

B. CZARKOWSKA-PACZEK¹, M. ZENDZIAN-PIOTROWSKA², I. BARTLOMIEJCZYK³, J. PRZYBYLSKI¹, J. GORSKI²

THE EFFECT OF ACUTE AND PROLONGED ENDURANCE EXERCISE ON TRANSFORMING GROWTH FACTOR- β 1 GENERATION IN RAT SKELETAL AND HEART MUSCLE

¹Department of Biophysics and Human Physiology, Medical University of Warsaw, Warsaw, Poland; ²Department of Physiology, Medical University of Białystok, Białystok, Poland; ³Department of Immunotherapy, Transplantology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland

The serum level of the transforming growth factor-beta1 (TGF- β 1) is elevated after acute bouts of exercise and prolonged training, as well as after myocardial infarction. However, the source of this increase remains unclear. Contracting skeletal muscles are known to be the source of many cytokines. To determine whether skeletal or heart muscles produce TGF- β 1 during exercise, we investigated the effect of a single bout of acute exercise on TGF- β 1 generation in skeletal and heart muscles in untrained rats (UT, n=30) and in rats subjected to prolonged (6-week) endurance training (T, n=29). The UT and T (a day after final training) groups were subjected to an acute bout of exercise with the same work load. Rats from both groups were sacrificed and skeletal and heart muscle samples were collected before (pre), immediately after (0 h), or 3 hours (3 h) after acute exercise. TGF- β 1 mRNA was quantified by RT-PCR in these samples, and basal TGF- β 1 protein levels were determined in skeletal muscle in the UTpre and Tpre subgroups by ELISA. Acute exercise caused a non-significant increase in TGF- β 1 mRNA in skeletal muscle in UT0h rats, in compare to UTpre rats. There was a significant decrease of TGF- β 1 mRNA in the T0h group (p=0.0013) in compare to Tpre rats. Prolonged training caused a significant increase in TGF- β 1 mRNA (p=0.02); however, the TGF- β 1 protein level decreased (p=0.02). In heart muscle, there was a significant decrease of TGF- β 1 mRNA in UT0h (p=0.01) and UT3h (p=0.04) compared to UTpre rats. TGF- β 1 mRNA levels were unchanged in T0h and T3h compared to Tpre; basal TGF- β 1 mRNA expression after training was also unchanged (UTpre vs. Tpre). We conclude that physical exercise is a potent stimulus for inducing TGF- β 1 gene expression in skeletal muscle, but does not increase the protein level. Thus, skeletal and heart muscle do not contribute to increased serum levels of TGF- β 1 after physical exercise.

Key words: *transforming growth factor-beta1, exercise, muscle, heart, hypoxia, reactive oxygen species*

INTRODUCTION

We previously found that acute bouts of exercise increase the serum level of TGF- β 1 in humans (1). Similarly, Hering *et al.* reported changes in serum TGF- β during 4 weeks of physical training: a significant increase was observed after the second week, followed by a decline to basal levels in the third and fourth weeks (2). However, the source of the increase in serum TGF- β after mechanical load remains unclear.

During physical exercise, skeletal muscle tissue is influenced by mechanical load. Contracting skeletal muscle acts as an immunogenic organ and is the source of many cytokines, termed myokines; these include interleukin-6, -8, -4, -13, and -15, which exert their effects both locally and in an endocrine manner (3-6). This raises the question of whether contracting muscle produces TGF- β 1 as well - this production would contribute to the increased level of this growth factor in serum and could contribute to adaptive changes in muscles as well.

The protective and therapeutic effects of physical exercise on cardiac disease are well known (7). Growth factors, including TGF- β 1, are also elevated in serum after myocardial infarction

(MI) (8) and it is thought that they play a role in cardiac muscle regeneration and remodeling. TGF- β 1 stimulates the differentiation of bone marrow stem cells into immature cardiomyocytes, and also enhances the differentiation of fibroblast into myofibroblasts, which produce collagen to aid scar formation after MI (9, 10). On the other hand, overproduction of TGF- β 1 plays a fundamental role in tissue fibrosis, and after MI contributes to pathogenic cardiac remodeling and cardiac failure (11-13). This raises the question of whether physical exercise elicits TGF- β 1 production in heart muscle, thus influencing TGF- β 1 serum levels and cardiac muscle morphology and function.

The goal of the present study was to investigate the influence of a single bout of acute exercise on TGF- β 1 production in skeletal and heart muscle tissue in rats that were untrained (control) or subjected to 6 weeks of prolonged physical training.

MATERIALS AND METHODS

This study used 59 male Wistar rats that were allowed to drink ad libitum, were fed Labofeed B, and were maintained on

a 12h/12h light/dark cycle. All procedures in the study were approved by the Ethical Committee of the Medical University in Warsaw and were performed according to EU regulations governing the treatment of laboratory animals.

At the start of the experiment, all animals were adapted to exercise, which consisted of running 10 min/day on a treadmill at a speed of 900 m/h for 5 successive days. Rats were then randomly assigned into two groups: trained rats (T, n=29), who underwent physical training for 6 weeks, and untrained rats (UT, n=30), who performed no exercise. The mean body mass of the rats on the day the experiment started was 127 ± 13.87 mg. Training for T group consisted of treadmill running 5 days a week as follows. On the first day of the first week, the rats exercised on a treadmill for 10 min/day at a speed of 1200 m/h, with the exercise time increasing by 10 min/day. In the second week, the rats exercised for 60 min/day at a speed of 1500 m/h, and in weeks 3-6, the exercise time remained constant at 60 min/day, and the speed was increased to 1700 m/h. The UT group remained at rest during the training period. Twenty-four hours after the last training session, rats from both groups (UT and T) were each randomly divided into 3 subgroups. In two subgroups, UTpre (n=10) and Tpre (n=9), samples of skeletal and heart muscle were collected under anesthesia (intraperitoneal chloral hydrate, 1 ml/100 g body mass) and stored at -80°C for subsequent analysis. The animals in all other subgroups performed an acute bout of exercise, comprising 60 min on the treadmill running at a speed of 1700 m/h. Samples of skeletal and heart muscle were collected from two additional subgroups, UT0h (n=10) and T0h (n=10), immediately after the exercise session. Samples were taken from the last two subgroups, UT3h (n=10) and T3h (n=10) 3 hours post-exercise. The mean body mass of the rats on the day the experiment ended was 379.75 ± 51.63 mg. All animals died as a consequence of sample collection. Basal TGF- β 1 protein levels were measured in skeletal muscle tissue in UTpre and Tpre groups.

mRNA isolation

Approximately 50 mg of skeletal or heart muscle tissue were homogenized in a TissueLyser bead mixer (Qiagen, Germany). Total mRNA isolation was performed using the EZ1 RNA Universal Tissue Kit and Biorobot EZ1 (Qiagen, Germany) according to the manufacturer's instructions. Total mRNA concentration was measured by spectroscopy at 260 nm using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). Samples were then frozen and stored at -80°C for further analysis.

Reverse transcription

Reverse transcription of total mRNA into cDNA was performed using the Thermomixer Comfort (Eppendorf,

Germany) with the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA) according to the manufacturer's instructions.

Real-time PCR to quantify TGF- β 1 mRNA

Detection of mRNA was performed using an ABI-Prism 7700 Sequence Detection System (Applied Biosystems, USA). PCR was performed using reverse-transcribed mRNA. The reaction mixture also included TaqMan Universal PCR Master Mix (polymerase and dNTPs), primers for the genes for TGF- β 1 and nuclease-free water (Applied Biosystems, USA) in a total volume of 20 μl . PCR amplification consisted of an initial step of 2 min at 50°C , followed by 20 min at 95°C , followed by 40 cycles of 15 s at 95°C and 1 min at 60°C . PCR for the reference gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was performed for each sample, and a 'no template control' (NTC) was performed for genes for TGF- β 1 and for GAPDH as a reference (normalizing) gene. According to Perez *et al.*, GAPDH is the optimal gene to use for this purpose in heart muscle (14).

The data were analyzed using the comparative cycle threshold (C_T) method. The C_T of each sample was normalized using GAPDH gene expression, and the results were reported as ΔC_T . The relative level of mRNA for TGF- β 1 was calculated by subtracting the normalized C_T values for genes in the experimental rat groups (exp) relative to medium control (ctr): $\Delta\Delta C_T = \Delta C_{T\text{-exp}} - \Delta C_{T\text{-ctr}}$; the relative changes in mRNA levels (expressed as 'fold changes') were calculated as $2^{-\Delta\Delta C_T}$ (15).

Protein quantitation

Each sample of skeletal muscle was homogenized in a TissueLyser bead mixer (Qiagen, USA) and centrifuged at 3000 rpm for 10 min. The supernatant was collected and frozen at -80°C until analysis. Total protein concentration was measured by spectroscopy at 562 nm on a Bio-Tek Power Wave XS spectrophotometer (Bio-Tek Instruments, USA) using bicinchoninic acid (BCA) Protein Assay Reagent (Pierce, Holland), according to the manufacturer's instructions. Activation of latent TGF- β was performed by incubation of the protein with 1 N HCl at room temperature for 10 min followed by neutralization with 1.2 N NaOH/0.5 M HEPES. Tissue TGF- β 1 protein concentration was measured immediately after activation and neutralization using the Quantikine Human TGF- β 1 Immunoassay (R&D Systems, USA). This assay recognizes both human and rat TGF- β 1.

Statistical analyses

Differences in mRNA levels between groups were analyzed using Kruskal-Wallis non-parametric ANOVA followed by post

Table 1. The expression of TGF- β 1 mRNA shown as $\Delta C_T \pm \text{SD}$, calculated after the normalization of C_T to GAPDH gene expression in skeletal (soleus) and heart muscle after an acute bout of exercise in rats that were untrained (UT, n=30) or subjected to training (T, n=29). Samples were collected and TGF- β 1 mRNA was estimated before the acute bout of exercise (UTpre, n=10 and Tpre, n=9), just after exercise cessation (UT0h, n=10 and T0h, n=10), and 3 hours post-exercise (UT3h, n=10 and T3h, n=10).

TGF- β 1 mRNA $\Delta C_T \pm \text{SD}$	Untrained rats (UT)			Rats subjected to training (T)		
	UTpre	UT0h	UT3h	Tpre	T0h	T3h
Skeletal muscle (soleus)	6.58 ± 1.09	6.05 ± 1.11	7.27 ± 1.19	4.96 ± 1.58	7.11 ± 1.12	5.97 ± 1.06
Heart muscle	5.22 ± 0.97	6.00 ± 1.13	6.01 ± 0.95	5.27 ± 1.20	5.43 ± 0.89	5.39 ± 0.92

hoc Duncan's Test. ΔC_T values were used for statistics. Pre- and post-training values of proteins in skeletal muscle tissue were compared using the Student's *t* test for independent samples. $p < 0.05$ was considered to be statistically significant.

RESULTS

TGF- β 1 mRNA levels were determined in skeletal and heart muscle samples from UT and T rats before an acute bout of exercise (UTpre, Tpre), immediately post-exercise (UT0h, T0h), and 3 hours post-exercise (UT3h, T3h). The mRNA levels were expressed as $\Delta C_T \pm SD$ (Table 1). The relative changes in TGF- β 1 mRNA in skeletal muscle (soleus) after an acute bout of exercise in UT and T rats are shown in Fig. 1 (panel A and B). In UT rats, there was a non-significant

increase in TGF- β 1 mRNA in subgroup UT0h, and a significant decrease in TGF- β 1 mRNA expression in UT3h ($p = 0.03$) compared to UT0h. In T rats, there was a significant decrease in TGF- β 1 mRNA in subgroup T0h ($p = 0.001$) and a non-significant increase towards pre-exercise values in subgroup T3h. The relative changes of TGF- β 1 mRNA in T0h and T3h in compare to UTpre are shown in panel C.

The relative changes in TGF- β 1 mRNA and protein levels in skeletal muscle (soleus) in UT versus T rats are shown in Fig. 2. There was a significant increase in TGF- β 1 mRNA in Tpre rats, who were trained for 6 weeks, compared to UTpre rats, who did not train ($p = 0.02$); however, the TGF- β 1 protein level decreased significantly from 0.044 ± 0.02 pg/ μ g total protein (UTpre) to 0.025 ± 0.01 pg/ μ g total protein (Tpre) ($p = 0.02$).

The relative changes in TGF- β 1 mRNA in heart muscle after an acute bout of exercise in UT and T rats are shown in Fig. 3.

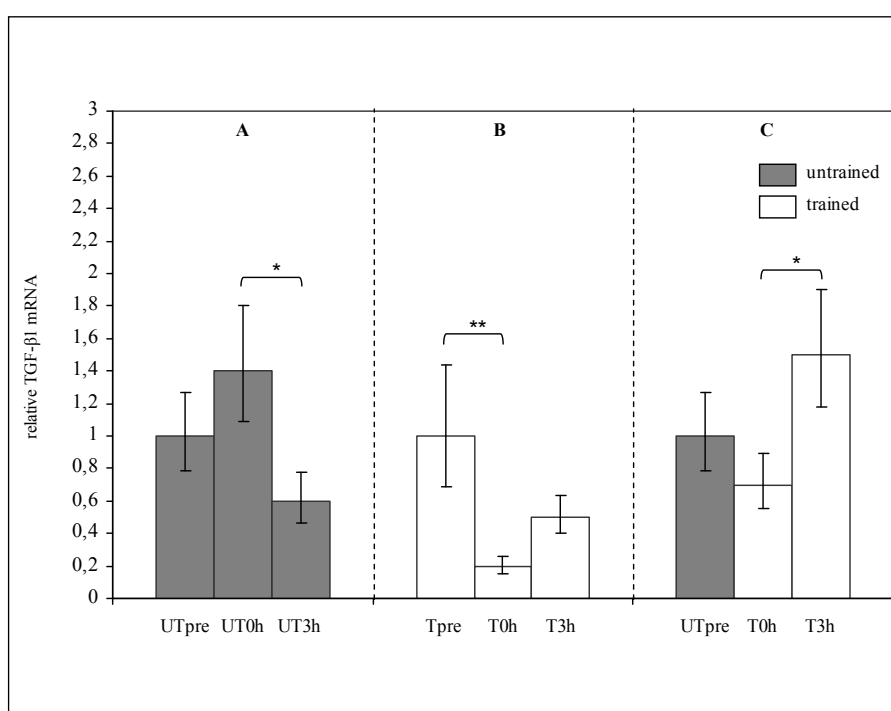


Fig. 1. The relative changes in TGF- β 1 mRNA in skeletal muscle (soleus) after an acute bout of exercise in rats that were untrained (UT, $n = 30$) (A) or subjected to training (T, $n = 29$) (B). Samples were collected and TGF- β 1 mRNA was estimated before the acute bout of exercise (UTpre, $n = 10$ and Tpre, $n = 9$), just after exercise cessation (UT0h, $n = 10$ and T0h, $n = 10$) and 3 hours post-exercise (UT3h, $n = 10$ and T3h, $n = 10$). In untrained rats, there was a non-significant tendency towards increased TGF- β 1 mRNA in UT0h rats, and there was a significant decrease in TGF- β 1 mRNA expression in UT3h rats (* $p = 0.03$) compared to UT0h rats. In rats subjected to training, a significant decrease in TGF- β 1 mRNA was observed in group T0h (** $p = 0.001$). A non-significant tendency to return to pre-exercise values was observed in group T3h. The relative changes of TGF- β 1 mRNA in T0h and T3h in compare to UTpre are shown in panel C.

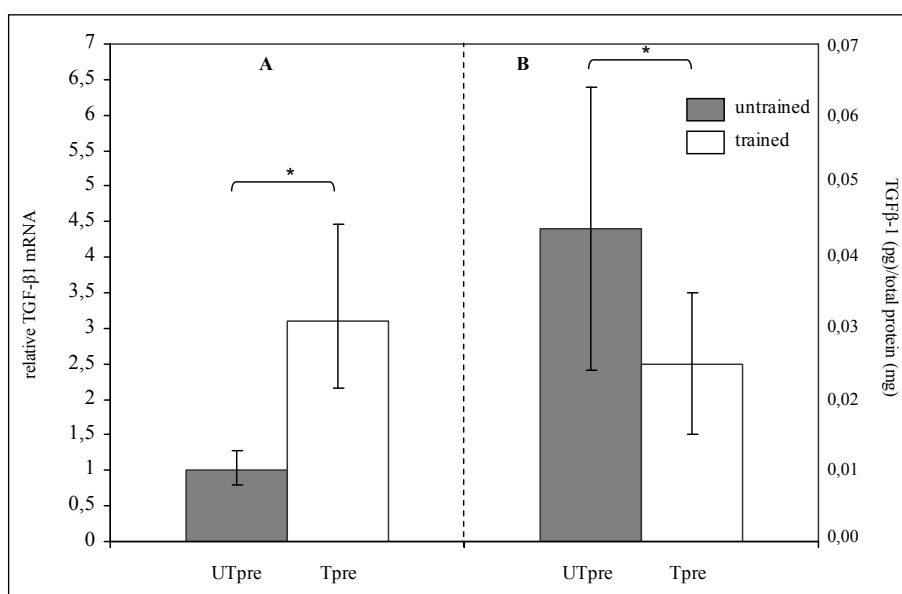


Fig. 2. Relative changes of TGF- β 1 mRNA (A) and protein (B) in skeletal muscle (soleus) in rats that were untrained (UTpre, $n = 10$) or subjected to training (Tpre, $n = 9$). There was a significant increase in TGF- β 1 mRNA (* $p = 0.02$) after 6 weeks of training (Tpre vs. UTpre); however, the TGF- β 1 protein level decreased significantly (* $p = 0.02$).

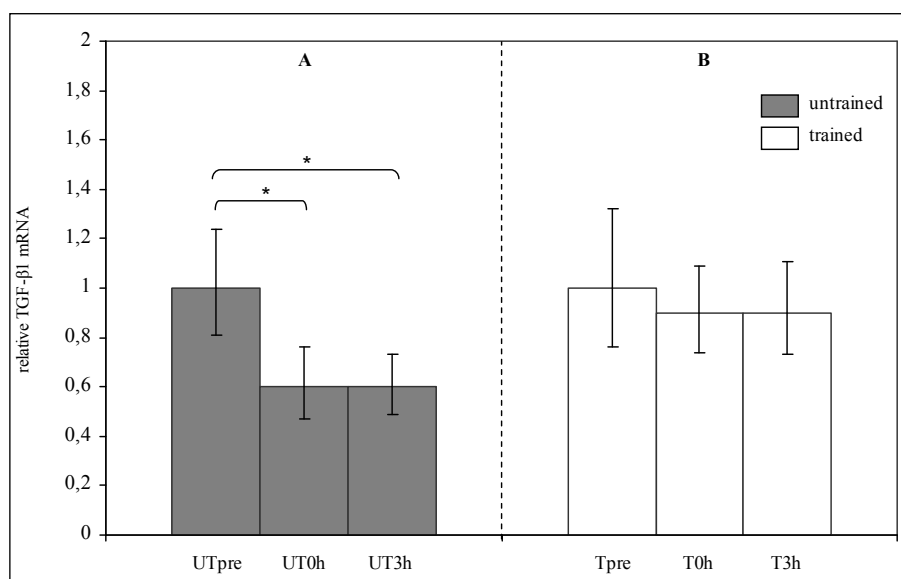


Fig. 3. The relative changes in TGF- β 1 mRNA in heart muscle after an acute bout of exercise in rats that were untrained (UT, n=30) (A) or subjected to training (T, n=29) (B). Samples were collected and TGF- β 1 mRNA was estimated before the acute bout of exercise (UTpre, n=10 and Tpre, n=9), just after exercise cessation (UT0h, n=10 and T0h, n=10), and 3 hours post-exercise (UT3h, n=10 and T3h, n=10). There was a significant decrease in TGF- β 1 mRNA in UT0h (*- p=0.01) and in UT3h (*- p=0.04) compared to UTpre. There were no changes in TGF- β 1 mRNA levels in the T0h or T3h subgroups in compare to Tpre.

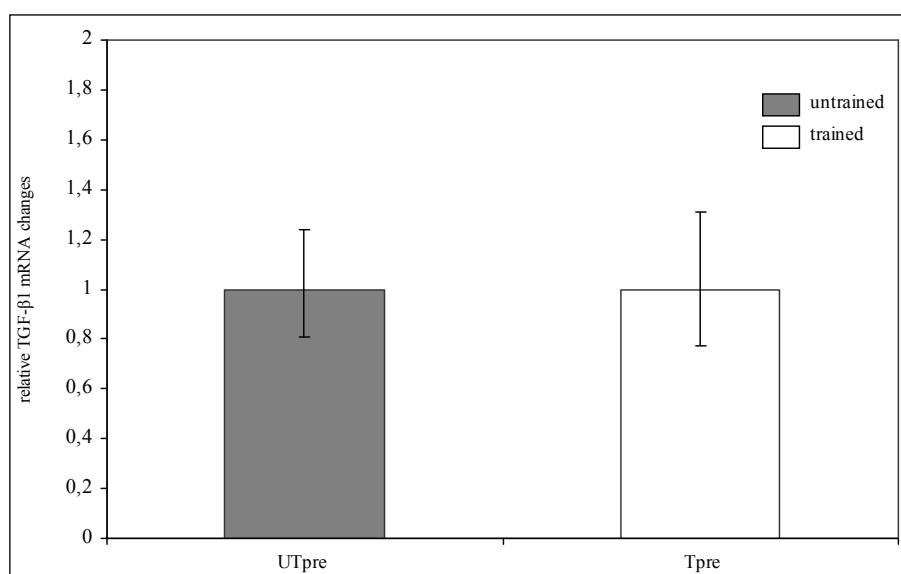


Fig. 4. The relative changes in TGF- β 1 mRNA in heart muscle after physical training (UTpre, n=10 vs. Tpre, n=9). Training did not change TGF- β 1 mRNA levels in heart muscle.

In UT rats, there was a significant decrease in TGF- β 1 mRNA in UT0h (p=0.01) and in UT3h (p=0.04) compared to UTpre. There were no changes in TGF- β 1 mRNA generation in heart muscle either after an acute bout of exercise in T rats or after 6 weeks of training (UTpre vs. Tpre) (Fig. 4).

DISCUSSION

There is very limited data concerning the influence of physical exercise on TGF- β 1 generation in tissues, including skeletal muscle. Animal studies suggest that different types of short training sessions increase TGF- β 1 mRNA expression in skeletal muscle, both daily and after the series of training sessions are completed (16, 17). These results are in line with those in our study: we also observed an increase in TGF- β 1 mRNA after 6 weeks of training. Unlike the present study, the earlier studies did not measure TGF- β 1 protein levels.

The signal for upregulation of TGF- β 1 mRNA in skeletal muscle is unclear. Recent studies suggest that the triggering mechanism could be oxidative stress, which accompanies physical

exercise (18, 19). In animal studies, Zhao *et al.* demonstrated that oxidative stress, as indicated by upregulated NADPH oxidase expression and suppressed superoxide dismutase expression, upregulates TGF- β 1 expression in cardiac muscle and in kidney. Co-treatment with antioxidants led to significantly attenuated TGF- β 1 gene expression. Hypoxia inducible factor-1 (HIF-1) increases TGF- β 1 gene expression as well. HIF-1 itself is elicited by tissue hypoxia, but also increases in response to other factors, including cytokines and cell stretching (20, 21).

It is possible that right after a bout of acute exercise in UT rats, there are many signaling events, each of which causes a small increase in TGF- β 1 mRNA; together, these small increases could add up to a significant increase in T rats. However, 6 weeks of training (which may increase antioxidant potency) and adaptation to physical exercise could result in diminished TGF- β 1 mRNA expression after an acute bout of exercise in T rats compared to UT rats (22).

The most interesting finding in the present study was the discrepancy between TGF- β 1 mRNA expression and protein expression in skeletal muscle samples. The level of protein expression depends not only on transcription, but also on

posttranscriptional and posttranslational control mechanisms. Their role in controlling protein expression has been confirmed by data indicating that only 20-40% of protein concentration is due to variations in mRNA levels (23). Translation is activated by several eukaryotic initiation factors, *i.e.* eIFs, which are affected by many types of stimuli, among them stress, starvation, amino-acid availability, hypoxia, energy status of the cell and oxidative burst. All mentioned stimuli accompany physical exercise. Thus, environmental changes could diminish translation of most mRNAs by inactivation of various eIFs (24). These mechanisms are all involved in 'global regulation' *i.e.* they affect protein biosynthesis in general. Translation of some defined groups of mRNA can also be controlled by sequence-specific RNA-binding proteins that interfere with eIF binding and by microRNAs. Regulation of the translation of specific mRNAs makes sense in terms of the cell responding to changes in environment. Yet another mechanism involved in translation regulation is the specific transport of a subset of mRNAs to particular regions of the cell where they decay. Decay programs depend on cellular and environmental conditions, and play important roles in processes such as inflammation and hypoxia (25). The decrease of protein level could be also the effect of posttranslational regulation, such as proteolysis, changing of protein activity and transport of protein off the tissue. In this study, our data did not allow us to determine the particular mechanism responsible for the observed decreases in TGF- β 1 protein level despite the increase in TGF- β 1 mRNA, however the problem deserves further research.

The observed decrease in the TGF- β 1 protein was especially interesting in the context of its role in regulation of myogenesis and promotion of fibrosis. Physical exercise elicits many changes in muscle composition and metabolism, collectively known as muscle plasticity (26, 27). Since TGF- β 1 influences myogenesis, regeneration, and healing after mechanical strain, its role in muscle plasticity could not be excluded (28).

Myogenesis is controlled by muscle regulatory factors, including MyoD and myogenin. They are the key to successful differentiation towards the myogenic lineage. TGF- β 1 increases MyoD degradation and decreases myogenin expression in satellite cells; it also suppresses expression of miR-24, a factor that promotes myogenesis. TGF- β 1 also controls myogenesis through upregulation of myostatin, predominantly in the course of terminal differentiation. In this way, TGF- β 1 inhibits muscle growth (29-32). During muscle adaptation to physical exercise, the opposite effect is observed: an increase in muscle mass is dependent on the intensity and the type of physical training in terms of muscle fiber hypertrophy and hyperplasia (27). In this context, we suggest the following explanation for our observations. Physical exercise elicits a signal for increasing TGF- β 1 mRNA expression. TGF- β 1 is very potent stimulator of collagen synthesis as well as an inhibitor of muscle cell differentiation. Overproduction of the TGF- β 1 protein could stimulate skeletal muscle fibrosis and inhibiting skeletal muscle growth. The TGF- β 1 protein could thus be harmful to the skeletal muscle. Because of the response of the cell to altered environmental conditions to meet the changing needs of the tissue, the level of TGF- β 1 protein is therefore down regulated (despite the increase in TGF- β 1 mRNA) by complex posttranscriptional regulatory mechanisms.

The role of TGF- β 1 in cardiomyocyte differentiation is not the same as in skeletal muscle differentiation. TGF- β induces cardiac differentiation of embryonic stem cells and regulates the progression of differentiation towards the cardiac lineage (32). TGF- β 1 is also a potent stimulator of extracellular matrix turnover and of collagen synthesis and angiogenesis. Upregulation of TGF- β in the myocardium has been demonstrated in several animal models of cardiac hypertrophy, suggesting that this growth factor could play a critical role in cardiac remodeling (33).

In this study, an acute bout of exercise caused a decrease in TGF- β 1 mRNA in heart muscle. As noted, oxidative stress may be one of the triggering mechanism for TGF- β 1 mRNA generation. It has been shown that after acute bouts of exercise, uncoupling-protein-2 (UCP-2) is upregulated in heart muscle in untrained rats (34). UCP-2 serves as an important defense mechanism against oxidative bursts and ROS production in heart muscle during early stages of exercise, when other defense mechanisms are still latent. It is possible, that this mechanism, *via* removing of ROS, influences the expression of TGF- β 1 mRNA in heart muscle. Prolonged training attenuates UCP-2 upregulation in response to acute exercise, because other mechanisms responsible for ROS removal are enhanced meanwhile. This could explain why subjecting rats to physical training and to acute bout of exercise after it in our experiment did not cause any changes in TGF- β 1 mRNA expression in heart muscle compared to UT rats.

Our observations conflict with the results of Calderone *et al.*, who reported an increase in TGF- β 1 mRNA in the left ventricular muscles of female Sprague-Dawley rats after 3- and 6-week voluntary running (35). They also observed a correlation between TGF- β 1 mRNA and the magnitude of cardiac hypertrophy. This discrepancy in results could be due to differences in exercise intensity. In our experiment, the rats ran a total of 44.5 km over 6 weeks (1 h of intensive run/day); in the experiment conducted by Calderone *et al.*, the rats ran 508 \pm 29 km (12 h intensive run/day). We designed the intensity and duration of the individual training sessions, as well as the overall training regimen as a whole, to mimic the exercise recommendations for humans for preventing cardiac disease and for rehabilitation after MI. In contrast, the other study involved physical exertion that was longer and more intense than that recommended for humans. Thus, different exercise intensities could induce different responses in cardiac cells. This clearly shows the necessity to establish not only recommended "doses" of exercise, but also to establish how much exercise represents an "overdose."

In conclusion, neither skeletal nor heart muscle tissue contribute to the increased level of serum TGF- β 1 after acute exercise or prolonged physical training. Although physical exercise is a potent stimulus for inducing expression of the TGF- β 1 gene in skeletal muscle, despite TGF- β 1 mRNA increase, TGF- β 1 protein decreases.

Acknowledgements: These studies were funded by the Warsaw Medical University (grant no.NZME/W1).

Conflict of interests: None declared.

REFERENCES

1. Czarkowska-Paczek B, Bartłomiejczyk I, Przybylski J. The serum levels of growth factors: PDGF, TGF-beta and VEGF are increased after strenuous physical exercise. *J Physiol Pharmacol* 2006; 57: 189-197.
2. Hering S, Jost C, Schulz H, Hellmich B, Schatz H, Pfeiffer AFH. Circulating transforming growth factor β 1 (TGF β 1) is elevated by extensive exercise. *Eur J Appl Physiol* 2002; 86: 406-410.
3. Petersen AM, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J Physiol Pharmacol* 2006; 57: 43-51.
4. Nielsen AB, Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Appl Physiol Nutr Metab* 2007; 32: 833-839.
5. Nielsen S, Pedersen BK. Skeletal muscle as an immunogenic organ. *Curr Opin Pharmacol* 2008; 8: 346-351.

6. Prokopchuk O, Liu Y, Wang L, Schmidtbleicher D, Steinacker JM. Skeletal muscle IL-4, IL-4R α , IL-13 and IL-13R α expression and response to strength training. *Exerc Immunol Rev* 2007; 13: 67-75.
7. Vona M, Codeluppi GM, Iannino T, Ferrari E, Bogousslavsky J, von Segesser LK. Effects of different types of exercise training followed by detraining on endothelium-dependent dilation in patients with recent myocardial infarction. *Circulation* 2009; 119: 1601-1608.
8. Czarkowska-Paczek B, Przybylski J, Marciniak A, *et al.* Proteolytic enzymes activities in patients after myocardial infarction correlate with serum concentration of TGF- β . *Inflammation* 2004; 28: 279-284.
9. Li TS, Komota T, Ohshima M, *et al.* TGF- β induces the differentiation of bone marrow stem cells into immature cardiomyocytes. *Biochem Biophys Res Commun* 2008; 366: 1047-1080.
10. Petrov VV, Fagard RH, Lijnen PJ. Stimulation of collagen production by transforming growth factor- β during differentiation of cardiac fibroblasts to myofibroblasts. *Hypertension* 2002; 39: 258-263.
11. Li S, Li X, Zheng H, Xie B, Bidasee KR, Rozanski GJ. Pro-oxidant effect of transforming growth factor- β 1 mediates contractile dysfunction in rats ventricular myocytes. *Cardiovasc Res* 2008; 77: 107-117.
12. Asazuma-Nakamura Y, Dai P, Harada Y, Jiang Y, Hamaoka K, Takamatsu T. Cx43 contributes to TGF- β signaling to regulate differentiation of cardiac fibroblasts into myofibroblasts. *Exp Cell Res* 2009; 315: 1190-1199.
13. Ellmers LJ, Scott NJ, Medicherla S, *et al.* Transforming growth factor- β blockade down-regulates the renin-angiotensin system and modifies cardiac remodeling after myocardial infarction. *Endocrinology* 2008; 149: 5828-5834.
14. Perez S, Royo LJ, Astudillo A, *et al.* Identifying the most suitable endogenous control for determining gene expression in hearts from organ donors. *BMC Mol Biol* 2007; 8: 114.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the method. *Methods* 2001; 25: 402-408.
16. Heinemeier KM, Olesen JL, Haddad F, *et al.* Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *J Physiol* 2007; 582: 1303-1316.
17. Gavin TP, Wagner PD. Effect of short-term exercise training on angiogenic growth factor gene responses in rats. *J Appl Physiol* 2001; 90: 1219-1226.
18. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor- β 1 in hypertensive rats. *Mol Cell Biochem* 2008; 317: 43-50.
19. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Kidney fibrosis in hypertensive rats: role of oxidative stress. *Am J Nephrol* 2008; 28: 548-554.
20. Milkiewicz M, Doyle JL, Fudalewski T, Ispanovic E, Aghasi M, Haas TL. HIF-1 α and HIF-2 α play a central role in stretch-induced but not shear-stress-induced angiogenesis in rat skeletal muscle. *J Physiol* 2007; 583: 753-766.
21. George DJ, Kaelin WG. The von Hoppel-Lindau protein, vascular endothelial growth factor and kidney cancer. *N Engl J Med* 2003; 349: 419-421.
22. Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic Biol Med* 2008; 44: 153-159.
23. Brockmann R, Beyer A, Heinisch JJ, Wilhelm T. Posttranscriptional expression regulation: what determines translation rates? *PLoS Comput Biol* 2007; 3: e57.
24. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 2009; 136: 731-745.
25. Halbeisen RE, Galgano A, Scherrer T, Gerber AP. Post-transcriptional gene regulation: From genome-wide studies to principles. *Cell Moll Life Sci* 2008; 65: 798-813.
26. Yu M, Stepto NK, Chibalin AV, *et al.* Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *J Physiol* 2003; 546: 327-335.
27. D'Antona G, Lanfranconi F, Pellegrino MA, *et al.* Skeletal muscle hypertrophy and structure and function of skeletal muscle fibers in male body builders. *J Physiol* 2006; 570: 611-627.
28. Kollias HD, McDermott JC. Transforming growth factor- β and myostatin signaling in skeletal muscle. *J Appl Physiol* 2008; 104: 579-587.
29. Budasz-Rwiderska M, Jank M, Motyl T. Transforming growth factor- β 1 upregulates myostatin expression in mouse C2C12 myoblasts. *J Physiol Pharmacol* 2005; 56: 195-214.
30. Sun Q, Zhang Y, Yang G, *et al.* Transforming growth factor- β -regulated miR-24 promotes skeletal muscle differentiation. *Nucleic Acids Res* 2008; 36: 2690-2699.
31. Li X, Velleman SG. Effect of transforming growth factor- β 1 on embryonic and posthatch muscle growth and development in normal and low score normal chicken. *Poult Sci* 2009; 88: 265-275.
32. Schabort EJ, van der Merwe M, Loos B, Moore FP, Niesler CU. TGF- β 's delay skeletal muscle progenitor cell differentiation in an isoform-independent manner. *Exp Cell Res* 2009; 315: 373-384.
33. Villarreal FJ, Dillman WH. Cardiac hypertrophy-induced changes in mRNA levels for TGF- β 1, fibronectin, and collagen. *Am J Physiol Heart Circ Physiol* 1992; 262: H1861-H1866.
34. Bo H, Jiang N, Ma G, *et al.* Regulation of mitochondrial uncoupling respiration during exercise in rat heart: role of reactive oxygen species (ROS) and uncoupling protein 2. *Free Radic Biol Med* 2008; 44: 1373-1381.
35. Calderone A, Murphy RJL, Lavoie J, Colombo F, Beliveau L: TGF- β 1 and prepro-ANP mRNA are differentially regulated in exercise-induced cardiac hypertrophy. *J Apply Physiol* 2001; 91: 771-776.

Received: April 25, 2009

Accepted: November 6, 2009

Author's address: Bozena Czarkowska-Paczek, MD, Department of Biophysics and Human Physiology, Medical University of Warsaw, Chalubinskiego Str. 5, 02-004 Warsaw, Poland; Phone + 48 22 628 63 34; Fax and Phone + 48 22 628 78 46; E-mail: dom_paczek@o2.pl