>
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<td>01/2012–05/2014</td>
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<td>298</td>
<td>165</td>
<td>125</td>
<td>10</td>
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biomedical research is conducted in a manner that offers equivalent benefit to both men and women.

**COUNTERPOINT (GLCY AND WKS):** Perhaps the Title IX approach to research funding is not the answer to “sex bias,” rather we should emphasize, but not mandate, the importance of recognizing and studying sex differences in both physiological and pathophysiological systems, particularly as initial discoveries are expanded into more detailed projects that may lead to the development of therapeutic agents or unique model systems. *AJP-Regul Integr Comp Physiol* has published several reviews on sex differences including on hypertension (35, 40), and we support the recent decision by NIH (2) to develop and disseminate training modules on experimental design for NIH staff, trainees, and grantees to educate the scientific community and raise awareness of this important biological variable.

**Summary:** This debate highlights the pros and cons of instituting a Title IX approach to eliminating sex bias in basic research. Both parties agree that both sexes should be studied during therapeutic development in preclinical research but that requiring each investigator to study both sexes is not scientifically justified and will make unnecessary and onerous demands that will waste precious resources and infringe on an individual’s ability to conduct rigorous investigator-initiated research. We disagree, however, on the extent to which both sexes need be studied and also on the mechanism to effect this change. K. Sandberg and J. G. Verbalis think that one’s sex is such a fundamentally important biological factor that it should be taken into account across all biomedical research utilizing animals and cells in an aggregate manner and that a Title IX funding approach by NIH would ensure that both men and women are not denied the benefits of biomedical research due to underrepresentation of one sex in experimental models of disease. G. L. C. Yosten and W. K. Samson believe a Title IX mandate is unwarranted because basic science at the early phases of discovery is not necessarily impacted by the sex of the species and that educating investigators as to the importance of studying sex differences will be an effective strategy in facilitating novel therapeutic discoveries that will benefit both men and women in health and disease. Both parties do agree that journals can contribute by serving as forum for discourse and education on this topic and K. Sandberg and J. G. Verbalis go one step further and recommend NIH use published papers citing federal funds to serve as a tangible metric for gauging the success of NIH initiatives designed to improve the male-female balance in biomedical research. This point-counterpoint discussion highlights the need for an informed discussion by basic scientists, who will be most affected by whatever policies are put into place by NIH to achieve the goal of reducing sex bias in preclinical as well as clinical research.

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**DISCLOSURES**

K. Sandberg is the Director of the Center for the study of Sex Differences in health, aging and disease, Georgetown University. J. G. Verbalis is a Principal Investigator on the Georgetown-Howard Universities Center for Clinical and Translational Research; G. L. C. Yost is a junior faculty member at the Saint Louis University School of Medicine. W. K. Samson is the Editor-in-Chief of the *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. The opinions expressed here represent those of the authors and not their respective institutions or the *American Journal of Physiology*.

**AUTHOR CONTRIBUTIONS**


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