

Editorial

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Breaking Female Hearts: The Gender Gap in Cardiovascular Research

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INTRODUCTION

Cardiovascular disease is the biggest killer of both men and women, causing 17.5 million deaths combined in 2012,¹ which is expected to rise to 23.4 million by 2030.² The leading cause of death in both sexes is ischemic heart disease, with hypertensive heart disease also appearing in the top ten and vascular complications the majority of the remainder.¹ Notably, mortality rates are increasing more rapidly in younger women compared to men.³ Given these observations, it might be expected that our knowledge of the cardiovascular system and treatment of its disorders would be based on studies performed on biological material and organisms of both male and female origin, in fairly equal proportions. However, this is simply not the case. In biomedical research, many publications do not specify the sex of the animals (presumably the reader should assume male), and where sex is specified the majority focus on males.⁴ In addition, drug studies are conducted mainly on males.⁵ Equal representation of men and women is also lacking in many large-scale clinical trials^{4,6}; somewhat surprising given that women represent a rather significant proportion of patients undergoing treatment in the clinic.

WHY?

Since most studies utilising mice or rats were performed exclusively on males, this became convention. Further studies required a continued use of males in order to relate the conclusions to previous data, and has compounded the issue. It may also have been assumed that the response of both sexes would be similar, thus negating the need for inclusion or use of females in any studies bar those on gender specific situations e.g. pregnancy. Another concern often mooted is that of confounding effects in females from the oestrous cycle. Very few studies have been conducted on this topic, so there is very little evidence to support this view. In fact, many support the opposite. One such study highlighted that the oestrus cycle can affect myocardial electrical and contractile function, but that females housed separately from males do not exhibit regular oestrous cycles and these changes are therefore negated to some extent.⁷ Thus under standard laboratory conditions, the oestrus cycle may be less relevant than assumed. Another study found that the oestrus cycle had no effect on response to ischemic injury (one of the most relevant to mortality in humans) in female rats.⁸ In addition, a meta-analytical study showed convincingly that in most settings (though in neuroscience rather than cardiac research), the female hormone cycle did not elicit any more variability in females than in males.⁹ To challenge convention, this is certainly an area that warrants further investigation in a heart specific context. Indeed, even if the oestrous cycle is found to be a significant factor should it be ignored? What if this affects factors such as presentation, symptoms and drug efficacy? This could be an important question to ask on clinical admittance to direct diagnosis and treatment in women.

Another issue is that performing studies on both sexes would require significantly more resource and investment. However, the end may justify the means. The importance of considering females at all levels; in studies at the molecular, cellular, organ and whole body level, from mice to humans, from target identification to translational therapies, is outlined below.

WHY DOES IT MATTER?

There is a clear and urgent need to consider both sexes when conducting basic research and the preclinical studies that follow. Mainly, this must be addressed due to sex-specific differences in both humans and mice. An increasing number of studies in mice demonstrate sex-specific differences in e.g. pathophysiological phenotypes, molecular mechanisms, degree of pathological response, response to drugs, and so on. All of these factors have the potential to strongly impact on the degree of success in clinical trials, and in guiding diagnosis and treatment in the clinic.

In several mouse strains where both sexes have been considered, pathological cardiac phenotypes are only evident in males, and where apparent in females, generally pathological remodelling and progression to heart failure is diminished.^{10,11} Cardiomyopathy induced by genetic means also appears to primarily affect males, and more severely so than females.¹⁰ Since cardiovascular disease affects both sexes similarly in terms of mortality in humans, these observations seem surprising. However, there are notable exceptions such as platelet-derived growth factor-C (PDGF-C) induced hypertrophy, and impaired contraction induced by overexpression of alcohol dehydrogenase, which are more pronounced in females.¹⁰ Interestingly, women are also more susceptible to alcohol-induced cardiac disease,¹² suggesting that a comorbidity of alcohol abuse in women is more of a cardiovascular disease risk factor than in men. In addition, many animal studies are based on isolated stresses and female responses may be as severe as males when presented with certain comorbidities. A study this year showed that while female hearts fared better in response to ischemia, this protection was lost in hearts with pre-existing hypertrophy.¹³ Male and female hearts were found to have different calcium handling properties, and in the setting of pre-existing hypertrophic cardiomyopathy responded differently to ischemic challenges. Thus the more pronounced functional recovery to ischemia typically seen in female hearts is blunted in the case of pre-existing hypertrophy.¹³

Drug metabolism, toxicity and efficacy can also differ significantly between males and females. In addition to presentation and outcomes, sex-specific distinctions exist in the pathophysiological mechanisms underlying cardiovascular disease.^{11,14} Within many heart failure models, gender also influences mortality, heart failure severity, and patterns of LV remodeling.¹⁵ Models of pressure overload exemplify gender differences in the patterns of hypertrophic remodelling; females are less inclined towards chamber dilatation and wall thickness increases consistent with a more compensated, concentric type of remodelling. Conversely, male hearts tend to display increased propensity towards eccentric hypertrophy.¹⁵

These observations are of particular relevance to the pharmaceutical industry since translation to the clinic is poor, and costs high. That females have rarely been considered in study design as well as in basic science research and pre-clinical trials, may in fact be an extremely significant (and simple) explanation for the poor translation from mouse to human. Data from studies on male mice are applied to both male and female humans. Given the emerging wealth of evidence to suggest that pathology and drug responses both in mouse and human females can be very different, this is an inherently flawed approach. Translational potential might be enhanced by testing novel therapies in models that include both sexes. It has been noted that rodent drug studies are conducted mainly on males, while most drugs removed from the market had greater adverse effects in women than in men.⁵ With drug related cardiac toxicity being the greatest cause for withdrawal from the market, it would certainly be prudent to include testing in female models in the pre-clinical pipeline, paying particular attention to drug related toxicity in both sexes.

Despite a substantial programme of supporting experimental and preclinical evidence, the outcome from the recent CIRCUS trial (Does Cyclosporine Improve Clinical Outcome in ST Elevation Myocardial Infarction) was disappointingly negative.¹⁶ The reasons for this have been debated,¹⁷ however sex related differences have not been discussed. This study comprised over 80% males in control and treatment groups.¹⁶ That the targeted patient group are predominantly men suggests this to be more of a gender specific disorder, or, that diagnosis and treatment is inferior in females. Indeed, a number of studies now show that to the latter.^{14,18} It might be that women were underdiagnosed, and/or that the 20% of women in the CIRCUS trial responded differently to treatment and thus 'skewed' or masked a more positive outcome in males. It would be interesting to see whether exclusion of female participants in this case might change the overall conclusion of the study. Conversely, greater efficacy might be achieved in women than in men.

WHY ARE RESPONSES DIFFERENT BETWEEN THE SEXES?

Less severe pathological responses often seen in female mice compared to males (discussed above) have been suggested to be moderated mainly by oestrogen, which can activate cardioprotective signalling pathways such as nitric oxide synthase (NOS) and Akt.¹⁰ While levels of Akt do not differ, the ratio of phosphorylated Akt to total Akt is significantly higher in female than male hearts, and increases further during reperfusion in females.¹⁹ Conversely, in some instances, oestrogen has been shown to inhibit pathways associated in some instances with pathological progression, e.g. p38 mitogen activated kinase (MAPK), which is selectively inhibited

by estrogen over the other MAPKs c-Jun N-terminal kinase (JNK), or extracellular signal regulated kinase (ERK1/2). However, this view is becoming increasingly uncertain; though undoubtedly a factor in certain settings, in others, it is not.^{10,11} Further, hormone replacement therapy was found to increase cardiovascular events in postmenopausal women,²⁰ indicating that the protective effects of oestrogen cannot be presumed.

Other pathways that may contribute to differential responses in hypertrophic and dilated cardiomyopathies include growth factors (Transforming growth factor beta; TGF β), cytokines (Tumour necrosis factor alpha; TNF α), transcription factors (MEF2) and the hormone modulator angiotensin-converting enzyme (ACE). All are generally elevated in male hearts either in the absence of stimulation, or exhibit a greater response on pathological stimulation compared to female counterparts.¹⁰

WHERE ARE WE HEADED?

In the United States, approximately half of the National Institute of Health (NIH) funded clinical studies include women, but this is not reflected in preceding experimental studies in cells or animals. The NIH initiated a big drive in 2014 to address this issue.²¹ Consideration of the role of sex and differential responses in study design had been already purported prior to this in the last decade by the European Union and the German Society of Epidemiology, followed by the Canadian Institutes of Health.⁴ Thus a worldwide drive to raise awareness and to support the inclusion of females in pre-clinical models and basic research is well underway. While significant impact has yet to be made, awareness and accumulation of evidence showing gender specific differences will likely influence design of preclinical studies and the basic science studies that support them in the future.

CONCLUSIONS

Given the major differences between the sexes in the underlying mechanisms of disease, along with diagnosis and treatment responses, clinical guidelines should take this into account. However, this is not currently common practice. For instance, the 2016 European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of acute and chronic heart failure give very little distinction between the sexes.²² This is no doubt hampered by the lack of knowledge of gender specific responses in the foundation of clinical science, i.e. basic research. Even at the level of clinical trials, women are still under represented.^{4,6} Some treatments have the potential to give more encouraging results in one sex over the other, and influence clinical trial design to focus on the most likely group to positively respond. However, in the absence of supporting evidence, clinical trials should include equal proportions of both males and females with the data reported in a sex specific manner. Perhaps, some studies could retrospectively be assessed in this way; failure of some trials might in fact be due to different responses between genders.

The prospect of conducting research on both sexes might seem daunting in terms of extra resources required, but in the long-term may impact more positively on our knowledge of the molecular mechanisms that underlie disease, and their application to clinical treatments and outcomes. Female mice seem relatively more protected against cardiac insults, internal or external when compared to males, at least in the absence of comorbidities. This in itself could provide an avenue for further exploration of cardio-protective signalling pathways and reveal further tailored targets for pharmacological intervention that might have greater efficacy in males. Further to this, future studies could more readily be conducted on different sexes at an earlier stage, before moving into *in vivo* models. Isolated cardiomyocytes for *in vitro* studies for example, do not have to come only from males. Further, human based platforms such as patient derived induced pluripotent stem cell derived cardiomyocytes from male and female patients could provide a fruitful avenue for such studies, and drug efficacy and toxicity should certainly be tested in these types of models. On the basis of this kind of research, subsequent trials could reasonably be focussed on models of selected at-risk female or male subgroups, most likely with enhanced translational potential.

The main question is, what are the signalling mechanisms that really underlie these differences and how can they be manipulated for more tailored treatments between men and women? Should we be thinking along the lines of personalised medicine on the basis of gender? Perhaps, on answering these questions, we can hope to bridge the gender gap in cardiovascular research.

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