Assessment of the Cardiovascular Risk of High-Fat-High-Fructose Diet in Hereditary Hypertriacylglycerolemic Rats and Venlafaxine Effect

Michaela SASVÁRIOVÁ[1], Lazaros SALVARAS[1], Dominika SEČKÁROVÁ MICHÁLIKOVÁ[2], Barbara TYUKOS KAPRINAY[2], Vladimír KNEZL[2], Zdenka GÁSPÁROVÁ[2], Tatiana STANKOVIČOVÁ[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava, Slovak Republic, [2]Centre of Experimental Medicine, Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology, Bratislava, Slovak Republic

Received June 7, 2024
Accepted September 17, 2024

Summary

Metabolic syndrome (MetS) represents a worldwide health problem, affecting cardiovascular and mental health. People with MetS are often suffering from depression. We used hereditary hypertriacylglycerolemic (HTG) rats as an animal model of MetS, and these were fed a high-fat-high-fructose diet (HFFD) to imitate unhealthy eating habits of people having several MetS risk factors and suffering depression. Male HTG rats were fed a standard diet (HTG-SD) or HFFD for eight weeks (HFFD8). Venlafaxine was administered for the last three weeks of the experiment (HFFD8+VE). Heart function was observed on the level of intact organisms (standard ECG *in vivo*), isolated hearts (perfusion according to Langendorff *ex vivo*), and molecular level, using the RT-PCR technique. The function of the isolated perfused heart was monitored under baseline and ischemia/reperfusion conditions. Analysis of ECG showed electrical abnormalities *in vivo,* such as significant QRS complex prolongation and increased heart rate. *Ex vivo* venlafaxine significantly reduced QT interval after ischemia/reperfusion injury. Baseline values of contractile abilities of the heart tended to be suppressed by HFFD. A significant reduction of LVDP was present in the HFFD8 group. Molecular analysis of specific genes involved in cardiac electrical (Cacna1c, Scn5a), contractile (Myh6, Myh7), metabolic function (Pgc1α) and calcium handling (Serca2a, Ryr2) supported some of the functional findings *in vivo* and *ex vivo*. Based on the present effect of venlafaxine on heart function, further research is needed regarding its cardiometabolic safety in the treatment of patients with MetS suffering from depression.

Keywords

Metabolic syndrome • Venlafaxine • ECG • Cardiac contraction • Ischemia/Reperfusion

Corresponding author

Tatiana Stankovičová, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovak Republic. E-mail: stankovicova@fpharm.uniba.sk

Introduction

Metabolic syndrome (MetS) represents a multifactorial disorder, affecting as much as 20 – 30 % of the general world population, and the prevalence is still rising [1]. MetS is characterized by four main symptoms: obesity, hyperglycemia, dyslipidemia and hypertension [2]. Patients with MetS are at high risk for the development of many other diseases, *e.g.* type 2 diabetes mellitus, cardiovascular diseases (CVD), or psychiatric disorders (mainly depression and psychoses). MetS increases the risk of the development of CVD 1.7 times [3].

The prevalence of MetS among patients with serious mental illness is 25 – 50 %, while it is 30.5-31.3 % among patients with depression [4]. Depending on the chosen therapy, it is necessary to take into consideration that MetS, depression and even many antidepressants themselves may contribute to the deterioration of the cardiovascular risk. Previous studies showed that some

components of MetS, such as obesity and dyslipidemia are more strongly associated with psychiatric disorders than others [5, 6].

It has been proposed, that antidepressants from the group of selective serotonin reuptake inhibitors (SSRIs) could be appropriate for the patients with MetS. They are pharmacodynamically specific and have minor side effects in comparison with less specific antidepressants, such as tricyclic (*e.g.* amitriptyline) or monoamine oxidase inhibitors (e.g. tranylcypromine). On the other hand, the majority of SSRIs (paroxetine, fluoxetine, citalopram, escitalopram) tend to negatively modify the lipid profile and increase the levels of triacylglyceroles and LDL cholesterol, potentially due to increased appetite [7].

Antidepressants from the group of selective serotonin and norepinephrine inhibitors (SNRIs), such as venlafaxine, are less described in the context of cardiometabolic safety. However, some clinical studies found a relationship between venlafaxine use and symptoms of MetS, such as dyslipidemia, increased blood pressure, and weight gain [8, 9].

In the present work, hereditary hypertriacylglycerolemic (HTG) rats were used as an animal model of human hypertriglyceridemia [10]. Hereditary HTG rats are characterized by hypertriglyceridemia, mild hypertension, and insulin resistance and they have some disturbances in glucose metabolism [11]. As patients with MetS have bad eating habits and often suffer from depression (and *vice versa*), we aimed to imitate this situation in HTG rats representing the animal model of MetS [12] fed unhealthy high-fat-high-fructose diet (HFFD) and focus on the cardiometabolic effect of the antidepressant venlafaxine.

Materials and Methods

Animals and experimental protocol

Male hereditary hypertriacylglygerolemic (HTG) rats (age 12 weeks, n=33) were used in this study. Rats were obtained from the Department of Toxicology and Breeding of Laboratory Animals, Dobrá Voda (SK CH 24016) of the Centre of Experimental Medicine, Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology (CEM SAS IEPT). The experiments were realized according to the approval of the Ethical Committee of CEM SAS IEPT and the State Veterinary and Food Administration of the Slovak Republic (No. 3853/18-221/3). An experimental model of metabolic syndrome used in these experiments was designed and prepared at CEM SAS, IEPT [12]. Rats were housed under standard conditions, with water and food *ad libitum* and a 12h light/dark cycle.

Rats from the tested groups were fed for eight weeks with the high-fat-high-fructose diet (HFFD), containing 1 % cholesterol, 7.5 % pork lard, and 10 % fructose. The standard diet (SD) for rodents and a modified HFFD were provided by the Department of Toxicology and Breeding of Laboratory Animals, Dobrá Voda, CEM SAS; IEPT (SK CH 24016). Animals were divided into 3 groups: controls fed SD (HTG-SD; n=14), rats fed HFFD (HFFD8; n=10), and rats fed HFFD with venlafaxine treatment (HFFD8+VE; n=9). Venlafaxine (VE; Chemos, Regenstauf, Germany) was administered to rats during the last three weeks of 8-week-lasting HFFD *p.o.* at a dose of 10 mg/kg/day (divided into 2 doses *per* day). The dose of venlafaxine was selected according to the work of Wang and co-workers [13] when the dose of 10 mg/kg ameliorated the depression-like behavior in a rat depression model.

Serum lipid profile and fasting blood glucose

The lipid profile was determined by diagnostics kits (ERBA Lachema, Brno, Czech Republic) at the end of the experiment. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerols (TAG) were examined. The absorbances of the resulting colored compound were measured spectrophotometrically at 500 nm on LabSystems 352 Multiskan MS Microplate Reader (ThermoFisher Scientific, U.S.). Glucose was measured by the glucometer (Contour Plus, Bayer, Germany) in the drop of blood taken from the tail vein after 14 hours (5:00 p.m. to 7:00 a.m.) of rat starvation.

ECG measurement

Standard ECG was monitored in conscious rats at the beginning of the experiment ("Onset" value) and at the end of the experimental protocol ("End" value), using SEIVA EKG Praktik Veterinary (SEIVA, Ltd., Prague, Czech Republic) [14]. Recordings were analyzed offline using the software SEIVA, Database Veterinary, Prague, Czech Republic.

Langendorff perfusion

After *in vivo* measurements, rats were anesthetized with diethyl ether and decapitated.

Hearts of rats (HTG-SD, n=8; HFFD8, n=5; HFFD8+VE, n=4) were isolated and perfused retrogradely through the aorta according to the Langendorff technique,

at the constant pressure (80 mmHg). Krebs-Henseleit solution (in mmol/l: NaCl, 118.00; KCl, 4.75; $CaCl_2$ x $2H_2O$, 2.50; MgSO4 x $7H_2O$, 1.20; KH_2PO_4, 1.18; $NaHCO_3$, 20.00; glucose, 11.10; Centralchem, Bratislava, Slovak Republic, and MikroCHEM, Pezinok, Slovak Republic) was used as a perfusion medium and was saturated by a gas mixture of 95 % O_2 and 5 % CO_2, pH 7.4; temperature 37 °C. A balloon (size 5; 0.1 ml) filled with water was inserted into the left ventricle to assess the basal diastolic pressure (80 mmHg). According to our perfusion protocol, a 10-minute stabilization phase was followed by a 30-minute ischemia and a 30-minute reperfusion. The ECG-like recording was monitored with a pair of electrodes positioned on the right atrium and left ventricle. Left ventricular pressure was measured with the electro-manometer (Tesla, Prague, Czech Republic). Other parameters – left ventricular developed pressure (LVDP), left ventricular diastolic pressure (LVPd), heart rate (HR), rate of contraction (+dp/dt) and relaxation (-dp/dt) were monitored with BioLab F software (Institute of Measurement Sciences, Slovak Academy of Sciences, Slovak Republic).

Sample preparation

Samples were collected from the left ventricular free wall weighted (approximately 100 mg) placed in the liquid nitrogen, and stored in the freezer at -80 °C. Samples from the hearts that underwent ischemia/reperfusion conditions were used (HTG-SD, n=8; HFFD8, n=5; HFFD8+VE, n=4).

RNA isolation and RT-PCR

Frozen samples from the left ventricle were homogenized in liquid nitrogen and total RNA was isolated by acid phenol-guanidinium thiocyanate-chloroform extraction (TriReagent, Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturer protocol. Electrophoresis in 2 % agarose gel (Agarose, Sigma-Aldrich, St. Louise, MO, USA) was used for the RNA quality control. Intact RNA samples were used for the reverse transcription with the High Capacity cDNA Reverse Transcription Kit with RNase inhibitors (Applied Biosystems, Grand Island, NY, USA). Quantitative real-time PCR (RT-PCR) was performed using SYBR Select Master Mix (Thermo Fisher Scientific, USA) on ABI Prism 7300 Real-Time PCR System (Applied Biosystems, USA). We focused on gene expression of myosin heavy chain 6 (Myh6), myosin heavy chain 7 (Myh7), cardiac ryanodine receptors 2 (Ryr2), sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (Serca2a), cardiac L-type calcium channel- α1C subunit (Cacna1c), sodium voltage-gated channel α subunit 5 (Scn5a), peroxisome proliferator-activated receptor gamma coactivator 1α (Pgc1α). Relative expression of selected genes was normalized to the expression of housekeeping genes: β-actin (Actb) and hypoxanthine phosphoribosyltransferase 1 (Hprt1). All of the used primers are shown in Table 1.

Data analysis

Data are expressed as mean ± the standard error of the mean (S.E.M.) or mean ± standard deviation (S.D.). Differences between HTG-SD, HFFD8, and HFFD8+VE were evaluated using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test. In ECG measurement *in vivo*, the Onset and the End values were compared by the Student *t*-test. Stabilization (STAB) and reperfusion (REP) values during ischemia/reperfusion injury *ex vivo* were compared by the Student *t*-test. The limit of $p<0.05$ was considered a statistically significant difference. The data were statistically evaluated using the GraphPad software version InStat V2.05.

Results

The basal metabolic characteristics of HTG rats are shown in Table 2. Eight weeks lasting HFFD induced an increase in the serum TC and a decrease in HDL-C and TAG values. The blood glucose value was not affected by HFFD. Venlafaxine treatment induced increased body weight gain compared to HTG-SD rats.

In vivo ECG measurements

The PQ interval was not significantly affected either by HFFD or venlafaxine treatment (Table 3). The QRS complex duration was prolonged in HTG rats fed either a standard diet or HFFD. In the venlafaxine group, the QRS complex duration was not significantly prolonged at the end of the experiment comparing onset value. A significantly prolonged QT interval was observed in the HTG-SD group, but this effect was diminished by HFFD.

Table 1. Primer sequences for RT-PCR of selected cardiac genes

Gene	GenBank accession number	Primer sequence (5´-3´) PCR	Product length (bp)
Myh6	NM_017239.2	Forward: GCCCTTTGACATCCGCACAGAGT Reverse: TCTGCTGCATCACCTGGTCCTCC	152
Myh7	NM_017240.1	Forward: GCGGACATTGCCGAGTCCCAG Reverse: GCTCCAGGTCTCAGGGCTTCACA	133
Scn5a	NM_001160162.1; NM_013125.2	Forward: TGTATGTCCTCAGCCCCTTC Reverse: ATGAACACGCAGTTGGTCAG	112
Ryr2	NM_001191043.1; NM_032078.2	Forward: ACTGCTGGGCTACGGCTAC Reverse: CTGAAGATGCGGAACCTCTC	99
Atp2a2 (Serca2a)	NM_001110139.2	Forward: CCCGAAACTACCTGGAGCCTGCA Reverse: ATGCACGCACCCGAACACCC	83
Ppargc1a (Pgc1a)	NM_031347.1, XM_039092488.1, XM_039092489.1, XM_039092490.1, XM_039092491.1, XM_039092492.1, XM_039092493.1, XM_039092494.1	Forward: AGTCACCAAATGACCCCAAGG Reverse: TATGAGGAGGAGTCGTGGGA	114
Cacna1c	NM_012517.2	Forward: GTTGCCCTGGGTGTATTTTG Reverse: GGCTTTCTCCCTCTCTTTGG	109

Table 2. Effect of high-fat-high-fructose diet and venlafaxine treatment on metabolic parameters of HTG rats

Parameter	HTG-SD *n*=14	HFFD8 *n*=10	HFFD8+VE *n*=9
Body weight (g) Onset of experiment	237.70 ± 11.62	238.20 ± 9.24	211.11 ± 12.33
Body weight (g) End of experiment	362.40 ± 11.81	382.00 ± 10.34	370.33 ± 9.72
Body weight gain (%) End of experiment	53.84 ± 3.74	61.24 ± 3.62	78.98 ± 8.47 *
Serum TC (mmol/l) End of experiment	1.07 ± 0.02	3.13 ± 0.11 *	3.39 ± 0.09 *
Serum HDL-C (mmol/l) End of experiment	0.75 ± 0.07	0.61 ± 0.01 *	0.55 ± 0.03 *
Serum TAG (mmol/l) End of experiment	4.50 ± 0.31	1.27 ± 0.10 *	1.90 ± 0.18 *
Fasting blood glucose (mmol/l) End of experiment	7.99 ± 0.48	8.45 ± 0.26	7.58 ± 0.27

Groups: hypertriacylglycerolemic (HTG) rats fed eight weeks of a standard diet (HTG-SD); HTG rats fed a high-fat-high-fructose diet (HFFD) lasting eight weeks (HFFD8); HTG rats fed HFFD eight weeks and treated with venlafaxine (VE; 10 mg/kg) last three weeks of diet (HFFD8-VE). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TAG), number (*n*) of rats in the group. Values are mean ± S.E.M. Significant difference *vs.* HTG-SD, *p<0.05, ANOVA, Tukey-Kramer comparison test.

In the HFFD8+VE group, the same QT duration was observed at the Onset and End of the experiment. HFFD significantly increased heart rate *in vivo*. An increase in heart rate was found in the HFFD8+VE group at the End of the diet.

Ex vivo ECG measurements

In an *ex vivo* experiment on isolated hearts perfused according to the Langendoff technique, only mild differences in monitored ECG parameters were observed among groups in the stabilization phase. Thus neither HFFD nor HFFD with venlafaxine treatment affects baseline ECG parameters as PQ, QRS, QT interval, and heart rate (Table 4). Then the hearts were subjected to ischemia/reperfusion. Our experimental ischemia/ reperfusion conditions induced only a weak, not quite significant, increase in heart rate due to HFFD in isolated perfused hearts (Table 4). However, venlafaxine induced marked shortening of the QT interval (**p*=0.047).

Table 3. Effect of high-fat-high-fructose diet and venlafaxine treatment on ECG parameters in hypertriacylglycerolemic (HTG) rats *in vivo*

	In vivo							
	PQ (ms)		**QRS** (ms)		**QT** (ms)		**HR** (beats/min)	
	Onset	**End**	**Onset**	**End**	**Onset**	**End**	**Onset**	**End**
HTG-SD *n*=14	46.04 ± 1.03	46.51 ±1.06	19.89 ±0.64	22.61 ± 0.53 *	58.09 ± 1.00	66.87 ± 1.00 *	459.45 ± 17.08	443.98 ± 13.92
HFFD8 *n* =10	45.54 ± 3.35	43.00 ± 1.07	20.94. ± 1.24	23.66 ± 0.51 *	55.54 ± 0.99	58.8 ± 2.01	462.29 ± 18.31	510.47 ± 9.27 *
HFFD8+VE *n*=9	45.07 ± 1.35	41.82 ± 1.06	21.69 ± 0.87	23.69 ± 0.82	59.64 ± 0.89	59.71 ± 0.91	466.84 ± 14.88	512.93 ± 6.96 *

Values at the beginning of the experiment (Onset), after an 8-week lasting high-fat-high-fructose diet (HFFD), or after HFFD with venlafaxine (VE) treatment at the end of the experiment (End) were compared. Differences among groups in baseline (Onset) values were compared. Groups: hypertriacylglycerolemic (HTG) rats fed eight weeks of a standard diet (HTD-SD), HTG rats fed eight weeks with HFFD (HFFD8), HTG rats fed eight weeks with HFFD and last three weeks of diet treated with VE (HFFD8+VE); *n* - number of rats in the group. Values are expressed as mean ± S.E.M. **p*<0.05 - a significant difference between Onset *vs.* End value; Student *t*-test.

Table 4. Effect of high-fat-high-fructose diet and venlafaxine treatment on ECG parameters *ex vivo*, in the phase of stabilization (STAB) and reperfusion (REP)

	Ex vivo							
	PQ (ms)		**QRS** (ms)		**QT** (ms)		**HR** (beats/min)	
	STAB	**REP**	**STAB**	**REP**	**STAB**	**REP**	**STAB**	**REP**
HTG-SD *n*=8	51.89 ± 26.47	45.48 ± 4.15	24.17 ± 7.07	19.12 ± 3,36	80.66 ± 19.82	78.68 ± 3.87	314.96 ± 55.38	317.81 ± 39.82
HFFD8 *n*=5	43.75 ± 7.89	49.75 ± 8.59	17.50 ± 4.36	18.25 ± 2.99	82.25 ± 19.94	75.50 ± 12.01	251.78 ± 8.32	281.81 ± 32.18
HFFD8+VE *n*=4	34.25 ± 14.60	40.25 ± 7.72	18.00 ± 2.44	15.00 ± 2.44	96.00 ± 19.62	68.00 ± 10.96 *	287.79 ± 40.10	294.43 ± 30.94

Groups: hypertriacylglycerolemic (HTG) rats fed eight weeks of a standard diet (HTD-SD), HTG rats fed eight weeks with HFFD (HFFD8), HTG rats fed eight weeks with HFFD and last three weeks of diet treated with VE (HFFD8+VE). Isolated rat hearts perfused according to the Langendorff technique, after 10 min stabilization (STAB) were exposed to 30 min ischemia (stop flow) followed by 30 min reperfusion (REP). Values are expressed as mean ± S.D. **p*<0.05 - a significant difference between STAB *vs.* REP value of QT interval; the Student *t*-test.

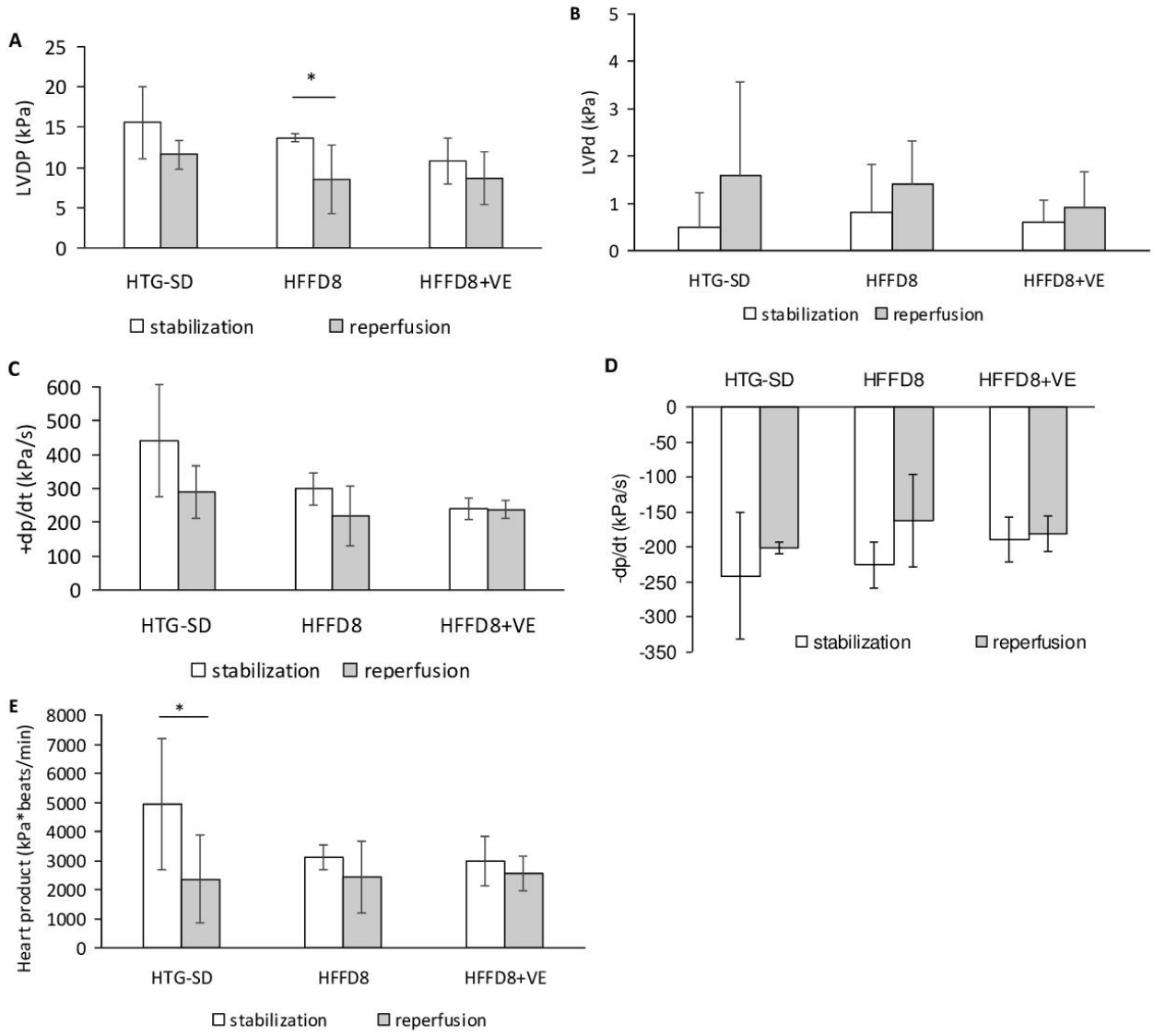

Fig. 1. Effect of high-fat-high-fructose diet (HFFD) and venlafaxine (VE) treatment on the contractile function of isolated heart perfused according to Langendorff. (**A**) Left ventricular developed pressure (LVDP), (**B**) left ventricular diastolic pressure (LVPd), (**C**) rate of contraction (+dp/dt), (**D**) rate of relaxation (-dp/dt), (**E**) heart product. Groups: hypertriacylglycerolemic (HTG) rats fed a standard diet (HTD-SD; *n*=8), HTG rats fed eight weeks with HFFD (HFFD8; *n*=5), HTG rats fed eight weeks with HFFD and last three weeks of diet treated with VE (HFFD8+VE; *n*=4). Values are expressed as mean ± S.D. *p<0.05 - a significant difference at stabilization and reperfusion value; Student *t*-test.

An analysis of contraction of isolated perfused hearts (Fig. 1) showed a tendency to the cardio-depressive effect induced by HFFD on the heart product (HP) and contraction rate (+dp/dt) comparing control animals in baseline values during the stabilization (STAB) phase. This trend persisted in venlafaxine-treated rats. Ischemia/reperfusion injury was the most present in the HFFD8, where a significantly reduced LVDP value was observed in the reperfusion period. In the control group fed a standard diet, a significant reduction in HP value was found. When hearts underwent ischemia/reperfusion conditions, venlafaxine treatment did not induce any changes in contraction parameters.

In molecular analysis (Fig. 2), we focused on specific parameters involved in the characterization of electric, contractile, and metabolic properties of the heart.

Myosin heavy chains: We found, that HFFD significantly suppressed the relative expression of Myh6. Venlafaxine together with HFFD increased Myh6 mRNA level to control values. The relative expression of Myh7 had a similar trend.

Intracellular Ca^{2+} regulation: Ryr2 was significantly down-regulated by HFFD, while venlafaxine increased the expression of Ryr2 to the control values. Relative expression of Serca2a was not changed either by HFFD or venlafaxine treatment.

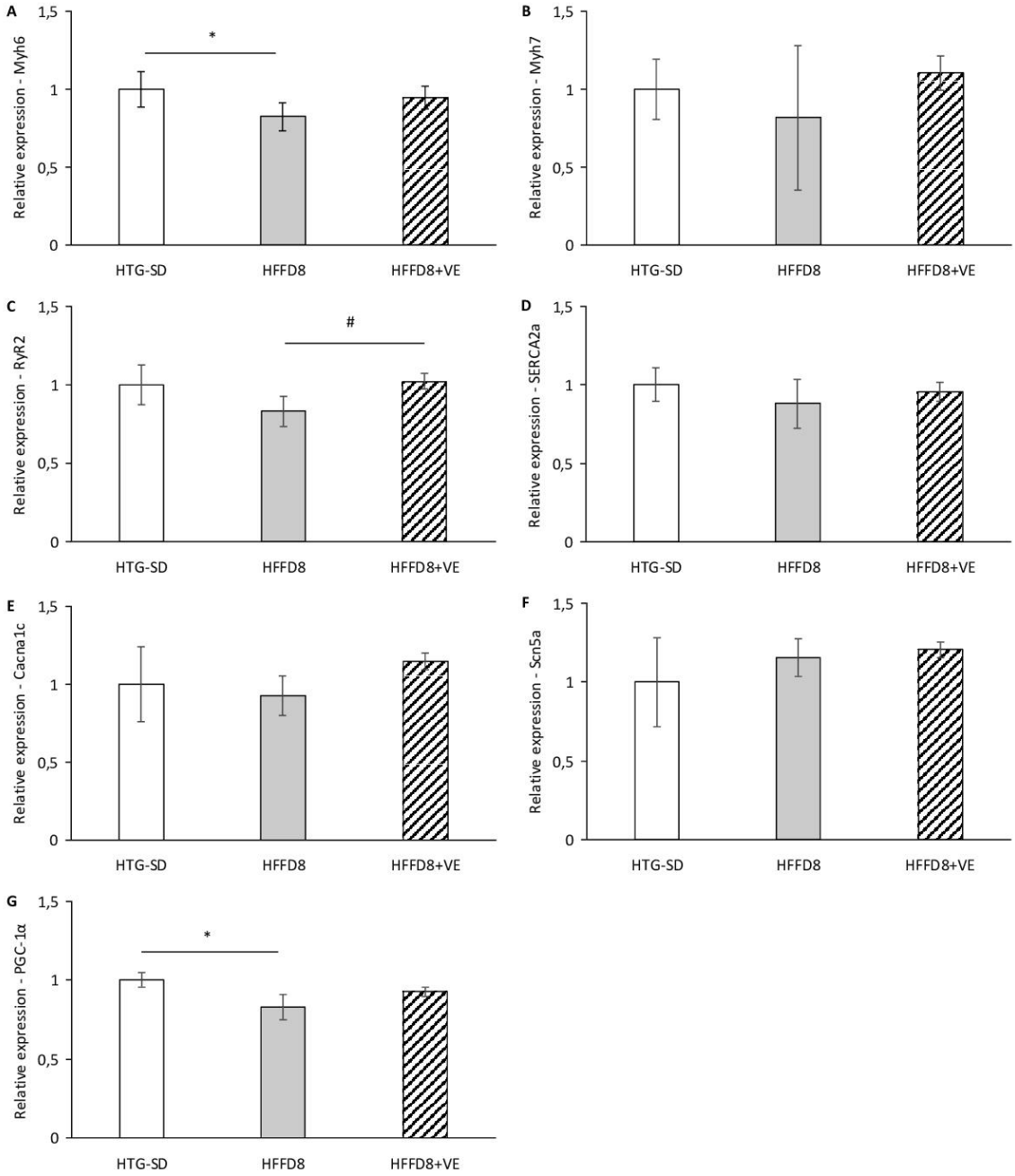

Fig. 2. Relative mRNA expression of specific cardiac genes, supporting the functional changes induced by a high-fat-high-fructose diet (HFFD) and venlafaxine (VE) administration. Groups: hypertriacylglycerolemic (HTG) rats fed eight weeks of a standard diet (HTD-SD; n=8), HTG rats fed 8 weeks with HFFD (HFFD8; n=5), HTG rats fed 8 weeks with HFFD and last 3 weeks of diet treated with VE (HFFD8+VE; n=4). Values are expressed as mean ± S.D. *p<0.05 - a statistically significant difference HTG-SD *vs.* HFFD8; $^{\#}$ p<0.05 HFFD8 *vs.* HFFD8+VE; ANOVA, the Tukey-Kramer comparison test.

Transmembrane ion transport: L-type Ca^{2+} channel (Cacna1c) tended to be up-regulated by the administration of venlafaxine in HFFD-fed rats. We observed a trend in the up-regulation of Scn5a ($Na_{v1.5}$) by HFFD, and this effect was potentiated by venlafaxine.

Cardiac metabolism: Pgc1α, a key player in mitochondrial biogenesis, was significantly down-regulated in the HFFD group compared to control. Venlafaxine treatment suppressed the effect of HFFD on the Pgc1α relative expression.

Discussion

Our study was focused on the assessment of the cardiovascular disturbances in the experimental model of MetS, induced in HTG rats by feeding HFFD representing

unhealthy eating habits in people with MetS, often suffering from depression, and the effect of venlafaxine administration was studied. An unhealthy sedentary lifestyle and a high-caloric diet result to metabolic diseases such as hypertension, visceral obesity, dyslipidemia, insulin resistance, hyperglycemia, non-alcoholic fatty liver disease, *etc*. Increased lipogenesis triggers atherogenic dyslipidemia and the pro-inflammatory cytokines release promoting chronic inflammation and thrombotic susceptibility with microvascular damage leading to cardiovascular disease [15]. We present here that in HTG rats used as an animal model of MetS [11-13] when fed HFFD a further increase in TC was achieved. Dyslipidemia is a condition related to cardiovascular disorders [15]. In this article, the damage of HTG rat heart function was studied *in vivo* and *ex vivo* supported by molecular analysis of gene experssion associated with electric, contractile, and metabolic properties of the heart.

We found that both, the modified high-fat-high-fructose diet and venlafaxine administration, seemed to accelerate the atrial conduction and shorten the PQ interval duration. *In vivo*, we observed a significant increase in QRS complex duration and heart rate due to HFFD. *Ex vivo*, QT interval tended to prolongate during the stabilization phase in the venlafaxine-treated group compared to the control group. Interestingly, exposure to the ischemia/reperfusion conditions did not cause any significant disturbances in the cardiac electrical activity of any group, apart from the marked shortening of QT interval in the venlafaxine group, which could be considered a beneficial effect comparing to QT interval prolongation in stabilization phase. Similarly, as we found, *in vivo* ECG recording in rats fed a fructose-fat diet showed QRS complex prolongation comparing control rats, and no significant changes in PQ or QT interval were observed [16]. Axelsen and co-workers [17] found, significantly slowed conduction velocity measured in strips from the right ventricle in rats fed a high-fat-high-fructose diet, compared to the control group. In experimental MetS induced by a high-carbohydrate diet, depression in heart work was observed in Langendorff-perfused hearts, together with increased heart rate and shortened QT interval duration. These hearts had significantly decreased cardiac output and increased end-diastolic pressure [18]. According to our results in the HFFD-fed group, QRS complex duration was prolonged in patients with obesity and hypertension, without any other cardiovascular disease or arrhythmias [19]. Teodorescu and co-authors [20] confirmed the association between prolonged QRS complex duration and sudden cardiac death in patients with coronary heart disease, independently of QTc interval prolongation.

Regarding treatment with venlafaxine, cardiovascular adverse effects were observed previously, mainly at higher doses, or in overdosed individuals. Case studies displayed dose-dependent venlafaxine-induced sinus tachycardia [21], prolonged QRS complex and QT interval in over-dosed patients [22], and hypertensive crisis in a patient with a low dose of venlafaxine [23]. On the other hand, venlafaxine in a dose of up to 300 mg/day did not prolong the QTc interval in older adults with major depressive disorder, as well QRS complex duration was unaffected, and only the PR interval was shortened [24].

In the present work, in the stabilization period, we showed that parameters of cardiac contraction tended to decreae by HFFD and venlafaxine administration compared to the control group. Ischemia/reperfusion conditions evoked the greatest injury in the HTG-SD group, but contractile dysfunction was elicited even due to HFFD. Venlafaxine did not potentiate ischemia/ reperfusion-induced changes.

To support observed heart dysfunction with associated cellular and molecular changes, the study of Dong and researchers [16] reported that five months of a high-fat diet in mice led to a decrease in the rate of contraction and relaxation, together with decrease in number of mitochondria and down-regulation of peroxisome proliferator activated receptor gamma coactivator-1alpha (Pgc1α). Increased levels of adipocyte-derived fatty acid-binding protein 4 (FABP4) inhibited cardiac contraction in a concentration-dependent manner [25]. Cardiodepressive substances from adipocytes could represent the link between cardiac dysfunction and MetS.

Venlafaxine showed potential to preserve the rate of contraction and relaxation on the values from stabilization. Gaur & Kumar [26] showed the protective function of venlafaxine against brain ischemia/reperfusion injury, resulting in mitochondrial dysfunction in mice, by decreasing the level of oxidative stress [26].

Some studies have found a relationship between mutations in Myh6 and dilated or hypertrophied cardiomyopathy, myocardial dysfunction, and heart failure [27]. In rodents, the ventricular α-MHC is a predominant form of myosin heavy chain, while β-MHC expression is diminished in postnatal development [28]. However, decreased expression of Myh6 could lead to disrupted contractile function of the heart. Our study showed significantly reduced expression of Myh6 in rats fed with

HFFD, but not with venlafaxine. This is consistent with findings about the dynamic of contraction and relaxation. With a decreased ventricular expression of Myh6, an increase in Myh7 expression is usually observed as a fetal form of myosin heavy chain, and is considered a marker of cardiac damage [29]. Our molecular analysis did not show an increase in Myh7 expression due to HFFD.

It has been observed, that the association between insulin resistance, obesity, and left ventricular contractile dysfunction may be due to changes in cardiac calcium handling, in rats with MetS [30]. Relative expression of ryanodine receptors 2 (Ryr2) was significantly decreased by the HFFD, but not in the presence of venlafaxine. A similar trend was observed in mRNA expression of sarcoplasmic/endoplasmic recitulum Ca^{2+} ATPase 2a (Serca2a), pointing to the fact, that HFFD could affect the intracellular Ca^{2+} regulation and cardiac contraction. The gene for transmembrane L-type calcium channels (Cacna1c) was slightly down-regulated by HFFD, but in the presence of venlafaxine, the expression of Cacna1c was even higher than in the control group. Components of MetS (insulin resistance, dyslipidemia) may compromise the gene expression in cardiomyocytes, including Ryr2 and Serca2a. The altered expression and function of Ryr2 is caused by overproduction of reactive oxygen species (ROS)-triggered glucotoxicity and apoptosis, contributing to systolic and diastolic dysfunction [30]. Dyslipidemia leads to lipotoxicity by the accumulation of fatty acids in cardiomyocytes, shortening of the action potential, disrupted opening of Ryr2 channels, diminished cycling of L-type calcium channel, and decreased stores of calcium in the sarcoplasmic reticulum [31,32]. Due to decrease in Serca2a expression, there is a lower Ca^{2+} uptake of the sarcoplasmic reticulum during relaxation and diastolic dysfunction. Down-regulation of Serca2 and Cacna1c was observed in gerbils fed a high-fat diet. Moreover, intra-myocardial lipid accumulation was found in these animals, what could result not only in contractile but also conduction disorders [33].

The initial phase of the action potential is driven by the entry of I_{Na} through $Na_{v1.5}$ channels encoded by Scn5a [34]. As observed in our study, increased expression of Scn5a in the presence of HFFD and venlafaxine could lead to accelerated ventricular depolarization. Metabolic alterations lead to disruption in I_{Na}, altered cardiac contractility, and potentially cause arrhythmias [35,36]. Venlafaxine is known to inhibit ventricular sodium channels [37], which can result in up-regulation of Scn5a expression, as seen in our experiments.

One of the key regulators involved in mitochondrial biogenesis is Pgc1α. The total absence of Pgc1α in mice resulted in cardiac contractile dysfunction [38]. Insulin resistance was manifested by a decrease in the number and size of skeletal muscle mitochondria, due to reduced expression of Pgc1α [39]. In our study, RT-PCR analysis showed significantly decreased expression of Pgc1α, which could point to disrupted energy metabolism in the myocardium. This would explain the changes in the process of contraction and relaxation. In the group with venlafaxine, we could see a trend of improvement, which corresponds with the fact, that rate of contraction and relaxation was not affected in this group, unlike the trend in the HTG-SD and HFFD8 groups. The potential protective role of venlafaxine was determined in the study by Wang and co-workers [40]. Administration of venlafaxine led to an increase in the expression of myocardial anti-apoptotic Bcl-xl and a decrease in proapoptotic Bax protein level in the animal model of chronic mild stress. Myocardial apoptosis is associated with heart diseases [41]. Venlafaxine is suggested to decrease the Bax/Bcl-xl ratio and may have a cardioprotective effect not only in individuals with depressive disorder but also suffering from MetS.

Conclusion

Hereditary HTG rats fed HFFD developed several metabolic and cardiac alterations. Electrical abnormalities were more visible on standard ECG *in vivo*. Analysis of isolated perfused hearts showed suppressed contractile ability of the heart affected by HFFD. Venlafaxine has the potential to protect the heart against ischemia/reperfusion injury by preserving the rate of contraction and relaxation in reperfusion on comparable values as in stabilization. Our findings were supported by the molecular analysis of specific cardiac genes involved in the electrical, contractile, and metabolic functions of the heart. Experimentally induced MetS seems to alter the metabolism of the heart itself and therefore disrupt its function. Venlafaxine did not improve the heart function affected by MetS. As a marked reduction of the QT interval in the *ex vivo* experiment was found, further research should verify its safety in the treatment of patients with MetS suffering from depression.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic VEGA 2/0018/23, APVV-22-0541, and APVV-19-0435.

References

1. Pucci G, Alcidi R, Tap L, Battista F, Mattace-Raso F, Schillaci G. Sex- and gender-related prevalence, cardiovascular risk and therapeutic approach in metabolic syndrome: A review of literature. Pharmacol Res 2017;120:34. https://doi.org/10.1016/j.phrs.2017.03.008
2. Kassi E, Pervanidou P, Kaltsas G, Chrousos G. Metabolic syndrome: definitions and controversies. BMC Med 2011;9:48. https://doi.org/10.1186/1741-7015-9-48
3. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med 2006;119:812-819. https://doi.org/10.1016/j.amjmed.2006.02.031
4. Alosaimi FD, Abalhassan M, Alhaddad B, Alzain N, Fallata E, Alhabbad A, Alassiry MZ. Prevalence of metabolic syndrome and its components among patients with various psychiatric diagnoses and treatments: A cross-sectional study. Gen Hosp Psychiatry 2017;45:62-69. https://doi.org/10.1016/j.genhosppsych.2016.12.007
5. Akbaraly TN, Kivimaki M, Brunner EJ, Chandola T, Marmot,MG, Singh-Manoux A, Ferrie JE. Association between metabolic syndrome and depressive symptoms in middle-aged adults: results from the Whitehall II study. Diabetes Care 2009;32:499-504. https://doi.org/10.2337/dc08-1358
6. Pyykkonen A-J, Raikkonen K, Tuomi T, Eriksson JG, Groop L, Isomaa B. Association between depressive symptoms and metabolic syndrome is not explained by antidepressant medication: results from the PPP-Botnia Study. Ann Med 2012;44:279-288. https://doi.org/10.3109/07853890.2010.543921
7. Goldstein MR, Mascitelli L, Pezzetta F. Is the increase in LDL cholesterol induced by selective serotonin reuptake inhibitor therapy a blessing in disguise? Med Hypotheses 2010;74:955-956. https://doi.org/10.1016/j.mehy.2009.11.041
8. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, Allen NB, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med 2013;11:200. https://doi.org/10.1186/1741-7015-11-200
9. Uguz F, Sahingoz M, Gungor B, Aksoy F, Askin R. Weight gain and associated factors in patients using newer antidepressant drugs. Gen Hosp Psychiatry 2015;37:46-48. https://doi.org/10.1016/j.genhosppsych.2014.10.011
10. Vrána A, Kazdová L. The hereditary hypetriglyceridemic nonobese rat: an experimental model of human hypertriglyceridemia. Transplant Proc 1990;22:2579
11. Zicha J, Pecháňová O, Čačányiová S, Cebová M, Kristek F, Török J, Šimko F, et al. Hereditary hypertriglyceridemic rat: a suitable model of cardiovascular disease and metabolic syndrome? Physiol Res 2006;55(Suppl.1):49-63. https://doi.org/10.33549/physiolres.930000.55.S1.49
12. Kaprinay B, Lipták B, Slovák L, Švík K, Knezl V, Sotníková R, Gáspárová Z. Hypertriacylglycerolemic rats fed high fat diet as a model of metabolic syndrome. Physiol Res 2016;65(Suppl.4):S515-S518. https://doi.org/10.33549/physiolres.933524
13. Wang C-H, Gu J-Y, Zhang X-L, Dong J, Yang J, Zhang Y-L, Ning Q-F, et al. Venlafaxine ameliorates the depression-like behaviors and hippocampal S100B expression in a rat depression model. Behav Brain Funct 2016;12:34-44. https://doi.org/10.1186/s12993-016-0116-x
14. Kráľová E, Mokráň T, Murín J, Stankovičová T. Electrocardiography in two models of isoproterenol-induced left ventricular remodeling. Physiol Res 2008;57(Suppl.2):S83S89, 2008. https://doi.org/10.33549/physiolres.931556
15. Hidalgo I, Fonseca-Coronado S, Ceballos G, Meaney E, Nájera N. Dyslipidemias, fatty liver, and cardiovascular disease. Cardiovasc Metab Sci 2022;33:134-139. https://doi.org/10.35366/107628
16. Dong F, Li Q, Sreejayan N, Nunn JM, Ren J. Metallothionein prevents high fat diet induced cardiac contractile dysfunction: role of peroxisome proliferator activated gamma coactivator 1alpha and mitochondrial biogenesis. Diabetes 2007;56:2201-2212. https://doi.org/10.2337/db06-1596

17. Axelsen LN, Calloe K, Braunstein TH, Riemann M, Hofgaard JP, Liang B, Jensen CF, et al. Diet-induced pre-diabetes slows cardiac conductance and promotes arrhythmogenesis. Cardiovasc Diabetol 2015;14:87. https://doi.org/10.1186/s12933-015-0246-8
18. Durak A, Olgar Y, Tuncay E, Karaomerlioglu I, Mutlu GK, Inan EA, Altan VM, et al. Onset of decreased heart work is correlated with increased heart rate and shortened QT interval in high carbohydrate fed overweight rats. Can J Physiol Pharmacol 2017;95:1335-1342. https://doi.org/10.1139/cjpp-2017-0054
19. Dzikowicz DJ, Carey MG. Obesity and hypertension contribute to prolong QRS complex duration among middle-aged adults. Ann Noninvasive Electrocardiol 2019;24:e12665. https://doi.org/10.1111/anec.12665
20. Teodorescu C, Reinier K, Uy-Evanado A, Navarro J, Mariani R, Gunson K, Jui J, et al. Prolonged QRS duration on the resting ECG is associated with sudden death risk in coronary disease, independent of prolonged ventricular repolarization. Heart Rythm 2011;8:1532-1567. https://doi.org/10.1016/j.hrthm.2011.06.011
21. Abozguia K, Chudley S, Gammage M. Dose-dependent venlafaxine induced sinus tachycardia. J Cardiol 2006;113:e9-10. https://doi.org/10.1016/j.ijcard.2006.01.072
22. Blythe D, Hackett LP. Cardiovascular and neurological toxicity of venlafaxine. Hum Exp Toxicol 1999;18:309-313. https://doi.org/10.1191/096032799678840165
23. Khurana RN, Baudendistel TE. Hypertensive crisis associated with venlafaxine. Am J Med 2003;115:676-677. https://doi.org/10.1016/S0002-9343(03)00472-8
24. Behlke LM, Lenze EJ, Pham V, Miller JP, Smith TW, Saade Y, Karp JF, et al. The effect of venlafaxine on electrocardiogram intervals during treatment for depression in older adults. J Clin Psychopharmacol 2020;40:553-559. https://doi.org/10.1097/JCP.0000000000001287
25. Lamounier-Zepter V, Look C, Alvarez J, Christ T, Ravens U, Schnuck W-H, Ehrhart-Bornstein M, et al. Adipocyte fatty acid-binding protein suppresses cardiomyocyte contraction. A new link between obesity and heart disease. Circ Res 2009;105:326-334. https://doi.org/10.1161/CIRCRESAHA.109.200501
26. Gaur V, Kumar A. Protective effect of desipramine, venlafaxine and trazodone against experimental animal model of transient global ischemia: Possible involvement of NO-cGMP pathway. Brain Res 2010;1353:204-212. https://doi.org/10.1016/j.brainres.2010.07.004
27. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. Circ Res 2000;86:386-390. https://doi.org/10.1161/01.RES.86.4.386
28. England J, Loughna S. Heavy and light roles: myosin in the morphogenesis of the heart. Cell Mol Life Sci 2013;70:1221-1239. https://doi.org/10.1007/s00018-012-1131-1
29. Locher MR, Razumova MV, Stelzer JE, Norman HS, Moss RL. Effects of low-level α-myosin heavy chain expression on contractile kinetics in porcine myocardium. Am J Physiol Heart Circ Physiol 2011;300:H869-H878. https://doi.org/10.1152/ajpheart.00452.2010
30. Balderas-Villalobos J, Molina-Muno T, Mailloux-Salinas P, Bravo G, Carvajal K, Gomez-Viquez NL. Oxidative stress in cardiomyocyte contributes to decreased SERCA2a activity in rats with metabolic syndrome. Am J Physiol Heart Circ Physiol 2013;305:H1344-1353. https://doi.org/10.1152/ajpheart.00211.2013
31. Battiprolu PK, Gillette TG, Wang ZV, Lavandero S, Hill JA. Diabetic cardiomyopathy: mechanisms and therapeutic targets. Drug Discov Today Dis Mech 2010;7:e135-143. https://doi.org/10.1016/j.ddmec.2010.08.001
32. Dinzer UD. Cardiac ryanodine receptor in metabolic syndrome: is JTV519 (K201) future therapy? Diabetes Metab Syndr Obes 2012;5:89-99. https://doi.org/10.2147/DMSO.S30005
33. Saharaoui A, Dewachter C, de Medina G, Naeije R, Bouguerra SA, Dewachter L. Myocardial structural and biological anomalies induced by high fat diet in Psammomys obesus gerbils. PLoS ONE 2016;11:e0148117. https://doi.org/10.1371/journal.pone.0148117
34. Lieve KV, Verkerk AO, Podliesna S, van der Werf C, Tanck MW, Hofman N, van Bergen PF, et al. Gain-of-function mutation in SCN5A causes ventricular arrhythmias and early onset of atrial fibrillation. J Cardiol 2017;236:187-193. https://doi.org/10.1016/j.ijcard.2017.01.113
35. Aromolaran AS, Boutjdir M. Cardiac ion channel regulation in obesity and the metabolic syndrome: Relevance to long QT syndrome and atrial fibrillation. Front Physiol 2017;8:431. https://doi.org/10.3389/fphys.2017.00431

36. Aromolaran AS, Colecraft HM, Butjdir M. High-fat diet-dependent modulation of the delayed rectifier K(+) current in adult guinea pig atrial myocytes. Biochem Biophys Res Commun 2016;474:554-559. https://doi.org/10.1016/j.bbrc.2016.04.113
37. Khalifa M, Daleau P, Turgeon J. Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocytes. J Pharmacol Exp Ther 1999;291:280-284.
38. Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, Ahmad F, et al. BasselDuby R, Rosenzweig A, Ingwall JS, Spiegelman BM.. Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. Cell Metab 2005;1:259-271. https://doi.org/10.1016/j.cmet.2005.03.002
39. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1α and PGC-1β) in muscle cells. J Biol Chem 2003;278:26597-26603. https://doi.org/10.1074/jbc.M301850200
40. Wang Y, Xiao Z, Liu X, Berk M. Venlafaxine modulates depression induced behaviour and the expression of Bax mRNA and Bcl-xl mRNA in both hippocampus and myocardium. Hum Psychopharmacol Clin Exp 2011;26:95-101. https://doi.org/10.1002/hup.1177
41. Ho YL, Chen CL, Hsu RB, Lin LC, Huang PJ. The correlation between expression of apoptosis related proteins and myocardial function reserve evaluated by dobutamine stress echocardiography in patients with dilated cardiomyopathy. J Am Soc Echocardiogr 2003;16:931-936. https://doi.org/10.1016/S0894-7317(03)00424-3