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Sex-based differences in myocardial contractile reserve

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Petre RE, Quaile MP, Rossman EI, Ratcliffe SJ, Bailey BA, Houser SR, Margulies KB. Sex-based differences in myocardial contractile reserve. Am J Physiol Regul Integr Comp Physiol 292: R810–R818, 2007. First published September 28, 2006; doi:10.1152/ajpregu.00377.2006.—Recent studies have identified sex differences in heart function that may affect the risk of developing heart failure. We hypothesized that there are fundamental differences in calcium (Ca) regulation in cardiac myocytes of males and premenopausal females. Isometric force transients (n = 45) were measured at various stimulation frequencies to define the force-frequency response (FFR) (0.5, 1.0, 1.5, and 2.0 Hz) during either changes in bath Ca ([Ca]o) (1.0, 1.75, 3.5, and 7.0 mM) or length-tension (20, 40, 60, and 80%, Lmax) in right ventricle trabeculae from normal male (MT) and premenopausal female (FT) cats. Force-Ca measurements were also obtained in chemically skinned trabeculae. Under basal conditions (0.5 Hz, 1.75 mM Ca, 80% Lmax) both MT and FT achieved similar developed forces (DF) (MT 11.0 ± 1.7 mN/mm2; FT 10.2 ± 1.1 mN/mm2). At low rates and lengths, there is no sex difference. At higher preloads and rates, there is a separation in DF in MT and FT. At basal [Ca]o, both MT and FT exhibited positive FFR (2.0 Hz, 1.75 mM Ca: MT 38 ± 3, FT 21 ± 4 mN/mm2); however, at higher [Ca]o, MT achieved greater DF (2.0 Hz, 7.0 mM Ca: MT 40 ± 3 and FT = 24 ± 4 mN/mm2). We detected no sex difference in myofilament Ca sensitivity at a sarcomere length of 2.1 μm. However, rapid cooling contractures indicated greater sarcoplasmic reticulum (SR) Ca load in MT at higher frequencies. Despite virtually identical contractile performance under basal conditions, significant sex differences emerge under conditions of increased physiological stress. Given the lack of sex differences in myofilament Ca sensitivity, these studies suggest fundamental sex differences in cellular Ca regulation to achieve contractile reserve, with myocardium from males exhibiting higher SR Ca load.

myocardium; force-frequency response; length-tension relationship; myofilament calcium sensitivity; sarcoplasmic reticulum calcium load

RECENT RESEARCH INDICATES that there may be differences in myocardial function in male and female hearts. Several observations support the hypothesis that there are sex-based differences in heart function and these differences will impact the risk for the development of heart failure. For example, females are more resistant to ischemia-reperfusion injury (27) and are less prone to heart failure during chronic pressure overload (16). This resistance is thought to be due in part to differences in nitric oxide synthase (NOS) expression and nitrosylation and sarcoplasmic reticulum (SR) loading ability (13, 27). Finally, pathological cardiac hypertrophy tends to be attenuated in female patients with ischemic heart disease or chronic pressure overload in animal models (12, 42). Despite these and other reported sex differences in myocardial responses to pathological stress, there has been few if any data directly addressing whether there are sex differences in the myocardial responses to physiological stress.

The current operating paradigm indicates that cardiac muscle has three primary mechanisms for adaptations to acute increases in physiological demand as occurs during exercise. Specifically, increases in resting muscle length, increases in frequency of contraction, and increases in adrenergic stimulation all tend to enhance contractility in normal mammalian myocardium. The inotropic response to increases in stimulation frequency is often referred to as the force-frequency response (FFR) and is typically associated with an increased amount of calcium (Ca) taken up and released by the sarcoplasmic reticulum during each contraction. The inotropic response to increased resting muscle length (preload) provides the basis for the Frank-Starling relationship and is closely related to length-dependent changes in myofilament Ca sensitivity. During exercise, or other physiological stress, increases in adrenergic tone further increase contractility and enhance myocardial relaxation via PKA-dependent phosphorylation of molecules, which alter both Ca cycling and myofilament Ca sensitivity.

The goal of this research was to determine whether there are differences in contractile reserve in male vs. female hearts. To avoid potentially confounding effects of sex differences in vascular responses (36) or cardiac morphology, we measured force-generating capacity directly in isolated thin myocardial trabeculae and normalized all force measurement by cross-sectional area. In this model system, we examined the FFR, the length-tension (L-T) relationship, myofilament Ca sensitivity, and SR Ca load. We also explored whether there might be sex

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Sex Differences in Myocardial Contractile Reserve

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Differences in the interaction between the FFR and L-T relationship or the peak force generating capacity of isolated trabeculae in the presence of excess Ca availability. Our findings indicate that although trabeculae from normal male and female feline hearts exhibit virtually identical contractile performance under basal conditions, significant differences emerge with increased physiological stress.

Methods

Tissue procurement. Normal myocardium was obtained from adult male and female cats (n = 32). Adult cats (Liberty Research, Waverly, NY) were cared for and used according to the 1996 Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and protocols were approved by the institution’s Institutional Animal Care and Use Committee. The cats were anesthetized using pentobarbital sodium (50 mg/kg ip), and the heart was rapidly excised and placed in retrograde perfusion of the aorta with a modified Krebs-Henseleit buffer (KHB) containing 20 mmol/l CaCl2, 0.25 mmol/l Na2-EGTA, and 0.11 mmol/l of MgCl2 at room temperature to facilitate clearing of the coronary arteries. The right ventricle free wall was removed for muscle strip experiments.

Multicellular muscle preparation and mounting. Trabeculae (average dimensions: 0.30 ± 0.02 mm wide, 0.27 ± 0.02 mm thick, and 1.89 ± 0.11 mm long) were dissected from the right ventricle and mounted in a slackened position in a custom-designed isometric muscle chamber (Scientific Instruments, Heidelberg, Germany) between a platinum/rhodium wire with a basket-shaped extension of a force transducer at one end and a hook connected to a microdisplacement device at the other, as previously described (37). Solution changes were exchanged with BDM-free KHB with increasingly higher Ca concentrations until the solution in the chamber contained 1.75 mmol/l of CaCl2. All solutions were kept at 37°C and bubbled with 95% O2-5% CO2 gas mixture to maintain a pH of 7.4. The solution in the muscle chamber was provided with a gas overlay. The trabeculae were continuously stimulated at 0.5 Hz, by 3.0 ms asymmetric pulses with an energy 20% above threshold (typically 3–7 V). After initiating stimulation in solution containing 1.75 mmol/l of Ca2+, the trabeculae were left for 45 min at a very low preload to equilibrate.

Subsequently, the trabeculae were released to a “slack” length [the length at which no developed force (DF) occurs], to balance the force transducer. Slowly, the trabeculae were stretched to a length (L0) where an active DF can first be identified. The muscle length at L0 was noted on the micrometer, and the muscle was further stretched to a length (Lmax) where maximal isometric force development occurred. The final length was set at 80% of the difference between Lmax and L0 [80% (Lmax − L0)]. At this length, the trabeculae exhibit stable isometric force measurements and moderate levels of resting tension. The trabeculae were paced at 0.5 Hz at 80% (Lmax − L0) for 30 min before the experiment. At this point, trabeculae were randomly chosen to undergo either FFR/L-T, FFR/extracellular Ca or L-T/extracellular Ca experiments. A separate sample of trabeculae were used for the force-Ca experiments.

Force-frequency and length-tension experimental design. Once stabilized (at 0.5 Hz), trabeculae from adult male and female cat hearts underwent FFR experiments. Steady-state twitches were recorded at 0.5, 1.0, 1.5, and 2.0 Hz. FFR experiments were performed at each of five muscle lengths, as determined by % of (Lmax − L0) [20, 40, 60, 80, and 100% (Lmax − L0)]. Muscles were stretched using the micromanipulator device. Muscles were allowed to equilibrate for a period of 15 min between length changes. Experiments were performed at a bath Ca of 1.75 mmol/l. At each concentration, steady-state twitches were recorded at 0.5, 1.0, 1.5, and 2.0 Hz. Muscles were allowed to equilibrate for a period of 15 min between solution exchanges. These experiments were all performed at 80% (Lmax − L0).

Calcium sensitivity experiments. Trabeculae for this protocol were dissected and mounted as explained above in a muscle bath system equipped to determine sarcomere length via laser diffraction. Sarcomere length was monitored and held constant throughout the experiment. Muscles were skinned for 60 min with a relaxation solution containing 1% Triton X-100. High EGTA relaxation solution contained (in mM): 53 KCl, 10 EGTA, 20 MOPS, 1 free Mg2+, 5 MgATP2−, 12 creatine phosphate, 10 IU/ml. Solutions had an ionic strength of 0.15 M and pH 7.0. After skinning, muscles were bathed in high EGTA relaxation solution, and sarcomere length was set at a length of 2.1 μm. Muscles were bathed in low EGTA relaxation solution prepared by substituting 0.1 mM EGTA for the 10 mM EGTA. Force generation was measured at a range of Ca2+ concentrations, ranging from 0.1 to 10 mM. The skinnning protocol was adapted from Arteaga et al. (4); the forces were normalized to maximal force generation, and the points were fitted with the Hill equation (4). Solutions were made using calculations obtained by the Maxchelator program (http://www.stanford.edu/~cpton/maxch.html).

SR Ca load experiments. Trabeculae for this protocol were dissected and mounted as explained above in a muscle bath system equipped with both warming and cooling circulating bath systems. Once stabilized (at 0.5 Hz), rapid cooling contractures (RCCs) were measured at a range of frequencies (0.5–2.5 Hz) by stopping electrical stimulation and rapidly cooling the trabeculae to −5°C, using the cooling circulating bath. Rapidly cooling the muscle simultaneously causes the SR to release all Ca while also prohibiting Ca transport mechanisms, including sodium-calcium exchange (NCX) and SR and sarcocellular Ca pumps. Upon rewarming, these mechanisms are reactivated and relaxation occurs.

Statistical analysis. Results are presented as means ± SE. In addition to DF, we evaluated the peak rates of force development (+dF/dt) and decline (−dF/dt). Data were analyzed using mixed-effects modeling to evaluate the effects of sex, stimulation frequency, and preload individually and to examine interactions between these factors (33). Differences were considered significant if the probability of the chance of occurrence (P) was < 0.05. Mixed-effects models were used, as they allow for the nonindependence among repeated measurements. Force-calcium relationships were assessed by fitting a sigmoidal curve to the data.

Results

FFRs. As illustrated in Fig. 1, isolated myocardial trabeculae exhibited significant increases in twitch force, as stimulation frequency was increased from 0.5 Hz to 2.0 Hz, while preload was held constant at L90. This observation holds true for trabeculae from both male and female hearts. Additionally, at a lower stimulation frequency (0.5 Hz), trabeculae from males produced force that was comparable to that produced by trabeculae from females. However, at higher stimulation frequencies (1.5 and 2.0 Hz), force produced by trabeculae from males was significantly higher than that produced by trabeculae from females, and there was a significant sex difference in the overall force-frequency response (P < 0.002). In these experiments, the peak force achieved by male trabeculae was 30% higher than that achieved by female trabeculae.

Force-frequency and length-tension interactions. We observed significant changes in the magnitude and slope of the force-frequency response when preload was varied. When data derived from males and females were pooled, the interaction...
between preload and frequency-dependent responses was highly significant ($P < 0.0001$). As illustrated in Fig. 2, when trabeculae from males were analyzed separately (Fig. 2A), stepwise increases in preload from $L_{20}$ to $L_{100}$ induced increases in force development at any given stimulation frequency: an intact Frank-Starling response ($P < 0.0001$). Representative raw twitch data are displayed in Fig. 3. We also observed that stepwise increases in preload were associated with progressive increases in the slope of the force-frequency relationship (Fig. 2B, left). In trabeculae from females, we

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**Fig. 1.** Force-frequency response. Representative examples of raw data from a force-frequency experiment in normal male (A) and female (B) trabeculae. Force-frequency relationships were determined between 0.5 Hz and 2.0 Hz. Experiments were performed in Krebs-Henseleit bicarbonate buffer containing 1.75 mM Ca$^{2+}$. Trabeculae were stretched to 80% ($L_{\text{max}} - L_o$), where $L_{\text{max}}$ is defined as the length at which maximum force development is generated and $L_o$ is defined as the shortest length that produces a contraction when stimulated at 0.5 Hz. Steady-state twitch tension is shown at 0.5 (black), 1.0 (red), 1.5 (green), and 2.0 (blue) Hz. C: average steady-state twitch tension in normal feline cardiac trabeculae (male and female) as a function of stimulation frequency. $*P \leq 0.001$ between groups.

**Fig. 2.** A: average steady-state twitch tension in normal male and female feline cardiac trabeculae as a function of stimulation frequency at varying lengths [20% (■), 40% (●), 60% (▲), 80% (▼), and 100% (●) ($L_{\text{max}} - L_o$)]. Trabeculae were studied at a bath Ca$^{2+}$ concentration of 1.75 mM. B: slope of the force-frequency response as a function of length [%($L_{\text{max}} - L_o$)] in male and female feline cardiac trabeculae. Experiments were performed in 1.75 mM Ca$^{2+}$.
again observed an intact Frank-Starling response at any given stimulation frequency \((P < 0.0001)\), but increases in preload were associated with smaller changes in the slope of the force-frequency relationship (Fig. 2B, right). As a result, the peak force developed at 100% \(L_{\text{max}}\) and 2.0 Hz was 37% greater in male trabeculae than in female trabeculae. When applied to the developed force data, our mixed-effects model indicated nearly significant interactions between sex, preload, and frequency \((P < 0.057)\).

As summarized in Table 1, we also examined the interaction between preload, stimulation frequency, and sex on several twitch parameters. For trabeculae from both males and females, we observed significant interactions between preload and stimulation frequency on the peak rates of force development \((+\text{dF/dt})\) and force decay \((-\text{dF/dt})\), as well as the time to peak force (TPF) development. However, as with developed force, the magnitude of the preload-dependent augmentation of responses to increased stimulation frequency was lower in trabeculae from females than it was in trabeculae from males. Indeed, our mixed effects model indicated a significant interaction between sex, preload, and frequency \((P < 0.057)\) for the changes in \(+\text{dF/dt}, -\text{dF/dt},\) and TPF. Together, these data indicate that under conditions favoring maximal performance (high preload and high stimulation frequency), cardiac muscle from normal female hearts produces less force, takes longer to reach peak force, and takes a longer time to relax compared with cardiac muscle from age-matched normal males.

Effects of extracellular Ca on the force-frequency relationship. To examine the peak force development in the setting of excess Ca availability, we examined force-frequency responses during graded increments in extracellular \(\text{Ca}^{2+}\) \([\text{Ca}^{2+}]_o\). As illustrated in Fig. 4, increases in \([\text{Ca}^{2+}]_o\) resulted in concentration-dependent increases in force development. The effect of increases in \([\text{Ca}^{2+}]_o\) on force development was observed in cardiac trabeculae from both normal males and normal females. We also observed a significant interaction between \([\text{Ca}^{2+}]_o\) and stimulation frequency such that higher \([\text{Ca}^{2+}]_o\) was associated with a shallower slope of the force-frequency relationship \((P < 0.0001)\). This effect resulted in a convergence of peak force development within each sex grouping, so that trabeculae reached a similar peak DF at high stimulation frequencies (2.0 Hz), despite substantial differences in \([\text{Ca}^{2+}]_o\). With preload fixed, these data define a ceiling of peak force generation: once saturating levels of bath \(\text{Ca}^{2+}\) are employed, increasing stimulation frequency generates little further increase in force. Conversely, once force is maximized via an isolated increase in stimulation frequency, increments in bath \(\text{Ca}^{2+}\) generates little further increase in force. Moreover, as with the interaction between preload and stimulation frequency, the interaction between \([\text{Ca}^{2+}]_o\) and stimulation frequency indicates that sex differences in contractility are mainly apparent under conditions favoring peak contractile performance, whereas male trabeculae exhibited a 40% higher peak force than female trabeculae (Fig. 5).

Myofilament calcium sensitivity in chemically skinned trabeculae. To help determine whether the differences in force-generating capabilities in male vs. female muscle strips are due to differences in Ca sensitivity, myofilament Ca sensitivity curves were constructed from skinned preparations of isolated trabeculae.
1. Effect of varying preload and stimulation frequency on the contractile properties of isolated trabeculae

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Values are presented as means ± SE. Freq, frequency; DF, developed force; TPF, time to peak force; DiaF, diastolic force; +dF/dt, contraction change in force per change in time; −dF/dt, relaxation change in force per change in time.

trabeculae from male and female hearts. As shown in Fig. 6, the curves are nearly identical, indicating that there is no difference in myofilament Ca sensitivity between normal male and female trabeculae.

**Fig. 4.** Average steady state twitch tension as a function of stimulation rate (frequency, Hz) were studied at bath calcium concentrations of 1.0 (■), 1.75 (○), 3.5 (▲), and 7.0 (▼) Hz. Male and female trabeculae were studied at a length of 80% (Lmax − L0).

**SR Ca load.** To determine whether the differences in force-generating capabilities in male vs. female muscle strips are due to differences in ability of the SR to load Ca, RCCs were employed at five different frequencies (0.5–2.5 Hz).
As shown in Fig. 7, SR Ca load increases with increasing stimulation frequency in male, but not female, trabeculae (P < 0.001).

DISCUSSION

These studies demonstrate that there are sex differences in the peak force-generating capabilities of the normal feline myocardium. Such sex differences are apparent at higher stimulation frequencies during conventional force-frequency experiments conducted at a fixed preload. We also observed sex differences in the interaction between preload and force-frequency responses such that preload-dependent increases in the slope of the force-frequency response are reduced in females. Blunted peak contractile responses in trabeculae from females include reduced peak rates of force generation and decline. In complementary experiments, exploring the effects of increases in [Ca²⁺]₀ upon myocardial contractility, we again observed lower peak force generation in cardiac trabeculae from females. Studies in skinned fibers demonstrate that these sex differences in peak force generation are not likely to be attributable to sex differences in myofibrillar Ca sensitivity. Finally, studies using RCCs to assess SR Ca load indicate that at higher frequencies, male trabeculae load more Ca with respect to basal Ca load. Together, our findings support the existence of fundamental sex differences in myocardial contractile reserve in a large animal model with excitation-contraction coupling and Ca-handling dynamics that closely mimic those observed in humans.

There is extensive evidence that in normal large mammalian tissue, increasing stimulation frequency results in a positive FFR (1, 30, 37, 38, 46). This is thought to be due in part to a frequency-dependent increase in Ca entry, which results in increased SR Ca uptake and increased SR Ca loading. This increase in SR load allows more Ca to be released for subsequent myofilament activation. From this perspective, our findings of equivalent baseline (low stimulation frequency) force generation in myocardium from males and females suggests similar SR Ca loading at baseline, while differences in force...
generation at high stimulation frequencies suggest the possibility of lesser increases in SR Ca load in trabeculae from females. This interpretation is supported by our experiments in which supraphysiological increases in $[\text{Ca}^{2+}]_o$ revealed lower peak twitch force in trabeculae from females than from males. We further illustrate this with our RCC data, in which the ability of the SR to load Ca increases with respect to increasing stimulation frequency in the male, but not female, trabeculae. These findings suggest that as the bath Ca concentration is raised, the SR reaches a plateau as to the amount of Ca it can store. In this construct, despite more Ca entry, the SR cannot sequester any additional Ca and the FFR plateaus. Because various combinations of increased $[\text{Ca}^{2+}]_o$, and increased stimulation frequency led to similar peak DF within each sex grouping, we speculate that the peak forces observed within each subgroup might reflect their relative abilities to increase SR Ca load. However, these studies alone do not exclude a possible role for sex differences in myofilament Ca sensitivity or viscoelastic properties of the myocardium (48).

Another fundamental feature of cardiac muscle is length-dependent activation such that an increase in stretch, and therefore and increase in sarcomere length, results in higher developed force. In the present studies, we observed no difference in the length-tension relationship between male and female hearts at low stimulation frequency. Substantial data indicate that myofilaments have greater Ca sensitivity at longer sarcomere lengths (2, 3, 29, 34). Several factors are thought to contribute to this increased Ca sensitivity, including increases in myofilament overlap (2, 3, 29, 34), titin-mediated effects (8, 9, 20, 26), decreases in interfilament lattice spacing (18, 19, 32, 35), or other mechanisms (31). Regardless of mechanism, the virtually identical force-Ca relationships that we observed in skinned trabeculae at a fixed sarcomere length (2.1 μm) also suggest an absence of sex differences in myofilament Ca sensitivity in normal feline myocardium.

Interactions between frequency- and length-dependent responses. Our data extend previous studies by systematically evaluating the interaction between the FFR and L-T relationship in normal myocardium. To our knowledge, no previous study has systematically explored this interaction in large mammalian (cat) trabeculae at physiological temperature (37°C). Rather than parallel upward shifts in the FFR curves as resting muscle length increased, we observed length-dependent increases in the slope of the FFR. In addition to peak force development, we observed more than additive interactions of rate and length upon the rates of force development ($+dF/dt$) and force decay ($-dF/dt$) in male, but not female, trabeculae. In previous studies, the fractional release of Ca from the SR with each stimulus has been shown to increase substantially with increased SR Ca load (7, 39). Such an increase in fractional release is typically associated with a faster rate of rise of the Ca transient (Ca availability) which, in turn, triggers a faster rate of force development and explains the more than additive effects that we observe. An alternative possibility is a synergistic interaction between Ca availability and Ca sensitivity. For example, increases in cytosolic Ca themselves could modulate length-dependent activation via Ca/calmodulin-dependent signaling processes (43). Whatever the basis for the more than additive interaction between preload and stimulation frequency, it appears that this synergistic effect is lower in female vs. male trabeculae, perhaps owing to a reduced maximal SR Ca load. Consistent with this idea, Chen et al. (10) reported that females had lower SR Ca loading compared with males after the addition of the β-adrenergic agonist isoproterenol (10). Similarly, in the presence of the beta-adrenergic agonist isoprenaline, isolated myocytes from male rats exhibited a greater increase in the baseline and peak Ca transient amplitude compared with myocytes from females (14).

There are several possibilities as to why the SR in female hearts may not be able to load Ca as effectively as its male counterpart. One possibility is that the conductance of the L-type Ca channel is lower in females in the presence of increased Ca availability. A recent study in mice suggests that an increase in Ca leads to increased S-nitrosylation of the L-type Ca channel in females via increased endothelial NO and neuronal NOS production, resulting in reduced Ca entry into the cell and therefore less Ca load of the SR (41). Alternatively, there could be differences in the balance of NCX and SR Ca of females compared with males, resulting in more Ca extrusion from the cell and less SR loading. This could be regulated in part by increased NCX abundance, which has been shown in the rat (11). Indeed, in transgenic mice overexpressing NCX, Ca overload occurred to a greater degree in male mice during metabolic inhibition (40). Alterations in the phosphorylation of phospholamban could also provide a potential mechanism for reduced loading of the SR in females. In this context, previous studies have suggested decreased phosphorylation of phospholamban in human donor female hearts compared with male hearts (15). SR Ca leak may contribute to a smaller load in the SR, leading to less systolic release. This may occur due to either backflux of Ca through the SR calcium ATPase (SERCA) or leak through the ryanodine receptor (RyR). In addition, the female myocytes could buffer Ca differently. For example, intra-SR buffering by calsequestrin might differ between males and females.

Recent studies have also highlighted the ability of nitric oxide (NO) and NOS to modulate cardiac contractility. Indeed, the localization of specific NOS isoforms to different organelles within cardiac myocytes suggests important functional distinctions (24). For example, NOS1 is found in the SR (6) and mitochondria (28), and NOS3 is localized to sarcomembrane caveolae, where it can produce NO which constrains β-adrenergic-mediated increases in contractility (6, 17, 22). In the SR, NO produced by NOS1 acts on the RyR channel to increase Ca influx (47). In addition, NO signaling can be altered by the interaction of NOS isoforms with reactive oxygen species (25). Given that previous studies have shown sex differences in myocyte NOS signaling between males and females (10, 13, 27), differences in the abundance or spatial distribution of NOS isoforms could also contribute to the sex differences in contractile reserve we have observed.

Implications of sex differences in contractile reserve. Studies from animal models and human hearts indicate sex differences in susceptibility and responses to pathological stress such as ischemia-reperfusion injury (5, 21, 44), volume overload (23), pressure overload (42, 45), and myocardial infarction (12). These studies have implicated differences in nitric oxide, PKC, and MAPK signaling as the basis for sex differences in myocardial susceptibility. In this context, the present studies also suggest that there are fundamental sex differences in contractile responses to physiological stress and that such differences might have implications for peak levels of cardiac
performance and responses to pathological stimuli. If confirmed, lower peak intracellular Ca levels during pathological stress could lessen the risk of cardiac ischemic injury in females. Moreover, sex differences in peak force development and/or Ca levels, could affect myocardial growth responses triggered by increases in wall stress or calcium-dependent signaling. From this perspective, the reduced level of peak contractile reserve observed in these studies does not appear to be a deficiency per se but rather a potential contributor to the relative resistance to pathological cardiac stimuli observed in females.

In summary, we have demonstrated sex-based differences in contractile reserve in cardiac tissue from normal feline hearts. However, these differences are not evident at less demanding levels of stimulation. The persistent sex differences in peak force generation with supraphysiological concentrations of extracellular Ca, the lack of a sex difference in myofilament Ca sensitivity, and the sex differences in RCCs at higher stimulation frequencies support the hypothesis that there is a sex difference in peak SR Ca load and/or release during dynamic stress. Our findings add to a growing body of evidence demonstrating previously unappreciated sex differences in normal myocardial biology and may provide clues to the mechanisms of sex differences in responses to pathological stimuli.

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