Trends in NHLBI-Funded Research on Sex Differences in Hypertension
Christine Maric-Bilkan, Zorina S. Galis

Although sex differences in blood pressure levels and the prevalence of hypertension are well recognized, the mechanisms responsible for this sexual dimorphism remain poorly understood. To gain a better understanding of the research funding trends in the field of sex differences related to hypertension and the main research topics funded by the National Heart, Lung, and Blood Institute (NHLBI), we analyzed the entire NHLBI award portfolio between fiscal years (FYs) 1991 to 2014. Using specific search terms to define “sex differences,” we interrogated the publicly available list of NHLBI-funded awards active in FY1991 to FY2014 in the National Institutes of Health (NIH) Research Portfolio Online Reporting (RePORTER) database. We further analyzed and categorized awards according to a disease focus and whether each award was directly focused on comparing males versus females, or examining the effects of sex hormones in either sex. The number of NHLBI awards related to “sex differences in hypertension” progressively increased from FY1991 to FY2014, for a cumulative total of 486 awards during the entire period analyzed. The overall dollar investment has also progressively increased from $0.5 million (FY1991) to $18.3 million (FY2014), for a cumulative total of $187 million during FY1991 to FY2014. When compared with global funding trends, NHLBI seems to be the largest funder of research on “sex differences in hypertension” in the world. Importantly, the increased NHLBI investment in sex differences in hypertension research has enabled the investigation of an increased diversity of scientific topics. Although NHLBI is funding an increasing number of awards related to “sex differences in hypertension,” there are still many unanswered questions related to the mechanisms underlying this disparity.

Despite progress in the awareness, prevention, detection, and treatment, hypertension remains a major public health issue affecting every 3 adults worldwide. Hypertension is the leading risk factor for cardiovascular disease, which in turn is a leading cause of death in both women and men. Because of its significance for human health, the National Heart, Lung, and Blood Institute (NHLBI) is committed to research on hypertension and its consequences.

Blood pressure levels are higher in men than in women below 40 to 50 years of age. After menopause, blood pressure levels increase in women so that after 65 years of age, hypertension becomes more prevalent in women compared with men. Premenopausal women with hypertension have an increased risk of developing hypertensive organ damage, such as microalbuminuria and left ventricular hypertrophy, compared with age-matched hypertensive men. These observations suggest that there are sex differences in the underlying mechanisms of hypertension and its associated complications.

Although hypertension is generally considered a disease of the aging population, due to lifestyle factors such as poor exercise and dietary habits, hypertension is a growing problem even at younger ages, in both men and women. In addition to unhealthy lifestyles, younger women also face risk of hypertension associated with pregnancy, including pre-eclampsia and gestational hypertension. Thus, in addition to understanding the factors that contribute to the sex differences in the development of hypertension, there are also sex-specific factors associated with hypertension that warrant further investigation.

Given the evidence that there are sex differences in the prevalence of hypertension and the need for better understanding the mechanisms underlying this sexual dimorphism, we examined currently funded research and historic funding trends at NHLBI in the field of sex differences related to hypertension.

Methods
The data related to NHLBI and NIH funding trends were obtained from the Research Portfolio Online Reporting Tool (RePORTER) (https://projectreporter.nih.gov/reporter.cfm) using the following combinations of search terms:

1. “Sex differences”: sex differences, sex differences, y chromosome, x chromosome, sex hormones, preeclampsia, pregnancy, and menopause.
2. “Sex differences in hypertension including pregnancy-related hypertension”: all keywords as in “Sex Differences”+hypertension.
3. “Sex differences in hypertension excluding pregnancy-related hypertension”: all keywords as in “sex differences”+hypertension, excluding preeclampsia and pregnancy.

These searches were restricted to fiscal years (FYs) 1991 to 2014. The abstracts and specific aims of each award were then classified according to disease categories, type of research (basic versus clinical), or inclusion of sex (male versus female or both). Basic research was defined as studies aimed at understanding the mechanisms of blood
pressure hypertension (whether in vitro, in animals, or in humans). Clinical research was defined as all applied research in humans, including clinical trials.

Global trends in FY2014 related to research on “sex differences in hypertension” (Online Figure IV) were obtained from Über Research, which is a global award database covering over 65 funders and over $700 billion in historical awards (http://www.überresearch.com). The search term used was sex differences+hypertension.

Results

Research on Sex Differences Funded by NHLBI

The number of awards related to “sex differences” in general (not specific to any one disease) funded by NHLBI progressively increased from 40 awards (FY1991) to 140 awards (FY2014), for a cumulative total of 2231 awards (including subprojects) during the entire period (FY1991–FY2014, Figure I). The overall dollar investment also increased from $6.1 million (FY1991) to $76.3 million (FY2014), for a cumulative total of $837 million during FY1991 to FY2014 (Figure 1). This is a 12.5-fold increase between FY1991 and FY2014.

In FY2014 (Online Figure I), 55% of the awards focused on cardiovascular disease, 19% on hypertension in pregnancy, 15% on hypertension unrelated to pregnancy, and 11% on other diseases (eg, pulmonary hypertension, sleep disorders, pregnancy complications other than hypertension, etc.). Our subsequent in-depth analyses focused on the field of hypertension (including versus excluding pregnancy-related hypertension).

General Trends in NHLBI-Funded Research on “Sex Differences in Hypertension Including Pregnancy-Related Hypertension”

The total number of awards (basic+clinical) funded by NHLBI related to “sex differences in hypertension including pregnancy-related hypertension” increased from 3 awards (FY1991) to 37 awards (FY2014), for a cumulative total of 480 awards during FY1991 to FY2014 (Figure 2A). The overall dollar investment also increased from $0.5 million (FY1991) to $18.3 million (FY2014), for a cumulative total of $191 million during FY1991 to FY2014 (Figure 2B). Up until FY2002, the number of awards related to “sex differences in hypertension including pregnancy-related hypertension” was similar for basic and clinical research (Figure 2A). Between FY2002 and FY2013, the number of clinical research awards was significantly lower than basic research, but that trend has been eliminated in recent years. The dollar investment in basic versus clinical research followed a similar trend as the number of awards (Figure 2B). In addition to the increase in the number of awards and dollar investments during FY1991 to FY2014, the variety of topic investigated in the field has also grown. On the basis of the frequency of keywords that appear in the text of the award application, in FY1991, much of the research in this field was centered on pregnancy (Online Figure II). In FY2014, in addition to pregnancy, frequent topics/keywords included: humoral factors in sex differences in blood pressure regulation, sex hormones in the regulation of endothelial function and blood pressure, hypertension in menopause, obesity–hypertension, and developmental programming of hypertension (Online Figure II).

During the same time period, more academic institutions got involved in research related to “sex differences in hypertension including pregnancy-related hypertension” as shown by the wider geographic distribution of funding (Online Figure III). Specifically, in FY1991, funding in the field was distributed among only 6 states (PA, NY, TX, WV, NC, and DC), whereas in FY2014, 25 states received funding. Of the 6 states funded in FY1991, only 4 continued to be funded in FY2014. The overall funding per state also increased during FY1991 to FY2014 (Online Figure III).

When comparing NHLBI’s investment in research related to “sex differences in hypertension” with that of other NIH Institutes, NHLBI is the leading institute followed by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK). According to the data obtained through Über Research, in calendar year 2014, NHLBI seems to be the worldwide granting leading agency for research related to “sex differences in hypertension” (Online Figure IV). Other leading international agencies dedicated to supporting research related to this filed are the Canadian Institutes of Health Research (CIRH) and the Medical Research Council (United Kingdom).

Research on “Sex Differences in Hypertension Excluding Pregnancy-Related Hypertension” Funded by NHLBI

Because pregnancy-related hypertension is specific for only one sex, to get a better understanding of true sex differences research in the field of hypertension, we further refined our analysis by excluding all pregnancy-related awards. These data showed similar overall trends as the analyses shown above in that the number of awards progressively increased from FY1991 to FY2014 (from 1 to 11 awards), for a cumulative...
total of 181 awards during FY1991 to FY2014 (Figure 3A). The overall dollar investment also progressively increased from $0.1 million (FY1991) to $6.1 million (FY2014), for a cumulative total of $76.6 million during FY1991 to FY2014 (Figure 3B).

Out of the awards related to “sex differences in hypertension excluding pregnancy-related hypertension,” ≈50% of awards examine the effects of sex hormones (either estrogens or androgens) on hypertension in only one sex. The other half focus on “sex differences” per se, that is, directly comparing males and females (Figure 3C). The number of these awards progressively increased from FY1991 (1 award) to FY2014 (8 awards), for a cumulative total of 109 awards during FY1991 to FY2014 (Figure 3C). The overall dollar investment also progressively increased from $0.1 million (FY1991) to $5.0 million (FY2014), for a cumulative total of $49.1 million during FY1991 to FY2014 (Figure 3D).

Figure 2. Research on “sex differences in hypertension including pregnancy-related hypertension” at National Heart, Lung, and Blood Institute (NHLBI) during FY1991 to FY2014. A, Up until FY2002, the number of awards related to “sex differences in hypertension including pregnancy-related hypertension” was similar for basic and clinical research. Between FY2002 and FY2013, the number of clinical research awards was significantly lower than the basic research, but that trend has been eliminated in recent years. B, The dollar investment in basic vs clinical research followed a similar trend as the number of awards. Data source: NIH RePORTER.

Figure 3. Research on “sex differences in hypertension excluding pregnancy-related hypertension” at National Heart, Lung, and Blood Institute (NHLBI) over FY1991 to FY2014. A, The number of awards progressively increased between FY1991 and FY2014, for a cumulative total of 181 awards during the entire period. B, The overall dollar investment progressively increased between FY1991 and FY2014, for a cumulative total of $76.6 million. C, Around 50% of awards examine the effects of sex hormones (either estrogens or androgens) on hypertension in one sex only, whereas the other half focus on “sex differences” per se, that is, directly comparing males and females. D, Total funding related to direct comparison of male and female research subjects, research on the effects of sex hormones in females or males only. Data source: NIH RePORTER.
Discussion

To examine current and historic funding trends related to “sex differences,” the first challenge was to define the term “sex.” On the basis of the definition adopted by the Institute of Medicine, sex is “being male or female according to reproductive organs and the functions assigned by chromosomal complement (XX for female and XY for male).” Thus, for the purposes of our search and analyses, we used the terms y chromosome, x chromosome, and sex hormones to define biological differences between males and females, that is, “sex differences.” According to the Institute of Medicine “sex refers to social and cultural factors related to being a man or a woman in a particular historical and cultural context.” Because of the fact that the term “sex” is often being incorrectly used as a synonym for “sex” in the scientific literature, we also used the term “sex” in our search to ensure that all the relevant awards were included in the analyses.

The analysis of NHLBI-funded awards related to “sex differences” in general revealed that there was a progressive increase in both the number of awards and the overall dollar investment during FY1991 to FY2014. This trend was paralleled by a similar increase in the number of publications related to “sex differences” in biomedical research and research related to diseases relevant to the mission of NHLBI during the past decade (unpublished data obtained from PubMed search using terms: sex differences, heart, lung, and blood published in the past 10 years). Although the focus of our analyses was hypertension, the analysis of “sex differences” in general provided an interesting insight into other diseases related to the mission of NHLBI in which sex differences are being examined. In FY2014, hypertension only comprised ≈15% of the total number of awards in the field of “sex differences,” and this roughly parallels the percentage of NHLBI-funded awards related to hypertension in general.

As with “sex differences” in general, the field of “sex differences in hypertension” (including or excluding pregnancy) has developed during the past couple of decades, both in the field of basic and clinical research. The field has not only grown in the number of awards and the dollar amount per award, but most importantly in the number and diversity of research topics being covered by these awards. Several of these NHLBI-supported awards have led to seminal observations in the field. For instance, using the 4 core genotype mouse model in which gonadal sex is separated from the sex chromosome complement, a study has shown that, in a gonadectomized state, blood pressure levels are higher in XX mice compared with XY mice. These observations challenged the dogma that blood pressure levels are lower in females merely because of the presence of estrogens or higher in males because of the presence of androgens. Rather, the study brought to attention that in addition to sex hormones, X- or Y-linked genes, parental imprinting, or X mosaicism contributes to sex differences in hypertension. Unrelated to sex differences, in 2007, an NHLBI-supported study by Guzik et al9 identified the importance of T cells in the development of angiotensin II–induced hypertension. This ground breaking observation marked a new direction in the research on blood pressure regulation. However, this study only included males and did not question whether T cells play the same role in the development of hypertension in females. More recently, a couple of NHLBI-supported studies showed that there are significant sex differences in the contributions of T cells to the regulation of blood pressure. Although angiotensin II infusion increases blood pressure after adoptive transfer of male T cells into male Rag-1−/− mice lacking T and B cells (as shown by Guzik et al9), female Rag-1−/− mice are resistant to male T-cell–mediated increases in angiotensin II–induced hypertension.10 Furthermore, male Rag-1−/− that received adoptive transfer of female T cells exhibited lower blood pressure levels after angiotensin II infusion compared with male Rag-1−/− mice, which had male T cells adoptively transferred.11 These studies indicated that both the sex of the host and the sex of the T cell are important factors in blood pressure regulation, and recently awarded grants from NHLBI are further investigating the mechanisms of sex differences in the immune modulation of hypertension.

Other NHLBI-supported studies related to “sex differences in hypertension” include studies examining the role of the renin–angiotensin and endothelin systems in blood pressure regulation, the contribution of sex hormones to fetal programming of hypertension, sex differences in the central regulation of blood pressure, obesity–hypertension, and sex differences in hemodynamic factors in essential hypertension in humans. Although these are the areas that are currently being funded by NHLBI, there are several research gaps that have been identified by members of the NHLBI Working Group on Sex Differences Research in Cardiovascular Disease.12

In addition to the increase in the number of awards and research topics related to “sex differences in hypertension,” the geographic funding distribution has also broadened during the past couple of decades, further contributing to the increase in diversity of research in the field. Interestingly, although most of the research on “sex differences in hypertension” at the “top 10” funded institutions is being funded through the R01 mechanism, several of these institutions have active research programs or centers with a focus on sex differences or women’s health (eg, University of Mississippi Medical Center: Women’s Health Research Center). This observation suggests that a critical mass of researchers with a common interest in sex differences research is an important factor for developing a successful research program in this field.

Although the field of “sex differences in hypertension” seems to be growing by all indices considered in our analyses, there are several caveats to this conclusion. Although the overall investment into research in the field has increased over the years, the absolute number of awards and dollars invested are only a small fraction of NHLBI’s total investment into research in general and hypertension in particular. Having said this, although the overall budget for “sex differences in hypertension” increased 12.5-fold between FY1991 and FY2014, the total NHLBI budget increased only 2.7-fold for the same time period (https://officeofbudget.od.nih.gov/history.html), suggesting that this is an emerging field. Of course, it is difficult, if not impossible, to determine the adequate number of awards for any one research field. However, given that hypertension affects both women and men similarly, it is reasonable to suggest that additional research into “sex differences
in hypertension” is warranted. Another caveat is the fact that only about half of the awards related to “sex differences in hypertension” truly focus on directly comparing males and females. Although there is certainly tremendous importance and relevance in examining the effects of gonadal hormones on blood pressure regulation in either sex, valuable knowledge on the underlying mechanisms of sex differences can be gained from direct comparisons of males and females. To encourage such studies, the NIH now mandates that sex as a biological variable be factored into the research design, analyses, and reporting in vertebrate animal and human studies (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15–102.html).

Conclusions
Over the past decade, NHLBI has continuously supported and contributed to the evolution of the field of “sex differences in hypertension.” Looking into the future, the increased scientific interest coupled with the new NIH guidelines are poised to expand our understanding about the important effects of sex in many fundamental physiological and pathological processes across the board and to contribute to the development of personalized strategies needed to prevent and cure human disease, including hypertension, in both men and women.

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References

Key Words: blood pressure ■ cardiovascular disease ■ hypertension ■ research ■ sex hormones
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Figure I. Research on “Sex Differences” at NHLBI in FY2014. In FY2014, 55% of the awards on “Sex Differences” at NHLBI focused on cardiovascular disease, 19% on hypertension in pregnancy, 15% on hypertension unrelated to pregnancy and 11% on other diseases (e.g. pulmonary hypertension, sleep disorders, pregnancy complications other than hypertension etc.). Our subsequent in-depth analyses focused on the field of hypertension (including vs. excluding pregnancy-related hypertension). Data source: NIH RePORTER.
Figure II. Research on “Sex Differences in Hypertension including Pregnancy-related Hypertension” at NHLBI over FY1991-FY2014. Based on the frequency of keywords that appear in the text of the award application, in FY1991, much of the research in this field was centered on pregnancy. In FY2014, in addition to pregnancy, frequent topics/keywords included: humoral factors in sex differences in blood pressure regulation, sex hormones in the regulation of endothelial function and blood pressure, hypertension in menopause, obesity-hypertension, and developmental programming of hypertension.
**Figure III.** Geographic distribution of NHLBI-funded research related to “Sex Differences in Hypertension including Pregnancy-related Hypertension” in the USA over FY1991-FY2014. In FY1991, funding in the field was distributed among only 6 states (PA, NY, TX, WV, NC and DC), while in FY2014, 25 states received funding. Out of the 6 states funded in FY1991, only 4 continued to be funded in FY2014. The overall funding per state also increased over FY1991-2014. Data Source: NIH RePORTER.

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Figure IV. Global funding of Research on “Sex Differences in Hypertension” in FY2014. NHLBI appears to be the world-wide granting leading agency for research related to “sex differences in hypertension”. Other leading international agencies dedicated to supporting research related to this filed are the Canadian Institutes of Health Research (CIRH) and the Medical Research Council (UK). Within the NIH, NHLBI is the leading institute followed by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK). Data source: Über Research.

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Aggregated funding amount:
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