Sex, sex steroids, and diabetic cardiomyopathy: making the case for experimental focus

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The intent of this article is to briefly outline the clinical context of sex-specific cardiac disease characteristics, to examine available experimental evidence that may offer insight into these sex differences, and to highlight potential areas for future research relating to diabetic cardiopathology. A commentary on the general features of diabetic cardiomyopathy in varied experimental settings is provided, information that is largely reliant on male-derived findings. Where experimental data are available, information relating to the sex-specificity of the cardiac impact of diabetes on the heart, in both type 1 diabetes (T1D) and type 2 diabetes (T2D), is highlighted. A case for greater experimental focus on mechanistic interrogation of the sexual dimorphism in expression of diabetic cardiomyopathy is emphasized.

Clinical Context

Physiologic and pathologic sex differences in systemic glucose regulation. There are significant differences in the regulation of systemic glucose levels in males and females even in the absence of diabetes. A clinical study examining sex-specific responses to dextrose infusion has shown that despite comparable elevations in blood glucose, systemic insulin is higher in females, suggestive of lower insulin-sensitivity (56). Insulin-stimulated glucose disposal (assessed with radiolabeled glucose) may be reduced in young adult females compared with age-matched males (7). Where insulin-sensitivity has been assessed via euglycemic clamp (constant insulin infusion titrated by glucose infusion to maintain stable blood glucose levels), sex differences are not necessarily identified (13, 60, 141).

The role of estrogen in the regulation of insulin-sensitivity is unclear. Oral contraceptive use has been associated with a 40% reduction in insulin-sensitivity (141). In contrast, aging in women (with associated reductions in gonadal estrogen production) is associated with a more marked reduction in insulin-sensitivity than observed in men (13). Thus insulin-sensitivity is reduced with estrogen increase and decrease in different circumstances.

Elevated fasting glucose is the primary diabetic diagnostic and may be used as the sole criteria in determining diabetes prevalence and incidence, as recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (52a). Although elevated fasting glucose is usual in diabetic males (68, 167), glucose dysregulation in females is more frequently characterized by a prolonged hyperglycemia following acute glucose loading (50, 68, 167, 197). It has thus been suggested that use of fasting blood glucose leads to underdiagnosis of diabetic females and therefore under-repre-
perspective in clinical trials (142). Sex hormones may play a part in manifestation of hyperglycemia in some patients; maintenance of higher estrogen levels in the luteal phase of the menstrual cycle has been associated with decreased insulin-sensitivity in some T1D women (196). Collectively, these observations support fundamental differences in insulin-dependent glucose homeostasis in women versus men.

Diabetes in women: exacerbated cardiovascular risk. There is general recognition of a sex difference in cardiovascular morbidity and mortality in nondiabetics (2a, 74, 182) and accumulating evidence that T1D or T2D may amplify this differential. The cardiovascular consequences of diabetes for women are particularly dire. Multiple combined T1D/T2D studies reveal increased cardiovascular risk in females, the Framingham Heart Study first identifying a fivefold increase in risk of heart failure in diabetic females compared with a twofold increase in risk in males (94).

Several large studies support a greater relative risk of heart disease (or a fatal cardiovascular event) with diabetes in females versus males (6, 57, 75, 124, 169). In general, cardiovascular disease presentation in women is delayed by about 10 years in comparison with men, but this relative protection is abrogated in diabetic women (126). Diabetic females are characterized by a higher risk for acute myocardial infarction than male diabetic counterparts (95, 107). For women the postmenopause increase in cardiac risk occurs in parallel with an increase in the incidence of insulin-resistance and diabetes (23, 94, 144, 183); both coronary and myocardial pathology involvement could be implicated. Whether menopause per se exacerbates risk to a greater extent in diabetics than nondiabetics is not yet clear.

Worsened postinfarction outcomes for diabetic women have been documented in studies of large cohorts, conducted in globally diverse locations and including women of varying ages (37, 58, 73, 115, 127, 158). In some studies, an increased overall risk for diabetic females (relative to males) has not always been evident, yet can be identified in particular study subgroups. In one study, increased mortality for all diabetic women 30 days following acute myocardial infarction was only maintained at 5 years follow-up for younger women (133). In another study, although the relative risk of cardiovascular disease-related death was greater in diabetic males than females, exclusion of participants experiencing previous events exposed a greater risk in females compared with males (100).

In women, risk of myocardial infarction is more sensitive to blood glucose levels than that in men (47), with a stronger correlation between hyperglycemia and acute event mortality in diabetic females (137). However, high fasting blood glucose is more event-predictive for men than women (174, 202), consistent with the observation that lower levels of hyperglycemia may be of more pathologic significance in females. Lipid metabolic disturbances and coincident hypertension appear accentuated in diabetic women, potentially contributing to greater cardiac risk (30, 80). There has been some suggestion that co-incident risk factors (e.g., age, smoking, hypertension, obesity) underlie the increased cardiovascular risk in diabetic females (93); however, meta-analyses suggest female vulnerability can persist even when these covariates are accounted for (86, 132).

Diabetes in women: a cardiac structural differential? The nature of adverse cardiovascular events may also differ between diabetic men and women, possibly reflecting underlying cardiac structural differences. An evaluation of diabetic patients undergoing percutaneous coronary intervention found that although the incidence of major adverse cardiac events was not different, diabetic females were more symptomatic (130). This may relate to increased occurrence of coronary micro- versus macrovascular disease in women compared with men in general (17). Increases in left ventricular mass and wall thickness are most marked in female patients with diabetes (94).

Diastolic dysfunction (preceding systolic abnormality) is an early sign of diabetic cardiomyopathy (15, 205), linked with increased chamber stiffness and abnormal filling (62, 113, 185). Diabetic hearts display increased diffusive interstitial collagen deposition, with perimysial and perivascular collagen accumulation (145, 166), which may contribute to diastolic stiffness. Intertitial fibrosis likely arises in part as a consequence of increased cardiomyocyte apoptosis and oncosis, as observed in biopsies from males and females (31, 61). Interestingly, diastolic dysfunction has been more closely correlated with impaired glucose tolerance than elevated fasting glucose levels (164). As already noted, for women, altered glucose tolerance rather than elevated glucose levels may be of particular pathologic relevance. Sex-specific fibrotic and/or diastolic stiffness characteristics are yet to be described.

Loss of myocardial collagen elasticity, through advanced glycation end-products (AGE) formation and cross-linking, is also implicated in the nogenesis of diabetic myocardial stiffness. Collagen and other proteins interact with glucose to form Shiff bases that are precursors of AGES (3, 179). The extent of AGE formation has been directly linked to glucose concentration (3, 23). In studies reporting pooled male and female data, serum AGE levels have been associated with left ventricular end-diastolic dysfunction in clinical T1D (11). Progression to heart failure has also been associated with serum AGE receptor levels in T2D and in nondiabetics (pooled sex cohorts) (191). More extensive investigation is required to identify potential sex-specific roles of AGE accumulation in cardiopathology.

Although in general clinical distinctions between male and female diabetic cardiopathologies have been drawn from pooled T1D and T2D cohorts (as discussed above), it is possible to glean further insight into the cardiac-specific impacts of these different disease states.

Type 1 diabetes: evidence of disease- and sex-specific cardiopathologies? For both men and women, T1D is associated with increased cardiovascular risk, with cardiovascular disease the leading cause of death from 30 years of age onward (104). At age 55, cumulative mortality in T1D men and women is ~35% (compared with 4–8% in nondiabetics) (102). Development of cardiac hypertrophy occurs early in the development of T1D (52), and diastolic dysfunction is the most common echocardiographic manifestation of T1D clinically (135, 156).

Some cardiac- and sex-specific data are available. Ischemic heart disease in young T1D women has been associated with exceptionally high mortality (105, 157), whereas in middle-aged and older cohorts this dimorphism may not be as apparent (104). A study of T1D children and adolescents identified significant increases in left-ventricular wall dimensions and diastolic
dysfunction in females but not males, with higher glycosylated hemoglobin in female diabetics, suggesting greater dysregulation of glucose handling in females (173). An early occurrence of diastolic dysfunction in young female T1Ds is especially notable. Because these young patients have no confounding hemodynamic and atherosclerotic complications, the argument for a primary myocardial defect (with female-specific exacerbation) in T1D is compelling.

**Type 2 diabetes: evidence of disease- and sex-specific cardiopathologies?** The incidence of T2D is considerably higher than T1D. For men and women combined, ~65% of T2D deaths are attributed to cardiovascular disease, involving increased coronary heart disease-related risk in particular (69). The prevalence of subclinical left-ventricular dysfunction in otherwise healthy T2D men and women is significant (54). With disease progression, increased left ventricle mass, wall thickness, and in some instances chamber dilation are reported in T2D populations (44). A component of this cardiac structural modeling may be attributed to coincident obesity, and not necessarily diabetes per se (65, 103).

Some data are available regarding sex-dependent characteristics of T2D cardiopathology. For T2D women, and for women with clinical indication of glucose-intolerance, there is a greater influence of angina on risk of coronary heart disease mortality (21). One study has estimated a substantial sex differential in a T2D-related hazard ratio for major coronary heart disease even after adjustment for other risk factors: 2.8 in males compared with 9.5 in females (91). As a cohort, T2D females exhibit greater symptomology although less obstructive coronary artery disease compared with diabetic males (176). Postinfarct prognosis for T2D women is poorer than for men, but increased mortality and morbidity may also be related to age differences, not simply attributable to sex (189). Consistent with findings from studies of pooled T1D/T2D populations, T2D females are more likely to have other cardiovascular risk factors (dyslipidaemia, obesity, hypertension), and the impact of these on the incidence of cardiovascular disease is more severe in women (91). T2D women are also reportedly less likely to receive treatment than men in comparable risk-factor settings (30, 86). This may have important outcome consequences and confound evaluation of underlying sex-specific differences.

In overview, clinical and population studies suggest diabetes induces significant and selective risk for women, particularly in relation to the occurrence and consequences of coronary disease, postinfarct outcomes, and progression to failure. There is some evidence from both T1D and T2D settings that younger women are more adversely affected by diabetes, exhibiting cardiac symptomatic jeopardy, even when the level of hyperglycemia may be lower than in men. This female vulnerability may partially reflect underlying sex differences in systemic glucose handling and a lower pathological threshold for plasma glucose elevation. Estrogen may have a role in long-term modeling and acute modulation of cardiac structure and function, although this is yet to be explicitly investigated clinically. In particular, more directed clinical research to evaluate sex-specific cardiac structural impacts of diabetes is warranted.

Despite accumulating clinical evidence of the sex-selective cardiac effects of diabetes, mechanistic insights have been limited. Dissecting the mechanisms underlying the cardiac-specific consequences of diabetic states has been the focus of a considerable experimental research effort, some of which address the sex question. The extent to which these experimental studies offer insight regarding mechanisms of diabetic cardiopathology in general, and sex-specific disease impact in particular, is considered below.

**Sex Difference In The Experimental Context?**

**Systemic glucose and insulin responses.** A number of experimental studies have described sex differences in insulin responses and systemic glucose handling - somewhat consistent with clinical findings. Healthy female rat hearts appear less sensitive to insulin than male hearts (190), and global deletion of the estrogen receptor (ERα) is associated with systemic hyperglycemia and glucose intolerance (154). Interestingly, however, ovariectomy per se did not modify insulin-resistance or blood glucose levels (63). Insulin and estrogen both exert regulatory influences on the Akt/Pi3K signaling pathway (depicted in the Fig. 1), and estrogen receptor activation modulates insulin signaling (66, 140). Given sex-specific differences in the systemic milieu of glucose and insulin control in non-diabetic experimental settings, sex disparity in diabetic responses might be anticipated. An interaction between insulin and estrogen signaling has the potential to modulate numerous downstream cardiac structural and functional characteristics associated with diabetic states.

In T1D, sex differences have been described in development of hyperglycemia in some animal models. The extent of hyperglycemia in response to pancreatic β-cell destruction with streptozotocin (STZ) is reported to be similar in male and female rats (45), whereas STZ combined with nicotineamide accentuates development of hyperglycemia in female rats.

**Perspectives**

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![Fig. 1. Identifying points of interaction for cardiac insulin and estrogen signaling pathways: potential mechanisms for sex-specific responses in diabetics. Receptor-bound insulin stimulates insulin receptor (IR) substrate 1 (IRS1) to upregulate Class I phosphoinositide 3-kinase (PI3K). A resultant phosphorylation and activation of Akt (also known as PKB) exerts downstream actions (highlighted in gray boxes) via multiple signaling intermediates to modulate cardiac modeling/performance (bold/italic text). Estrogen can modulate heart function/morphology by several mechanisms. Analogous to insulin signaling, estrogen can regulate the PI3K/Akt pathway by binding to membrane-associated estrogen receptors (ER) to activate PI3K. Additionally, estrogen binding to nuclear-localized ER modulates transcription and expression of channels/exchangers involved in determining intracellular Ca levels. mTORC1, mammalian target of ramacycin complex 1; Bad, Bcl-2 associated death promoter; GSK3β, glycosyn synhase kinase 3b; NOS, nitric oxide synthase.](http://ajpheart.physiology.org/content/early/2013/11/20/ajpheart.00141.2013/F1.large)
Higher glucose levels were attributed to greater β-cell destruction (190), which may be related to anti-apoptotic β-cell sparing effects of testosterone (136). In mice, STZ-induced hyperglycemia has been shown to be more or less marked in females, or equivalent in males and females depending on the strain studied (24, 25, 117, 128). Notably, hyperglycemia in the T1D experimental setting has been observed to be less prominent in females of the widely used C57Bl/6 strain (128).

In the context of T2D, occurrence of systemic insulin-resistance is reportedly less marked in female rodents exposed to elevated dietary fructose (63), elevated dietary sucrose (79), or within a hyperinsulinemic setting (64). This more modest female insulin-resistance/hyperglycemia is likely sex steroid influenced, since ovariectomy abrogates protection against hyperinsulinemia (63). In a T2D genetic model (Goto-Kakizaki), although plasma glucose was not sex dependent, disturbances in plasma lipids were observed only in females (43).

Thus ambiguities relating to systemic diabetic characterization of women (relative to men) are observed to some degree in experimental settings. Experimental manipulations may not necessarily produce equivalent systemic phenotypes in females versus males, and presumption that all individuals within a treatment cohort possess similar disease states (as gauged by glycemic state or insulin resistance) is questionable. Beyond questions of a sex differential in systemic insulin-sensitivity, the structural and functional cardiac implications of the diabetic state have also received considerable experimental attention.

The diabetic heart: structural aspects. Fibrosis and extracellular matrix remodeling. Experimental studies have enabled detailed and mechanistic characterization of diabetes-associated cardiac fibrosis and stiffness-clinical hallmarks of diabetic cardiomyopathy. These studies outlined below (and tabulated in Table 1) involve either distinct male cohorts or female cohorts, consist of pooled male/female data, or do not specify the sex of the animals investigated. Investigations directly addressing sex-specificity of diabetic cardiopathy are lacking. As observed clinically, increased cardiac fibrosis and collagen deposition is a characteristic of experimental T1D (96, 112, 121) and T2D (40, 120, 207) models. In vivo and in vitro studies provide some mechanistic insights regarding differences in fibrosis. Work in the STZ T1D model shows that stimulation of TGF-β in a hyperglycemic context promotes fibroblast collagen production and suggests that heightened ANG II signaling is frequently involved (10, 34, 171). Acting through the AT-1 receptor, angiotensin decreases matrix metalloprotein-2 activity in diabetic (STZ) mice, suppressing collagen degradation and promoting cardiac fibrosis (195). In the T2D experimental setting, upregulated TGF-β-receptor signaling has also been observed (i.e., in the Otsuka Long Evans Tokushima Fatty rat), with collagen deposition a precursor to hyperglycemia (123). As human studies suggest, apoptotic cardiomyocyte loss is evident when there is fibrotic infiltration in both T1D and T2D experimental models (5, 55). In vivo and in vitro studies indicate a pro-apoptotic influence of oxidative-stress associated with altered myocardial lipid metabolism, and involvement of the renin-angiotensin system (14, 22, 32, 118, 148, 149).

Increased myocardial AGE formation has been directly observed in experimental models of T1D and T2D (4, 155). Experimental approaches have identified AGE (and AGE receptors) as pro-inflammatory triggers exacerbating oxidant damage (53). Experimental evaluations of myocardial stiffness at the tissue and subcellular level have been undertaken. Interestingly, effects of ovarian steroid withdrawal in T1D female rodents have been assessed (16), although no comparison between males and females was undertaken. Passive stiffness and collagen content of excised ventricular tissue was increased in STZ-treated rats, although not in ovariectomized animals, suggesting a profibrotic effect of estrogen. The responsiveness of contractile myofilaments to activator Ca2+ was unchanged by STZ treatment alone (16).

Collectively, although experimental studies have advanced our understanding of the mechanisms involved in remodeling processes contributing to diastolic dysfunction in diabetic myocardium, characterization of sex-specific myocardial impacts of diabetes awaits future investigation.

Cardiac hypertrophy. Cardiac hypertrophy is not always evident in experimental models of diabetes. Even when heart weight may be altered, normalizing to body weight can be problematic. The majority of data derives from male studies (with some studies pooling male and female data, or not specifying sex) and are summarized below and in Table 1. Several studies have made sex-specific comparisons and are addressed in more detail (Table 2).

Rodent models of T1D are typically untreated, resulting in severe hyperglycemia and a reduction in or failure to gain bodyweight (dependent upon age). In males this can result in higher heart weight-to-body weight ratios (78, 84, 109, 125, 131, 144, 146, 168, 170, 175, 183, 199, 201), independent of heart weight changes, although in some cases, no differences are observed in heart weight or normalized heart weight (88, 150, 153, 198). Increased heart weight (nonnormalized) is not common in T1D models (25). The pattern of STZ-induced reduction in heart weight presenting as increased (183) or unchanged (204) cardiac weight index was also observed in females, although increased heart weight that persists after normalization was also observed (25). In male T1D rodents, ventricular cardiomyocyte size is reported to be higher (88, 98, 112, 150, 177, 198) or unchanged (38, 77, 101, 144, 153, 160, 161, 194) or less commonly lower (168), and left ventricular wall thickness is reported to be reduced (121, 153) or unchanged (76, 172). In studies of females, cardiomyocyte size was unchanged (25, 77, 81). One study has identified decreased cardiomyocyte size, although it is not clear which sex was studied (168).

In T2D animal models the situation regarding cardiac hypertrophy appears even more complex, sometimes confounded by animal obesity rather than body weight loss. Tibial length is sometimes preferred to body weight for normalization purposes, but with genetic models of diabetes this may also be altered relative to control (40). Many studies in males report no change in heart weight in T2D models, including Zucker rats (83, 129, 193), leptin-receptor-deficient (lepre/-) mice (72, 138, 139), leptin-deficient (lep/-) mice (116), fructose-fed animals (27, 29, 120), sucrose supplemented rats (187), Goto-Kakizaki diabetic rats (82), high-fat fed rats (134), and Otsuka Long-Evans Tokushima Fatty rats (99). When normalized to body weight, this lack of effect on cardiac size persists in the Zucker rats (8, 83, 192), fructose-fed animals (27, 29, 120), and Otsuka Long-Evans Tokushima Fatty rats (99).
In some Zucker studies heart weight is decreased relative to controls (8, 39, 192), and cardiac weight index can also be decreased in Zucker rats (184). Defining a hypertrophic response in the Zucker model is particularly problematic due to maturational variations in body weight difference relative to lean controls.

In other T2D models, cardiac weight increased in GLUT-4 knockout mice (1, 46, 85), the \textit{lepr/lepr} mouse (111), and the \textit{lep/lep} mouse (48). This increase was maintained after normalization in the \textit{lepr/lepr} mouse (111) and in some (46, 85, 92, 151) but not all (1) GLUT-4 knockout studies. Increased heart weight emerges only after normalization in Goto-Kakizaki rats (26, 41) and fructose-fed rats (206) and in one study of Zucker diabetic fatty rats (39).

In the T2D context, where cardiomyocyte size has been evaluated, studies report unchanged (39, 82, 120) or increased dimensions (1, 46, 48, 59, 111, 206); more rarely, decreased cell dimensions have been observed (83).

As with T1D studies, fewer studies in T2D have been undertaken in females. In those that have investigated, females without direct comparisons to males, heart weight was increased in GLUT-4 knockout mice (1), \textit{lep/lep} mice (33), \textit{lepr/lepr} mice (87), and in a high-fat/low STZ model (207) or unchanged in a model of multiple low-dose STZ (81). Increased normalized heart weight was also noted in \textit{lepr/lepr} mice (87) and high-fat/low STZ (207), whereas normalized heart weight was decreased in GLUT-4 knockout mice (1).

Sex-specific comparisons in relation to hypertrophy occurrence have been made in a limited number of investigations and are summarized in Table 2 (which presents only those studies that incorporate a direct sex comparison). In T1D, two studies of STZ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Effect</th>
<th>T1D</th>
<th>References</th>
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<tr>
<td>Heart weight</td>
<td>F ↓</td>
<td></td>
<td>183, 204</td>
<td>1, 33, 87, 207</td>
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<td></td>
<td>M ↓</td>
<td></td>
<td>84, 109, 125, 131, 144, 146, 170, 175, 180, 201</td>
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<td></td>
<td>M ↑</td>
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<td>28, 140</td>
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</tr>
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<td></td>
<td>M ↔</td>
<td></td>
<td>78, 88, 150, 153, 165, 198, 199</td>
<td>27†, 29, 59, 72, 82, 83, 99, 116†, 120, 134†, 138, 139, 187, 193</td>
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<tr>
<td></td>
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<td>168ª</td>
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<tr>
<td>Cardiac weight index</td>
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<td>87, 207</td>
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<tr>
<td></td>
<td>M ↓</td>
<td></td>
<td>98, 180</td>
<td>1, 138, 184†</td>
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<tr>
<td></td>
<td>M ↑</td>
<td></td>
<td>12, 25, 78ª, 84, 109, 125ª, 131, 146, 147, 177, 199</td>
<td>26†, 39, 41ª, 46, 85, 92, 111, 206</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>M/F ↔</td>
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<td>151</td>
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<tr>
<td></td>
<td>NS ↑</td>
<td></td>
<td>168ª</td>
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<td>Cardiomyocyte size</td>
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<tr>
<td></td>
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<td></td>
<td>25, 77, 101, 144, 153, 160, 161</td>
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<tr>
<td></td>
<td>NS ↔</td>
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<td></td>
<td>40</td>
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<tr>
<td>Diastolic function</td>
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<td>33ª, 87ª, 207ª</td>
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<td></td>
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<td>1ª, 208ª</td>
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<td></td>
<td>M ↑</td>
<td></td>
<td>162ª, 172ª, 177ª, 200ª, 201ª</td>
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<td></td>
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<td>1ª, 26ª, 99ª, 134ª, 138ª, 139ª, 143ª, 187ª, 193ª</td>
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<td></td>
<td>MF ↔</td>
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<td></td>
<td>71ª</td>
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<td>NS ↓</td>
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<td>Systolic function</td>
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<td>207ª</td>
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<td></td>
<td>M ↓</td>
<td></td>
<td>112ª, 121ª, 125ª, 131ª, 143ª, 147ª, 150ª, 153ª, 161ª, 180ª, 200ª, 201ª</td>
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<td></td>
<td>NS ↓</td>
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<td>178ª</td>
<td>40ª</td>
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</table>

Male only, female only, or pooled sex studies. T1D, type 1 diabetes; T2D, type 2 diabetes; F, data from females; M, data from males; M/F, data pooled from male and female; NS, sex not specified. Arrows specify alterations relative to age-matched, control animals. ↓, Diabetes-induced increase; ↑, diabetes-induced decrease; ↔, no effect of diabetes; **bradycardia will impact on cardiac function; † left ventricular values alone; cardiac weight index based on LV/tibia or LV/body weight; “based on biventricular weight; *in vivo function assessed; ‡ex vivo function assessed.
diabetes-induced increase; F, diabetes-induced decrease; F ∼ s M, no effect of diabetes; +, effect was greater in females; −, effect was lesser in females; †inferred from data presented (not directly compared in study); *contractility will be influenced by alterations in heart rate; ‡heart rate not reported.

Table 2. Sex-specific features of cardiac responses observed in experimental models of type 1 and type 2 diabetes

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Duration (weeks)</th>
<th>Age at expt (weeks)</th>
<th>Blood Glucose</th>
<th>Heart Weight</th>
<th>Heart Weight Normalized to Body Weight</th>
<th>Ventricular Developed Pressure</th>
<th>Ventricular Rate of Contraction</th>
<th>Ventricular Relaxation/Compliance</th>
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<td>Rat STZ</td>
<td>6</td>
<td>18–20</td>
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<td>Rat STZ</td>
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<tr>
<td>Rat STZ</td>
<td>5</td>
<td>?</td>
<td>↑ ↑</td>
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<td>Rat GK Genetic</td>
<td>24–28</td>
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<td>⇩ ⇩ ⇩ = ⇩ ⇩ ⇩ = 43</td>
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<td>Rat Sucrose diet</td>
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<td>24–28</td>
<td>⇩ ⇩ ⇩ = ⇩ ⇩ ⇩ = 18</td>
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<tr>
<td>Rat Sucrose diet</td>
<td>35</td>
<td>24–28</td>
<td>⇩ ⇩ ⇩ = ⇩ ⇩ ⇩ = 19</td>
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<td>Mouse High-fat diet</td>
<td>5–6</td>
<td>24–28</td>
<td>⇩ ⇩ ⇩ = ⇩ ⇩ ⇩ = 114</td>
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<td>Mouse High-fat diet</td>
<td>11–12</td>
<td>24–28</td>
<td>⇩ ⇩ ⇩ = ⇩ ⇩ ⇩ = 204</td>
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Table entries consist of studies involving male and female experimental groups (rodent strains; Rat, Wistar; Mouse, C57Bl6). M, diabetes-induced change in male; F, diabetes-induced change in female; F versus M, extent of change in female compared with male. Symbols indicate alterations relative to age-matched, control animals: ↑, diabetes-induced increase; ↓, diabetes-induced decrease; +, no effect of diabetes; −, effect was greater in females; −, effect was lesser in females; †inferred from data presented (not directly compared in study); *contractility will be influenced by alterations in heart rate; ‡heart rate not reported.

Biochemical treatment (rats, 5-wk diabetes duration) report that both male and female animals achieve similar levels of hyperglycemia, with no change in cardiac weight index in either sex (152, 181). In the T2D (mild) sucrose-fed rat, a sex comparison did not find any differential responses, and there were no effects of diet on cardiac weight index in males or females (18). In contrast, an investigation of the genetic T2D Goto-Kakazaki rat model identified a markedly greater increase in cardiac weight index in females, although hyperglycemia was equivalent in both sexes (43). The females in this model exhibit more deranged plasma lipids than males, and it is possible metabolic signals deriving from altered lipid-handling have a greater role in mediating cardioc hypertrophy in female diabetics.

To summarize the diversity of findings from an extensive range of model types (involving either exclusively male cohorts or pooled sex groups), it is not possible to ascribe different observations regarding occurrence of cardiac hypertrophy to model-specific factors, or to the duration of treatment. The explanations for variable effects on myocardial mass remain unclear, although hypertension, obesity, and insulin status are likely confounders. One possible explanation for differences between the clinical and basic research settings is that diabetes in humans is actively managed, but not so in experimental models, and hypertrophic responses to secondary diabetic complications are clinically minimized. This proposition awaits further exploration. Only a very small number of studies have pursued explicit sex-comparisons, providing an indication that when hypertrophy occurs it is more pronounced in females (somewhat consistent with clinical findings). Much more work is required in this area, and given that the Framingham study indicates that, after age, cardiac hypertrophy per se constitutes the most significant cardiac risk factor (110), this should be an important area for future investigation.

The diabetic heart; functional aspects. There have been extensive experimental efforts directed toward characterizing cardiac dysfunction in progression of diabetic cardiomyopathy. Research has moved beyond the perception of diabetes as a single condition of glucose dysregulation, with attention now directed toward distinguishing differential mechanisms in T1D and T2D that contribute to cardiomyopathic disease development (89). As discussed above, clinical investigations employing imaging techniques have established the occurrence of relatively early diastolic dysfunction, at a nonsymptomatic stage, as a predilect to later progression to systolic dysfunction. In the experimental setting there is scope to more precisely dissect the components of cardiac diastolic and systolic dysfunction using different in vivo and ex vivo approaches. With only a few notable exceptions (highlighted at the end of each of the following sections, and presented in Table 2), these experiments have been undertaken in males only, whereas some papers pool data from both sexes, and others fail to specify sex (as identified in Table 1). In general, as reflected by the paucity of female data contained in both Tables 1 and 2, there is a considerable sex-specific knowledge gap to be addressed.

Type 1 Diabetes and Cardiac Function. In males, impaired diastolic function in vivo is generally observed in rodent models of T1D (predominately STZ induced). Reduced echocardiographic E:A ratios (88, 98, 121, 150, 160, 177) and decreased end-diastolic volume (35, 49, 108) have been reported. Catheterization of the left ventricle in males has revealed impaired/delayed relaxation (78, 88, 172, 177) and increased left ventricular end-diastolic pressures (112, 150), although such changes are not always identified (88, 108). Analysis of pressure-volume relationships reveals increased left ventricular stiffness in males (143). In vivo assessment of animal T1D models has generally identified significant reductions in heart rate (2, 84, 162, 178, 200), but not always (70, 160). This contrasts with observations of equivalent heart rate in diabetic and nondiabetic patients (135) and may reflect greater (untreated) glucose dysregulation in experimental models. Evidence of diastolic dysfunction (decreased E:A ratio) was found in young adult female STZ-treated rats; however, this study did not include male comparators (90, 204).
In vivo systolic dysfunction is also reported in many investigations of T1D in males, characterized by reduced ejection fraction as assessed via echocardiography (162, 177), reduced left ventricular systolic pressure development (88, 150, 200), and a less steep end-systolic pressure volume relationship (143), although bradycardia observed in this latter study may negatively influence contractility via the Treppe effect. In males, decreased maximum velocity of shortening has also been reported (88, 153), and fractional shortening may be decreased (111, 121, 178) or unchanged (12, 78, 88, 108, 150, 172). Anesthetic choice may be an important consideration in assessment of cardiac dysfunction via echocardiography. In the STZ model of diabetes, reduced cardiac output is observed with ketamine or emidate, but not propofol (36).

In ex vivo perfused hearts from males, systolic contractile dysfunction is usually evident with T1D. Reductions in systolic pressure and rate of pressure development have been reported in hearts from STZ-treated male animals (125, 131, 147, 165, 175, 180) and in OVE26 mice, a less well-characterized model of pancreatic β-cell dysfunction secondary calmodulin overexpression (201). In the ex vivo setting, although relaxation parameters can be very accurately assessed, it is difficult to establish the presence of diastolic dysfunction under baseline conditions. Dysfunction can assessed by examining end-diastolic pressure/volume relationships and the kinetics of relaxation. In studies of females, systolic function was decreased as assessed by isolated heart function in STZ-treated rats (90, 183) associated with reduced fractional shortening and cardiac output (204).

Potential sex-specificity of cardiac functional changes in T1D has received minimal attention, with available findings summarized in Table 2. Ex vivo evaluation of young (6 wk) STZ-treated rats identified less impairment of pressure development (+dP/dt) in female compared with male hearts (152). Other isolated heart studies in adult STZ-treated rats (12–14 wk) show left ventricular pressure development is less adversely affected in females despite similar hyperglycemia in both sexes (181, 203).

At present, experimental correlates of the increased female jeopardy observed clinically in T1D have yet to be identified. In particular, available experimental evidence does not allow mechanistic resolution of the important issue of exacerbated diastolic dysfunction. It may be that relatively short-term STZ treatment (without insulin supplement) in female animal models does not recapitulate the clinical state and that extended duration studies are required.

**Type 2 Diabetes and Cardiac Function.** In males, diastolic dysfunction, evaluated via in vivo echocardiography (altered E:A ratio), is observed in Zucker rats (184), Otsuka Long Evans Tokushima Fatty rats (159), and lepr/lepr mice (33). Diastolic dysfunction could not be identified via echocardiography in lepr/lepr males. The E:A ratio can appear normal, whereas hearts transition from mild (elevated E:A ratio) to severe (decreased E:A ratio) diastolic dysfunction (62), and a single time point determination may not be definitive. Millar catheter assessment of left ventricular function is suggestive of diastolic and systolic dysfunction in male Zucker rats (184), although reduced heart rate in these animals may contribute to altered contractility. Millar catheter assessment of male lepr/lepr mice did not identify diastolic dysfunction (138). A similar lack of effect on diastolic function has been observed in male GLUT-4 knockout mice (1), Zucker diabetic fatty rats (193), or in sucrose-fed rats (187), although evidence of increased left ventricular stiffness in male Zucker rats has also been revealed by examining changes in the relationship between pressure and volume (143). Diastolic dysfunction was evident as a reduced slope of relaxation in hearts isolated from GLUT-4 knockout mice (85), lepr/lepr mice (48), and fructose-fed rats (27). In studies of females, diastolic dysfunction was evident in lepr/lepr mice (33, 87), lep/lep mice (208), and in a low-dose STZ/high fat model (207), but not in the GLUT-4 knockout (1).

In males, in vivo systolic dysfunction has been identified in numerous T2D models. High-fat (134) or high-sucrose (187) diet treatment in male rats reduces fractional shortening and ejection fraction. In male lepr/lepr mice, left ventricular developed pressure was found to be decreased in one study (97) and fractional shortening decreased in others (20, 139, 159, 188). The male Torii (spontaneous nonobese diabetic, Sprague-Dawley derived) rat exhibits reduced ejection fraction and fractional shortening (106), and the male Goto-Kakizaki rat is reported to display reduced fractional shortening at 8 but not 20 wk (26). In male Otsuka Long Evans Tokushima Fatty rats, fractional shortening was not reduced (even though diastolic dysfunction was apparent) (99). The male Zucker rat exhibits decreased cardiac output; however, coincident reductions in heart rate complicate interpretation of changes in contractile measures (39). Male mice fed a high-fat diet from birth exhibit decreased ejection fraction and elevated end-systolic volumes, suggestive of systolic dysfunction (114). No difference in systolic function was detected echocardiographically in male lep/lep mice (116) lepr/lepr mice (138) or Goto-Kakizaki diabetic rats (26).

Heart rate reduction has not been generally identified in T2D models. Some studies in male Zucker rats report no changes in heart rate (8, 28, 186), whereas others report significant heart rate reductions (39, 59, 184, 192). Heart rate was also not reduced in male lepr/lepr mice (97), leptin-deficient (lep/lep) mice, (67, 116), and male mice fed a high-fat diet (114). Male Goto-Kakizaki rat heart function was examined in unpaced, isolated hearts, and both reduced and unchanged rates were reported (42, 43). The basis of this disparity in outcomes is unclear.

Ex vivo experiments assessing isolated heart function in T2D models also reveal reduced left ventricular pressure development, contractility, and lusitropy in isolated myocardium from male lepr/lepr mice (9, 188). Isolated working lepr/lepr hearts also display reduced contractility (cardiac output, cardiac work, and left ventricular pressure development), although reduced heart rate in the male diabetic mouse likely contributes to contractility differences in such studies (71, 72). Isolated hearts from male Zucker rats (71, 192) were found to perform less contractile work, likely due in part to reduced heart rate. Hearts isolated from male Goto-Kakizaki rats demonstrated reduced systolic function despite comparable heart rates (42), whereas GLUT-4 knockout mice do not exhibit reduced contractile function (1). In females, reduced fractional shortening and ejection fraction has been observed in a low-dose STZ/high-fat model of T2D (207), but function was unchanged in female lepr/lepr mice as assessed by echocardiography (33, 87) and is also unchanged in female GLUT-4 knockout mice (1).
Sex-specific comparisons in T2D models, as for T1D models, are also modest in number (Table 2). A high-fat diet (45%) for 12 wk in rats induced equivalent weight gain in both sexes, but hyperinsulinemia, hyperglycemia, and functional decrement (reduction in ejection fraction) were only observed in males (114). In a different rat dietary study, fructose feeding for 8 mo produced a more marked reduction in ex vivo cardiac contractile function in females (measured as heart rate x pressure product), although glycemic status and heart rate reduction was the same for both sexes (18, 19). This differential was not evident earlier in disease development, and age may be an important factor in recapitulating diabetic functional effects in experimental settings. As noted above, diastolic dysfunction was observed in female leptin-receptor-deficient (lepr/lepr) mice (87) but was not established in a different study involving lepr/lepr males (139). This possible sex difference requires further verification.

It is apparent from an experimental perspective that there is limited information available regarding the comparative functional attributes of T2D male and female hearts. The diversity of experimental models is itself a challenge, and it may be that cardiopathology development in T2D takes on sexually dimorphic characteristics only in older animals. Current knowledge does not permit conclusive statements; much more information is required to begin to make informed links between clinical and experimental T2D disease differences in males and females.

Conclusions and Future Directions

More than three decades ago the Framingham study provided evidence that cardiovascular risk is elevated for all diabetics and that this jeopardy is particularly and substantially accentuated for women. Numerous studies have subsequently documented worsened cardiac outcomes for women. Given that estrogen and insulin both engage a common intracellular signaling pathway critical to cardiomyocyte function (PI3K/Akt), a sex-hormone/diabetic-disease interaction seems plausible. However, aspects of female cardiovascular pathophysiology that may exaggerate cardiovascular diabetic risk remain to be identified and may include increased vulnerability to coronary microvascular disease, age-dependent impairment of insulin-sensitivity and differential susceptibility to hyperglycemia.

Since Framingham, considerable progress has been made in developing and analyzing a multitude of experimental models of diabetes. The evidence (as reviewed) indicates that animal models of both T1D and T2D recapitulate to varying extents aspects of diabetic and systolic dysfunction, together with structural pathology including fibrosis, loss of compliance, and in some instances ventricular hypertrophy. Perplexingly, little of this work has explored the important sexual dimorphism of diabetic cardiomyopathy. Only a small number of experimental studies have addressed this question; thus prospects for gaining important mechanistic insights from further experimental enquiry are considerable. Recent attention has been focused on the lack of literature involving sex-specific aspects of pathophysiological states in general (122), and this deficit is especially pronounced in relation to diabetic cardiomyopathy.

In particular, longitudinal animal imaging studies to examine sex-specific and critical functional transition milestones are yet to be undertaken. Systemic parameters may not necessarily be indicative of similar cardiopathologic impact in females and males, and a more nuanced approach to evaluating relationships between systemic and cardiac responses is required. Given the recognized importance of early occurrence of diastolic dysfunction in diabetes, preceding symptomatic progression to systolic dysfunction, it would be expected that the use of animal models to pursue these studies could prove very valuable. There is evidence in humans that the echocardiographic diastolic signatures for healthy women and men differ (163). Exploration of the basis of this physiological difference, and of the sex-specific pathology of diastolic dysfunction in animal diabetic models, should be especially informative.

Understanding how cardiac dysfunction can be distinguished at the in situ/ex vivo level in T1D and T2D states, and in particular between females and males, remains a challenge. The early pathology observed in T1D in relatively young women presents a problem that might be effectively addressed in the experimental realm. For T2D, working with older animals and evaluating the continuum of hyperglycemic states on cardiac function is entirely feasible. The case for experimental pursuit of mechanistic insight into sex differences, and sex-steroid influences in the etiology of diabetic cardiomyopathy, is particularly compelling and provides important incentive for future investigation with clear therapeutic potential.

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AUTHOR CONTRIBUTIONS

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