Ensuring that women are adequately represented in clinical trials is recognized as essential for sex equity in health. However, the use of female animal models and sex-based reporting have not been equally enforced in preclinical stages of research, which often precede and inform clinical trials. In 2014, the National Institutes of Health announced that it would require that sex be considered as a biological variable in applications for preclinical research funding, yet a reluctance to include female animal models in preclinical experiments persists. Inappropriately inferring experimental findings to both sexes when a single sex is studied or when sex is not specified has the potential to disadvantage women by skewing our understanding of disease processes toward male-predominant patterns and by reducing the likelihood of female-specific therapeutics advancing to the clinical realm. We therefore systematically examined all preclinical cardiovascular studies published in American Heart Association journals with archives spanning at least 10 years (Circulation, Circulation Research, Hypertension, Stroke, and Arteriosclerosis, Thrombosis, and Vascular Biology [ATVB]) for evidence of sex bias.

Full articles published between July 2006 and June 2016 and reporting original data from in vivo experiments in nonhuman mammals on pathophysiology, genetics, or therapeutic interventions directly relevant to a specific cardiovascular disorder in humans were included. Studies on physiological or genetic characteristics were included if they proposed potential therapeutic applications or implications of the study findings. Each journal article was independently reviewed and prespecified data were extracted by using standardized case report forms by 2 authors, including the cardiovascular disease investigated, animal model(s) used and their sex, whether study samples were sex matched (in studies using both sexes), whether at least 1 study result was reported by sex, and whether the use of a single sex was reported as a limitation (in single-sex studies). Interrater agreement was calculated using the Cohen $\kappa$-statistic and percentage agreement. Discrepancies were resolved by consensus or independent adjudication. Categorical variables were compared via $\chi^2$ tests. Temporal patterns were evaluated via Pearson correlation or Cochrane-Armitage trend tests. All analyses were performed by using SAS 9.4 (SAS Institute Inc) with the use of an $\alpha$-level of 0.05 to define statistical significance.

Of 28,636 articles screened, 3,396 met inclusion criteria and were analyzed. Interrater agreement for study inclusion before resolution was $94.5\%$ ($\kappa=0.72$; 95% confidence interval, 0.70–0.73). The sex of the animals used was not reported in 20.0% of studies. Males were exclusively used in 71.6% of studies in which sex was reported, whereas females were exclusively used in 12.9% and both sexes in 15.5%. Sex matching of animals was reported in 17.1% of studies that included both sexes. Restricting this analysis to the 988 studies of therapeutic interventions did not appreciably change these distributions. When stratified by the cardiovascular disease studied, males were exclusively used significantly more often than females or both sexes in all cases, with the exception of atherosclerosis and arrhythmia. When stratified by the animal model...
used, males were exclusively used significantly more often when mice, rats, rabbits, or a combination of animal models was used (accounting for 95.0% of studies).

The number of preclinical studies published yearly over the past decade has remained stable; however, there has been a significant increase in the proportion using exclusively males and a significant decrease in the proportion using exclusively females. There have not been significant changes in the proportions of studies using both sexes or reporting the sex of the animals used (Figure). These trends were unchanged when studies in which sex was not specified were excluded.

Sex-based reporting of results occurred in 35.1% of studies in which sex was reported using the liberal criterion of any results being reported in reference to the sex of the animals used at least once. There was no appreciable difference in the prevalence of sex-based reporting before and after the publication of planned National Institutes of Health policies to improve sex inclusion in preclinical research1 (34.9% versus 35.7%, respectively). Of the 2297 studies in which only 1 sex was reportedly used, 1.6% mentioned this aspect of the study design as a potential limitation.

Although the need to include women in clinical trials is now a well-established requirement to enhance external validity, analogous standards have not gained a foothold in preclinical stages. Reasons put forward for the preferential use of males include increased variability attributable to fluctuating gonadal hormone levels throughout the estrous cycle and sample size implications of planning sex-based analyses.2,3 Evidence to support these concerns is limited for most experimental settings, however.3–5

Our review of preclinical research published in leading cardiovascular journals over the past 10 years demonstrates that sex bias is prevalent and increasing, contrasting with advances made in clinical research designs and reporting. Concerted efforts on the part of researchers, journal editors, peer reviewers, universities, industry, professional organizations, and patient advocacy groups are urgently needed to address this discrepancy.

DISCLOSURES
None.

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FOOTNOTES
Circulation is available at http://circ.ahajournals.org.

REFERENCES

Figure. Temporal patterns in the sex of animals used in preclinical cardiovascular research over a 10-year period.

A, Absolute number of preclinical studies published and sex of animals used as a function of time. r denotes Pearson correlation coefficient for the total number of studies over time. B, Sex of animals used in preclinical studies over time (%). P trend calculated for the proportion of studies using exclusively males, exclusively females, both sexes, and not reporting the sex of the animals used over time.
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F. Daniel Ramirez, Pouya Motazedian, Richard G. Jung, Pietro Di Santo, Zachary MacDonald, Trevor Simard, Aisling A. Clancy, Juan J. Russo, Vivian Welch, George A. Wells and Benjamin Hibbert

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