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Wnt1 gene expression in the heart left ventricle as a response to the various durations of the intensive exercise: An experimental study

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Objective. Myocardial fibrosis is a devastating condition causing millions of deaths yearly. Several factors, such as aging, cause myocardial fibrosis. The Wnt/β-catenin pathway is one of the critical intracellular signaling for the development of cardiac fibrosis. Molecular and cellular mechanism of myocardial fibrosis induced by intensive exercise is not well-understood. The current study evaluates the effects of short- and long-term intensive exercise on the *Wnt1* gene expression in a heart left ventricle in an animal model.

Methods. Twenty-one male Wistar rats (mean weight 250±50 g) were divided into three groups (n=7): 1) control group (C); 2) short-term regular intensive exercise group (S-RIE, high-intensity exercise for one month six days weekly for 60 min with speed of 35 m/min), and 3) long-term regular intensive exercise group (L-RIE, high-intensity exercise for six months six days daily for 60 min with speed of 35 m/min). The heart left ventricle was isolated at the end of the experiment, and the relative gene expression of the *Wnt1* gene was measured by the Real-Time PCR.

Results. The L-RIE group showed a significant increase in the *Wnt1* expression compared to the S-RIE and the control group. Although no difference was observed in the *Wnt1* mRNA level in the S-RIE group compared to the control group, *Wnt1* mRNA level increased in the L-RIE group compared to the S-RIE group.

Conclusion. The exercise duration was of a great importance in the *Wnt1* gene expression. Regular intensive exercise may be involved in the formation of the myocardial fibrosis by increasing the expression of the *Wnt1* gene.

Keywords: myocardial fibrosis, *Wnt1* gene, regular intensive exercise, high-intensity exercise

Myocardial fibrosis plays a key role in progression of heart failure (de Leeuw et al. 2001). The diffuse and disproportionate accumulation of type I and III collagen fibers in myocardial interstitium leads to a myocardial fibrosis development. Previous study has demonstrated that myocardial fibrosis occurs due to myocardial injuries caused by an intrinsic cardiac disease, arterial hypertension, diabetes mellitus or chronic kidney disease (Gonzalez et al. 2018). Some studies have reported proliferation of cardiac fibroblasts and increase of extracellular matrix (ECM) components in myocardial fibrosis, which are promoted by activation of multiple signaling pathways (Li et al. 2016; Su et al. 2017; Han et al.

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2018). Thus, ECM components and secretion of growth factors by cardiac fibroblasts increase during the myocardium fibrosis (Deb and Ubil 2014).

Wnt proteins act as signaling molecules to accelerate cell proliferation, differentiation, and migration (Wang et al. 2014). This pathway is inactive under physiological conditions and can be activated through several endogenous or exogenous pathogens. Wnt signaling comprises two highly-conserved pathways (canonical and non-canonical) that participate in various cellular functions (Angers and Moon 2009; Kim et al. 2013). β-catenin is an intracellular transducer of extracellular signals that activates downstream target genes including the Wnt1-inducible signaling pathway protein (WISP-1) (Xu et al. 2000). Thus, WISP-1 induces fibroblast proliferation, ECM deposition, and cardiomyocyte hypertrophy (Colston et al. 2007). On this basis, the canonical Wnt pathways may play a pivotal role in the activation of myocardial fibrosis (Blyszczuk et al. 2017; Dzialo et al. 2018). Recently, studies have shown that the onset and progression of kidney, lung, liver, heart, and skin fibrosis are in correlation with abnormal activation of the Wnt signaling pathway (Surendran et al. 2002; Tzouvelekis et al. 2015).

Exercise can induce either beneficial or detrimental effects on dystrophic skeletal and cardiac muscles. However, the effect of exercise depends on several factors including reputation, intensity, duration, and type of exercise (Markert et al. 2011). Previous reports have shown an association between regular intense exercise and structural, functional, and electrical changes of the heart, known as the "athlete's heart" (La Gerche et al. 2009). Considering the demand for very high cardiac outputs and sustained oxygen supply, the most profound cardiac remodeling typically occurs in endurance athletes (La Gerche et al. 2012). As a result, the volume of all four heart chambers, thickness of the ventricular wall, and muscular mass would be increased.

The current evidence indicates that there may be an overlap in the spectrum between physiological and pathological hypertrophy, which might be associated with lifelong endurance training. However, the molecular and cellular mechanisms of intensive exercise inducing myocardial fibrosis, are not yet entirely understood. The current study is aimed to evaluate the effects of short- and long-terms of intensive exercise on the expression of *Wnt1* gene in the male Wistar rats.

Table 1
Primer sequence and characteristics used in the quantification of the target genes

Gene	Primer		Annealing temperature (°C)
Wnt1	Forward	5'-CGTGGACTCTGGGGAGAAG-3'	62.0
	Reverse	5'-CAGCCGCATCCAACACGT-3	59.0
Gapdh	Forward	5'-CATAGACAAGATGGTGAAGGTCG-3'	57.9
	Reverse	5'-CCGTGGGTAGAGTCATACTGG-3'	58.3

Materials and Methods

Animals. In the present study, twenty-one adult male Wistar rats with a weight range of 250±50 g were received from the Institute of Pasteur, Tehran, Iran. During the experiment, the temperature at the breeding environment was kept at 22±2 °C with a 12:12 h light-dark cycle (7:00 a.m. to 7:00 p.m.) and the rats have free access to water and typical food.

Design of the experiment. The rats were randomly divided into three groups (n=7/group): 1) control group (C), which underwent no exercise, 2) short-term regular intensive exercised group (S-RIE) that performed high-intensity exercise (35 m/min) on a treadmill for four weeks, and 3) long-term regular intensive exercised group (L-RIE), that underwent high-intensity exercise (35 m/min) on a treadmill for twenty-four weeks (Khajehnasiri et al. 2018).

The exercise protocol. Rats in S-RIE and L-RIE groups were exercised for 1 h/day, 6 days/week at a speed of 35 m/min on the treadmill (Khajehnasiri et al. 2018). As a warm-up, the machine's speed was increased from 5 m/min to 35 m/min within the initial 5 min. Moreover, the speed was gradually reduced to 5 m/min in the last 5 min, to return them to the initial state. Rats in control groups were placed on the treadmill as long while the machine was off (Khajehnasiri et al. 2019).

Sample collection. Anesthesia was inducted in all rats 24 h after the last day of exercise via intraperitoneal injection (i.p.) of ketamine 100 mg/kg and xylazine 5 mg/kg (Petersen and Pedersen 2005). Then the chest was incised and the beating heart detached from the blood vessels and placed in cold normal saline 9%. Finally, the left ventricle was isolated from the heart and all the specimens were stored at –80 °C until assayed by Real-Time PCR.

The RNA extraction, cDNA synthesis, and qRT-PCR. Total RNAs extraction (RNXPluse, Cinagene, Cat.#: RN7713C), DNaseI treatment (Thermo Scientific,

USA) and cDNA synthesis (Bio fact cDNA synthesis kits) were performed on the left ventricle samples according to the manufacturer's instructions. To evaluate *Wnt1* transcripts, triplicate reactions were carried out on the cDNA samples. PCR conditions and primer sequences are summarized in Table 1. Melting curve analysis showed a single amplification peak for each reaction. The Ct value of target was normalized to the number of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) copies for each sample. The relative expression was calculated using the arithmetic formula $2^{-\Delta CT}$ (Xu et al. 2000).

Statistical analysis. The data were controlled to the normality test and values were expressed as mean±standard error (SE). Comparisons between groups were analyzed by one-way analysis of variances (ANOVA) and a subsequent Tukey post hoc test for pairwise comparisons using SPSS software version 16.0. For the significance level, $p<0.05$ was considered.

Ethical statement. This experiment was approved by the Animal Care Committee of the Shahid Beheshti University, Tehran, Iran (approval number: D/920/1010) and carried out in compliance with the recommendations of the Care and Use of Laboratory Animals (National Academy Press, 1996, Washington, USA).

Results

Previous articles have shown the effect of intensive exercise on cardiac fibrosis. The Wnt/β-catenin signaling pathway plays an essential role in cardiac development and cardiac tissue homeostasis in adults (Duan et al. 2012). In the present study, long-term regular intensive exercise group showed a significant increase in the *Wnt1* mRNA level expression ($p=0.016$) in the heart left ventricle compared to the control group. However, no difference was observed in the *Wnt1* mRNA level in the heart left ventricle in the short-term regular intensive exercise group ($p=0.800$) compared to the control group. Furthermore, the *Wnt1* mRNA level in the heart left ventricle of the long-term regular intensive exercised group significantly increased ($p=0.042$) compared to the short-term regular intensive exercised group (Figure 1).

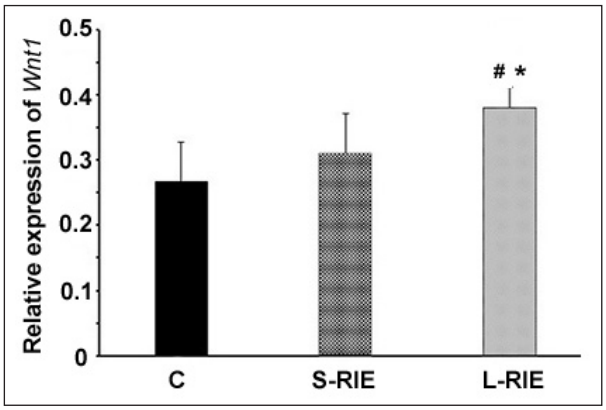

Figure 1. The effects of short- and long-term intensive exercise on the *Wnt1* gene expression in a heart left ventricle of adult male Wistar rats. Data are presented as mean±standard error (SE) and were analyzed by one-way analysis of variances (ANOVA) and Tukey post hoc test. *$p<0.05$ vs. the control group; #$p<0.05$ vs. the S-RIE group. Abbreviations: C – control group; L-RIE – long-term regular intensive exercise group; S-RIE – short-term regular intensive exercise group.

Discussion

The results of the present study demonstrate that the long-term regular intensive exercise significantly increased the *Wnt1* mRNA level in the heart left ventricle of rats compared to the control group. In addition, the increased *Wnt1* mRNA level the rat heart left ventricle after the long-term regular intensive exercise compared to the *Wnt1* mRNA level after the short-term regular intensive exercise suggests that *Wnt1* gene expression correlates with the duration of exercise. It can be assumed that exercise duration significantly modifies the *Wnt1* gene expression and contributes to cardiac fibrosis. These results are in consistency with previous studies showing that changes in exercise intensity and duration have different effects on the expression of selected gene (Benite-Ribeiro et al. 2016; Foulquier et al. 2018).

Long-term intensive exercise contributes to tissue deformation, which is mainly induced by *Wnt1*. These structural changes in gene expression, cellular and interstitial structure changes lead to wide spectrum of conditions ranging from physiological to pathological processes. Although the underlying mechanisms of myocardial fibrosis remain still unclear, the genetic background, silent myocarditis, pulmonary artery pressure overload, and repeated exercise-induced injuries in athletes contribute to the onset of myocardial fibrosis (van de Schoor et al. 2016). Thus, *Wnt1* gene expression in the long-term exercise group maybe a reason for the initiation of destructive pathways in the myocardium.

The Wnt/β-catenin signaling pathway plays an essential role in cardiac development and adult cardiac homeostasis. Abnormal regulation of this signaling pathway is associated with various heart diseases, including myocardial hypertrophy, fibrosis,

arrhythmia, and infarction (van de Schoor et al. 2016; Foulquier et al. 2018). Fibrosis can be due to either healing after myocardial infarction or other cardiac disease, but results in an arrhythmia by preventing electrical wave propagation. Inappropriate activation of Wnt signaling through overexpression of Wnt ligands or beta-ketone nuclear accumulation is suggestive of that Wnt signaling alone might be sufficient to trigger the fibrogenic gene program expression in fibroblasts (Lorenzon et al. 2017). During myocardial infarction, Wnt signaling increases in epicardium, and translocation of fibroblasts to epithelial-mesangial would be initiated (Tao et al. 2016). Besides, Wnt signaling inhibits the apoptotic processes involved in the transition from hypertrophy to heart failure. It seems that activation of Akt and subsequent inhibition of GSK3β protect cardiac cells against apoptosis in patients with heart failure.

In general, Wnt/β-catenin signaling is a very complex system involved in many different types of cardiac pathologies with entirely different effects (Lorenzon et al. 2017). Changes in Wnt signaling and other pathways connected with β-catenin may affect the expression of the connexin gene in the heart. The Wnt1 is a potent and specific stimulator of Cx43 expression in cardiomyocytes and increases Cx43 mass forming up cleft junctions. It has been shown that abnormal placement of connexins in laboratory animals and humans may be associated with cardiomyopathy. The so called "gap junction model" is also thought to result in cardiac arrhythmias, including ventricular tachycardia (Ai et al. 2000). The effect of Wnt1 on early Wnt/β-Catenin signaling and induction of Cx43 expression in cardiomyocytes supports the hypothesis of irregular signaling contribution to altered impulse propagation and arrhythmia in the cardiomyopathy (Lorenzon et al. 2017). Of 19 genes known from *Wnt* gene family, *Wnt2*, *Wnt5b*, *Wnt11*, and *Wnt9a* are expressed only in the healthy heart, whereas *Wnt3a*, *Wnt1*, and *Wnt5a* are expressed during cardiac injury (Tao et al. 2016). Some studies have shown that *Wnt1* gene expression increased up to seven-fold in epicardium and fibroblasts at the site of injury during acute ischemia (von Gise and Pu 2012; Tao et al. 2016). Another study confirmed increased *Wnt1* expression after infection in epicardium and fibroblasts leading to ECM proliferation and secretion (Dawson et al. 2013).

The exercises, like marathons, iron men's racing, and long-distance cycling, decrease left ventricular output and increase cardiac biomarkers. Although, these changes are transient and for a duration of a week, the prolonged overload during exercise contributes to heterogeneous and disproportionate participation of cardiac fibroblasts. As a consequence, collagen proteins and growth of myocytes and cords during exercise-induced hypertrophy are not accompanied by sufficient vascular growth. Thus, myocyte function will be disrupted due to lack of optimal blood supply. Overall, these pathologic changes might lead to cardiac damages, such as fibrosis (Lakhan and Harle 2008; van de Schoor et al. 2016). The results of magnetic resonance imaging using gadolinium enhancement revealed the presence of scattered myocardial fibrosis, especially in the atria, ventricular wall, and the left ventricle. It should be noted that not all endurance athletes develop myocardial fibrosis (Lakhan and Harle 2008; van de Schoor et al. 2016). Wilson et al. (2011) have shown that myocardial fibrosis was present in 6–12% of endurance athletes who had exercised throughout their lifetimes. Of course, some studies have ruled out exercise-induced fibrosis. It has been hypothesized that athletes often have ventricular hypertrophy, which is not associated with fibrosis, since this hypertrophy disappears during exercise interruption (Galanti et al. 2016). Evidence for myocardial fibrosis in athletes is based exclusively on observational studies that complicate insights into the possible underlying mechanisms. Therefore, further studies are needed to determine the exact mechanism of fibrosis in athletes (Wilson et al. 2011).

Our study has limitations. Since, we were not able to induce all physiological conditions similar to those seen in humans, because there are probably multiple factors contributing to these conditions.

Conclusion. The outcomes of the current study suggest that training duration increases the *Wnt1* gene expression, which also activates the Wnt/β-catenin pathway, and might lead to myocardial fibrosis. Given the differences between model laboratory animals and athletes, further research using more appropriate exercise protocols and research on humans is needed.

Conflict of interest: *The authors declare no conflict of interest.*

References

Ai Z, Fischer A, Spray DC, Brown AM, Fishman GI. Wnt-1 regulation of connexin43 in cardiac myocytes. J Clin Invest 105, 161–171, 2000.

Angers S, Moon RT. Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol 10, 468–477, 2009.
Benite-Ribeiro SA, Putt DA, Santos JM. The effect of physical excise on orexigenic and anorexigenic peptides and its role on long-term feeding control. Med Hypotheses 93, 30–33, 2016.
Blyszczuk P, Muller-Edenborn B, Valenta T, Osto E, Stellato M, Behnke S, Glatz K, Balser K, Luscher TF, Distler O, Errikson U, Kania G. Transforming growth factor-β-dependent Wnt secretion controls myofibroblast formation and myocardial fibrosis progression in experimental autoimmune myocarditis. Eur Heart J 38, 1413–1425, 2017.
Colston JT, de la Rosa SD, Koehler M, Gonzales K, Mestril R, Freeman GL, Bailey SR, Chandreasekar B. Wnt-induced secreted protein-1 is a prohypertrophic and profibrotic growth factor. Am J Physiol Heart Circ Physiol 293, 1839–1846, 2007.
Dawson K, Aflaki M, Nattel S. Role of the Wnt-Frizzled system in cardiac pathophysiology: a rapidly developing, poorly understood area with enormous potential. J Physiol 591, 1409–1432, 2013.
de Leeuw N, Ruiter DJ, Balk AH, de Jonge N, Melchers WJ, Galama JM. Histopathologic findings in explanted heart tissue from patients with end-stage idiopathic dilated cardiomyopathy. Transpl Int 14, 299–306, 2001.
Deb A, Ubil E. Cardiac fibroblast in development and wound healing. J Mol Cell Cardiol 70, 47–55, 2014.
Dzialo E, Tkacz K, Blyszczuk P. Crosstalk between the TGF-β and WNT signalling pathways during cardiac fibrogenesis. Acta Biochim Pol 65, 341–349, 2018.
Duan J, Gherghe C, Liu D, Hamlett E, Srikantha L, Rodgers L, Regan JN, Rojas M, Willis M, Leask A, Majesky M, Deb A. Wnt1/βcatenin injury response activates the epicardium and cardiac fibroblasts to promote cardiac repair. EMBO J 31, 429–442, 2012.
Foulquier S, Daskalopoulos EP, Lluri G, Hermans KCM, Deb A, Blankesteijn WM. WNT signaling in cardiac and vascular disease. Pharmacol Rev 70, 68–141, 2018.
Galanti G, Stefani L, Mascherini G, Di Tante V, Toncelli L. Left ventricular remodeling and the athlete's heart, irrespective of quality load training. Cardiovasc Ultrasound 14, 46, 2016.
Gonzalez A, Schelbert EB, Diez J, Butler J. Myocardial interstitial fibrosis in heart. Biological and translational perspectives. J Am Coll Cardiol 71, 1696–1706, 2018.
Han A, Lu Y, Zheng Q, Zhang J, Zhao Y, Zhao M, Cui X. Qiliqiangxin attenuates cardiac remodeling via inhibition of TGF-β1/Smad3 and NF-κB signaling pathways in a rat model of myocardial infarction. Cell Physiol Biochem 45, 1797–1806, 2018.
Khajehnasiri N, Khazali H, Sheikhzadeh F. Various responses of male pituitary-gonadal axis to different intensities of long-term exercise: Role of expression of KNDY-related genes. J Biosci 43, 569–574, 2018.
Khajehnasiri N, Khazali H, Sheikhzadeh F, Ghowsi M. One-month of high-intensity exercise did not change the food intake and the hypothalamic arcuate nucleus proopiomelanocortin and neuropeptide Y expression levels in male Wistar rats. Endocr Regul 53, 8–13, 2019.
Kim W, Kim M, Jho EH. Wnt/β-catenin signalling: From plasma membrane to nucleus. Biochem J 450, 9–21, 2013.
La Gerche A, Taylor AJ, Prior DL. Athlete's heart: the potential for multimodality imaging to address the critical remaining questions. JACC Cardiovasc Imaging 2, 350–363, 2009.
La Gerche A, Burns AT, Taylor AJ, Macisaak AI, Haidbuchel H, Prior DL. Maximal oxygen consumption is best predicted by measures of cardiac size rather than function in healthy adults. Eur J Appl Physiol 112, 2139–2147, 2012.
Lakhan SE, Harle L. Cardiac fibrosis in the elderly, normotensive athlete: case report and review of the literature. Diagn Pathol 3, 12, 2008.
Li X, Han D, Tian Z, Gao B, Fan M, Li C, Li X, Wang Y, Ma S, Cao F. Activation of cannabinoid receptor type II by AM1241 ameliorates myocardial fibrosis via Nrf2-mediated inhibition of TGF-β1/Smad3 pathway in myocardial infarction mice. Cell Physiol Biochem 39, 1521–1536, 2016.
Lorenzon A, Calore M, Poloni G, De Windt LJ, Braghetta P, Rampazzo A. Wnt/β catenin pathway in arrhythmogenic cardiomyopathy. Oncotarget 8, 60640–60655, 2017.
Markert CD, Ambrosio F, Call JA, Grange RW. Exercise and Duchenne muscular dystrophy: toward evidence-based exercise prescription. Muscle Nerve 43, 464–478, 2011.
Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98, 1154–1162, 2005.
Su SA, Yang D, Wu Y, Xie Z, Zhu W, Cai Z, Shen J, Fu Z, Wang Y, Jia L, Wang Y, Wang JA, Xiang M. EphrinB2 regulates cardiac fibrosis through modulating the interaction of Stat3 and TGF-β/Smad3 signaling. Circ Res 121, 617–627, 2017.
Surendran K, McCaul SP, Simon TC. A role for Wnt-4 in renal fibrosis. Am J Physiol Renal Physiol 3, 431–441, 2002.
Tao H, Yang JJ, Shi KH., Li J. Wnt signaling pathway in cardiac fibrosis: new insights and directions. Metabolism 65, 30–40, 2016.

Tzouvelekis A, Bonella F, Spagnolo P. Update on therapeutic management of idiopathic pulmonary fibrosis. Ther Clin Risk Manag 11, 359–370, 2015.

van de Schoor FR, Aengevaeren VL, Hopman MT, Oxborough DL, George KP, Thompson PD, Eijsvogels TM. Myocardial fibrosis in athletes. Mayo Clin Proc 91, 1617–1631, 2016.

von Gise A, Pu WT. Endocardial and epicardial epithelial to mesenchymal transitions in heart development and disease. Circ Res 110, 1628–1645, 2012.

Wang Y, Li YP, Paulson C, Shao JZ, Zhang X, Wu M, Chen W. Wnt and the Wnt signaling pathway in bone development and disease. Front Biosci 19, 379–407, 2014.

Wilson M, O'Hanlon R, Prasad S, Deighan A, Macmillan P, Oxborough D, Godfrey R, Smith G, Maceira A, Sharma S, George K, Whyte G. Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athlete. J Appl Physiol 110, 1622–1626, 2011.

Xu L, Corcoran RB, Welsh JW, Pennica D, Levine AJ. WISP-1 is a Wnt-1- and beta-catenin-responsive oncogene. Genes Dev 14, 585–595, 2000.