

The effects of moderate physical exercise on cardiac hypertrophy in interleukin 6 deficient mice

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Abstract

Purpose: Prolonged physical training leads to compensatory changes in cardiovascular system. One of the most important of them is cardiac hypertrophy. The knowledge, which factors contribute to cardiomyocyte hypertrophy caused by physical exercise is still incomplete. Interleukin 6 (IL6) secreted by contracting skeletal muscles may affect cardiac hypertrophy and remodeling. The aim of the study was to investigate the role of IL6 in exercise induced cardiac hypertrophy.

Material and methods: Female mice lacking functional IL6 gene C57BL6/J^{IL6-/-tm1Kopf} (IL6KO) and age and sex matched controls C57BL6/J (WT) were subjected to 6 week swimming regime. Twenty-four hours after the last training session the mice were sacrificed, hearts were excised and weighed. Two other groups of sex and strain matched mice (9 in each group) not subjected to physical training, were sacrificed and served as controls. Weights of the heart and the left ventricle were related independently to the body weight and the tibia length as measures of hypertrophy. Statistical analysis was performed using multifactorial ANOVA and the Fisher test.

Results: There was significantly higher heart/body weight ratio in both groups of mice which were trained as compared to the respective sedentary animals [F(3,30)=31.085 p<0.001] There were, however, no significant differences between respective WT and IL6KO groups. Similar relations were found for the left ventricle and also when the weights of the heart and the LV were related to the tibia length.

Conclusion: IL6 is not necessary for cardiac hypertrophy induced by prolonged moderate physical exercise in mice. Additional study is warranted to elucidate this phenomenon.

Key words: interleukin 6, physical exercise, cardiac hypertrophy, mice.

Introduction

Human physiology have evolved to be optimized for repetitive and strenuous physical exercise. Overcrowded world and contemporary western civilization radically diminished the amount of movement performed by individuals. Therefore new epidemics have appeared: type 2 diabetes and obesity. Both of them are strongly related to lacking exercise. Also cardiovascular diseases – the leading cause of death in the developed countries are strongly associated with lacking physical exercise. Prolonged physical training leads to the development of adaptive changes in cardiovascular system and its regulatory mechanisms [1]. Cardiac hypertrophy is vital for meeting increased oxygen demand during strenuous exercise. However, in contrast to the changes occurring due to increased load in pathological conditions like hypertension or heart failure, the physiologic hypertrophy is associated with orchestrated growth of cardiomyocytes, capillaries and connective tissue [2]. Therefore, there is no increase of apoptosis and no increase of fibrosis [2]. Moreover, fetal gene program activation in heart, so typical for any pathological hypertrophy, is also absent after physical training. There is much effort put into research that should dissect molecular mechanisms regulating physiological (during developmental growth, in pregnancy or after exercise) and pathological hypertrophy [3]. Yet, relatively little is known about humoral factor involved in inducing changes in the heart during exercise [4].

Interleukin 6 (IL6) is a prototypical pleiotropic cytokine, involved in inflammatory response, lymphocyte survival and

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Table 1. Cardiac hypertrophy parameters in sedentary and swimming mice

	WT		IL6 KO	
	Sedentary	Swimming	Sedentary	Swimming
Heart weight to body weight ratio (mg/g)	4.2±0.17	4.78±0.31 **	3.99±0.26	4.86±0.12**
LV weight to body weight ratio (mg/g)	3.27±0.2	3.81±0.27 **	3.27±0.21	3.85±0.12 **
Heart weight to tibia length ratio (mg/mm)	5.09±0.38	5.59±0.39 *	4.99±0.42	5.66±0.54 *
LV weight to tibia length ratio (mg/mm)	3.96±0.32	4.45±0.32 *	4.0±0.37	4.49±0.44 *

differentiation, metabolic regulation and cytoprotection [5]. Human studies revealed its association with higher risk of congestive heart failure (CHF), atherosclerosis and worse prognosis after myocardial infarction [5,6]. On the other hand, there are numerous publications presenting protective actions of this cytokine [7]. It has not been fully clarified whether IL6 has positive or detrimental influence on cardiovascular system. Experiments performed on animal models have shown that its transduction pathway is involved in cardiomyocyte hypertrophy [8,9]. There is a strong evidence that this cytokine regulates cardiac lipid metabolism and expression of fatty acid transporters [10]. It is secreted by contracting skeletal muscles and may induce metabolic changes in other organs. IL6 is thought to be responsible for the alterations in insulin sensitivity and anti-inflammatory effects of exercise [11,12]. Due to its abundance and pleiotropic actions some authors call this cytokine even “an exercise factor” [13].

We have undertaken this study in order to investigate whether IL6 deficiency may affect the exercise induced cardiac hypertrophy in mice.

Material and methods

Female mice lacking functional IL6 gene C57BL6/J^{IL6^{-tm1Kopf}} (IL6KO) and respective wild type C57BL6/J (WT) mice were kept in constant temperature of 22°C ±1°C in 12:12 dark-light cycle with constant access to chow and water.

Eight female 8-10 week old mice IL6KO and eight age and sex matched WT controls were subjected to 6 week swimming regime according to previously described protocol [14]. In brief: swimming sessions took place daily on the same time of the day. Mice swam in small tanks (surface cir. 500 cm², 18 cm depth) filled with tap water that maintained constant temperature 30-32°C. The first session lasted 20 minutes and this interval was increased daily by 10 minutes to 90 minutes per session on the eighth day. This session duration was maintained for the remaining 5 weeks. After each session mice were put into clean cages maintained in temperature 27°C and were allowed to dry. Afterwards the animals returned to their cages. Two groups (IL6KO and WT) of strain and sex matched animals (9 in each group) were handled similarly with the exception that they were put into tanks without water – they were considered as sedentary controls. Twenty-four hours after the last training session mice were sacrificed, hearts were excised and weighed,

tibia length was measured. Weights of heart and left ventricle were related independently to body weight and tibia length. All results were multiplied by 1 000 and then presented.

The experimental procedures were carried out according to the European Council Directive of 24 November 1986 (6/609/EEC) and were approved by the Local Ethics Committee in Białystok.

Statistical analysis was performed using multifactorial ANOVA and Fisher post-hoc test.

Results

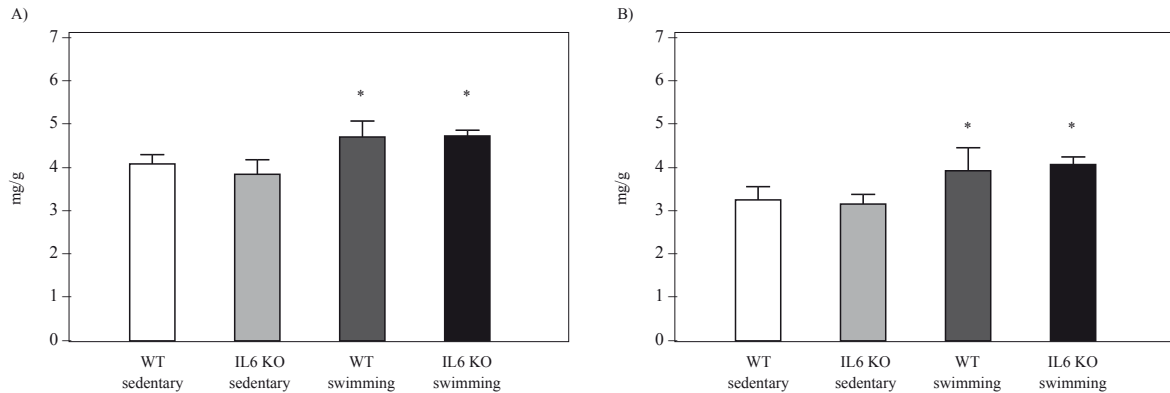
We did not notice any significant differences between sedentary IL6KO and WT animals neither in heart weight to body weight ratio nor in LV weight to body weight ratio. In contrast, mice after 6 week swimming regime presented statistically significant higher heart weight to body weight ratio [ANOVA F(3,30)=31.085; p<0.001] and LV weight to body weight ratio [ANOVA F(3,30)=26.105; p<0.001] than their sedentary counterparts (*Tab. 1*). Surprisingly, we did not observe any differences between IL6KO and WT animals neither in basal conditions nor after prolonged physical training (*Fig. 1*).

In order to confirm the abovementioned findings we have related the heart weight and LV weight to the tibia length as a measure independent of changes in fatty tissue encountered in the C57BL6/J strain. The results of this analysis confirmed earlier findings. The heart weight to tibia length ratio and left ventricle weight to tibia length ratio was significantly higher in mice after 6 week swimming regime than in sedentary controls [ANOVA F(3,30) = 5.2688; p<0.001 for total heart and F(3,30)=5.183; p<0.001 for LV]. Further post-hoc comparison made with Fisher post hoc test confirmed that both trained groups (IL6KO and WT) had higher parameters of cardiac hypertrophy than both sedentary groups (p<0.05) (*Fig. 2*). Also in this analysis we did not see any significant differences between respective IL6KO and WT animals in any abovementioned parameter.

Discussion

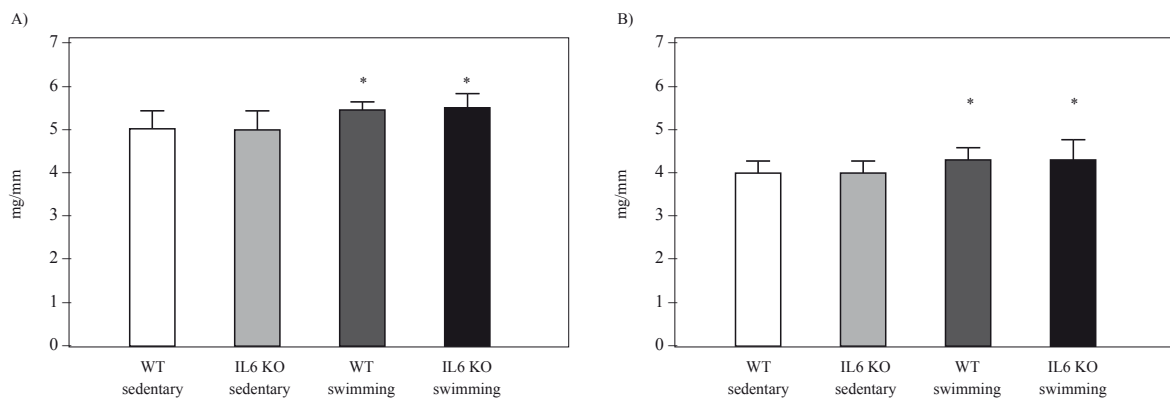
Cardiac hypertrophy is a physiological adaptive mechanism to the increased blood volume or pressure. In order to cope with the increased load late diastolic pressure increases what may

Figure 1. Heart weight to body weight ratio (A) and left ventricle weight to body weight ratio (B) in interleukin 6 deficient mice (IL6 KO) and in wild type animals (WT) subjected to 6 week swimming regime (swimming) and in respective sedentary controls (sedentary). The difference between groups is statistically significant (ANOVA $F(3,30)=31.085$ for total heart and $F(3,30)=26.105$ for LV)



* $p < 0.001$ vs both sedentary groups (Fisher post hoc test)

Figure 2. Heart weight to tibia length ratio (A) and left ventricle weight to tibia length ratio (B) in interleukin 6 deficient mice (IL6 KO) and in wild type animals (WT) subjected to 6 week swimming regime (swimming) and in respective sedentary controls (sedentary). The difference between groups is statistically significant (ANOVA $F(3,30)=5.2688$ for total heart and $F(3,30)=5.183$ for LV)



* $p < 0.05$ vs both sedentary groups (Fisher post hoc test)

be associated with the left ventricle (LV) dilation. In such circumstances increasing thickness of the LV walls will decrease the wall tension, hence will diminish oxygen consumption. On the other hand, when LV must develop higher systolic pressure there is also need for more actin-myosin bridges that develop as new sarcomeres arise during hypertrophic growth [15]. There are several physiological conditions that require orchestrated cardiac hypertrophy: developmental growth, pregnancy and nursing associated changes in the cardiovascular system and prolonged physical training [2,16]. In this circumstances a parallel growth of cardiomyocytes, connective tissue and vascular bed occurs, what determines optimal organ function. Interestingly, a different situation occurs when there is hypertrophy caused by pathological states like hypertension, loss of contractile tissue after myocardial infarction or hormonal dysregulation [17]. Then, cardiomyocyte hypertrophy usually is associated with insufficient expansion of the microvasculature and hence increased growth of fibrotic extracellular matrix. The following consequence of the pathological hypertrophy is activation of

the fetal gene expression pattern, induction of hypoxia-sensitive genes, activation of apoptotic cardiomyocyte death and finally heart failure [17].

Recently it has been suggested that transduction cascades inducing either type of hypertrophy overlap in some critical points. These include activation of PKC, ERKs, calcineurin, GSK- β 3 and GATA-4 dependent mechanisms [3,18]. There is, however, an increasing body of research presenting different factors involved in these two types of cardiac hypertrophy. $G\alpha_q$ and $G\alpha_{11}$ proteins seem to be necessary for the pathologic hypertrophy, as mice harboring their knockout were incapable of increasing heart weight in response to the aortic banding [14,19]. Angiotensin II blockade diminishes pathologic hypertrophy with no effect on physiologic one induced by swimming [20]. There are also factors involved exclusively in physiologic hypertrophy. Phosphoinositide 3-kinase subunit (p110 α) and Akt have been shown to be necessary for the exercise induced cardiac hypertrophy and even to counteract heart changes caused by increased pressure [2,3,14]. Precise dissection of the

intracellular mechanisms involved in the physiologic and pathologic hypertrophy is necessary to find the pivotal points where the cell fate is conveyed from physiologic adaptation to pathologic state and in effect apoptosis. With such knowledge we, hopefully, will be able to fix the flawed hypertrophic response and “redirect” it to the physiological pathway in patients with the pathologic conditions like hypertension, valvular heart disease or myocardial infarction. The results of experimental studies have already suggested first answers. The physical training may, to some extent, correct the pathological gene expression profile in hearts of hypertensive animals [21]. This may, at least partially, explain the beneficial effects of exercise in patients with hypertension or heart failure [4].

Interleukin 6 is a cytokine secreted during exercise by a contracting muscle [22]. Its actions may include influence on insulin transduction pathway, carbohydrate metabolism, inflammatory reaction and cognitive processes [23-25]. Some authors have already called this cytokine “an exercise factor” due to its pleiotropic effects during physical training [13]. It has been reported that during exercise IL6 stimulates endogenous glucose production [11,13] and regulates lipid oxidation [26]. This cytokine may also act anti-inflammatory as it attenuates TNF α secretion and stimulates production of IL10 and interleukin 1 receptor antagonist (IL1ra) – two potent anti-inflammatory agents [12,27]. Another interesting study revealed that IL6KO mice present higher myocardial expression of CD36 fatty acid transporter as well as higher concentration of free fatty acids, diacylglycerols and ceramide [10]. These results suggest that IL6 may decrease the amount of ceramide in heart, an effect that is also evoked by exercise [28].

There is support for the notion that IL6 transduction pathway is closely associated with cardiac hypertrophy [9]. It has been shown that simultaneous overexpression of both subunits of IL6 receptor is sufficient to induce cardiac hypertrophy in mice [29]. On the other hand, IL6 deficiency does not change the weight and dimensions of the left ventricle after myocardial infarction [24]. In this paper we show for the first time that this cytokine is not necessary for exercise induced cardiac hypertrophy as well. The presented data confirmed that after six week swimming regime female mice present higher heart weight to body weight ratio than their sedentary counterparts, regardless of the presence of functional IL6 gene.

One of the limitations of the study is that we have relied only on relatively crude parameters, which the ratios are. Unfortunately, we were not able to perform *in vivo* studies like echocardiographic or magnetic resonance studies. Nevertheless, this preliminary report is the first one to communicate that IL6 deficiency does not change the physiological cardiac hypertrophy in mice. Faldt et al. have shown, that IL6KO mice have lower maximal exercise capacity than the wild type controls [26]. We have chosen a training pattern that utilizes repetitive submaximal exercise with possibly diminished stress for animals. Therefore, we did not encounter animals from our experiment groups that were unable to perform according to protocol. We cannot, however, exclude that there might be differences in hypertrophy when more strenuous exercise is used. Moreover, we have used female animals due to more reproducible swimming induced cardiac hypertrophy [30]. Faldt et al. do not state

which sex they used for endurance experiments [26]. Although there were no changes in heart and LV weights we also cannot rule out, that cardiac metabolism during exercise is changed in IL6KO mice.

We can only hypothesize what other factors may take over the function of IL6 and trigger the heart hypertrophy after physical exercise. It has been shown that IL6KO mice present higher expression of other IL6-family cytokines like leukaemia inhibitory factor (LIF) as well as bigger abundance of renin – angiotensin system components [24]. It has been established that both these cascades may affect cardiac hypertrophy. Another possible explanation of the lack of changes in physiological cardiac hypertrophy between IL6KO and WT mice would be the effect of TNF α . A cytokine involved in hypertrophy that is up-regulated in IL6KO mice [27,31]. To support this hypotheses, however, further studies are required.

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