Objective: Several studies have suggested that testosterone exerts a growth-promoting effect in the heart. Limited data are available regarding interactions between possible endocrine/paracrine effects in response to exercise training. Therefore, we examined supraphysiological testosterone-induced heart hypertrophy and cardiac insulin-like growth factor (IGF)-1 content in sedentary and exercise-trained rats.

Design: Male Wistar rats (n=33) were randomly allocated to groups with a 6-week endurance training with or without testosterone, and sedentary animals with or without testosterone. The hormone (20 mg/250 g body weight) was administrated once a week for six weeks. After six weeks the animals were anesthetized, euthanized and the heart was excised and weighed. The left ventricle was separated for biochemical analyses.

Results: Testosterone-treated animals showed significantly higher cardiac IGF-1 content compared to untreated control and trained groups (p=0.01). The administration of supraphysiological testosterone significantly increased the heart weight to body weight ratio (HW/BW, p<0.01). A significant positive correlation was seen between IGF-1 levels and the HW/BW ratio (p=0.002; r=0.50) and between serum total testosterone levels and HW/BW (p=0.000; r=0.79).

Conclusions: The results demonstrate that increased cardiac IGF-1 content in response to higher serum testosterone might be responsible for heart hypertrophy observed in both sedentary and endurance-trained animals.

Keywords: heart, growth factors, anabolic steroids, exercise training

1 Introduction

Cardiac adaptation to physical training involves a complex process of left ventricular hypertrophy (LVH), increase in coronary reserve, and improvement of left ventricular myocardial contractility [1, 2]. It has been observed that cardiac hypertrophy in endurance-trained experimental animals was associated with a higher endurance time, a significant increase in the relative frequency of oxidative type 1 fibers and higher muscle oxidative capacity compared to untrained groups [3]. Despite the difficulties of transforming data from animals to humans, animal models confirmed the role of exercise-induced physiological cardiac hypertrophy and this adaptation may reflect the role of aerobic training efficacy.

The primary stimulus for cardiac hypertrophy is mechanical stress, which induces a growth response in the overloaded myocardium [4, 5]. The mechanical loading regulates intracellular signals of specific gene expression for myocardial hypertrophy with participation of both endocrine and paracrine factors [6, 7, 8].

Athletes abusing anabolic androgenic steroids (AAS) with the aim of physical performance improvement may exhibit cardiac hypertrophy. Echocardiographic studies have derived conflicting results regarding the effects of AAS on left ventricular mass and function in athletes [9, 10]. The results of the studies indicate a prevalence of both physiological and pathological cardiac hypertrophy in athletes taking anabolic steroids.
Effects of exercise training and anabolic androgenic steroid on cardiac hypertrophy and IGF-1

2 Methods

2.1 Experimental animals and hormonal treatment

Young male Wistar rats, from Mossakowski Medical Research Center (Warsaw, Poland), aged 5-6 weeks and weighing 100-120 g were used in the experiments. The rats were housed at 12:12 h (light: dark) cycle, ambient temperature of 22-24°C and 45-65% relative humidity. Food and water were provided ad libitum. All rats were randomly allocated to four groups. The following inclusion criteria were used:
- untrained controls (UTr, n= 8)
- untrained rats with testosterone administration (UTr + TP, n= 8)
- endurance trained group (Tr, n= 9)
- endurance trained group with testosterone administration (Tr + TP, n= 8)

The rats allocated to the groups with testosterone administration (UTr + TP and Tr + TP) were given intramuscular injections of testosterone propionate (TP, Testosteronum propionicum; Jelfa, Poland) at a dose of 20 mg/250 g BW dissolved in sesame oil at the ratio 1:2 (v/v) once a week for 6 weeks, alternatingly into the right and left hindlimb. The rats of UTr and Tr groups were given the same volume of sesame oil according to the same schedule. The TP dose (20 mg/250 g BW) was administered due to exogenous administration and endogenous secretion may stimulate myocardial and ventricular hypertrophy, both in training-related and pathological conditions. It has been reported that chronic anabolic T treatment led to a specific physiologic pattern of myocardial hypertrophy with a significant increase in left ventricle (LV) weight, with an upregulation in alpha/beta myosin heavy chain (MHC), and an increase in IGF-1 expression [22, 24, 25]. Several lines of evidence, however, have suggested that testosterone induced a reduction in circulating IGF-1 levels and may have a direct, IGF-1 and GH-independent effect on growth [15].

Limited data are available regarding interactions between anabolic hormone concentrations and IGF-1 expression in the heart in response to exercise training. To the best of our knowledge, no study has investigated the effect of AAS use on cardiac IGF-1 content in response to exercise training.

This study is a sequel to that of Sadowska-Krepa et al. [26], which analyzed the effects of endurance training and different testosterone propionate (TP) dosage on the activities of selected antioxidant enzymes in the LV of male Wistar rats. The main observation made in that study was that the higher-dose TP treatment significantly increased the heart weight to body weight (HW/BW) ratio, however, a more detailed description of the mechanism of heart hypertrophy in response to supraphysiological dose of testosterone was not presented.

We hypothesized that myocardial hypertrophy might develop as a result of androgen receptors stimulation (supraphysiological dose of testosterone) or as a secondary effect via up-regulation of IGF-1 in the tissue. Therefore, we examined the effects of high-dose testosterone treatment on left ventricular remodeling and cardiac IGF-1 content in sedentary and endurance trained young male rats.
based on body weight, in concentrations corresponding to highest doses taken by the athletes [27].

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. All animal procedures were approved by the IVth Local Ethics Committee for Animal Experimentation (Certificate No. 38/2011) from Mossakowski Medical Research Center (Warsaw, Poland).

2.2 Endurance training

During the first week, the animals of the Tr and Tr + TP groups were run-tested on a motorized rodent treadmill (Fig. 1.), 3 × 5-min intervals, with 15-min breaks, for three successive days to adapt them to the training environment. The exercise training was done once a day for 5 days a week, from the second to the sixth week of the experiment. The treadmill speed was 16 m/min with 0° inclination during the first week. Then the speed was increased by 4 m/min weekly over the next 3 weeks, and was kept at 28 m/min for the remaining training sessions. The session duration started at 40 min/day and was increased by 5 min daily during the first 4 weeks; during the last 2 weeks, the rats ran for 1 h daily. A previous study confirmed that six weeks of endurance training shifts anaerobic threshold to higher exercise intensity and this effect is associated with increased muscle oxidative capacity, and a significant increase in HW/BW ratio in rats [28].

2.3 Blood and tissue preparation

At 6 weeks, the rats were weighed for the analysis of differences in BW in response to training and/or TP treatment. Then the rats were anesthetized and euthanized by decapitation, the trunk blood was collected, left to clot at room temperature for at least 30 min, then serum was separated by centrifugation, aliquoted and kept frozen (at −80°C) until analysis. In order to determine HW and LV weight, the heart was removed from the thoracic cavity and dissected to separate the LV. To evaluate cardiac hypertrophy, the HW was normalized to BW of the animal and the HW/BW ratio was calculated (mg/g). The HW/BW ratio was used as an index of cardiac hypertrophy as reported by Rocha et al. [11]. For analytical assay, the heart was perfused with Krebs-Henseleit buffer supplemented with glucose (10mM), and the LV was cut into pieces that were snap-frozen in liquid nitrogen and stored at −80°C until analyses. LV samples were homogenized in ice-cold buffers according to instructions provided by the manufacturer of the diagnostic kits using an Ultra-Turrax T8 homogenizer (IKA Labortechnik, Staufen, Germany). The homogenates were used immediately for the determination of IGF-I. LV tissue IGF-I content was determined according to D’Ercol et al. [29] in duplicate using mouse/rat IGF-I double antibody radioimmunoassay (DSL-2900; Diagnostic System Laboratories, Webster, TX, USA). Total serum testosterone was determined with a radioimmunoassay kit (Testosterone RIA, DSL-4100, Diagnostic System Laboratories Inc. Webster, TX, USA).

2.4 Statistical analysis

All data are presented as means ± standard deviations (SD). A two-way analysis of variance (ANOVA) with endurance training and TP treatment as the main factors, followed, when appropriate, by Tukey’s honest significant difference means comparison tests, was used to test the significance of intergroup differences. Pearson correlation coefficients were analyzed to determine the intervariable relationships. All analyses were performed using the Statistica v. 10 software package (StatSoft, Tulsa, OK, USA). The level of significance was set at p<0.05.

3 Results

3.1 Hormone Levels

A two-way ANOVA revealed significant effects of endurance training on serum total testosterone (TT) (p=0.001; F=10.4) and of TP administration on serum TT (p<0.001; F=583.0). As compared to untreated rats with and without training, serum TT concentrations were significantly higher in rats given high-dose of TP (p<0.001). Compared to sedentary
Effects of exercise training and anabolic androgenic steroid on cardiac hypertrophy and IGF-1

3.2 Body and Heart Weights

Testosterone-treated animals had significantly lower BW compared to untreated rats (p<0.01). It should be emphasized, however, that BW was significantly higher in the untrained (UTr) compared to the trained (Tr) animals (p<0.01). Significant effects of training and TP administration also were observed with respect to HW and the HW/BW ratio. Heart weight significantly increased in response to TP treatment and endurance training compared to non-supplemented groups (p<0.05) (Table 1).

The HW/BW ratio was significantly higher in trained animals on TP treatment compared to untrained, and trained but TP untreated rats. High TP doses also increased the HW/BW ratio in untrained rats (p<0.01; Table 1). A strong positive correlation between serum TT concentrations and cardiac IGF-1 content (p<0.001; r=0.70) (Table 2); however, endurance training itself did not affect IGF-1 concentrations. The ANOVA revealed a significant effect of training (p=0.007; F=8.4) and TP administration on BW (p<0.001; F=23.7).

Table 1. Effects of testosterone propionate (TP) treatment and endurance training (Tr=trained, UTr=untrained) on body weight (BW), left heart ventricle (LV) and heart weight (HW), LV tissue insulin-like growth factor-1 (IGF-1) content and serum total testosterone (TT) levels in male Wistar rats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>UTr</th>
<th>UTr + TP</th>
<th>Tr</th>
<th>Tr + TP</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW [g]</td>
<td>348.8</td>
<td>288.8**</td>
<td>293.9##</td>
<td>273.6</td>
<td>Training: F1,32=8.4 p=0.007</td>
</tr>
<tr>
<td></td>
<td>(22.3)</td>
<td>(9.5)</td>
<td>(33.3)</td>
<td>(9.2)</td>
<td>TP treatment: F1,32=23.7 p&lt;0.001</td>
</tr>
<tr>
<td>HW [g]</td>
<td>0.81</td>
<td>0.90</td>
<td>0.86</td>
<td>0.99*</td>
<td>Training: F1,32=5.9 P=0.02</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.06)</td>
<td>(0.11)</td>
<td>(0.09)</td>
<td>TP treatment: F1,32=12.5 P=0.001</td>
</tr>
<tr>
<td>HW/BW ratio [mg/g]</td>
<td>2.36</td>
<td>3.13**</td>
<td>2.98*</td>
<td>3.60**##</td>
<td>Training: F1,32=33.6 p=0.000</td>
</tr>
<tr>
<td></td>
<td>(0.17)</td>
<td>(0.26)</td>
<td>(0.62)</td>
<td>(0.32)</td>
<td>TP treatment: F1,32=94.3 P&lt;0.001</td>
</tr>
<tr>
<td>TT [nmol/l]</td>
<td>5.06</td>
<td>93.51***</td>
<td>17.74#</td>
<td>91.10*###</td>
<td>Training: F1,32=10.4 p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(3.54)</td>
<td>(1.04)</td>
<td>(3.1)</td>
<td>TP treatment: F1,32=583 p&lt;0.001</td>
</tr>
<tr>
<td>IGF-1 [ng/g wet weight]</td>
<td>36.9</td>
<td>59.9**</td>
<td>33.6</td>
<td>54.3**#</td>
<td>Training + TP: F1,32=46.5 p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(9.4)</td>
<td>(14.5)</td>
<td>(7.3)</td>
<td>(14.9)</td>
<td>TP treatment: F1,32=26.6 p&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as means and standard deviations (SD) in parentheses.

*p<0.05; **p<0.01 Tr + TP and UnTr + TP vs. the respective TP untreated group
#p<0.05; ##p<0.01; ###p<0.001 vs. UnTr

Fig.2. Serum total testosterone (TT) concentrations in response to testosterone propionate (TP) treatment and endurance training (Tr=trained, UTr=untrained) in male Wistar rats. *p<0.05; **p<0.01 Tr + TP and UnTr + TP vs. the respective TP untreated group

Fig.3. Cardiac insulin-like growth factor 1 (IGF-1) content in response to testosterone propionate (TP) treatment and endurance training (Tr=trained, UTr=untrained) in male Wistar rats. **p<0.01 Tr + TP and UnTr + TP vs. the respective TP untreated group

#p<0.05 vs. UnTr
significant positive correlation was seen between cardiac IGF-1 content and the HW/BW ratio (p=0.002; r=0.50) (Fig. 4); HW and HW/BW ratio were found to be correlated with total serum testosterone concentrations in all experimental subjects (Table 2).

![Fig. 4. Correlation between cardiac insulin-like growth factor 1 (IGF-1) content and heart rate to body weight (HW/BW) ratio.](image)

Table 2. Correlations between cardiac insulin-like growth factor [1] (IGF-1) content, total testosterone (TT) level and body weight (BW), heart rate to body weight (HW/BW) ratio.

<table>
<thead>
<tr>
<th>Relationships</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1 vs. BW</td>
<td>r= −0.3 p=0.04</td>
</tr>
<tr>
<td>IGF-1 vs. HW/BW</td>
<td>r= 0.50 p=0.002</td>
</tr>
<tr>
<td>TT vs. BW</td>
<td>r= −0.64 p=0.000</td>
</tr>
<tr>
<td>TT vs. HW/BW</td>
<td>r= 0.79 p&lt;0.001</td>
</tr>
<tr>
<td>IGF-1 vs. TT</td>
<td>r= 0.70 p&lt;0.001</td>
</tr>
</tbody>
</table>

4 Discussion

The major findings of this study are that high TP administration was associated with an increase in cardiac IGF-1 content in response to serum testosterone and might have been responsible for heart hypertrophy observed in both sedentary and trained animals. Cardiac hypertrophy of young male rats was only slightly affected by six weeks endurance training but the most important stimulus for cardiac hypertrophy was the combined effect of high testosterone doses and IGF-1 stimulation. The contribution of IGF-1 to heart hypertrophy was similar in the testosterone treated sedentary and trained animals and cannot be attributed to training induced IGF-1 release.

In our previous study, we found the existence of cardiac hypertrophy in response to high - dose of testosterone treatment and potential risk to cardiac health from androgen use either alone or in combination with endurance training in young rats [26]. For this purpose, in the present study we decided to investigate whether the cardiac hypertrophy induced by a high - dose of testosterone can affect the production of cardiac IGF-1, the main growth factor that mediates the signaling pathways responsible for cardiac hypertrophy.

Physiological heart hypertrophy is a process of adaptation to an increased hemodynamic or pressure overload. The primary stimulus for myocardial remodeling is mechanical stress, however, other mechanisms might also contribute. A high prevalence of heart hypertrophy was related to subjects body surface, type, and intensity of training [30, 31, 32]. Therefore, animals selected for our experiment had comparable baseline body weight. All animals participated in an identical 6-week program of treadmill running, which was documented to shift anaerobic threshold to higher exercise intensity and this effect is associated with increased muscle oxidative capacity, and the significant increase in HW/BW ratio [28]. On this basis, we believe that our experiment mainly reflects the testosterone- and/or training-induced effect of IGF-1 on cardiac cells.

Cellular changes in cardiac hypertrophy involve increased cardiomyocyte volume and expression of gene and protein synthesis associated with the activation of a variety of cell signaling molecules. Although the effects of exercise training and IGF-1 on the cardiovascular system have been studied before [17, 18, 33], this is, to the best of our knowledge, the first study to analyze the relationships between this cardiac growth factor and heart hypertrophy in response to endurance training with and without AAS use.

Several studies confirmed an important role of anabolic steroids in IGF-1 production and IGF-1 mRNA expression in the myocardium [6, 7, 19, 26]. IGF-1 is an important growth factor which, via its receptors, mediates physiological cardiac growth and cell differentiation by the activation of phosphoinositide 3-kinase (PI-3K) and mitogen-activated protein kinase (MAPK) signaling pathways. It also prevents cardiomyocyte apoptosis and increases inotropic properties by sensitizing myofilaments to Ca$^{2+}$ calcium ions. In the left ventricle, volume overload was accompanied by an increase in IGF-1 mRNA expression and significant IGF-1 tissue concentration with corresponding increase of myocyte diameter [14, 34, 35].

In our study, however, IGF-1 increased in high-dose TP treated animals as compared to those without testosterone administration; hence, it might be postulated that serum testosterone elevation in response to AAS abuse in young rats might stimulate greater tissue accumulation of IGF-1 in
the heart, IGF-1 may have a direct effect on cardiac growth (presented as HW/BW ratio) in rats. It cannot be excluded that the effects of IGF-1 in the heart following TP treatment depend on higher serum IGF-1 level in experimental animals. This finding is in agreement with the results of Hobbs et al. [21] that 6-week testosterone administration increased serum IGF-1 concentrations in healthy men. The IGF-1 response to supraphysiological doses of testosterone and treatment with exogenous steroids (e.g., nandrolone decanoate) may have differently influenced the IGF-1 system. Testosterone (T enanthate; 300 mg im, each week) administration increased serum IGF-1 concentrations in healthy men after 6 weeks of treatment; however, treatment with exogenous steroids (nandrolone decanoate; 300 mg im, each week) did not change serum levels of IGF-1 but did decrease the level of the serum IGF-binding protein-3, potentially increasing the level of bioavailable IGF-1 in the nandrolone subjects [21]. In contrast to these studies in humans, testosterone administration to juvenile rats, has been reported to significantly decrease circulating levels of IGF-1 [15]. We did not analyze the serum IGF-1 levels, however, previous results confirmed that there was a negative correlation between TT levels in rats treated with high doses of testosterone and serum IGF-1 [15]. Regardless of the fact that high doses of testosterone might diminish serum IGF-1 concentrations, we conclude that changes in IGF-1 expression in cardiac tissues may have major impacts on heart hypertrophy.

Our study revealed a tendency of IGF-1 to decrease in trained compared to sedentary untreated animals (untrained). Although previous studies indicated that serum IGF-1 elevation, apparently caused by muscle work, might play a crucial role in stimulating physiological heart hypertrophy in athletes [36], our experiment did not confirm these findings. It should be emphasized that, while earlier experiments suggested an exercise intensity-related increase in serum IGF-1 [17, 33], other research yielded opposite results [37].

The findings of the present study confirmed the metabolic role of testosterone. We found that testosterone-treated rats had significantly lower body weight and significantly higher heart weight and HW/BW ratio compared to untreated rats. The heart weight was significantly increased by TP treatment, but not in response to endurance training. We hypothesized that the decrease in BW of TP treated animals could be attributed to the testosterone-induced increase in adipose tissue lipolysis. Additionally, exercise training probably induced favorable changes in body composition in trained rats with and without TP use [26, 38, 39]. This is evidenced by a marked elevation of the HW/BW ratio, which was, however, more prominent in trained and TP treated animals.

It should be noted that, the use of the HW/BW ratio as a hypertrophic index has limitations and the fluctuations in BW as occurs with aging makes BW an unreliable reference for normalizing heart weight [35]. However, in the experiment in which the age of the animals did not differ we suggested that cardiac hypertrophy can be more accurately quantified by relating HW to BW [3, 40, 41].

Animal experiments have shown structural myocardial alterations and heart hypertrophy in response to high AAS doses and physical training [42, 43, 44]. Of importance is that treatment of rats with supraphysiological doses of AAS induced pathological myocardial hypertrophy, and exercise training combined with AAS caused a loss of the beneficial effects on LV function induced by exercising [11]. TP treatment resulted in an increase in serum TT in the examined rats, but it was suggested that the significant increase of TT was associated with higher susceptibility of free testosterone, which is a biologically active hormone.

Our study is of considerable importance as AAS are increasingly used in both therapeutic and non-therapeutic practices. In this regard, escalating attention has focused on the effects of steroid hormones on physiological and pathological cardiac growth. If AAS is associated with an exaggerated cardiac hypertrophic response to training, athletes may be more prone to cardiac failure [45, 46]. Special attention should be paid to athletes who have been using them without prescription, with the purpose of increasing muscle mass or to improve physical performance.

Interestingly, cardiac IGF-1 contents in the trained animals with high doses of testosterone were marginally lower compared to treated sedentary rats. We can only speculate that weaker stimulation of IGF-1 in the myocardium in response to endurance training may retard AAS-induced pathological cardiac hypertrophy. Considering our findings in the light of previous reports, it is tempting to postulate that TP treatment was the causative factor for increasing IGF-1 after high intensity training. Since the trained rats performed the testing trials at the same absolute workload, our data indicate that TP supplementation was a crucial causative stimulus in the TT and IGF-1 response to intense endurance training. Additionally the significant effects of cardiac hypertrophy were evidenced by a significantly higher HW/BW ratio in trained TP-treated animals.

Our study has several limitations, such as the lack of myocardial function measurements and photographic evidence of the registered changes on the cardiac tissue treated in respect to the control. Indeed, we cannot exclude
an effect of higher serum IGF-1 concentration in response to exercise training. Nevertheless, using the same model it has been suggested that six weeks of endurance training significantly increased the LV wall thickness in the trained compared with sedentary rats [41]. The training induced physiological heart hypertrophy was confirmed by echocardiography variables and enhancement of ejection fraction and fractional shortening [41, 47, 48]. However, the protocol presented in our study has some major advantages, e.g. higher HW and HW/BW ratio in testosterone treated rats.

To summarize, this study shows that increased cardiac tissue IGF-1 content in response to higher serum testosterone might be a causative factor responsible for heart hypertrophy observed in both sedentary and endurance-trained animals.

Abbreviations

AAS anabolic androgenic steroids
BW body weight
GH growth hormone
HW heart weight
IGF-1 insulin-like growth factor 1
LV left ventricle
LVH left ventricular hypertrophy
TP testosterone propionate
Tr trained rats
UTr untrained rats
TT total testosterone

Conflict of interest: Authors state no conflict of interest

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