Journal of Cell and Molecular Research (2021) 13 (1), 65-71 DOI: 10.22067/jcmr.2021.71395.1010

Research Article

The Effect of Androgen Deprivation on the Expression of Connexin-43 mRNA in the Heart

Mahnaz Ghowsi [1*], Nazli Khajehnasiri [2], Sajjad Sisakhtnezhad [1]

[1]*Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran*
[2]*Department of Biological Sciences, Faculty of Basic Sciences, Higher Education Institute of Rab-Rashid, Tabriz, Iran*

Received 10 July 2021 Accepted 20 September 2021

Abstract

Connexin-43 (Cx-43) plays axial roles in the propagation of action potentials and contractile coupling in the heart. Down-regulation of Cx-43 in the heart is associated with arrhythmia, dilated cardiomyopathy, and heart failure. To date, no studies have examined the effects of androgen deprivation therapy (ADT)-induced hypogonadism on the expression of Cx-43 in the heart. This study investigated the effects of testosterone deprivation and its replacement with testosterone enanthate on the expression of Cx-43 mRNA and the muscle-specific miRNAs miR-206 and miR-1, as two potential regulators of the Cx-43 protein expression in the ventricular tissue. Accordingly, 21 male *Wistar* rats were divided into three groups: I) Normal control, II) ORX-S: castrated rats serving as animal models for ADT and receiving the sesame oil as a solvent of testosterone enanthate for ten weeks, and III) ORX-T: these animals were castrated, receiving testosterone enanthate (25 mg/kg) for ten weeks. The relative expression of Cx-43 mRNA, miR-206, and miR-1 was determined by qRT-PCR. Cx-43 mRNA was found to be decreased in the ORX-S group. The Cx-43 mRNA was up-regulated after the administration of testosterone enanthate. There were no significant changes in miR-206, and miR-1 levels in the ORX-S and ORX-T groups compared to the controls. Our results indicated that testosterone should be regarded as an important factor in the regulation of Cx-43 mRNA expression in the heart, and testosterone deprivation may down-regulate the Cx-43 mRNA expression; however, it doesn't alter miR-1 and miR-206 levels. These results suggest that ADT-induced hypogonadism may put males at risk for cardiac dysfunctions.

***Keywords*:** Connexin-43, miR-206, miR-1, Heart, Testosterone, Androgen deprivation therapy

Introduction

Intercellular gap junction proteins form a membrane-spanning channel allowing a forthright intercellular passage of small molecules and inorganic ions between cells; this then leads to electrical and metabolic coupling between two adjacent cells, regulating the ionic conduction and metabolic coupling between cells (Kamal et al., 2020). In cardiac tissue, gap junctions are involved in the formation of cell-to-cell communication pathways, helping propagation a wave of electrical stimulation between myocytes; this leads to the synchronous contraction of atria and ventricles (Kurtenbach et al., 2014). Also, they provide the fast exchange of ions and small molecules such as ATP, glutathione, cAMP, IP3, and glucose between myocytes (Michela et al., 2015). In cardiomyocytes, connexin-43 (Cx-43) is the most abundant isoform of gap junction channels (Jansen et al., 2010). New findings indicate that the canonical muscle-specific miRNAs (myomiRs) miR-1, -133, and -206 are involved in the development of skeletal and cardiac muscles and their health maintenance. They can down-regulate the targeted mRNA to degrade or inhibit translation. They also regulate the members of different cell signaling pathways in the muscle cells. Therefore, they play important role in myogenesis, muscle development, and muscle remodeling (Liu and Olson, 2010; McCarthy, 2011; Mitchelson and Qin, 2015). MiR-1, together with a few other miRs, serve a significant role in the development of embryonic stem cells and cardiomyocyte progenitor cells; it also plays important role in the expression of some cardiac transcription factors; as a result, it can change cardiac function (Kura et al., 2020). In normal cellular conditions, both miR-1/ -206 help myogenic differentiation and homeostasis (Mitchelson and Qin, 2015).

In vitro reports have shown that overexpression of miR-206 could reduce apoptosis of cardiomyocytes, and down-regulation of miR-206 could raise apoptosis of these cells(Yan et al., 2020).

*Corresponding author's e-mail address:
Ghaousi.mahnaz@razi.ac.ir

Recent findings on the irregular expression and functional significance of miR-1 have shown that miR-1 is dysregulated in some types of cardiac diseases, particularly arrhythmia and heart failure (Duan et al., 2014). There are two binding sites for the expression miR-206 and miR-1 in the 3′-untranslated region of Cx-43 mRNA; their activity is necessary for the down-regulation of Cx-43 (Oyamada et al., 2013). MiRs-1 and -206 have similar mature sequences, and their seed sequences are precisely identical. In addition, to sharing some common target genes, they have some independent target genes (Mitchelson and Qin, 2015).

The increased or decreased concentrations of androgen both raise the risk of heart failure. In this regard, testosterone abuse in athletes can significantly elevate the possibility of unexpected heart failure (Huang et al., 2016). The influence on the contractile function of the myocardium, endothelial function, and alterations in skeletal muscles are among the suggested mechanisms by which androgen may affect cardiac function (Huang et al., 2016; Malkin et al., 2009). On the other hand, various studies conducted on the men with prostate cancer who had received androgen deprivation therapy (ADT) indicated that the risk of developing heart failure and cardiovascular mortality in these subjects was high. They have concluded that androgen deprivation might promote heart failure (Keating et al., 2006; Shahani et al., 2008). Besides, there is some evidence showing that the down-regulation of Cx-43 protein is associated with arrhythmia, dilated cardiomyopathy, and heart failure (Chang et al., 2017). Thus, changes in Cx-43 expression and its distribution may be involved in developing heart failure and heart dysfunctions. ADT is one treatment for inhibiting testosterone production in the testes, and although new drugs targeting androgen receptors have been developed for many years, ADT is the mainstay of treatment for prostate cancer (Mitsuzuka and Arai, 2018). To date, no studies have examined the effect of ADT-induced hypogonadism on the expression of Cx-43 in cardiac tissue. Therefore, in the present study, the effects of hypogonadism caused by orchiectomy on an animal model for ADT and testosterone replacement on the mRNA expression of Cx-43 were evaluated. Also, the expression of miR-1 and miR-206 as two critical factors involved in the regulation of Cx-43 protein expression were determined.

Materials and Methods

Experimental Animals

Twenty-one male *Wistar* rats (190–200 g) were maintained at an animal facility (Razi University, Kermanshah, Iran). All experimental protocols were approved by the Local Animal Care and Use Committee of Razi University, Kermanshah, Iran and they were according to the research guidelines on animal experimentation (Kermanshah, Iran, permit number: IR.RAZI.REC.1399.017). Rats were kept on a 12-h light/dark cycle with free access to standard pelletized rat food and tap water. The animals were randomly divided into three groups (n=7). These included: I) the normal control group with no treatment during ten weeks (group NC). II) the castrated group (group ORX-S, to eliminate circulating testosterone, testes of these animals were removed via bilateral orchiectomy surgery). The animals belonging to this group were subcutaneously injected with sesame oil every day for ten weeks. III) the castrated group received testosterone enanthate 25 mg/kg/day by the subcutaneous injection as testosterone replacement therapy for ten weeks (group ORX-T). The sesame oil was used as a solvent for testosterone. For orchiectomy, the animals were anesthetized and maintained using a combination of ketamine and xylazine. The skin of the scrotal region was shaved and washed using the sterile technique. The orchiectomy surgery was done using the scrotal approach technique (Foley, 2005).

At the end of treatments, the rats were anesthetized and the blood samples were collected from their hearts by cardiac puncture. Also, a sample from the left ventricular tissue was snap-frozen in liquid nitrogen and stored at –80◦C. The sera samples were separated from the collected blood, and the serum total testosterone contents were determined using an enzyme-linked immunoassay (ELISA) kit (Cusabio, Wuhan, China). Both the intra-assay coefficient of variation and the inter-assay coefficient of variation (CV) for the testosterone kit were < 15%.

The RNA Isolation and Real-time PCR Analysis of CX-43 mRNA, miR-1, and miR-206 Expression in the Ventricular Tissue

Approximately 50 mg of the ventricular tissue samples were homogenized in TRIzol reagent (Life Technologies, U.S.A.); the total RNA samples were isolated according to the manufacturer's instructions. Each of the total RNA samples was quantified using a Nanodrop.

Reverse transcription into cDNA was then performed using the cDNA synthesis kit (Fermentas, California, U.S.A.) according to the manufacturer's guidelines. To eliminate the genomic DNA, RNA was treated with DNaseI (RNase-free) (Aminsan, Tehran, Iran). 2 µl cDNA, 3 µl water, 6 µl 2 X SYBR Green Master mixes (SYBR premix Ex Taq TMII (Takara Holdings Inc., Kyoto, Japan), and primer pairs at five pmol concentrations were mixed in a final volume of 12 µl; the PCR reactions were done in triplicates by using the Corbett Research RG 3000 thermal cycler (CR CORBETT, Australia). The forward and reverse primers used for gene expressions were as follows: Cx-43 forward: 5′ TGTGATGAGGAAGGAAGAGAAG 3′, reverse: 5′ AGAGGATGGTGATGATGTAGGT 3′; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward: 5′ AAGTTCAACGGCACAGTCAAGG3′, reverse: 5′ CATACTCAGCACCAGCATCACC33′.

Reverse transcription and quantitative expression analyses of rno-miR-1(*Rattus norvegicus*-miR-1), miR-206, and the endogenous control U6 were performed using real-time PCR, as described previously (Kramer, 2011). The primer sequences used for stem-loop RT-PCR were as follows: the mir stem-loop RT primer 5′ GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACNNNN 3′; miR-1 forward primer 5′TGCTTCGGCAGCACATATAC 3′; rno-miR-206 forward primer 5′-TGGAATGTAAAGAAGTAT-3′; Universal reverse primer 5′CCAGTGCAGGGTCCGAGGTA -3′; U6 forward primer 5′TGCTTCGGCAGCACATATAC 3′; U6 reverse primer AGGGGCCATGCTAATCTTCT3′. The primers were provided by Sinaclon (Tehran, Iran). The cycling condition was one cycle of 10 min 95°C, 40 cycles of 95°C for 10 s, and 60°C for 1 min.

To calculate the relative gene expression, the comparative CT method was applied (Schmittgen and Livak, 2008). The expression of Cx-43 was normalized to *Gapdh* gene; also, the expression of miR-1 and miR-206 were normalized to that of the U6 snRNA gene.

Statistical Analysis

Statistical analyses were carried out using SPSS, version 16. The level of significance for comparison was set at $P < 0.05$. To determine the differences between groups, the data were analyzed by ANOVA, which was followed by Tukey's test. Data are presented as means with a standard error of the mean (mean ± SEM). The GraphPad Prism 5 software was then applied for plotting the figures.

Results

Castration decreased the concentration of testosterone in the ORX-S group. In the ORX-T group, testosterone replacement could not restore the testosterone levels up to normal levels of the control rats (Figure 1a). The expression levels of Cx-43 in the ORX-S group (5.118e-006 ± 2.562e-006) were significantly lower than those of the control group (3.105e-005 ± 4.571e-006) (P = 0.001). These levels in the ORX-T group (2.009e-005 ± 3.815e-006) were similar to those of the control group (P = 0.138) (Figure 1b). Meanwhile, the expression levels of Cx-43 in the ORX-T group were higher than those of the ORX-S group (P = 0.038) (Figure 1b). The miR-1 expression levels were not, however, significantly different between control group (0.004998 ± 0.001207) and the ORX-S (0.002695 ± 0.001160) and ORX-T groups (0.005500 ± 0.002538) (P =0.634 and p = 0.978, respectively). The miR-1 levels in the ORX-T and ORX-S groups were similar (P = 0.515) (Figure 1c). The expression levels of miR-206 in the ORX-S (0.005034 ± 0.002442) and ORX-T groups (0.005484 ± 0.001900) were not significantly different from those of the control group (0.007303 ± 0.0006013) (P = 0.661 and p = 0.764, respectively). Meanwhile, the miR-206 levels in the ORX-T and ORX-S groups were similar (P = 0.983) (Figure 1d).

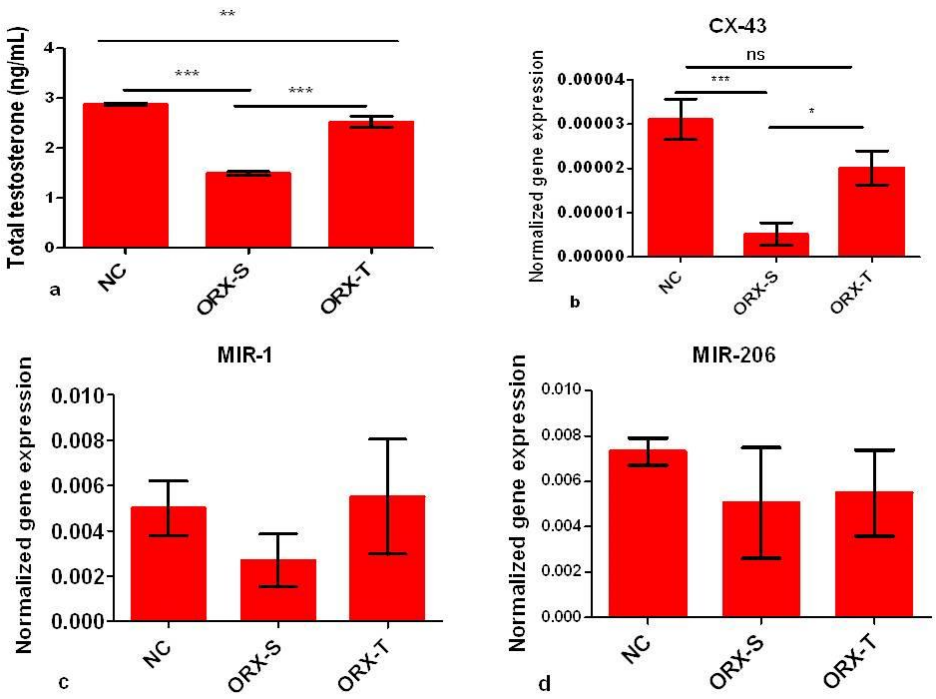

Figure 1. Total testosterone concentration (a); expression of CX-43 mRNA (b), miR-1 expression (c), and miR-206 expression (d) in the left ventricular tissue of rats. NC group, normal control rats; ORX-S group, Castrated rats; ORX-T group, castrated rats that received testosterone enanthate 25 mg/kg/day. Data are expressed as mean ± SEM; One-way ANOVA followed by Tukey's *post hoc* test. ***: $P < 0.001$;**: $P < 0.01$; *: $P < 0.05$. Abbreviations: CX-43, Connexin-43; miR-1, miRNA-1; miR-206, miRNA-206.

Discussion

In the present study, to investigate the effects of testosterone deficiency on the Cx-43 mRNA, miR-206, and miR-1 expression, a rat model of ADT, was established by following the orchiectomy surgery. Also, the expression of Cx-43, the most abundant connexin expressed in the adult working myocardium (Jansen et al., 2010), was evaluated. Our results showed the marked influence of testosterone deprivation on the expression of Cx-43; so, the expression of Cx-43 mRNA in the castrated rats was decreased in comparison to the controls. In line with our results, one study done by Zhang et al. (2016) reported that the alteration of testosterone could influence the expression of Cx-43 in Leydig cells (Zhang et al., 2016). Also, another study showed that excess androgen levels reduced Cx-43 expression and impaired the gap junction intercellular communication between human granulosa cells through androgen receptors (Wu et al., 2010). In the hormone-responsive tissues, the expression, modification, stability, and localization of connexin can be influenced by nuclear hormone receptors and their ligands (Firestone and Kapadia, 2012). In this regard, the current study showed that testosterone is involved in the expression of Cx-43 in heart. These findings were consistent with another study reporting that the treatment with flutamide, an androgen receptor blocker, changed Cx-43 expression in the pig's uterus (Wieciech et al., 2014). Animal and human studies also implicated that estrogen receptor subtypes (ERα and ERβ) and the progesterone receptor could regulate the functions of the gap junctions in the reproductive tissues through transcriptional or non-transcriptional mechanisms (Firestone and Kapadia, 2012). Considering the roles of connexin in the cell-cell interactions for carrying out different cellular and physiological processes, a disruption in the connexin expression can result in the malfunction of the intercellular communication and cardiac tissue, which may cause the onset of physiological disorders; because, it has been shown that the down-regulation of the major cardiac gap junction protein Cx-43 in many cases is associated with arrhythmia, dilated cardiomyopathy and heart failure (Chang et al., 2017). In the present study, the treatment of castrated rats with testosterone enanthate increased the Cx-43 mRNA expression. Consistent with our finding, the results of a previous study showed that the phosphorylated-Cx-43/ total-Cx-43 ratios were significantly reduced in the

castrated rats, and testosterone replacement increased this parameter in the cardiac ischemia-reperfusion injury (Pongkan et al., 2015). The pattern of alteration in the Cx-43 mRNA expression studied here suggests that the changes of serum testosterone levels may have noticeable effects on the Cx-43 expression in men. It has been reported that male hypogonadism or testosterone deficiency syndrome (TDS) is one of the frequent endocrine disorders among middle-aged to older men. Also, it has been estimated that the serum testosterone leve in nearly 37.8% of American males who are > 45 years old and visit a physician in the USA are < 300 ng/dL. Also, in men, ADT is associated with a marked decrease in testosterone levels (Xu et al., 2016). The impact of androgen imbalance was supposed to cause cardiovascular disorders (Pongkan et al., 2015) such as heart failure (Keating et al., 2006; Shahani et al., 2008). One study done by Pastor-Perez et al. showed that 28% deficiency of testosterone and less circulating testosterone concentrations in the body could be related to exercise capability in male patients with chronic heart failure (Manzano-Fernández et al., 2011). One study also reported that the arrhythmia score was significantly increased in the castrated rats, thus indicating the high susceptibility to arrhythmia and testosterone replacement markedly decreased the arrhythmia score (Pongkan et al., 2015).

Recently, some studies have shown that miRNAs play some roles in the regulation of connexin expression (Oyamada et al., 2013). MiR-1 is the most abundant miR in the adult mouse heart since embryonal development (Kura et al., 2020). It has also been revealed that the expression of MiR-1 is dysregulated during cardiac hypertrophy and heart failure (Duan et al., 2014). MiR-1, by affecting the expression of some cardiac transcription factors like myocardin, Nkx2.5, serum response factor (SRF), WNT and fibroblast growth factors (FGFs) signaling pathways, and targeting cyclin-dependent kinase-9 (Cdk9), histone deacetylase 4 (HDAC4), SRY-Box transcription factor 6 (Sox6), FZD7 (Frizzled-7) and FRS2 (fibroblast growth factor receptor substrate 2), can alter cardiac function (Kura et al., 2020). The activity of miR-1 is necessary for the down-regulation of Cx-43 (Oyamada et al., 2013). Yang et al. also showed that in the cultured myocytes, the treatment with miR-1 isolated from neonatal rats decreased Cx-43 (Anderson et al., 2006). Also, miR-206 was found to be an important factor in the regulation of Cx-43 expression, and it could negatively target Cx-43 (Anderson et al., 2006). Mature microRNAs have 22 nucleotides that can prevent the expression of the seed region of miRNA and 3′ untranslated regions of mRNA (Oyamada et al., 2013). In the normal rodent hearts, the miR-206 level is very low; during pathological conditions such as myocardial infarction, miR-206 is induced (Dong et al., 2009; Shan et al., 2009). One study on mice showed that down-regulation of both miR-133 and miR-1 was involved in cardiac hypertrophy in mice (Care et al., 2007). Also, in another study on hypertrophic rat cardiomyocytes, under both in vitro and in vivo conditions, it was shown that the down-regulation of miR-1 mediated the induction of pathologic cardiac hypertrophy (Curcio et al., 2013). MicroRNAs participated in the post-transcriptional regulation of gene expression (Huang et al., 2019).

It has also been shown that miR-1-2 by straight targeting Iroquois homeobox 5 (Irx5), which is a repressor of the potassium channel Kcnd2, could increase arrhythmias. Also, miR-1 is involved in regulating the Cx-43 expression and activity. miR-1 has remarkable effects on a range of ion channels, Ca^{2+}-handling, and contractile proteins. Also, miR-1 increases phosphorylation of L- type and ryanodine receptor 2 (RyR2) channels and enhances the immature Ca^{2+} transient amplitude and kineticsand the excitation-contraction coupling (Duan et al., 2014). Besides, it has been reported that in infarct rat hearts, the removal of miR-1 alleviates arrhythmogenesis (Yang et al., 2007).

KCNJ2 encodes the K^+ channel subunit Kir2.1, and GJA1 encodes Cx-43. Overexpression of miR-1 via post-transcriptionally repressing *KCNJ2* and *GJA1* makes slow conduction and depolarize the cytoplasmic membrane; the effects could be a cause for its arrhythmogenic potential (Duan et al., 2014). Although, there are response elements for testosterone in the promoter region of myomiRs (Nielsen et al., 2014), it seems that our hypothesis, that expression of miR-1, and miR-206 may be regulated in the cardiac tissue by the alteration of testosterone concentrations, was not true, as we observed that the relative expression of miR-206 and miR-1 in heart of rats did not change significantly by alterations in the testosterone concentration. Also, we found that the expression of miR-1 and miR-206 in the ventricular tissue of castrated rats receiving testosterone replacement was not affected, as compared to the controls. Our findings were by one study done by Oyamada (2013), showing that the ectopic expression of miR-206 decreased the expression of Cx-43 protein, but it didn't alter the expression of Cx-43 mRNA (Oyamada et al., 2013). In the present study, testosterone replacement with testosterone enanthate caused the up-regulation of

Cx-43 mRNA. Evidence obtained by in vitro and *ex vivo* studies has also shown that testosterone might have protective effects on the heart through androgen receptors (Pongkan et al., 2015).

To summarize, the current study delineated the effects of testosterone deprivation and the influence of testosterone replacement on the ventricular expression of Cx-43 mRNA and myomiRs miR-1 and miR-206 as two potential regulators of Cx-43 protein expression in the heart of rats.

Castration was associated with the down-regulation of the expression of Cx-43 mRNA in rats; however, it didn't affect miR-1 and miR-206. Our results, thus, suggest that an alteration in the Cx-43 mRNA expression may be a mechanism by which androgen deficiency may affect cardiac function.

If down-regulation of Cx-43 mRNA (induced by the low levels of circulating testosterone) contributed to cardiac function in male subjects for whom a significant decrease in serum testosterone levels was recorded, more research is needed to carefully monitor the cardiac function of the patients receiving ADT therapy. These results, suggest that ADT may put men at risk for heart dysfunction. Future clinical studies are, however, required to determine the effects of ADT on the cardiac tissue and function in ADT-recipient patients.

Conflicts of Interest

The authors declare no conflict of interest

References

Anderson C., Catoe H. and Werner R. (2006) MIR-206 regulates connexin43 expression during skeletal muscle development. Nucleic Acids Research 34: 5863-5871.

Care A., Catalucci D., Felicetti F., Bonci D., Addario A., Gallo P., et al. (2007) MicroRNA-133 controls cardiac hypertrophy. Nature Medicine 13: 613-618.

Chang K.-T., Cheng C.-F., King P.-C., Liu S.-Y. and Wang G.-S. (2017) CELF1 mediates connexin 43 mRNA degradation in dilated cardiomyopathy. Circulation Research 121: 1140-1152.

Curcio A., Torella D., Iaconetti C., Pasceri E., Sabatino J., Sorrentino S., et al. (2013) MicroRNA-1 downregulation increases connexin 43 displacement and induces ventricular tachyarrhythmias in rodent hypertrophic hearts. PloS One 8: e70158.

Dong S., Cheng Y., Yang J., Li J., Liu X., Wang X., et al. (2009) MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. Journal of Biological Chemistry 284: 29514-29525.

Lian D., Xiong X., Liu Y. and Wang J. (2014) miRNA-1: functional roles and dysregulation in heart disease. Molecular BioSystems 10: 2775-2782.

Firestone G. L. and Kapadia B.J. (2012) Minireview: regulation of gap junction dynamics by nuclear hormone receptors and their ligands. Molecular Endocrinology 26: 1798-1807.

Foley PL. (2005) common surgical procedures in rodents', laboratory animal medicine and management. office of animal research education and compliance, University of Virginia, Charlottesville, VA, USA: International Veterinary Information Service, Ithaca NY (www. ivis. org).

Huang C.-K., Lee S. O., Chang E., Pang H. and Chang C. (2016) Androgen receptor (AR) in cardiovascular diseases. The Journal of Endocrinology 229: R1.

Huang Y. M., Li W. W., Wu J., Han M. and Li B. H. (2019) The diagnostic value of circulating microRNAs in heart failure. Experimental and Therapeutic Medicine 17: 1985-2003.

Jansen J. A., van Veen T. A., de Bakker J. M. and van Rijen H. V. (2010) Cardiac connexins and impulse propagation. Journal of Molecular and Cellular Cardiology 48: 76-82.

Kamal D. A. M., Ibrahim S. F. and Mokhtar M. H. (2020) Effects of testosterone on the expression of Connexin 26 and Connexin 43 in the uterus of rats during early pregnancy. In Vivo 34: 1863-1870.

Keating N. L., O'Malley A. J. and Smith M. R. (2006) Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. Journal of clinical oncology 24: 4448-4456.

Kramer M. F. (2011) Stem-loop RT-qPCR for miRNAs. Current Protocols in Molecular Biology. 95: 15.10. 1-15.10. 15.

Kura B., Kalocayova B., Devaux Y. and Bartekova M. (2020) Potential clinical implications of miR-1 and miR-21 in heart disease and cardioprotection. International Journal of Molecular Sciences 21: 700.

Kurtenbach S., Kurtenbach S. and Zoidl G. (2014) Gap junction modulation and its implications for heart function. Frontiers in Physiology 5: 82.

Liu N. and Olson E. N. (2010) MicroRNA regulatory networks in cardiovascular development. Developmental Cell 18: 510-525.

Malkin C.J. and Channer K. (2009) Testosterone in chronic heart failure. Advances in the Management of Testosterone Deficiency 37: 183-196.

Manzano-Fernández S., Pastor-Pérez F. J., Barquero-Pérez Ó, Pascual-Figal DA, Goya-Esteban R, Rojo-Álvarez J. L., et al. (2011) Short-term variability of heart rate turbulence in chronic heart failure. Journal of Cardiac Failure 17: 735-741.

McCarthy J. J. (2011) The MyomiR network in skeletal muscle plasticity. Exercise and Sport Sciences Reviews 39: 150.

Michela P., Velia V., Aldo P. and Ada P. (2015) Role of connexin 43 in cardiovascular diseases. European Journal of Pharmacology 768: 71-76.

Mitchelson K. R. and Qin W.-Y. (2015) Roles of the canonical myomiRs miR-1,-133 and-206 in cell development and disease. World Journal of Biological Chemistry 6: 162.

Mitsuzuka K. and Arai Y. (2018) Metabolic changes in patients with prostate cancer during androgen deprivation therapy. International Journal of Urology 25: 45-53.

Nielsen S., Hvid T., Kelly M., Lindegaard B., Dethlefsen C., Winding K., et al. (2014) Muscle specific miRNAs are induced by testosterone and independently upregulated by age. Frontiers in Physiology 4: 394.

Oyamada M., Takebe K. and Oyamada Y. (2013) Regulation of connexin expression by transcription factors and epigenetic mechanisms. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828: 118-133.

Pongkan W., Chattipakorn S. C. and Chattipakorn N. (2015) Chronic testosterone replacement exerts cardioprotection against cardiac ischemia-reperfusion injury by attenuating mitochondrial dysfunction in testosterone-deprived rats. PloS One 10: e0122503.

Schmittgen T. D. and Livak K.J. (2008) Analyzing real-time PCR data by the comparative CT method. Nature Protocols 3: 1101-1108.

Shahani S., Braga-Basaria M. and Basaria S. (2008) Androgen deprivation therapy in prostate cancer and metabolic risk for atherosclerosis. The Journal of Clinical Endocrinology & Metabolism 93: 2042-2049.

Shan Z.-X., Lin Q.-X., Fu Y.-H., Deng C.-Y., Zhou Z.-L., Zhu. J.-N., et al. (2009) Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. Biochemical and Biophysical Research Communications 381: 597-601.

Wieciech I, Grzesiak M., Knapczyk-Stwora K., Pytlik A. and Słomczyńska M. (2014) Influence of the antiandrogen flutamide on connexin 43 (Cx43) gene and protein expression in the porcine placenta and uterus during pregnancy. Folia Biologica (Kraków) 62: 367-375.

Wu C.-H., Yang J.-G., Yang J-J., Lin Y.-M., Tsai H.-D., Lin C.-Y., et al. (2010) Androgen excess down-regulates connexin43 in a human granulosa cell line. Fertility and Sterility 94: 2938-2941.

Xu M., Hu C., Khan H.-h., Shi F.-h., Cong X.-d., Li Q., et al. (2016) Argirein alleviates stress-induced and diabetic hypogonadism in rats via normalizing testis endothelin receptor A and connexin 43. Acta Pharmacologica Sinica 37: 246-254.

Yan Y., Dang H., Zhang X., Wang, X., and Liu, X. (2020) The protective role of MiR-206 in regulating cardiomyocytes apoptosis induced by ischemic injury by targeting PTP1B. Bioscience Reports 40: BSR20191000.

Yang B., Lin H., Xiao J., Lu Y., Luo X., Li B., et al. (2007) The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nature Medicine 13: 486-491.

Zhang J., Jin S., Zhao J. and Li H. (2016) Effect of dibutyl phthalate on expression of connexin 43 and testosterone production of leydig cells in adult rats. Environmental Toxicology and Pharmacology 47: 131-135.