Improving Translational Research in Sex-specific Effects of Comorbidities and Risk Factors in Ischemic Heart Disease and Cardioprotection: Position Paper and Recommendations of the ESC Working Group on Cellular Biology of the Heart

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Short title: Sex and comorbidities in ischemic heart disease

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Abstract

Ischemic heart disease (IHD) is a complex disorder and a leading cause of death and morbidity in both men and women. Sex however affects several aspects of IHD, including pathophysiology, incidence, clinical presentation, diagnosis as well as treatment and outcome. Several diseases or risk factors frequently associated with IHD can modify cellular signalling cascades, thus affecting ischemia/reperfusion injury as well as responses to cardioprotective interventions. Importantly, the prevalence and impact of risk factors and several comorbidities differ between males and females, and their effects on IHD development and prognosis might differ according to sex. The cellular and molecular mechanisms underlying these differences are still poorly understood, and their identification might have important translational implications in the prediction or prevention of risk of IHD in men and women. Despite this, most experimental studies on IHD are still undertaken in animal models in the absence of risk factors and comorbidities, and assessment of potential sex-specific differences are largely missing. This ESC WG Position Paper will discuss: a) the importance of sex as a biological variable in cardiovascular research, b) major biological mechanisms underlying sex-related differences relevant to IHD risk factors and comorbidities, c) prospects and pitfalls of preclinical models to investigate these associations, and finally d) will provide recommendations to guide future research. Although gender differences also affect IHD risk in the clinical setting, they will not be discussed in detail here.

Keywords

Cardioprotection; sex differences; ischemic heart disease; ischemia and reperfusion; translational research; comorbidities.

Abbreviations list (alphabetical order):

ACS: acute coronary syndromes

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

CVD: cardiovascular diseases

COPD: chronic obstructive pulmonary disease

e-NOS: endothelial nitric oxide synthase

GFR: glomerular filtration rate

HIV: human immunodeficiency virus

IHD: ischemic heart disease

IR: ischemia and reperfusion

LVH: left ventricular hypertrophy

MI: myocardial infarction

OSA: obstructive sleep apnoea

PAD: peripheral artery disease

PCI: percutaneous coronary interventions

ROS: reactive oxygen species

T3: triiodothyronine

VCD: 4-vinylcyclohexene diepoxide

1. Introduction

Ischemic heart disease (IHD) is the leading cause of death and morbidity in both men and women in Europe, even if age-standardized incidence and prevalence of IHD are lower in females than males.[2] Several differences in pathophysiology, clinical manifestations, treatment and effect of cardiovascular drugs due to sex have been reported as recently reviewed.[3-9]

Apart from genetic predisposition and age, risk factors including abnormal lipid profile, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, alcohol intake, and lack of regular physical activity are associated with occurrence of myocardial infarction (MI) worldwide in both sexes and at all ages.[10] However, several other diseases and lifestyle-related factors are also frequently associated with IHD, even if mechanistic links to IHD risk have not been proven yet.[11-13] The prevalence of some cardiovascular risk factors and comorbidities is different in male or female IHD patients (Figure 1), and these conditions, as well as their treatments, can also differently impact IHD risk according to sex.[14-16] Thus, sex-specific health promotion efforts may be needed to improve IHD prognosis in both women and men.[16]

It is well known that the presence of risk factors, comorbidities or specific health behaviours may also differently affect myocardial response to ischemia and reperfusion (IR) in males and females. Indeed, several animal models can be used to investigate either the mechanisms underlying sex differences, or the effects of risk factors, comorbidities and their medications.[17, 18] Consistent with clinical observations, sex-specific responses to myocardial IR injury have been observed in preclinical studies.[19] Several sex-related changes have been implicated in these differences, including androgens,[20] estrogens, nitric oxide, calcium handling (including mitochondrial permeability transition),[21-23] reactive oxygen species (ROS) formation,[24] which leads to changes in apoptosis and autophagy[25] as well as programmed necrosis,[26] to name some of them.[19] Unfortunately, current pharmacological approaches directed at attenuation of IR injury have failed to translate into clinical treatments in both males and females.[27] Possible explanation for these

disappointing results is that IHD is a complex disorder depending on a number of etiologic factors, and is frequently associated with other systemic disease states.[18, 28] Furthermore, these conditions might exert different effects in males and females. Despite this evidence, preclinical studies usually only include young and healthy male animals and/or derived tissues and cells, thus neglecting the possible effects of sex-related variables.

This ESC WG Position Paper will a) discuss biological mechanisms underlying the interaction between sex and most common IHD risk factors or comorbidities; b) discuss the advantages and challenges of preclinical studies investigating the interplay between sex, IHD, risk factors, comorbidities and associated co-medications; c) provide recommendations on strategies to enhance identification, characterization, validation and publication of studies addressing sex-related differences in comorbidities and IHD.

2. Mechanisms underlying sex-related differences in IHD

Sex classification of sexually-reproducing organisms is made according to their chromosomal complements, functional reproductive organs and levels of sex steroids.[29] Whether sex differences in IHD are due to sex, hormones, or sex and hormone interactions at various life stages is still not well known.[4, 29] Additional factors, like prenatal environment may also be crucial. In addition to sex, defined by biological factors, gender differences related to social, environmental, and community factors can also affect IHD risk.[3, 30] For example, gender can account for differences in health-seeking behaviours and thus clinical outcomes in women affected by IHD.[3] Since gender recapitulates the social and cultural role of individuals within a given society, it is usually developed in response to environment and cultural settings (including family interactions, media, peers, and education), it can change among different societies,[31] and it is very complicated to dissect and study gender differences by using preclinical studies. However, in a Canadian study of young adults with Acute Coronary Syndromes (ACS) using a newly developed composite measure

of gender, feminine gender was associated with increased risk of recurrent events independent of female sex.[32] Since it is beyond the scope of this manuscript, mechanisms underlying gender-related differences will not be discussed further in the current article.

2a. Sex chromosomes

i) Y chromosome

Compared to the X and autosomal chromosomes, the Y chromosome encodes for very few genes, divided into male-specific genes and genes with an X chromosome analogue. So far, only 71 protein-coding genes have been described, and the best known is *Sry*, gene coding for Testis Determining Factor, a transcription factor needed for testis development and testosterone production in male foetal life. Knowledge of the function of the additional male specific Y chromosome derived genes is scarce.[33, 34] Sex-related difference in IHD epidemiology make it reasonable to ask what role the non-gonadal effects of the Y-chromosome play. Importantly, the upregulation of inflammatory genes and downregulation of autoimmunity promoting atherosclerosis in men, has been linked to Y chromosome genes.[35, 36] In addition, gene and chromosome manipulation in mice has made it possible to move testis determining gene Sry from the Y chromosome to an autosome, and thereafter produce offspring with gonadal sex uncoupled from sex chromosom identity. Cardioprotection studies in these mice have shown that XY combination results in smaller MI compared to XX combination independent of gonadal sex and hormonal status through development.[37]

ii) X chromosome

Despite the difference between males and females in total number of genes due to the much larger X chromosome, dosage compensation is secured by inactivation of one of the X chromosomes in female cells. Some genes, however, seem to escape inactivation, thereby partially explaining

phenotypic diversity. Random inactivation of one X chromosome makes the female heart a mosaic of two different cardiomyocytes (one with the maternal X chromosome and one with the paternal X chromosome). [38-40] When it comes to the question of whether genes on the X chromosome have a role in IHD, associations between different forms of ischemic injury, specific X chromosomal gene variants or dosing remain to be studied.[41]. In contrast to large studies of sets of single nucleotide polymorphisms on defined chromosome loci of autosomal chromosomes, studies so far found no association between IHD and X chromosomal variants.[41] However, most studies had limited power to detect sex differences, since they mainly enrolled males.[42]

2b. Gonadal hormones and their receptors

Systemic or tissue-specific levels of gonadal hormones (estrogens, progestogens, androgens) change through different stages of life in a sex-specific pattern, and are believed to have significant impact on IHD. Several experiments involving gonadectomy prior to IR demonstrated that both female and male hearts benefit from exogenous supplementation of estradiol or testosterone, respectively.[43-46] Estradiol protects the isolated heart against IR injury via non-genomic estrogen receptors either by stimulating G protein-coupled estrogen receptors, resulting in activation of phosphoinositol 3 kinase-dependent and mitochondrial adenosine triphosphate (ATP)-sensitive potassium channels survival pathways,[47, 48] or through non-nuclear estrogen receptors leading to endothelial nitric oxide synthase (e-NOS) activation and cardioprotective S-nitrosylation of key mitochondrial proteins.[49] Preclinical studies indicate that acute administration of progesterone has a non-genomic cardio-depressive effect involving modulation of calcium handling, including Sarco-Endoplasmic Reticulum Calcium ATPase expression[50] and action potential duration[51]; anti-apoptotic effects have also been suggested, and might provide cardioprotection.[52] The role of testosterone has been controversial, and synergistic effects or co-dependency of estradiol and testosterone might also be crucial.[53, 54] Non-gonadal expression of aromatase is higher in males

than females,[55, 56] and significant conversion of androgens to estrogens takes place in the heart. Recent experimental studies indicate a dose-dependent cardioprotective effect of testosterone, but also additive cardioprotection when combined estrogen and testosterone treatment is used.[43] However, results from clinical studies of IHD after testosterone supplementation to elderly men with low endogenous levels of testosterone are inconclusive.[53, 57, 58]

2c. Pre-natal environment and foetal programming

Preclinical and epidemiological studies suggest that susceptibility to IHD can be the result of foetal programming via limitation of the final cell number in the heart, reduced vessel density and by epigenetic modification of gene expression. Sex dimorphisms could be due to foetal hormonal differences (testosterone in males) and other less well-characterized dissimilarities.[59-63] Pre- and perinatal complications like hypoxia, foetal malnutrition and maternal hypothyroidism have repeatedly been linked experimentally to increased susceptibility to IR injury of the adult heart.[63-66] Later studies confirmed the presence of DNA hypermethylation leading to reduced expression of cardioprotective protein kinase Cε, e-NOS, adenosine monophosphate kinase, and heat-shock protein 70.[67, 68] Reduced adult expression of heart mitochondrial respiratory chain proteins has also been reported after prenatal hypoxia,[69] potentially increasing vulnerability to ischemia. A limited number of studies included both sexes, and some but not all of these reported larger MI in adult male compared to female hearts after pre- or perinatal stress.[63, 66, 70]

3. Sex-specific effects of comorbidities and other confounding factors in IHD

According to sex distribution, comorbidities can be considered “general” when similarly distributed among men and women or sex-related when disproportionately represented in or exclusively limited to one sex. Divergence in prevalence (or lack of this) between males and

females for major comorbidities and confounding factors are schematically indicated in Figure 1 and discussed below. In the general population, association of IHD to single or frequently multiple diseases (and relative treatments) can impact on IHD development, IR injury and protection from it. However, much less information is currently available regarding the role of sex, and in particular whether the effects of comorbidities in IHD differ between men and women, and if so what are the underlying mechanisms. Importantly, prevalence of comorbidities and their sex-specific prognostic effect on IHD might change after stratification for age. For several risk factors or comorbidities common to males and females, no data are currently available regarding sex-specific effects of them on IHD risk (Table 1). Moreover, there are significant differences in the clinical treatment of several comorbidities in men and women that may be further complicated by the different efficacy profile of some drugs used for treatment of these comorbidities as recently extensively reviewed,[5, 71-73] and by the confounding effect of drugs that are indicated only for women (e.g. contraceptives, hormone replacement therapy).

Various preclinical models have been used to study most comorbid diseases possibly affecting IHD risk and prognosis. However, there is a critical information gap between preclinical and clinical research in this area since the majority of animal experiments are conducted on young and healthy animals of one sex only, even though the confounding effect of several risk factors and comorbidities on IHD has been known for decades.[13, 28, 74] Even more, in most animal models of comorbidities, drug treatments as done in human is lacking. The combination of multidisciplinary approaches in both male and female experimental models has the potential to unravel novel mechanisms underlying sex-related differences, but it has been rarely attempted.

3a. Age and lifestyle

i) Age

Women are affected by IHD at a later age than men.[75] On the other hand, young women have a particularly high risk of mortality following MI.[75] More women than men die each year of IHD, and the hearts of postmenopausal women are more vulnerable to ischemic insult compared to premenopausal women,[1] suggesting that aging has an effect on sex-specific differences in IHD. Ovariectomy significantly increases infarct size, but it increases by aging in female rats, independent of plasma estradiol levels.[76] Ischemic preconditioning is well-known to reduce infarct size in young male rats, but both in aged hearts and female hearts the protective effect is less evident.[28] There are also age-dependent, sex-specific differences in extracellular matrix and coronary resistance vessels, which may affect adaptation to work load.[1, 77-79]

ii) Smoking

Smoking is currently more common in males compared to females, but it has been repeatedly reported to increase IHD risk more in females than males.[80-82] Also, passive smoking exposure since birth increases risk of higher cholesterol levels in late adolescence especially in females.[83] Experimental studies on IHD and smoking including both sexes are few; however, a nicotine-induced reduction in estrogen levels has been proposed as an explanation for the increased ischemic brain damage in females.[84]

iii) Physical inactivity

Although most studies have been undertaken in men, women benefit at least as much as men from being physically active both prior to cardiac events and as part of rehabilitation.[85-89] Unfortunately, available data are limited due to adjustment for age and sex prior to presentation of clinical trial results.[87] After short-term forced exercise, sex-dependent differences in cardioprotection have been observed in preclinical models.[90] In sedentary female rats, infarct size was smaller than in age-matched sedentary males, and males benefited more from the preischemic exercise protocol.[90]

iv) Stress

Psychosocial and metabolic chronic stresses modify the atherosclerotic process, the related acute cardiovascular events[91] and other disorders such as Takotsubo cardiomyopathy differently in males and females.[92] The underlying mechanisms involve, among possible other factors, enhanced haematopoiesis and different responses of immune cells to glucocorticoid release,[93] with consequent changes in leukocyte homing to atherosclerotic plaques in response to enhanced sympathetic activation.[91] In addition, young women post-MI have a 2-fold higher likelihood of developing mental stress-induced myocardial ischemia, presumably due to increased proclivity to microcirculatory abnormalities.[94]

3b. Endocrine and metabolic diseases

i) Obesity, metabolic syndrome, diabetes

Although prevalence of obesity varies greatly within and between countries, overall, more women are obese than men, but an increased body mass index has the same deleterious effects on IHD risk in women and men across diverse populations.[95] In contrast, sex may modify the prevalence and incidence of IHD in the context of type 1 and 2 diabetes and metabolic syndrome.[96-99] Sexual disparity in the diagnosis of cardiovascular risk factors for IHD as well as the management and treatment of ACS are involved in the loss of "female advantage" in metabolic disorders[97, 99], beside any significant sex difference in the effects and complications of diabetes itself.[100-107]

ii) Hyperlipidaemia

The management of dyslipidaemia is known to be different in men and women.[108] Interestingly, in a community-based study conducted in the United States among subjects with high risk for IHD, hyperlipidaemia was more aggressively treated in white men compared to white women or black men and women.[109] In the community based Tromsø Study in Norway, higher serum total

cholesterol implied higher relative risk of MI in men than women.[110] Various experimental models of hyperlipidemia confirm increased myocardial injury due to ischemia, but the cofounding role of sex differences has not been studied yet.

iii) Thyroid disease

Although observational and experimental studies suggest that thyroid hormones might have a possible therapeutic role modifying the course of IHD,[111, 112] it remains yet unknown whether such effect translate into efficacy and safety in the clinical setting and whether they vary by sex.[113] Thyroid hormones have inotropic actions mediated through the modulation of calcium re-uptake and, in particular triiodothyronine (T3), modulates inflammatory response, apoptosis, mitochondrial function and hence progression to heart failure.[114, 115] Under experimental conditions, thyroid status markedly affects the acute response to myocardial IR.[116]

iv) Osteoporosis

IHD and osteoporosis have been seen as two independent conditions, but recent evidences may change this view.[117-119] Proposed shared mechanisms are reduced sex hormone production, elevated Follicle Stimulating Hormone in women, hyperlipidaemia, inflammation, reduced blood flow in intraosseous and coronary vascular beds, increased homocysteine level, and reduced vitamin K or D levels.[120-125] The most commonly used animal models of induced osteoporosis are based on gonadal hormone deficiency in rats or mice, addition of glucocorticoids,[126] aged or female gonadectomized Apo $E^{-/-}$ mice. All these models also increase susceptibility to myocardial IR.

3c. Cardiopulmonary and vascular diseases

i) Hypertension

a) Arterial hypertension

Hypertension approximately doubles the risk of IHD. Although recent reports have found that overall hypertension is more prevalent in men, its sex-specific prevalence varies according to age, and while in subjects <40 years old it is more prevalent in men, in subjects older than 65 years it is more prevalent in women.[127] Specific relations between IHD, hypertension and sex are also influenced by age. Surprisingly, in perspective of human clinical data, the number of experimental studies examining IR in hypertensive hearts in both sexes is limited.[128, 129]

Left ventricular hypertrophy (LVH) is more prevalent in women when the recommended definitions of LVH are currently used.[130, 131] Patients with LVH are more vulnerable to IR,[132-134] and some therapeutic strategies reducing LVH, including antihypertensive drugs, may exert beneficial effects not completely related to their hypertension-lowering effect.[132, 135] Male and female hypertrophic rat cardiac myocytes exhibit different responses to experimental IR, suggesting that sex-specific strategies should be attempted to optimize post-ischemic treatment of male and female patients with LVH.[136]

b) Pulmonary hypertension

Recent studies highlight the high prevalence of mechanical left coronary artery compression by a dilated pulmonary artery in patients with pulmonary arterial hypertension, an effect which would explain, at least in part, the angina and angina-like symptoms observed in a large number of patients with the disease.[137] The difference in prevalence of pulmonary hypertension may be explained by chromosomal, sexual hormone and/or immune system differences. Preclinical studies have identified a partly paradoxical role of estrogen and/or testosterone depending on experimental model and sex.[138, 139]

i) Atrial fibrillation

Atrial fibrillation and IHD are frequently associated in the aging population. Men have a 1.5-2 fold higher lifetime risk of incident atrial fibrillation than women, and major risk factors for atrial fibrillation are IHD, hypertension and obesity.[140, 141] Myocardial ischemia can trigger atrial fibrillation, and atrial fibrosis can sustain re-entry circuits.[142, 143] Moreover, atrial fibrillation can induce or aggravate myocardial ischemia through several mechanisms, including microcirculatory abnormalities.[144] Significant sex differences in pulmonary veins and left atrium action potential characteristics have been reported in rabbits, and they may contribute to sex-related arrhythmogenesis.[145] Available experimental models in this area of research might be used to test susceptibility to electrical induction of atrial fibrillation in conjunction with acute myocardial ischemia or post-infarct remodelling, however the role of sex in these models is still unclear.[146, 147]

ii) Heart valve disease

Aortic stenosis is frequently associated with IHD and its risk factors.[148] Compared to men, women with severe aortic stenosis have less valve calcification and more valve fibrosis, suggesting that pathophysiology of aortic stenosis and potential drug targets may differ according to sex.[149] In contrast, men with aortic stenosis develop more fibrosis, maladaptive hypertrophy and ventricular dilatation than women.[150, 151] Several small and large animal models of calcific aortic valve diseases are currently available that might be useful to improve understanding of the basic biology, determine the contributions of comorbidities to IHD development and the efficacy of early interventions.[152]

iii) Peripheral arterial disease

As with IHD, the prevalence of peripheral arterial disease (PAD) at younger ages is higher in men compared to women, but increases after menopause.[62] Preclinical studies of PAD as comorbidity to IHD are limited, as is the inclusion of both sexes in such studies.

iv) Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is frequently associated with IHD[153]. Their coexistence is associated with worse outcomes than either condition alone. Pathophysiological links between COPD and IHD include common risk factors, predominantly smoking, and systemic inflammation during COPD exacerbations. Sex-specific knowledge about the influence of COPD and its treatments on IHD and vice-versa remains incomplete.[154] Information from preclinical models is also limited.

v) Obstructive sleep apnoea

Obstructive sleep apnoea (OSA) increases cardiovascular risk, including IHD.[155] Intermittent hypoxia due to obstructive sleep apnoea may promote atherosclerosis,[156-158] and it seems to increase the risk of IHD in men, with an apparently weaker relationship in women.[159, 160] Information from preclinical models is limited.

3d. Neuro-psychological diseases

i) Stroke

A relationship between endogenous sex hormones (estrogens and androgens) and ischemic stroke or IHD has been suspected. Similar to experimental MI, in animal models of stroke premenopausal female rodents show reduced infarct size compared to male or menopausal female rodents, and estrogen administration reduces infarct size. Estrogen supplementation immediately after ovariectomy exerts neuroprotective effects, whereas it shows no beneficial effects when administered 10 weeks after ovariectomy.[161] Protective effects are mediated via estrogen receptors-α and downstream cellular signaling[162] or increase in astrocyte-specific insulin-like

growth factor-1 expression and improved mitochondrial metabolism.[163] Information from preclinical models combining IHD and stroke is limited.

ii) Degenerative brain disease

IHD is a risk factor for dementia or cognitive impairment, with an increased risk of dementia in women with IHD.[164, 165] Also, prevalence of dementia subtypes and cognitive impairment differ between men and women.An overall 3:2 difference for Altzheimers disease dementia with women more often affectd also before older age was reported. It has been hypothesized that anti-platelet/anti-thrombotic therapies could reduce the risk of dementia in IHD patients.[166] However, the protective effect of anti-platelet agents was not the same in men and women, reinforcing the importance of sex-related pathophysiological differences.

iii) Clock disruption

Circadian rhythms are driven by internal molecular clocks regulating sleep-wake cycles, heart rate, feeding, body temperature, blood pressure, hormone secretion, metabolism and bone marrow function[167, 168] reflected in diurnal clinical manifestation of diseases like MI with increased incidence of in the early morning.[169, 170] Disturbances of the normal activity and resting phase have adverse effects on cardiovascular parameters, healing responses and remodeling.[171-173] Sex- and estrogen cycle-dependent variations in circadian rhythmicity of plasma corticosterone levels in rats have been reported.[174] Female clock mutant mice were found to be protected from the development of metabolic changes and cardiomyopathy that was observed in male mice with the same mutation.[175] This protection could be mediated by ovarian hormones via differentially regulated metabolic pathways, but its importance in IHD remain to be determined.

iv) Depression and anxiety

Depression and anxiety disorders are common in male and female IHD patients, are linked to higher mortality and morbidity rates[176] and increased mortality in coronary artery disease patients.[177] Depression represented a cardiovascular risk factor comparable to obesity and high cholesterol levels in a study focusing on males only.[178] With respect to mechanisms, an experimental study in rats revealed a sexual dimorphism in the molecular response to stress, involving sex-specific differences in brain-derived neurotrophic factor (BDNF) and cyclic adenosine monophosphate response element-binding protein.[179] A point mutation of the BDNF protein caused a defect in the coagulation cascade in mice and was significantly associated to MI.[180] Interestingly, occurrence of a polymorphism in BDNF is associated to either depressive symptoms or female sex[181] therefore suggesting a direct link between change in BDNF activity and increased susceptibility to IHD in women carrying this specific variant.

3e. Gastro-intestinal tract diseases

Inflammatory bowel disease has been consistently associated with an increased risk of IHD.[182] In addition, the correlation between alterations in gut microbiota composition and IHD is gaining increasing attention.[183, 184] Interestingly, comorbidities such as obesity and type 2 diabetes are associated with alterations in gut microbiota.[185] Animal models of intestinal inflammation might be extremely helpful to dissect the molecular mechanisms underlying these interactions.[186] Several animal and human studies have shown sex-related differences in gut microbiota composition.[187-189] However, whether gut symbiosis can attenuate the effects of risk factors or reduce post-ischemic events,[190] and whether sex plays a role in these processes is still unclear.

3f. Kidney and urinary tract diseases

Disorders of the kidney and urinary tract are comorbidities with sex-specific effects in cardiovascular diseases.[191, 192] In patients with decreased glomerular filtration rate (GFR), IHD is the most common cardiovascular cause of death whereby men are more often affected than women.[193] Interestingly, uric acid levels together with GFR levels are strong predictors of IHD, particularly in women.[194-197] However, a Korean study of renal function and clinical outcomes after ST-segment elevated MI revealed no sex difference in 1-year mortality.[198] Although many animal models have been developed to study the causes and treatments of chronic kidney disease in humans,[199] most models do not develop chronic kidney disease-associated cardiovascular disease[200] except for the adenine diet model that produces rapid-onset kidney disease and cardiovascular disease.[201] Subtotal nephrectomy plus permanent coronary ligation in rats resulted in more organ damage than each condition separately,[202] however, nephrectomy did not affect the cardioprotective effect of preconditioning.[203] The role of sex in these conditions is still unknown.

3g. Immune system and blood diseases

i) Infection(s)

Infectious agents, including viruses, bacteria, and parasites, can be associated with atherosclerosis and IHD. While the association for some, like helicobacter pylori, chlamydia pneumonia, and cytomegalovirus is strong, others like influenza still need clarification. Nevertheless, large randomized prospective trials, evaluating the efficacy of antibiotic treatment for the secondary prevention of IHD have not demonstrated a reduction in the rate of events. Differences between sex in the association between infections and IHD and in response to treatment remain largely unknown.[204]

ii) Human immunodeficiency virus

Infection by Human immunodeficiency virus (HIV) and the use of some antiretroviral drugs are associated with an increased risk of cardiovascular disease that goes beyond the risk explained by traditional cardiovascular risk factors including social status. Although most studies in HIV-positive patients mainly included male subjects, HIV infection has been associated with up to twice as high risk of IHD in females as in males.[205-207] Lower body weight, slower drug metabolism and hormonal control may explain sex-related differences in antiretroviral associated toxicities and contribute to differences in outcome of co-existing IHD.[208] Furthermore, the use of IHD-related therapeutic interventions is lower in HIV-positive females than males with similar risk profiles.[209]

iii) COVID-19

The COVID-19 pandemic with debut in 2019 is another example. Age, sex and cardiovascular comorbidity significantly affected outcome (morbidity and mortality) (PMID: 32171076, PMID: 32320003). For unknown reasons middle-aged males are more vulnerable compared to middle-aged females, and the mechanism behind this finding and the connection with CVD and IHD remain to be investigated. Obviously long-term recovery and risk of IHD are unknown and need to be investigated separately in males and females.

iv) Inflammation and rheumatic diseases

Several systemic inflammatory diseases are associated with increased risk of IHD.[37, 210-214] Chronic inflammatory diseases can promote coronary microvascular dysfunction and hereby contribute to the development of myocardial ischemia and cardiovascular events even in the absence of obstructive epicardial IHD.[37, 215, 216] Autoimmune diseases are on average more frequent in women,[217] and are also characterized by cardiovascular inflammation promoting development of hypertension, LVH as well as atherosclerosis.[218, 219 220-222]These cardiovascular changes may regress in response to immunomodulatory therapy.[223] Inducible, spontaneous or engineered mouse models of chronic inflammatory diseases are available, reflecting the sex bias in susceptibility to

the specific diseases,[224-228] and the higher vulnerability to atherosclerosis.[229-231] Among those mouse models, only one spontaneously develops MI,[232] and the incidence of degenerative coronary vascular disease with MI is more pronounced in male versus female mice.[233] To the best of our knowledge, no studies are available evaluating the outcome of MI or IR in models of chronic inflammatory diseases, neither including evaluation of sex, even if clinical studies suggest sex-specific impact of rheumatic diseases on cardiovascular risk.[234, 235]

v) Anaemia

In a cohort study including over 17000 patients undergoing elective percutaneous coronary interventions (PCI)[38], pre-PCI anaemia was associated with higher prevalence of bleeding and stroke, while post-PCI anaemia had higher incidence of death, MI, target vessel revascularization, bleeding, and major adverse cardiovascular events.[220] However, no sex-related differences in outcome were found in anaemic patients compared to non-anaemic patients of either sex.[220-222]

3h. Cancer

Oncological patients are susceptible to experience cardiovascular diseases (CVD),[236, 237] due to the clustering of cardiovascular risk factors in cancer[238, 239] or cardiovascular toxicity of anticancer therapies.[240, 241] Proposed mechanisms linking IHD, sex hormones and cancer are obtained from preclinical and cellular studies, for example by regulation of hypoxia inducible factor 1α.[242-245] Experimental models combining cancer with anti-cancer therapies are needed beyond observational cohort studies. Although experimental cancer models exist, reflecting the sex bias in prevalence or severity of the specific cancer,[246, 247] so far they only focused on tumour effects, without addressing the occurrence of IHD. Mouse models of anti-cancer therapies associated with cardiotoxicity, but not specifically with IHD, are available and illustrate sex bias in susceptibility to cardiac toxicity.[248]

3i. Special conditions exclusive for a specific sex

i) Pregnancy, lactation and contraceptives

IHD is usually rare in pregnancy, although it is becoming more common for several factors, including lifestyle changes and increased maternal age, associated to stress, smoking, diabetes and chronic hypertension.[249] MI in pregnancy or the early postpartum period is associated with higher risk,[249, 250] while data on the effects of pregnancy after MI are scarce.[251] Consistent with these clinical observations, hearts of late pregnant rodents are more prone to IR injury compared to non-pregnant rodents.[252, 253] Despite this, some cardioprotective mechanisms are activated during pregnancy. For example, the pregnancy-related hormone relaxin has been shown to exert multiple beneficial cardiovascular effects during myocardial infarction, including suppression of arrhythmia and inflammation, and reversal of fibrosis[254] and amniotic fluid stem cells play a cardioprotective role following MI.[255] While higher parity is associated with a higher risk of IHD later in life, breastfeeding duration inversely impacts on IHD risk.[256, 257] Oxytocin, a main breastfeeding hormone, is cardioprotective against ischemia/reperfusion injury, mainly through the activation of pro-survival pathways.[258-260]

Oral contraceptive therapies based on estrogens are known to increase thrombotic events, however, there is scant evidence related to the adverse effects of contraception types among women with already existing IHD.[261, 262] Moreover, little is known on the confounding effects of contraceptives in women with comorbidities such as e.g. obesity on cardiovascular risk.[263]

3j. Comorbid diseases exclusive for a specific sex

i) Pregnancy-related disorders

Women with a history of common pregnancy complications or pregnancy-related disorders, including hypertensive disorders or gestational diabetes, peri-partum cardiomyopathy and persistence of weight gain after delivery are at increased risk for CVD later in life.[264, 265] Since a large proportion of women worldwide become pregnant once or twice over their lives,[265] evaluation of pregnancy outcome and in general reproductive factors may provide an unique and early opportunity to prevent IHD in women.[266] Abnormal placental development and function underlie most pregnancy disorders, including spontaneous preterm birth, foetal growth restriction and preeclampsia. Even women between 45 and 55 years of age with former preeclampsia show severe subclinical atherosclerosis.[267] In addition to its crucial role in maternal and foetal circulatory systems, the placenta is hormonally, metabolically and immunologically active.[268] Several animal models involving rodents, guinea pigs, sheep and non-human primates have been useful to address the role of placenta in foetal growth disorders, preeclampsia or other maternal diseases during pregnancy.[268-271] Using surgical, genetic, and pharmacological approaches, animal models have been also developed to recapitulate the maternal symptoms of preeclampsia and other hypertensive disorders of pregnancy,[272] as well as gestational diabetes.[273-275] To our knowledge, combination of these systems with IHD models has never been systematically attempted.

ii) Endocrine-related conditions and disorders

a) Polycystic ovary syndrome

Women with polycystic ovary syndrome[38] are characterized by hyperandrogenism, infertility and an unfavourable cardiometabolic profile in early life.[276] Data on IHD and mortality in peri- and post-menopausal women with polycystic ovary syndrome appear to be controversial, even if they seem to be at an elevated risk.[277-280] Available animal models of hyperandrogenism and ovarian morphology changes can be used to investigate polycystic ovary syndrome,[281] and might be crucial to determine the molecular mechanisms underpinning these effects.

b) Menopause

Similar to humans, rats and mice cease oestrus cycling with aging, but the age may vary with strain or other variables. To investigate the mechanisms underlying menopause and pre-menopause, 4-vinylcyclohexene diepoxide (VCD), a chemical toxin that causes ovarian failure by targeting pre-antral follicles can be used.[282, 283] VCD treatment blocks the production of female ovarian hormones, while production of androgens is preserved, representing a better model to analyse menopause rather than the loss of all ovarian hormones as would result from ovariectomy. VCD can be also administered to young adult animals to mimic early ovarian failure. Timing of gonads removal in animal models (indicated as castration if shortly after birth, prior to sexual development or gonadectomy if performed after puberty) may be critical in the development or progression of IHD. Menopausal hormone replacement therapies to prevent and treat symptoms of menopause have a complex risk-benefit pattern as they may also modify the risk for IHD in certain subpopulations of women.[284, 285] Sufficient clinical data for individual risk-benefit considerations of these treatments are missing.[286]

c) Erectile dysfunction

Vascular erectile dysfunction[38] is a strong predictor of IHD, and cardiovascular evaluation of a patient presenting with erectile dysfunction is now recommended.[287] Erectile dysfunction shares common pathways and risk factors with IHD.[288] Phosphodiesterase-5 (PDE5) inhibitors, usually reserved as treatments of erectile dysfunction and pulmonary arterial hypertension, have been shown to reduce MI size and suppress ischaemia-induced ventricular arrhythmias.[289]

d) Androgenetic alopecia

Alopecia has been associated with an increased IHD risk and there appears to be a greater risk with degree of baldness.[290-292] Alopecia is also associated with an increased risk of hypertension,

hyperinsulinemia, metabolic syndrome and dyslipidemia.[290-292] The precise mechanisms underlying these effects are currently unknown and deserve further investigation.

4. Preclinical research to assess sex-specific effects of comorbidities in IHD: opportunities and challenges

Preclinical models are crucial to test hypotheses on sex differences in cardiovascular research and to study the importance of and differences among signalling cascades.[293, 294] Similar to humans, animal models display cardiac remodelling and sexually dimorphic characteristics with respect to IR injury.[293] Here, mitochondria – which are mainly derived from the mother only – play an important role in mediating IR injury and protection from it, but also to explain the biology of sex differences.[295, 296] Experimental animal studies have reported sex differences in various aspects of mitochondrial function, some of which may explain, in part, the cardioprotection against IHD observed in pre-menopausal women. Cardiac mitochondria from female animals show decreased uptake of calcium,[297, 298] improved respiratory function,[299, 300] less oxidative stress,[299, 301, 302] greater resistance to calcium-induced mitochondrial permeability transition pore opening[303, 304] and less mitochondrial fragmentation,[305] when compared to mitochondria from male animals. Post-translational modification of mitochondrial proteins (such as aldehyde dehydrogenase and α-ketoglutarate dehydrogenase) modified ROS handling and played an important role in female cardioprotection.[302]

While animal studies are therefore of utmost importance for a better understanding of the underlying causes for sex differences in IHD, current research approaches present major limitations (summarized in Table 2). To more easily allow translation of animal data, inclusion of males and females and the use of a wider range of models, incorporating more realistic environmental and comorbid conditions are required.[28, 306] Moreover, unbiased studies can provide a general overview and avoid reductionist approaches.[307, 308] Species-specificity issues and

technical/methodological caveats should be also considered, to allow a better alignment of animal studies with IHD patients' real world, and a focus on human biology and therapeutic goals. Whenever possible global or tissue-specific knockout mice or overexpression of crucial genes involved in the modulation of gonadal sex or sex hormones should be considered to study the mechanisms underlying sex-dimorphic effects of comorbidities on IHD. The following sections will address opportunities and challenges related to these aims.

4a. Use of male and female cells, tissues, organs or organisms

Although the study of both sexes individually is important to validate scientific hypothesis or test novel therapeutic approaches, direct comparison of results in both sexes might present even greater advantages. While most signalling pathways might be commonly shared in cells or tissues derived from male or female animals, specific gene and protein expression or modifications might be affected by sex.[309] Therefore, focusing on only one sex might prevent the identification of important biological effects or promote their misinterpretation.

4b. Comorbidity models

Several animal models are currently available to reproduce comorbidities as well as sex-related conditions such as peri-menopause and menopause, to test novel therapeutic interventions and health-promoting strategies.[310-312] Combination of these models might allow the identification of sex-dimorphic effects of specific comorbid diseases on IR injury and protection from it and their underlying mechanisms. Unfortunately, not all comorbidities identified in humans can be currently mimicked in animal models, and in almost all animal studies on the effects of comorbidities in IR injury and protection from it, adequate treatment of comorbidities by state-of-the-art therapy is lacking. [28]

4c. Sex-related candidate mechanisms

Once sex dimorphisms on the effects of comorbidities on IR injury and protection from it are identified, the relative contributions of sex hormones and sex chromosomes should be determined.[313, 314] Since peripheral or "activational" effects of gonadal hormones cause the majority of sex differences, gonadectomy is usually the first experiment performed in this context, preferably in both sexes. Gonadectomy allows to determine whether the sex difference depends on the secretion of gonadal hormones in adulthood. Then, further experiments will be needed to determine relevant hormones and their downstream mechanisms of action. In addition to the exogenous administration of sex hormones, estrogen and androgen receptor knockout mice are also available.[315-317] For example, estrogen receptor-beta knockout mice have been widely used to investigate the effects of these hormones on IHD.[316, 318-321]

In case sex differences persist after gonadectomy, then permanent changes caused by gonadal hormones eventually acting at early stages of development (long-lasting, differentiating "organizational" effects) need to be assessed. If these effects also do not explain the sex difference, then extra-gonadal mechanisms related to sex chromosomes might be considered. This simplified sequential experimental approach addresses essential questions and provides the first step for finding the mechanisms explaining sex-biased effects of diseases in preclinical models. To determine whether a phenotype depends on gonadal hormones or sex chromosomes different mouse models could also be used, including the Four Core Genotypes and the XY* mouse model (advantages and limitations have been previously reviewed elsewhere).[313, 322]

4d. Species differences

Results obtained from animal species may not translate directly to women for several reasons. Firstly, the frequency of oestrous cycle in female experimental animals is species dependent. In particular, rodents present different duration of oestrous cycle and very different estrogen levels, they are poly-ovulatory while women are mono-ovulatory. Moreover, although the initial stages of follicular growth seem to be comparable between humans and rodents, differences in the later stages cannot be excluded.[323] Among small mammals, mice are the most commonly used because of the possibility to perform in vivo genetic modifications.[324] As outlined above, mice also allow the manipulation of the hormonal state and specific sex-chromosome genes and thus to discriminate between sex chromosomes, gonadal status and hormonal effects.[29]

Rats have also been used to study sex differences. However, estradiol levels do not fall as low in female rats after cessation of oestrous cycling as in women following menopause, and this represents a critical issue when using rats as a model of menopause.[325] Also, remarkable differences have been described after MI between mice and rats, when comparing males and females.[326, 327]

In large animals provided by commercial suppliers (in particular pigs), the presence of gonads should be confirmed, since some male animals may be castrated at birth. In other cases, animals might be sexually immature at the time of study (for example piglets smaller than 100 kg used in research), making extrapolation of data to adult animals problematic. Moreover, mostly female pigs are used for studies of IHD due to easier handling of these animals.[328] Finally, while preclinical models may identify biological sex differences when they exist, the complex social, psychological, environmental, community factors and constraints leading to gender peculiarities are impossible to examine in animal models.

4e. Technical caveats

The bias deriving from the preferential use of only animals of one sex is often based on practical rather than scientific concerns. Since in many fields there is a significantly larger body of literature and data sets on male mice, this further encourages the use of this sex in preclinical studies. In addition, male mice are larger and easier to be surgically manipulated, and they lack oestrous cycles. In contrast, females are smaller (requiring lower weight-adjusted drug dosages), less aggressive, easier to handle, and they generally are less expensive. However, the use of female mice with synchronized oestrus cycles strongly complicates research design.

Although most primary or stabilized cell lines are derived from animals of unknown sex, the sex of the cell/tissue donor can be determined identifying specific fragments of the X and Y chromosomes. With respect to cardiomyocyte-like cell lines, both H9C2 [38] and HL-1 origin from female mice. In addition, it is important to consider the hormonal environment of cultured cells, in particular culture media composition, since it might contain sex steroid hormones and in vitro exposure of cells to hormones may affect cellular pathways/signals of interest over several passages. Conversely, charcoal treatment could be used to eliminate or reduce hormones levels.

Sex steroid hormones initiate rapid actions that do not require gene transcription (non-genomic actions) as well as effects on gene transcription (genomic actions). Thus, duration of hormone exposure is a critical consideration in study design. Moreover, since systemic actions of hormones might significantly affect hemodynamic state, the use of in vivo animal models followed up by isolated heart perfusion studies might be helpful to eliminate in vivo confounding factors related to extracardiac hemodynamic, particularly in the pregnancy state.

Several conditions related to animal feeding, housing or breeding need accurate evaluation. Retired breeder females may be used for studies of aging, but this approach has some limitations, since it is currently unknown whether presence and number of previous pregnancies can affect over time cardiovascular function. Thus, comparisons between multiparous animals and age-matched nulliparous females or males might be inaccurate.

Housing conditions, including light/dark cycles, temperature, absence of vibrations or external noise, are crucial to maintain oestrous cycling in female rats and mice. Females housed together frequently synchronize their cycles. Disruption of sleep/wake cycles, isolation, lack of physical activity or handling conditions may increase stress imposed on animals, influence sex hormone-related pathways and therefore should be taken into account. Finally, chow composition and the possible presence of phytoestrogens should be ruled out.

4f. Documentation, costs and duration of research

ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting animal research propose to include sex of the animals among the items to be described as the minimum information in all scientific publications.[329] Similarly, revised recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals clearly report the importance of describing variables of the source population including sex.[330] However, these recommendations are not always fulfilled, even if requested by most scientific journals.

While preliminary studies can identify sex-dependent effects of comorbidities on IHD, only subsequent more complex, long and costly studies may identify the precise mechanisms underlying observed sexual dimorphisms. Combination of several available animal models will require time and a learning curve to identify the best conditions and segments of investigation. It is possible that new animal models will be needed, and these requirements might further increase costs and prolong duration of research.

Furthermore, experimental preclinical studies involving aging or pregnant animals usually present several ethical and regulatory difficulties in most countries. Duration of research in these cases is usually longer, and severe ethical restrictions apply to respect animal welfare. In addition, although studies in non-human primates represent a pre-requisite of studies in humans, costs and hurdles related to project managing are even higher and make them prohibitive for most basic science

investigators and small companies developing novel therapies for IHD. These considerations should be taken into account by investigators, Scientific Societies and Funding agencies in order to provide financing through dedicated calls or considering rewards/bonuses/incentives covering higher costs and longer duration of research.

5. Conclusions and recommendations

IHD is an epidemic and global disease affecting men and women, frequently associated with multi-morbidity in the adult and aging population. Within scientific and medical communities there is now increasing awareness that many IHD mechanisms differ between sexes, and sex differences in IHD risk factors and types of IHD have been identified. Despite this evidence, studies specifically investigating sex-specific implications of comorbidities in IHD are largely missing at all levels of research. Extremely narrowly focused studies may bias research directions and eventually miss essential aspects of human disease, including sex-related differences and their relation to comorbid disease. To overcome these hurdles, it would be necessary to account for sex, comorbidities and their treatments in a virtuous circle tightly linking preclinical, translational and clinical research (schematically illustrated in Figure 2). According to this hypothetical model, relevant clinical questions could be addressed through available preclinical models, investigating the presence of sexual dimorphisms and their underlying mechanisms. Next, the relevance of obtained results should be tested in larger animals and using in silico modelling or human derived cells or tissues, in order to finally translate results into large real-world populations of IHD patients.

Based on these considerations, the ESC WG on Cellular Biology of the Heart and invited experts provide the following Recommendations (Table 3):

1. Some confusion regarding sex or gender nomenclature still exists in the literature, and the two terms are sometimes incorrectly considered interchangeable. Proper terminology should be

always used, particularly in preclinical research involving animals, cells and tissues, that can explore biological mechanisms related to sex, but are unable to address the complex socio-cultural phenomena underlying gender differences.

2. To test whether sex is an independent biological variable, experimental protocols should include both sexes, possibly analysed simultaneously (not separately or under different conditions or timing). If not possible, results should be cautiously interpreted, or this should be highlighted as a study limitation.

3. In order to facilitate comparisons between published data, all relevant experimental details (including age, strain, sex, anaesthesia, model, timing of intervention) should be clearly provided, preferentially in parts of the text searchable in databases (e.g. title and abstract). Publishers and Editors should require a report on sex and age of experimental animals or cell lines included in full papers of biomedical research.

4. Since several preclinical models are currently available to reproduce most conditions, risk factors and comorbid diseases that might affect IHD risk and prognosis differently according to sex, an interdisciplinary approach could be useful, combining IHD and comorbidities preclinical models in male and female animals.

5. Reviewers of grant applications and manuscripts for studies addressing IHD and the different comorbidities should consider whether a potential sex-specific effect has been accounted for. If the Authors propose to generalize results based on investigations in only one sex, this should be very well motivated and potential limitations should be discussed.

6. Educational programs in cardiology and basic cardiovascular research should include elements encouraging students and young doctors to be aware of the sex differences in biology and medicine.

7. Considering the widespread, global presence of IHD and multimorbidity in the adult and aging population, research should not be limited only to the most common comorbidities in IHD but address a wider spectrum of diseases present in an adult population of both sexes and their

relative comedications. Such research adds to the basic understanding of IHD independently from the role of sex and comorbidities.

8. Research addressing sex-specific effects of comorbidities in IHD is expected to have great scientific and clinical impact, but presents several technical, methodological, economical and scientific challenges. These considerations should be taken into account by Investigators, Scientific Societies and funding agencies in order to provide financing through dedicated calls or considering rewards/bonuses/incentives covering higher costs and longer duration of research to reach this goal.

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Table 1 - Effects of general risk factors or comorbidities on IHD risk in women

Increasing risk	Decreasing risk	Unknown or unclear
Aging	Physical activity	Thyroid diseases
Smoking		Osteoporosis
Stress		LVH
Obesity		Pulmonary hypertension
Hyperlipidaemia		Atrial fibrillation
Hypertension		Heart valve diseases
Diabetes		PAD
Depression		COPD
HIV		OSA
Inflammatory diseases		Brain diseases
		Clock disruption
		Gastro-intestinal diseases
		Kidney diseases
		Anemia
		Cancer

Abbreviations used: LVH=left ventricular hypertrophy; OSA=obstructive sleep apnoea; PAD= peripheral artery disease; COPD= chronic obstructive pulmonary disease, HIV= human immunodeficiency virus.

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Table 2 - Major limitations of current research approaches to investigate the role of sex and comorbidities in IHD

- Mechanistic preclinical studies investigating sex dimorphic aspects highlighted by clinical studies are rare.
- IHD research studies are rarely combined with experimental models reproducing major comorbidities, and the role of sex is usually neglected.
- Methodological information on age/sex/hormonal status of the research material (cells/tissue/organs) or animals is often incomplete in full research papers, hampering comparisons and reproducibility.
- Simultaneous comparison of both sexes is rarely performed in preclinical studies.
- Sexual maturity, parity or reproductive senescence of experimental animals are usually under-evaluated in preclinical research.
- Castration/gonadectomy or exogenous administration of hormones are rarely employed to assess the role of sex on specific intracellular signalling pathways.
- Due to species specificities, results obtained from animal studies may not be translated directly to women.
- Complexity, duration and costs.

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2 **Table 3 - Recommendations**

1.	Correct nomenclature should be always used when describing sex- or gender-related differences in IHD.
2.	Experimental studies investigating IHD should include subjects from both sexes and, if not possible, results should be cautiously interpreted.
3.	For any observed sexual dimorphic phenotype in IHD, it should be determined whether it is dependent on the hormonal state and if it is specific to or modified by genetic sex.
4.	All relevant experimental details including age, strain and sex should be clearly provided, preferably also in the searchable parts of the MS, e.g. abstract and title.
5.	Combination of IHD and comorbidities in preclinical models in male and female animals should be encouraged.
6.	Peer-review of studies investigating IHD and comorbidities should always consider whether potential sex-specific effects have been accounted for.
7.	Educational programs in Cardiology and basic cardiovascular research should include elements addressing sex differences in Biology and Medicine.
8.	Research should include a wide spectrum of diseases present in an adult population of both sexes and consider the sex-related effects of comedications.
9.	Scientific Societies and Funding agencies should provide financing through dedicated calls or consider rewards/bonuses/incentives covering higher costs and longer duration of research in this area.

Figure legends

Figure 1

Distribution of major risk factors, special conditions and comorbidities in patients with IHD according to divergence (or lack of this) between males and females. Sex-specific prevalence represented in this Figure was derived from epidemiological data available in the literature. Abbreviations used: LVH=left ventricular hypertrophy; OSA=obstructive sleep apnoea; PAD= peripheral artery disease; COPD= chronic obstructive pulmonary disease.

Figure 2

Proposed flow-chart to investigate the role of sex and comorbidities in IHD in a virtuous circle tightly linking preclinical, translational and clinical research. For abbreviations, please see abbreviations list.

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Conflict of Interest

PF is the founder and CEO of Pharmahungary Group, a group of R&D companies.

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Figure 1

PREVALENCE OF MAJOR RISK FACTORS, SPECIAL CONDITIONS AND COMORBIDITIES IN MEN AND WOMEN WITH ISCHEMIC HEART DISEASE

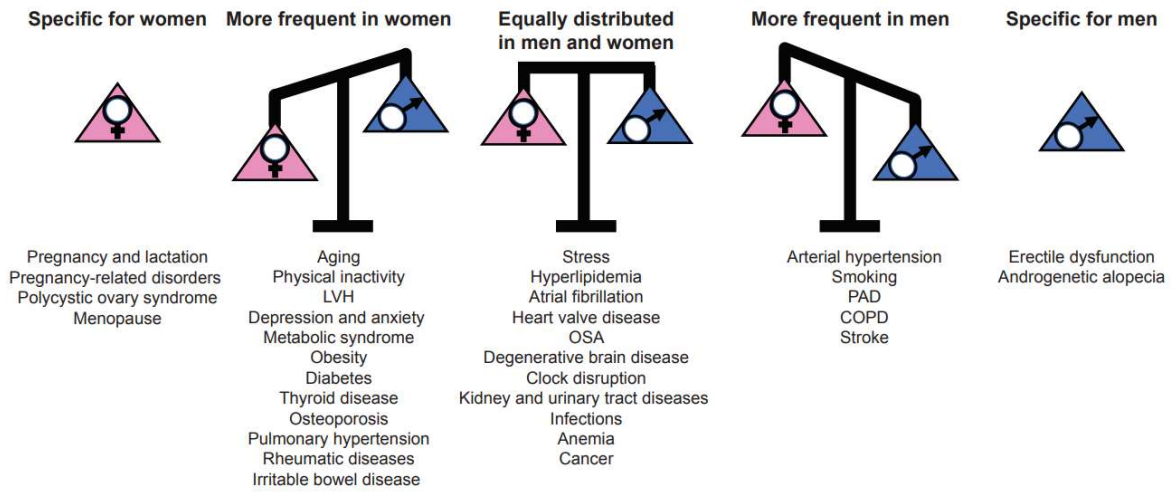

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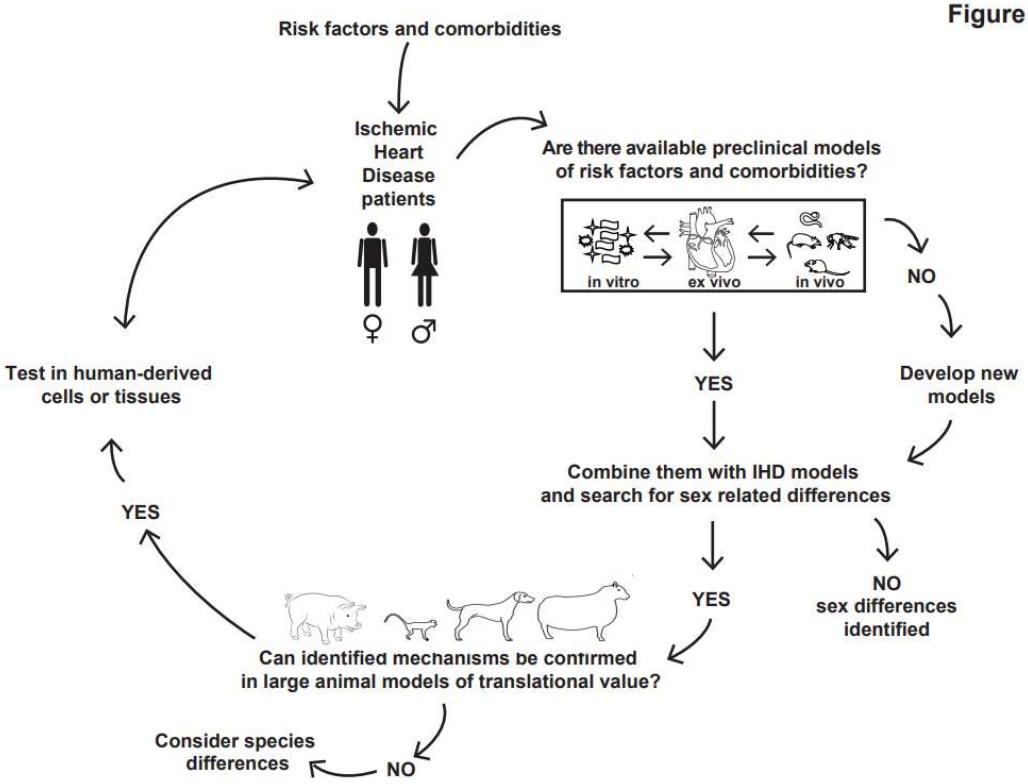
Figure 2


Risk factors and comorbidities
Ischemic Heart Disease patients
♀ ♂
Are there available preclinical models of risk factors and comorbidities?
in vitro
ex vivo
in vivo
NO
Develop new models
YES
Combine them with IHD models and search for sex related differences
NO sex differences identified
YES
Can identified mechanisms be confirmed in large animal models of translational value?
NO
Consider species differences
YES
Test in human-derived cells or tissues

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