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Cardiac myocyte contractility, calcium dynamics, and morphology in Zucker diabetic fatty female and male rats – lacking data in this model of obesity and diabetes: A review

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Diabetes mellitus (DM) represents an increasing global health burden with type 2 diabetes mellitus (T2DM) accounting for most cases. T2DM is associated with microvascular and macrovascular complications including myocardial ischemia and contributes to the development of a distinct form of cardiac dysfunction referred to as diabetic cardiomyopathy (DCM). Importantly, DCM contributes to 50–80% of mortality in patients with diabetes independent of other vascular comorbidities and is characterized by structural and functional alterations of the myocardium. Notably, these outcomes exhibit sex-specific differences in patients with T2DM. Despite this, most preclinical rodent studies fail to incorporate female subjects. This is truly the case of the Zucker diabetic fatty (ZDF) rat model, where the left ventricular contractile and morphological properties have been investigated almost exclusively in males. This review aims to: a) synthesize existing data on myocyte shortening and morphology in ZDF male and female rats; b) identify critical gaps in current knowledge and define priorities for future experiments; and c) emphasize the urgent need for longitudinal studies assessing cardiomyocyte contractility and structural remodeling in both sexes. Cardiac myocyte contractility and calcium dynamics data are entirely lacking in ZDF female rats, while findings in males remain limited and, in some cases, inconsistent. For morphological and ultrastructural alterations in cardiac myocytes information is scarce for both ZDF males and females. Overall, this review highlights the need for comprehensive longitudinal investigations spanning the early and advanced stages of DCM including progression to heart failure in both male and female ZDF rats.

Keywords: cardiac myocyte, contractility, calcium transients, ultrastructure, Zucker diabetic fatty rats, sex differences

Diabetes mellitus (DM) is a rapidly rising global health concern with type 2 diabetes mellitus (T2DM) representing the majority of DM cases. T2DM is marked by high glucose levels mainly due to insulin resistance (Cho et al. 2018). Due to these factors, the T2DM is associated with micro- and macro-vascular complications such as myocardial ischemia leading to specific and intrinsic cardiac dysfunction – diabetic cardiomyopathy (DCM). DCM accounts for 50–80% deaths in patients with diabetes independent on other vascular co-morbidities (Marfella et al. 2021) and is associated with abnormal cardiac structure and performance resulting from endothelial dysfunction, atherosclerosis, coagulation, and fibrosis deficiency in energy metabolism and disruption of cardiac myocyte morphology and contractility. Even with optimal glycemic control in patients with diabetes, DCM cannot be prevented (Zarek-Starzewska et al. 2025).

The authors would like to dedicate this research article to the Endocrine Regulations 60th anniversary.

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Several rodent models have been introduced to study T2DM including the ob/ob (Zhang et al. 1994) and db/db (Coleman 1978) mutant mice, the spontaneously diabetic model Otsuka Long-Evans Tokushima Fatty rat model (Kawano et al. 1994) as well as the Zucker diabetic fatty (ZDF) rat (Clark et al. 1983). The latter model has been derived from the genetically based obesity Zucker fatty (ZF) rat model, in which obesity is inherited in an autosomal Mendelian recessive manner. The ZF rat has a missense mutation (Gln269Pro, fatty, fa) in the highly conserved region of the leptin receptor gene (Phillips et al. 1996). Mutated version of the leptin receptor exhibits decreased plasma membrane expression, reduced leptin binding affinity, and diminished receptor signaling capability (da Silva et al. 1998). As a result, the animals carrying the fa/fa genotype develop obesity due to hyperphagia (uncontrolled hunger) as a consequence of disrupted hypothalamic satiety pathways. In this model, hyperinsulinemia is present; however, blood glucose remains at the normal levels (Takaya et al. 1996). From a ZF rat colony, a new strain with a propensity to develop T2DM has been developed by selective breeding. Homozygous fa/fa male rats of this strain fed Purina #5008 diet develop DM as early as 10 weeks of age reaching 100% incidence by 21 weeks of age, while none of the fa/+ male rats develop DM (Yokoi et al. 2013). The phenotypic characteristics of the diabetic ZDF rat strain are distinct from those of normoglycemic ZF rats. The ZDF rat strain with a high reproductive efficiency when fa/fa males are mated with fa/+ females serves as a useful animal model of T2DM (Yokoi et al. 2013; Sultan et al. 2020). The advantage of the ZDF rat model is further supported by the fact that the close genetic and phenotypic relationship between ZF and ZDF rats allows a direct comparison between these strains facilitating the distinction between the effects of obesity and hyperglycemia on the development of cardiac pathology. In addition, because females do not develop diabetes when maintained on the Purina #5008 diet, this model enables the investigation of sex-specific differences in the development of diabetes in obese animals.

Sex-specific differences in the impact and progression of T2DM are well established. Although the overall prevalence of T2DM is higher in men, diabetic women exhibit a disproportionately greater cardiovascular risk indicating a more adverse trajectory toward cardiomyopathy and heart failure (Kannel et al. 1974). Evidence from randomized clinical trials further shows that the cardioprotective advantage observed in pre-menopausal women compared to age-matched men is abolished in the presence of diabetes suggesting that estrogen does not confer protection against diabetes-associated cardiovascular risk (Norhammar and Schenck-Gustafsson 2013). Despite this, most pre-clinical rodent studies available do not include female animals, creating a significant gap in understanding sex-dependent mechanisms underlying diabetes-induced myocardial dysfunction. This is particularly evident in the ZDF rat model, where the left ventricular contractile and morphological properties have been evaluated almost exclusively in males.

With specific regard to cardiomyocyte contractility and morphology, most studies have focused on male ZDF rats at stages of fully developed hyperglycemia and obesity typically between 12 and 20 weeks of age. To date, only one study has analyzed sex differences in myocyte morphology in ZDF rats (Lum-Naihe et al. 2017) and data on cardiomyocyte contractility in female ZDF rats remain entirely lacking.

Accordingly, this review aims to: a) summarize the current data available for cardiomyocyte shortening and morphology in male and female ZDF rats; b) identify key gaps in current knowledge and outline priorities for future research; and c) emphasize the need for longitudinal studies in both sexes to assess cardiomyocyte contractility and structural remodeling, identify early markers of the DCM, and characterize disease progression to heart failure. To this end, the review is structured to present contractility data in a chronological framework followed by an overview of morphological findings.

Contractility and calcium transients

Very early stages (6–13 weeks). For the ZDF male rats, the earliest study exists for 6-week-old animals, where none of the ventricular myocyte contractility and calcium transient-derived parameters were different from those of lean littermates (Fulop et al. 2007). Howarth et al. (2011) have reported an elevation of time to peak as well as time to half relaxation of myocyte shortening in single myocytes isolated from the left ventricle of ZDF rats. This has been associated with increased resting Ca^{2+} levels as indicated by increased resting Fura-2 fluorescence and increased time to peak of the calcium transients. In addition, the authors performed electrophysiological experiments demonstrating lower density and decreased inactivation of L-type Ca^{2+} currents in ZDF animals compared to controls despite the L-type calcium channel alpha subunit being upregulated in ZDF rats. Moreover, the authors assessed myocyte myofilament sensitivity and sarcoplasmic reticulum

(SR) Ca^{2+} load, for both, no changes were observed (Howarth et al. 2011). For the ZDF females, the data are completely absent.

Early stages (14–20 weeks). There are five original articles available for this period for the ZDF male rats. Daniels et al. (2018) have reported data on right ventricle trabeculae contractility and isolated right ventricular myocyte calcium dynamics in 20-week-old animals. Using the force transducer, the group reports depressed developed force maximum rate of contraction and maximum rate of relaxation at all stimulation frequencies ranging from 2 to 6 Hz in the diabetic group when compared to lean controls. However, in isolated myocytes, there were no changes in calcium transients assessed as $[Ca]_i$ twitch (F/F_0) and the mean time constant of Ca^{2+} decay (Tau). In addition, the estimation of isolated myocyte SR Ca^{2+} load induced by caffeine application showed no differences between the experimental groups (Daniels et al. 2018). Skinned isolated left ventricular cardiomyocyte fibers were used to determine isometric force development in the work of the van den Brom group at the age of 14 weeks. Here, drop in maximal force and maximal rate of force development were observed without a change in calcium sensitivity. Moreover, there was no change in sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA) type 2a protein expression (van den Brom et al. 2009). This is in contrast to the increased mRNA SERCA2a level observed by the Fredersdorf et al. (2012) at 19 weeks of age, which was accompanied by enhanced Ca^{2+} uptake into the SR in left ventricular homogenates using a $^{45}Ca^{2+}$ uptake assay. Bhatt et al. (2014) have documented only subtle changes in the Ca^{2+} transients in 14-week-old animals with the increased time to 50% Ca^{2+} transient decay in right ventricular cardiomyocytes, while other parameters of Ca^{2+} transients and cell shortening remained unchanged. In addition, no difference has been found by this group for the yield of contractile work for right ventricular trabeculae comparing the ZDF and lean groups (Bhatt et al. 2014). Finally, for this period of age, specifically at 20 weeks, Ng et al. (2022) have reported a shift in myofilament Ca^{2+} sensitivity in isolated left ventricle cardiomyocytes without change in maximal and passive force or the Hill coefficient. Moreover, this work detected a decrease in SERCA2a protein expression; however, without changes in the SERCA2a maximal Ca^{2+} uptake rate and Michaelis constant (K_m) when comparing the groups. There are no data available for the ZDF female rats to date.

Middle stages (21–34 weeks). In ZDF male rats aged 22 weeks, Fulop et al. (2007) have demonstrated slowed relaxation of cardiomyocyte shortening without differences in peak fractional shortening or time to peak contraction. This was accompanied by a decrease in calcium transient amplitude and prolonged calcium transient decay (Fulop et al. 2007). At a slightly older age, Sultan et al. (2022a) have found increased resting cytoplasmic Ca^{2+} concentration as well as prolonged decay of the calcium transients estimated by Fura-2 ratio in left ventricular myocytes isolated from 28-week-old animals. Kinetic parameters and the amplitude of myocyte shortening were without significant changes (Sultan et al. 2022a). Intriguingly, Sultan et al. (2022b), in another work performed on a smaller number of cells, have reported no changes in either calcium transients or in cell shortening parameters at the same age. In addition, they have observed no difference in the SR Ca^{2+} content induced by caffeine application. In addition, a recent article from this group have observed prolonged action potential duration and a drop in the action potential upstroke velocity (dV/dt_{max}). However, the properties of the L-type Ca^{2+} current ($I_{(Ca,L)}$) as well as voltage-gated Na^+ currents were not changed when comparing the ZDF to the ZL group at the age of 28 weeks (Shmygol et al. 2025). In slightly older animals at the age of 30–34 weeks, Howarth et al. (2012) have reported a prolonged time to peak of calcium transients and reduction in amplitude of the L-type Ca^{2+} current across a wide range of test potentials in ZDF left ventricular myocytes compared to controls. The other parameters of calcium transients were unaltered as were those of cell shortening SR Ca^{2+} transport and myofilament sensitivity to Ca^{2+}. No data exist for this time period for the ZDF female rats.

Late stages (more than 34 weeks). Information for ZDF males at this age is very limited. The only work on data related to calcium transients and shortening of ventricular myocytes is the significantly reduced expression of SERCA2a at the mRNA level in the left ventricle of ZDF rats compared to controls at 45 weeks of age (Daniels et al. 2012). Currently, no information is available on contractility and calcium transients in ZDF female rats older than 34 weeks.

Summarized information for the myocyte calcium transients and contractility is presented in Table 1.

Morphological changes in cardiac myocytes

Very early stages (9–13 weeks). For the ZDF male rats, the knowledge is very sparse. In the work by Siwy et al. (2012), no difference in cardiomyocyte area calculated from the histological sections of the

Table 1
Chronological development of cardiac myocyte calcium transients and contractility alterations in the Zucker Diabetic Fatty (ZDF) rats

Stage	Weeks	Parameters	Reference
Very Early	6	**Myocytes – Shortening:** • Peak fractional shortening (PK) ↔ • Area under the contractile phase (A_C/PK) ↔ • Area under the relaxation phase (A_R/PK) ↔ **Myocytes – Ca^{2+} transients:** • Amplitude ↔ • Total area under Ca^{2+} transient ↔	Fulop et al. 2007
	9–13	**Myocytes – Shortening:** • Resting cell length ↔ • **Time to peak** ↑ • **Half relaxation** ↑ • Amplitude ↔ **Myocytes – Ca^{2+} transients:** • **Resting Fura 2 ratio** ↑ • **Time to peak** ↑ • Decay ↔ • Amplitude ↔ **Myocytes:** • Myofilament Ca^{2+} sensitivity ↔ **Myocytes – L-Type Ca^{2+} current:** • **Density** ↓ • **Inactivation duration** ↑ **Myocytes – SR - Ca^{2+} transport:** • Fractional release ↔ • Time to peak ↔ • Area under the curve ↔ • Recovery of Ca^{2+} transient ↔	Howarth et al. 2011
Early	14–15	**Right ventricular trabeculae:** • Yield of contractile work ↔ **Right ventricular myocytes – Shortening:** • Relative fractional shortening ↔ • Time from baseline to 50% peak (TTP50%) ↔ • Time to 50% relengthening (TR50%) ↔ **Right ventricular myocytes – Ca^{2+} transients:** • Ca^{2+} transient amplitude ↔ • Time from baseline to 50% peak (TTP50%) ↔ • **Time to 50% transient decay (T50%)** ↑	Bhatt et al. 2015
	14	**Skinned myocyte fibers:** • **Max. force (F_{max})** ↓ • Passive force (F_{pas}) ↔ • **Max. rate of force development $K_{tr\ max}$** ↓ • Myofilament Ca^{2+} sensitivity ↔ • SERCA 2a protein expression ↔	van den Brom et al. 2009
	19	**Ventricle homogenates:** • **SERCA 2a mRNA expression** ↑ • **SR Ca^{2+} uptake rate ($^{45}Ca^{2+}$)** ↑	Fredersdorf et al. 2012
	20	**Right ventricle trabeculae – contractility:** • **F_{dev}** ↓ • **Max. rate of contraction (dF/dt_{max})** ↓ • **Max. rate of relaxation (dF/dt_{min})** ↓ **Right ventricle myocytes – Ca^{2+} transients:** • $[Ca]_i$ twitch (F/F0) ↔ • Mean time constant of Ca^{2+} decay (Tau) ↔ **Right ventricle myocytes – SR - Ca^{2+} load:** • Caffeine (ΔF/F0) ↔	Daniels et al. 2018

Table 1
Continued . . .

Stage	Weeks	Parameters	Reference
Early	20	**Skinned myocytes – myofilament Ca^{2+} Sensitivity:** • **pCa50** ↑ • Max. force ↔ • Passive force ↔ • Hill coefficient ↔ **Ventricle homogenates – protein expression:** • **SERCA 2a** ↓ • NCX ↔ **Ventricle homogenates – SERCA Ca^{2+} uptake (Fura-2 fluorescence ratio):** • Max. Ca^{2+} uptake rate ↔ • K_m ↔	Ng et al. 2022
Middle	22	**Myocytes – Shortening:** • Peak fractional shortening (PK) ↔ • Area under the contractile phase (A_C/PK) ↔ • **Area under the relaxation phase (A_R/PK)** ↑ **Myocytes – Ca^{2+} transients:** • **Amplitude** ↓ • **Total area under Ca^{2+} transient** ↑	Fulop et al. 2007
	28	**Myocytes – Shortening:** • Amplitude ↔ • Time to peak ↔ • Time to half relaxation ↔ **Myocytes – Ca^{2+} transients:** • Amplitude ↔ • **Resting Fura 2 ratio** ↑ • Time to peak ↔ • **Time to half relaxation** ↑ **Myocytes:** • Myofilament Ca^{2+} Sensitivity ↔	Sultan et al. 2022a
	28	**Myocytes – Shortening:** • Amplitude ↔ • Time to peak ↔ • Time to half relaxation ↔ **Myocytes – Ca^{2+} transients:** • Amplitude ↔ • Resting Fura 2 ratio ↔ • Time to peak ↔ • Time to half relaxation ↔ **Myocytes – SR- Ca^{2+} transport:** • Amplitude ↔ • Time to peak ↔ • Time to half decay ↔ • Fractional release ↔	Sultan et al. 2022b
	28	**Myocytes – Action potential:** • **Duration** ↑ • **Action potential upstroke velocity (dV/dt_{max})** ↓ • Resting potential ↔ • Threshold potential ↔ **Myocytes – L-Type Ca^{2+} current:** • I_{Ca} density ↔ • I_{Ca} /I_{Ca}-max ↔ **Myocytes – voltage gated Na^+ current:** • I_{Na} density ↔ • G/G_{max} ↔	Shmygol et al. 2025

Table 1
Continued . . .

Stage	Weeks	Parameters	Reference
	30–34	**Myocytes – Shortening:** • Amplitude ↔ • Time to peak ↔ • Time to half relaxation ↔ **Myocytes – Ca^{2+} transients:** • Amplitude ↔ • Resting Fura ratio ↔ • **Time to peak ↑** • Time to half decay ↔ **Myocytes:** • Myofilament Ca^{2+} Sensitivity ↔ • Myocytes – L-Type Ca^{2+} current: • **Density ↓** • Inactivation duration ↔ **Myocytes – SR- Ca^{2+} transport:** • Amplitude ↔ • Time to half decay ↔ • Fractional release ↔	Howarth et al. 2012
Late	45	**Ventricle homogenates – mRNA expression:** • **SERCA 2a ↓**	Daniels et al. 2012

Significant changes in ZDF rats vs. controls are marked in bold. Unless not stated otherwise, the data have been obtained from left ventricle. The complete table represents solely data from ZDF male rats. Abbreviations: NCX – sodium-calcium exchanger; SERCA – sarcoplasmic/endoplasmic reticulum calcium ATPase; SR – sarcoplasmic reticulum.

left ventricle at the age of 8 weeks, has been reported. For the ZDF female rats, there are no data available to date with respect to myocyte morphological and ultrastructural changes.

Early stages (14–20 weeks). This age range is the most studied period from the morphological point of view. In case of ZDF males, no myocyte hypertrophy estimated by mid-left ventricular wall myocyte cross-sectional area (CSA) from the immunohistological sections at the age of 14 weeks was observed (Marsh et al. 2007). Similar results have been reported by the group of Olsen et al. (2013), no change in right ventricle myocyte length and myocyte CSA has been observed after analysis from longitudinal immunohistochemistry sections as well as from the mean volume fraction of the healthy myocytes (Masson's Trichrome staining) at the age between 12 and 17 weeks. In contrast, at the age of 19 weeks, Fredersdorf et al. (2004) have reported increased left ventricular myocyte volume from the immunohistological tissue morphology analysis. In this study, the increase in the myocyte volume originates mainly as a rise in myocyte width (diameter) with unaltered length (Fredersdorf et al. 2004). Next, the morphological analysis revealed increased lipid droplet density both in the left ventricular myocytes as assessed by both transmission electron microscopy (TEM) at the age of 14 weeks (Zhou et al. 2000) and BODIPY-based histological staining at the age between 12 and 17 weeks in the right ventricular myocytes (Olsen et al. 2013). There is only one study by Lum-Naihe et al. (2017) available to date that directly compare ZDF male and female animals with their lean (ZL) counterparts. At the age of 19 weeks, the cardiomyocyte CSA in the ZDF female group exhibited significant cardiomyocyte hypertrophy among the four groups, as assessed from the histopathology analysis using *Helix pomatia* agglutinin staining, while cardiomyocyte hypertrophy was not present in ZDF males compared to ZL males (interestingly, CSA was significantly higher in ZL males than ZL females). Additionally, the authors have performed a qualitative analysis of the left ventricular myocyte ultrastructure via TEM demonstrating that both ZDF male and ZDF female rats had structural disorganization of the myocardium, mitochondrial clustering, and disrupted spatial orientation of mitochondria in relation to the sarcomere compared to their lean counterparts (Lum-Naihe et al. 2017).

Middle stages (21–34 weeks). There are two studies available for this period in ZDF male animals, one of them providing data demonstrating cardiomyocyte hypertrophy and the other one focused on myocyte ultrastructure. At the age of 32 weeks, left ventricular

cardiomyocyte area increased significantly in ZDF animals when compared to lean controls (Siwy et al. 2012). In the latter study, Sultan et al. (2022a) have found decreased sarcomere length, but no change in mitochondria count in ZDF left ventricular myocytes after quantitative analysis of TEM slides. In addition, qualitative analysis showed an increase in lipid droplet abundance on TEM slides at 28 weeks of age (Sultan et al. 2022a). No data exist from the ZDF female rats for this stage.

Late stages (more than 34 weeks). For this period, similarly to the previous paragraph, the available data are limited. In case of ZDF males at the age of 45 weeks, no left ventricular myocyte hypertrophy has been found assessed as cardiomyocyte CSA from immunohistochemically stained tissue sections (Daniels et al. 2012). More recently, a report of Kozma et al. (2022) has identified left ventricular hypertrophy at the cellular level assessed as increased cardiomyocyte diameter at the age of 60 weeks (Kozma et al. 2022). No study has been released regarding the ZDF female cardiac myocyte morphological analysis for the age more than 34 weeks.

The overview of the morphological and ultrastructural observations in the ZDF female and male rat cardiac myocytes is presented in Table 2.

Summary

Diabetes mellitus (DM) increases the risk of cardiac dysfunction independently of coronary artery disease and hypertension contributing to a largely

Table 2
Chronological development of cardiac myocyte morphological parameters in the Zucker Diabetic Fatty (ZDF) rats

Stage	Week	Parameter	Reference
Very Early	9–13	**Myocytes:** • Area ↔	Siwy et al. 2012
Early	12–17	**Right ventricle myocytes:** • Length ↔ • CSA↔ • Volume fraction of healthy myocytes ↔ • **Lipid droplet density** ↑	Olsen et al. 2013
	14	**Myocytes:** • CSA↔	Marsh et al. 2007
	14	**Myocytes:** • **Lipid droplets density** ↑	Zhou et al. 2000
	19	**Myocytes:** • **Volume** ↑ • **Width** ↑ • Length ↔	Fredersdorf et al. 2004
	20	**Myocytes:** • ***CSA in ZDF females compared to ZDF males*** ↑ • ***CSA in ZDF females compared to ZL (control) females*** ↑ • ***Both ZDF males and ZDF females: disorganization of myocardium, mitochondrial clustering, disrupted spatial orientation of mitochondria in relation to the sarcomere***	Lum-Naihe et al. 2017
Middle	28	**Myocytes:** • **Sarcomere length** ↓ • Mitochondria count ↔ • **Lipid droplet** ↑	Sultan et al. 2022a
	32	**Myocytes:** • **Area** ↑	Siwy et al. 2012
Late	45	**Myocytes:** • CSA ↔	Daniels et al. 2012
	60	**Myocytes:** • **Diameter** ↑	Kozma et al. 2022

Significant changes in ZDF rats vs. controls are marked in bold. Unless not stated otherwise, the data have been obtained from left ventricle. The data from ZDF females are presented in Italics, otherwise the data have been obtained from ZDF male animals. Abbreviations: CSA – cross sectional area; ZL – Zucker lean.

asymptomatic yet progressive deterioration of cardiac function. T2DM is associated with microvascular and macrovascular complications including myocardial ischemia as well as a distinct intrinsic cardiac disorder termed diabetic cardiomyopathy (DCM), which is characterized by structural and functional alterations of the myocardium independent of other comorbidities. DCM accounts for a substantial proportion of mortality in patients with diabetes and remains insufficiently prevented by glycemic control alone reflecting underlying myocardial remodeling processes initiated at the cardiomyocyte level. Both clinical and experimental studies indicate significant sex-specific differences in the prevalence, pathophysiology, and outcomes of diabetes-associated cardiovascular disease. Specifically, diabetic women exhibit a disproportionately higher cardiovascular risk despite the higher prevalence of T2DM in men. This disparity is present even in pre-menopausal women, suggesting limited cardioprotective effects of estrogen in the diabetic state. Despite these observations, mechanistic insights into sex-dependent differences in DCM progression remain limited, particularly at the cellular level.

Moreover, this gap is further compounded by the underrepresentation of female subjects in preclinical research, including the widely used Zucker diabetic fatty (ZDF) rat model, for which the majority of data have been derived from male animals despite its long-standing use. ZDF rats were developed by Peterson et al. (1990) by a selective inbreeding of males with spontaneously developed diabetes to produce a model for non-insulin-dependent diabetes mellitus with consistent diabetes development. One of the reasons for this may be the fact that ZDF females only develop diabetes if fed a high-fat diet (Lum-Naihe et al. 2017).

The ZDF rat model has been used for more than 30 years in research specifically focused on cardiomyocyte contractility. However, data in ZDF animals remains limited. In ZDF males, we can conclude that myocyte contractile performance is preserved, although slight differences have been observed in some cases. Specifically, in very early stages the shortening and calcium transient kinetic parameters are slowed, associated with impairments in L-type calcium currents. For the early and middle stages, these parameters are either well preserved, in some cases slightly changed or the results are contradictory when comparing the data from different groups. For the late stages, the information is completely absent. As documented in Table 1, no data are available for the ZDF females regarding myocyte contractility and calcium transients up to date. In addition, no study exists that directly compares cardiac myocyte contractility in ZDF females to ZDF males at the same age and under equal experimental conditions.

The available information is even sparser with regard to the ventricular myocyte morphological and ultrastructural remodeling (Table 2). From the chronologically sorted available data for ZDF males, cardiomyocyte hypertrophy develops around the 19th week of age and continues to the middle and late stages although at the late stages some groups report no change in myocyte CSA (Daniels et al. 2012). Intriguingly, at 20 weeks of age, ZDF female rats have developed myocyte hypertrophy, whereas the males do not (Lum-Naihe et al. 2017). This demonstrates sex-specific differences in myocyte hypertrophy and again underscores the need for direct comparison between female and male individuals at a defined stage of DCM. At the early stages (12–17 weeks), the lipid droplet abundance is reported in ZDF males (Olsen et al. 2013) and it is also present in the middle stages (Sultan et al. 2022a). Ultrastructural studies are mostly absent – there are only two works reporting mitochondrial abnormalities, one of them reporting qualitative changes in mitochondria both in female and male ZDF animals (Lum-Naihe et al. 2017) at the early stage; however, the other works found no difference in mitochondria count via morphometrical analysis at the middle stage (Sultan et al. 2022a). Information about other organelles as endoplasmic reticulum (ER)/SR, ribosomes, dyads, lysosomes, Golgi apparatus, sarcolemma, t-tubules and their mutual communication are completely absent both for female and male ZDF rats throughout their lifespan.

All the facts stated above require further profound investigations:

1. Specifically aiming to reveal differences in single myocyte contractility, calcium transients, morphology, and ultrastructure in ZDF females, as these data are completely missing.
2. Directly comparing ZDF females to ZDF males at a specific age in regard to cardiac myocyte contractility, calcium transients, and morphology as this information is limited to a single study (Lum-Naihe et al. 2017).
3. Focused on the late stages as well as designed to specifically analyze the progression to the heart failure in both female and male ZDF animals. Cardiac myocyte contractility and calcium transient data for the late stages are completely missing for both sexes. For morphological parameters, little information is available for the

left ventricular myocyte hypertrophy (Daniels et al. 2012; Kozma et al. 2022); however, detailed morphological and ultrastructural reports for the late stages are fully absent. Importance of studying late stages depicts the fact that the ZDF male rats at the age of 12 months have 60% mortality (Wachal et al. 2021); however, the case of the death of the animals in this study has not been described and thus in general remains to be elucidated.

4. Securing standardized longitudinal experimental conditions and unified parameter settings to reveal the progress of the DCM stages from the early onsets up to the late stages with progression to the heart failure. On one hand, as it is documented by the presented review of available literature, cardiac myocyte contractility and calcium transients seem to be more or less preserved from the very early (Fulop et al. 2007; Howarth et al. 2011) up to the middle stages (Howarth et al. 2012) at least in ZDF male animals. Hence, contractility and calcium transients are unlikely to serve as early markers of the disease progression. Therefore, other molecular biology (protein, DNA, mitochondrial DNA, mRNA), morphological (for example mitochondria, ER/SR, sarcolemma, t-tubules, lysosomes and their mutual communications) and/or functional (as mitochondrial respiration, substrate utilization, dyad re-organization, organellar dynamics and others) parameters should be considered as early markers of the DCM progression in this experimental model for their further translation to the human disease.

Conclusion

The contractility and calcium dynamics data are completely absent in ZDF female rats. In ZDF male animals, the data are either limited or in some cases contradictory. For the morphological and ultrastructural alterations of the cardiac myocytes, up-to-date information is almost completely absent. This review points out the knowledge gaps and underlines the necessity for longitudinal studies ranging from early and late stages of the DCM, including progression to the heart failure for both ZDF female and male animals.

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