

# Voluntary exercise and testosterone therapy caused increase in percentage of Myh6 and expression of oxidative stress marker Cybb in left ventricles of rats

## Nárast percentuálneho zastúpenia Myh6 bol sprevádzaný nárastom expresie markera oxidačného stresu Cybb v ľavých komorách potkanov medikovaných testosterónom

Original research article

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**Abstract** **Aim:** The aim of this study is to identify a possible damage to heart ventricles caused by supraphysiological doses of testosterone, voluntary physical activity or their combination.

**Methods:** In the 8-week long experiment, 10–12 weeks old male Wistar rats were administered testosterone depot in dose of 100 mg/kg (TES, n = 15) or vehiculum (CON, n = 12) once a week subcutaneously. Next groups injected with testosterone (SPOTES, n=12) or vehiculum (SPO, n=12) were running in exercise wheels *ad libitum*. Gene expressions in left and right ventricles of the heart were measured by quantitative reverse transcription polymerase chain reaction method.

**Results:** In left ventricles of the testosterone groups, we observed a mild but significant increase in the percentage of Myh6 myosin heavy chain isoform and higher expression of NADPH oxidase subunit Cybb (\*p < 0.05).

**Conclusions:** Testosterone affected the expression of genes related to contractile apparatus and oxidative stress in the left ventricle but not in right ventricle of the heart of rats. The observed level of physical activity did not have a compelling effect on the expression of measured genes.

**Slovak abstract** **Ciele:** Cieľom tejto práce je identifikovať možné poškodenie komôr myokardu vplyvom vysokých dávok testosterónu, nenútenej fyzickej aktivity, alebo ich kombinácie.

**Metódy:** Samcom potkanov kmeňa Wistar vo veku 10-12 týždňov bol po dobu 8 týždňov 1x týždenne s.c. aplikovaný depot testosterónu v dávke 100 mg/kg (TES, n=15) alebo vehikulum (CON, n=12). Skupinám s aplikovaným testosterónom (SPOTES, n=12) alebo vehikulum (SPO, n=12) bolo umožnené behať v závesnom koliesku *ad libitum*. V myokarde ľavých a pravých komôr srdca boli metódou RT-qPCR stanovené génové expresie.

**Výsledky:** V ľavých komorách testosterónových skupín bol pozorovaný mierny, ale signifikantný nárast percentuálneho zastúpenia izoformy ťažkých reťazcov myozínu Myh6 a vyššia expresia podjednotky NADPH oxidázy Cybb (\*p<0.05).

**Záver:** Testosterón ovplyvnil expresiu génov súvisiacich s kontraktilným aparátom a oxidačným stresom v ľavej, ale nie v pravej komore srdca potkanov. Pozorovaná miera fyzickej aktivity nemala významný vplyv na expresiu sledovaných génov.

**Keywords** Testosterone – Physical activity – Heart ventricles – Oxidative stress – Myosin heavy chains

**Kľúčové slová:** testosterón – fyzická aktivita – srdcové komory – oxidačný stres – ťažké reťazce myozínu

## INTRODUCTION

Abuse of anabolic steroids has been linked to a variety of cardiovascular side effects accompanied by elevated oxidative stress markers (Emer et al., 2016). Elevated testosterone concentrations on molecular level can induce cardiomyocyte hypertrophy (Altamirano et al., 2009). Cardioprotective effects of testosterone replacement therapy include improved

ejection fraction and heart rate variability in orchietomised rats (Pongkan et al., 2015) and improvements in exercise capacity and symptoms in men with heart failure (Pugh et al., 2004). Exercise affects a variety of bodily functions, most importantly the function of cardiovascular system (Tibenska et al., 2010) (Tibenska & Medekova, 2014). Adequate physical

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activity has positive impact on the cardiovascular system, such as prevention of heart disease (Myers, 2003) or physiological hypertrophy (Woodiwiss et al., 2000). It can also increase oxidative stress (Powers & Jackson, 2008) or cause sudden death in athletes with underlying heart condition (Finocchiaro et al., 2016). Heart damage manifests itself by shift in major myosin heavy chain gene expression – decrease of alpha isoform (Myh6) and increase of beta isoform (Myh7) (Lowes et al., 1997). Chemical gonadectomy promotes *in vivo* and *ex vivo* cardiac dysfunction in sedentary rats associated with Myh7 upregulation, but voluntary wheel running provided protection from this process (Hydock et al., 2007). In case of heart damage in sports, focus is primary on the left ventricle, as main circulatory blood ejection component (Baggish & Wood, 2011), but undetected right ventricle impairment can precede left ventricle damage (de Groote, 2016).

## METHODS

### Experimental Design

In the 8-week long experiment, 10–12 weeks old male Wistar rats were administered testosterone depot (Agovirin Depot 25 mg/ml, BB Pharma a.s., Czech Republic) in dose of 100 mg/kg (TES, *n* = 15) or vehiculum (CON, *n* = 12) once a week subcutaneously. Other groups were injected with the same dose of testosterone depot (SPOTES, *n* = 12) or vehiculum (SPO, *n* = 12) and were running in exercise wheels *ad libitum*. Animals were sacrificed by CO<sub>2</sub> asphyxiation and tissue samples were obtained from left and right ventricles of the heart.

### Gene expression

Total RNA was isolated from samples of left and right ventricles of the heart using Tri-Reagent (Sigma-Aldrich, USA). We verified the quality of total RNA by 2% agarose gel electrophoresis and the quantity by spectrophotometry. Subsequently, total RNA was reverse-transcribed to cDNA (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA), and real-time polymerase chain reaction (PCR) was performed using SYBR Green detection (qPCR kit SYBR Select Master Mix, Life Technologies, USA) on StepOnePlus™ Real-Time PCR System (Life Technologies, USA) according to the manufacturer's instructions. Expression of Myh6 (myosin heavy chain 6, alpha), Myh7 (myosin heavy chain 7, beta) and Cybb (cytochrome b-245, beta polypeptide) was determined using the gene-specific primers. The following primer sequences were used: Myh6 (forward: „GCCCTTTGACATCCGCACAGAGT” reverse: „TCTGCTGCATCACCTGGTCCTCC”), Myh7 (forward: „GCGGACATTGCCGAGTCCCAG” reverse: „GCTCCAGGTCTCAGGGCTTCA”), Cybb (forward: „TGGGAGACTGGACTGAGGGGCTA” reverse: „GGCTGTACCAAAGGGCCCATCAA”). B2m (beta-2-microglobulin) and Hprt1 (hypoxanthine phosphoribosyltransferase 1) were used as endogenous

reference genes. All primers were verified to yield a single PCR product with the correct molecular weight, and the absence of signal was confirmed when reverse transcription was omitted.

### Statistical analysis

All variables are reported as mean ± standard deviation (SD). Mean PCR efficiency estimates (*E*) per amplicon and quantification cycle (*C<sub>q</sub>*) values per sample were determined using LinRegPCR software (Ruijter et al., 2009, version 2015.0) and efficiency corrected relative expression ratios were calculated (all reactions had *E* > 1.8 and *C<sub>q</sub>* < 35). The Shapiro–Wilk test was used to determine the normality of data. Normally distributed data (parametrical) were tested by analysis of variance (ANOVA) with post-hoc Tukey's honest significant difference tests. Non-parametrical data were tested by using the Kruskal–Wallis test with post-hoc Wilcoxon test and Benjamini–Hochberg correction for multiple comparisons. Results with *p* < 0.05 were considered as statistically significant. The data were handled by GraphPad Prism (GraphPad Software, Inc., version 6), R programming language and software environment for statistical computing and graphics (R Foundation for Statistical Computing, version 3.2.4) and Microsoft Excel (Microsoft, version 15.20).

## RESULTS

We observed no statistically significant difference in gene expressions of Myh6 and Myh7 in both left and right ventricles of the study groups (data not shown). However, after percentage calculation by formula  $\text{Myh6 \%} = \frac{\text{Myh6}}{\text{Myh6} + \text{Myh7}}$ , we observed a mild but significant increase in percentage of Myh6 in left ventricles of testosterone groups (\**p* < 0.05) (Fig. 1). Higher expression of Cybb was also observed in the same compartment and study groups (\**p* < 0.05) (Fig. 2). No significant changes were observed in right ventricles. Voluntary, mild physical activity did not have a substantial effect on the measured results.

## DISCUSSION

Supraphysiological doses of testosterone have deleterious effects on heart function in cases of abuse such as doping (Emer et al., 2016), but improvement of heart function was observed during testosterone replacement therapy for hypogonadism (Pongkan et al., 2015) and heart failure (Pugh et al., 2004). Our results indicate possible beneficial effect of supraphysiological doses of testosterone in healthy male rats, as cardiac myosin heavy chain gene expression shift in favour of Myh6 isoform may increase cardiac output, which is important in situations of increased demand (Lowes et al., 1997; Hydock et al., 2007). This change was accompanied by increased expression of oxidative-stress-related gene Cybb, which corresponds with findings of Emer et al. (2016). Increased physical activity was described as

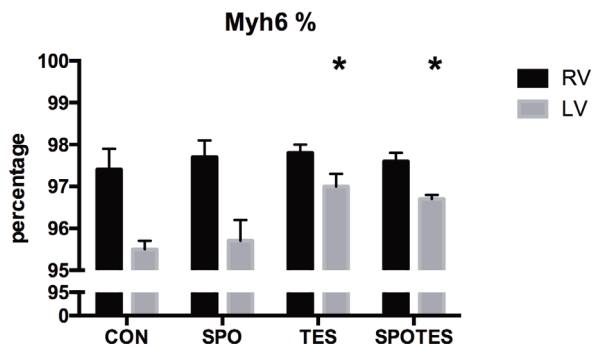


Figure 1. Percentage of Myh6 in right and left ventricle of the heart. average  $\pm$  SD; \* $p < 0.05$  vs. CON

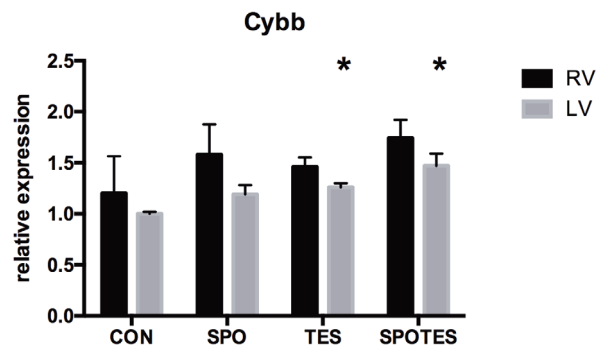


Figure 2. Percentage of Cybb in right and left ventricle of the heart. average  $\pm$  SD; \* $p < 0.05$  vs. CON

impairing factor in heart function (Finocchiaro et al., 2016) and cause of increased oxidative stress (Powers & Jackson, 2008), but in adequate amount and intensity showed cardioprotective effects (Myers, 2003). Rats in our experiment have shown no impairment of heart function on molecular level, according to the knowledge of Myh6 percentage as an important factor in the determination of cardiac output (Korte et al., 2005); on the contrary, it had tendency to improve. Major cardiac myosin heavy chains are known to be expressed approximately uniformly in both heart ventricles with greater than 95% in rats (Zammit et al., 2000). Although gene expressions were in general higher in the right ventricle compared to the left ventricle, recorded differences manifested in the left ventricle

only, which is known to be a source and primary focus in case of heart damage in sports (Baggish & Wood, 2011). However, physical activity did not have a significant effect on measured parameters. Supraphysiological doses of testosterone altered gene expression of contractile apparatus and oxidative stress in left, but not in right, ventricle of the heart.

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