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Glycogen and Collagen Fibres in Myocardium of Endurance Trained Rats Following Nandrolone Decanoate Treatment

S. Delchev, K. Georgieva, Y. Koeva, P. Atanassova*

Department of Anatomy, Histology and Embryology, Medical University, Plovdiv
**Department of Physiology, Medical University, Plovdiv*

The pattern of substrate use by the heart (fatty acids, lactate, and glucose) and its utilization varies according to the duration and intensity of workload and hormonal status. Sex hormones play a substantial role in regulating glycogen metabolism during exercise. The myocardial collagen matrix surrounds myocytes and functions as a medium between them and the circulatory system. This study investigates single and combine effect of endurance training and anabolic androgenic steroids (AAS) treatment on glycogen and collagen content in rat myocardium. AAS administration resulted in increased glycogen in cardiomyocytes and increased perivascular collagen fibres in sedentary rats. The training effects were not associated with alterations in either the glycogen or collagen content. Combining both factors - training and AAS, was manifested as a reduction in the glycogen amount in the cardiomyocytes and lack of changes in the collagen fibres around the vessels, as compared to the single effect of AAS.

Key words: glycogen, collagen, myocardium, endurance training, Nandrolone Decanoate.

It is known that glycogen stores, located primarily in the liver and skeletal muscles provide a ready source of energy for use during exercise. The pattern of substrate use by the heart (fatty acids, lactate, and glucose) and its utilization varies according to the duration and intensity of workload and hormonal status. To date, it is well documented that sex hormones like estrogens and testosterone play a substantial role in regulating glycogen metabolism during exercise [9]. Many athletes use supraphysiological doses of anabolic androgenic steroids (AAS) aiming to increase the glycogen content in tissues and improvement of their athletic performance [2].

Also, an increase in collagen concentration is observed as an integral part of extracellular matrix remodeling in myocardium in response to a variety of pathologies [8]. Little is known about the influence of AAS and exercise on the glycogen content and interstitial collagen in the myocardium.

The aim of the present study was to investigate single and combine effect of endurance training and AAS treatment on glycogen content and collagen fibers in rat myocardium.

Material and Methods

Forty male Wistar rats (initial body weight 200-220g) were randomly distributed into two main groups (n=20) - sedentary (S) and trained (T). The exercised rats trained on motor-driven treadmill with submaximal loading (70-75% VO_2 max) 5 days per week for 8 weeks. During the first 2 weeks the duration of the daily training session increased every second day. At the end of 2nd week the rats run 40 min daily and this loading was maintained to the end of the experiment. After second week from the beginning of the experiment half of the trained and sedentary rats received weekly either 10 mg·kg^{-1} Nandrolone Decanoate (ND) or Placebo (PL) i.m. for the last 6 weeks.

All the groups: S+Pl; S+ND; T+Pl; T+ND were subjected on submaximal running endurance (SRE) and VO_{2max} tests several times. The day after the last test was performed the animals were decapitated after thiopental anesthesia and material from the left heart ventricle of each animal was taken immediately. Samples of the middle third of the outer left ventricle wall were obtained and fixed in Bouin's fixative for twenty-four hours at room temperature and than embedded in paraffin. Part of paraffin sections (5 мм thick) was investigated histochemically for glycogen using PAS method (McManus, 1948) and the other part - for collagen using Azan method (Heidenhain, 1915).

The staining saturation of the PAS reaction and distribution of collagen were assessed by specialized software "DP-Soft" (Olympus, Japan) on 'Microphot' (Nikon, Japan) microscope, equipped with a Camedia-5050Z (Olympus, Japan) digital camera. Measurements were applied on longitudinal sections at equal magnification (×400).

Data was evaluated for statistically significant differences by two-way ANOVA followed by Games-Howell post hoc test. Significance was accepted at $P<0.05$. The results are presented as mean ± SEM.

Results and Discussion

In the sedentary control group PAS staining showed small amounts of glycogen in the cardiomyocytes. There was significant main effect of ND treatment on the glycogen content in comparison with placebo (15.75±0.42 vs. 9.19±0.42, $P<0.001$). Endurance training had significant main effect on myocardial glycogen ($P<0.001$). Trained rats presented lower glycogen than sedentary (10.39±0.42 vs. 14.55±0.42). There was significant interaction between training and AAS treatment ($P<0.001$).

ND treatment significantly increased glycogen content in cardiomyocytes of the sedentary animals compared with placebo ($P<0.001$) (Fig. 1). In the trained groups ND expressed similar effect on glycogen increasing it's content in T+ND group compared to training per se ($P<0.01$).

There was no significant difference between the trained (T+Pl) and control group (S+Pl). Combination of both factors resulted in an intermediate glycogen content, which in T+ND group compared to the steroid control was significantly lower (12.38±0.90 vs. 19.12±0.48, $P<0.001$).

Cardiomyocytes in the myocardium of the control group (S+Pl) were separated by small amounts of collagen in the interstitium (Fig. 2, A). In the perivascular areas collagen was slightly presented. There was no significant difference in the percentage of collagen fibres in the endomysium between all the groups ($P>0.05$)

An increase in the amount of collagen fibres surrounding the blood vessels of greater caliber was observed in the myocardium of untrained, ND-injected animals

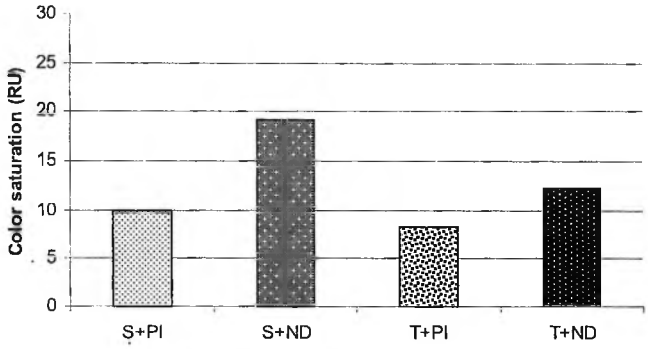

Fig. 1. Glycogen in the cardiomyocytes (in relative units)
*P<0.001, S+ND vs. S+Pl; **P<0.01, T+ND vs. T+Pl; #P<0.001, T+ND vs. S+ND

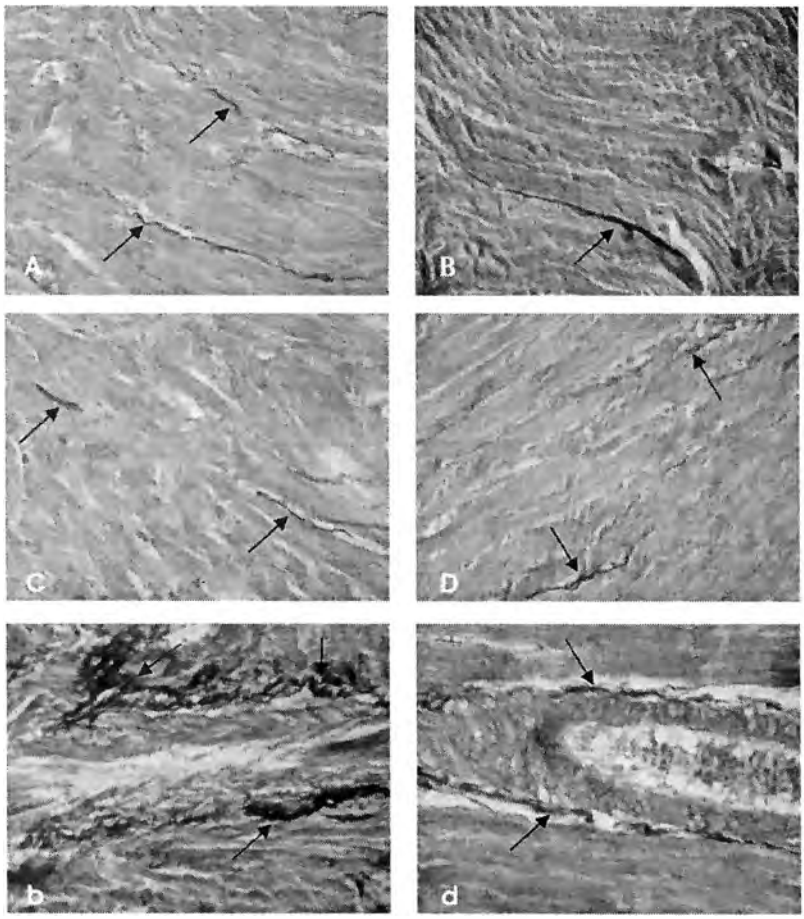

Fig. 2. Collagen fibres in the myocardium, Azan staining
A — sedentary+Placebo; **B, b** — sedentary+Nandrolone Decanoate; **C** — training+Placebo; **D, d** — training+Nandrolone Decanoate. Arrows indicate collagen fibres, × 400

(Fig. 2, b). In the myocardium of rats subjected to a submaximum training (T+Pl), no increase in the amount of collagen was observed - both around the vessels and in the intercellular spaces, as compared to the control (Fig. 2, d). Combining both factors did not alter the amount, neither of collagen in the interstitium, nor around the vessels, as compared to the control.

Endurance training did not lead to changes in the glycogen content in the cardiomyocytes. These results are consistent with the results obtained by other authors who also did not find any changes [2]. The content of connective tissue (mainly collagen) in the spaces between the cardiomyocytes also did not change. Similar results were reported by other research teams [6, 8, 10].

AAS treatment in untrained animals resulted in considerable glycogen increase in the cardiomyocytes. Being a derivative of testosterone (Ts), ND is capable of activating the processes of synthesis of enzymatic, structural, receptor and contractile proteins. Glycogenin, a protein that has been recently found in the muscles and the heart, is capable of initiating glycogen synthesis autocatalytically [1]. We hypothesize that ND influenced glycogenin activity in synthesizing a new glycogen. Another possible mechanism increasing glycogen content is the activation of lipoprotein lipase, as well as the predominant utilization of fats as a substrate [4]. On the other hand, glycogen accumulation could be interpreted as a pathologic tendency [5]. An increase of glycogen content in cardiomyocytes was found in left ventricle biopsies from patients, with coronary heart disease in the presence of areas with permanently reduced oxygen supply [3]. The increase in the collagen fibres in the perivascular areas is difficult to assess at this duration of AAS application. If this tendency is preserved with a more prolonged AAS treatment, this would definitely result in impairment of coronary blood flow and oxygen delivery.

On the other hand, endurance training led to decrease in Ts serum levels in the animals of the same experiment [7]. This fact could also account for the lack of changes in the glycogen and collagen content in the trained group.

Combining both factors — training and AAS — was manifested as a reduction in the glycogen amount in the cardiomyocytes, as compared to the single effect of AAS application. The levels remained higher, as compared to the independent effect of training. Similar results were obtained by Cunha et al. [2], but they found no glycogen increase in the myocardium of the steroid control. The lack of changes in the collagen content around the vessels in the group of trained animals that received an anabolic steroid (T+ND), is a sign of the beneficial influence of training, as compared to the single effect of AAS treatment.

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