

# Myocardial Disorders in BDNF-Deficient Rats: Limited Recovery Post-Moderate Endurance Training

Norbert Grzelak<sup>1</sup>, Dominik Kaczmarek<sup>2</sup>, Krystian Marek Poziemba<sup>2</sup>, Włodzimierz Mrówczyński<sup>1</sup> 

<sup>1</sup>Department of Neurobiology, Poznań University of Physical Education, Poznań, Poland; <sup>2</sup>Department of Physiology and Biochemistry, Poznań University of Physical Education, Poznań, Poland

Correspondence: Włodzimierz Mrówczyński, Department of Neurobiology, Poznań University of Physical Education, Poznań, Poland, Email [mrowczynski@awf.poznan.pl](mailto:mrowczynski@awf.poznan.pl)

**Introduction:** The study aimed to determine whether heterozygous BDNF-deficient (BDNF-knockout, SD-BDNF) rats exhibit pathological changes in the myocardium and to assess whether a 5-week moderate-intensity endurance training program can reverse adverse changes in the heart muscle.

**Methods:** Experiments were conducted on four groups of rats: control wild-type, control BDNF knockout, trained wild-type and trained BDNF knockout. Knockout rats were selected due to the presence of symptoms resembling metabolic syndrome in serum and liver while 5-week moderate endurance training was used as an intervention targeted at restoring heart function. Measurements of BDNF/Trk-B concentrations and molecules levels and activities, such as cardiac specific enzymes like creatine kinase and creatine kinase myocardial band, lipids as total cholesterol, low-density lipoprotein and triglycerides, metabolic enzymes including alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and lactate dehydrogenase and interleukin-1 were carried out in myocardium homogenates.

**Results:** In BDNF-deficient rats, the myocardium showed significantly reduced lipid concentrations, decreased metabolic and cardiac enzyme activity, and elevated Trk-B levels, all of which are indicative of myocardial ischemia or hypoxia. These changes in critical biomarkers were consistent with those earlier observed in the livers of BDNF-deficient rats, suggesting a link between the liver and cardiac function. Moderate endurance training led to an increase in creatine kinase activity in the myocardium of trained rats, suggesting increased production and utilization of energy required for myocardial contraction in trained wild-type and knockout populations of rats.

**Discussion:** BDNF-deficient rats exhibited numerous myocardial abnormalities, most of which were not reversible after moderate-intensity endurance training. These findings provide a basis for a deeper understanding of the mechanisms underlying myocardial disorders in BDNF-deficient rats, which appear to be a suitable model for studying various aspects of metabolic disorders.

**Keywords:** BDNF, Trk-B, gene-deficiency rats, heart, endurance training

## Introduction

It has been shown that brain-derived neurotrophic factor (BDNF) is a pleiotropic protein belonging to the neurotrophic factor family.<sup>1</sup> It was first discovered in the mammalian brain,<sup>2</sup> purified and described as a key factor involved in neuronal growth, development and survival.<sup>3</sup> This neurotrophic factor plays an important role in development and synaptic plasticity,<sup>4</sup> particularly in relation to neurons located in brain areas such as the hippocampus, visual cortex, substantia nigra and striatum.<sup>5</sup> Furthermore, extensive studies have shown that the action of BDNF is mediated by its specific binding to the tropomyosin-related kinase B receptor (Trk-B), which initiates a cascade of downstream signalling pathways, finally causing physiological changes in nervous tissue.<sup>6</sup>

Recent studies have shown that BDNF and its the tropomyosin-related kinase B receptor (Trk-B) are also expressed in various types of cardiac cells such as cardiomyocytes<sup>7-9</sup> and endothelial cells lining the coronary arteries.<sup>10</sup> Studies

indicate that the BDNF/Trk-B axis is crucial for maintaining normal cardiovascular function<sup>11</sup> by promoting cardiomyocytes survival,<sup>12</sup> improving cardiac function related to cardiac contraction and relaxation<sup>8</sup> and regulating the development of large blood vessels in heart tissue.<sup>10</sup> On the other hand, dysfunction of BDNF/Trk-B axis is associated with cardiovascular disease and related disorders,<sup>13</sup> while low levels of circulating BDNF have been recognized as an indicator for diagnosing adverse myocardial changes.<sup>14</sup> For example, BDNF levels in peripheral blood (serum or plasma) were significantly lower in patients with ischaemic heart disease or acute coronary syndrome,<sup>15</sup> with unstable angina,<sup>16</sup> at risk of stroke and vascular brain injury,<sup>17</sup> and with chronic heart failure<sup>18</sup> compared to healthy controls. Furthermore, Kaess et al<sup>19</sup> showed in a large human sample that low blood BDNF levels were associated with higher mortality and increased incidence of cardiovascular events. In turn, studies in Trk-B knockout mice have shown that the absence of the Trk-B receptor leads to impaired cardiac contraction and diastole.<sup>8</sup>

On the other hand, endurance running is considered as health-promoting therapy against numerous cardiovascular and heart diseases, as confirmed studies highlighting its beneficial effects.<sup>20</sup> This type of training increases the aerobic capacity of the exercised heart,<sup>21</sup> leading to a significant reduction in the risk of cardiovascular mortality.<sup>22</sup> Moreover, some research indicated a link between exercise training and increased BDNF expression in skeletal muscle, heart, and brain.<sup>23–26</sup> Nakano et al<sup>27</sup> suggested that BDNF production is dependent on muscle function and activity resulting from adequate skeletal muscle training in patients with heart failure. Lee et al<sup>28</sup> showed that 4-week treadmill running training significantly increased BDNF protein expression in the left ventricle of rats after myocardial infarction. Moreover, 12 weeks of voluntary wheel running in young and aged rats resulted in higher levels of BDNF in heart when compared to the sedentary counterparts.<sup>29</sup>

The first purpose of the study was to determine pathological changes in the myocardium of heterozygous knockout rats (BDNF-knockout, SD-BDNF), while the second was to evaluate the effect of a 5-week moderate-intensity endurance training program as a potential intervention to counteract adverse changes occurring in the myocardium of BDNF-deficient rats. Hence, the levels of BDNF, Trk-B and key metabolic biomarkers were measured in myocardial homogenate and compared between groups of rats with different BDNF genotypes (typical/knockout) and lifestyles (untrained/trained). Our hypothesis assumed that animals with different genotype and lifestyle would show various BDNF/Trk-B content and variations in levels of common myocardial biomarkers.

## Materials and Methods

### Animals

Heart biomarkers were measured in 41 male Sprague-Dawley rats aged 4–5 months, which were obtained from a breeding colony at SAGE Labs (St. Louis, MO, USA). 21 animals had the normal *BDNF* genotype (*BDNF*-wild type), while the remaining 20 rats had the heterozygous *BDNF* genotype (*BDNF*-knockout, SD-BDNF).<sup>30</sup>

Rats were randomly divided into the following four groups: control rats with the normal genotype (*BDNF*<sup>+/+</sup>, n=11), control rats with the knockout genotype (*BDNF*<sup>+/-</sup>, n=11), trained rats with the normal genotype (*BDNF*<sup>+/+</sup>T, n=10) and trained rats with the knockout genotype (*BDNF*<sup>+/-</sup>T, n=9). Prior to the experiments, the animals were kept in standard laboratory cages, with two rats of the same genotype housed together and provided with ad libitum access to food and water. The rats were housed in a room with controlled conditions, including an inverted 12-hour light/dark cycle, humidity of 55 ± 1% and temperature of 22 ± 2°C. To collect hearts, all animals were euthanized by an overdose of sodium pentobarbital (180 mg kg<sup>-1</sup>).

### Ethical Approval

The experiments conducted on animals complied with the regulations of the Polish Animal Protection Act, the European Union guidelines and the ARRIVE guidelines. Experimental protocols were reviewed and approved by the Local Ethical Committee in Poznań, with the number 58/2018 assigned.

## Endurance Training Protocol

A moderate-intensity endurance training was implemented to modulate the concentrations and activity of the tested biomarkers in heart tissue. Training was conducted using an electric treadmill designed for small rodents (Exer-6M, Columbus Instruments). The training programme consisted of a preliminary week followed by 5 weeks of the main training. Each training session lasted 45 minutes and the rats trained 5 days per week. During the training period, treadmill speed was gradually increased, reaching 25 m/min at the end of the total training time.<sup>31</sup>

## Tissue Collection

Following an overdose of sodium pentobarbital as an anaesthetic, cardiac muscle was collected. The tissue collected from the heart muscle was then weighed and transferred into cryogenic vials (NUNC/Thermo Fisher Scientific, USA). To preserve the tissue, the vials were rapidly frozen using liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  until further measurement.

## Homogenization

The rats were killed by an overdose of sodium pentobarbital, a thoracotomy was performed to expose the myocardium, and the heart muscle was collected. To obtain pure heart muscle except for structures from the atria and ventricles, the most external parts of the heart were excised. The removed tissue sections were weighed and an appropriate amount of Phosphate-buffered saline (PBS) with the addition of Halt Protease Inhibitor Cocktail EDTA-free (100X) (Thermo Fischer Scientific, USA) was added in proportion to the tissue weight. The tissue sections were then homogenized using a dispersion homogenizer (VWR VDI 12, Singapore) in four cycles. Each cycle consisted of homogenization for 30 seconds followed by a one-minute cooling period on ice. The homogenization speed was set at 30.000 revolutions per minute (rpm) to ensure effective fragmentation of the tissue. After homogenization, the homogenate was centrifuged at 5.000 rpm for five minutes. This centrifugation step separated the cellular debris and larger particles from the supernatant. The resulting supernatant, containing the desired myocardial components, was carefully collected and stored at  $-80^{\circ}\text{C}$  for further analysis and measurement.

## Enzyme-Linked Immunosorbent Assay

To assess the concentrations of BDNF (sensitivity test: 0.035 ng-mL<sup>-1</sup>, cat. no.: SRB-T-81493), Trk-B (sensitivity test: 12.337 pg/mL; cat. no.: 201-11-0426) and IL-1 (sensitivity test: 10.135 ng/L; cat. no.: 201-11-0108), immunoenzymatic assays (ELISAs) were performed according to the manufacturer's instructions provided by Sunredbio, China. The absorbance of the resulting solution was measured at 450 nm using a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA).

## Biochemistry Analyses

Measurements of LDL concentrations (test sensitivity: 3.9 mg/dl; cat. no.: 7-280 [Cormay, Poland]), CHOL (test sensitivity: 1.95 mg/dl; cat. no.: 7-204 [Cormay, Poland]), TG (test sensitivity: 1.4 mg/dl; cat. no.: 7-253 [Cormay, Poland]), ALAT (sensitivity test: 8 U/L; cat. no.: 7-216 [Cormay, Poland]), ASAT (sensitivity test: 7 U/L; cat. no.: 7-214 [Cormay, Poland]), LDH (sensitivity test: 6.6 U/L; cat. no.: 7-239 [Cormay, Poland]), GGT (sensitivity test: 8 U/L; cat. no.: 7-224 [Cormay, Poland]), CK (sensitivity test: 7.4 U/L; cat. no.: 7-220 [Cormay, Poland]) and CK-MB (sensitivity test: 4 U/L; cat. no.: [Cormay, Poland]) were performed using a clinical chemistry analyzer (Accent 220S, Cormay, Poland).

## Statistical Analysis

Presented data were expressed as mean  $\pm$  standard deviation (SD) for all studied factors. To compare all the studied groups, a two-way analysis of variance (ANOVA) was performed. If the interaction between the independent variables was found to be statistically significant, Tukey's post-hoc test was carried out. All statistical analyses were conducted using Statistica 13 software (StatSoft, Poland, Cracow).

## Results

### BDNF and Trk-B

There was no significant difference in cardiac BDNF levels between populations of rats with the normal genotype (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) and rats with the knockout genotype (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) (Figure 1A and Table 1). Despite this observation, heart Trk-B levels were significantly higher in the knockout rat population (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) compared to the typical genotype rat population (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) (Figure 1B and Table 1). On the other hand, BDNF and Trk-B levels in the heart of the trained population (*BDNF*<sup>+/+T</sup> and *BDNF*<sup>+/-T</sup>) were not significantly different compared to the control population of rats (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/-</sup>) (Figure 1A, B and Table 1).

### IL-1

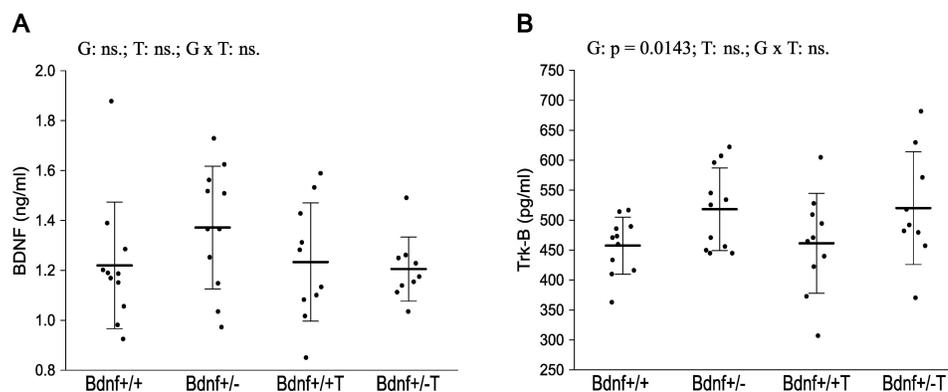
There were no changes in IL-1 levels when comparing the typical (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) and knockout (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) (Figure 2 and Table 1) as well as the sedentary (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/-</sup>) and trained (*BDNF*<sup>+/+T</sup> and *BDNF*<sup>+/-T</sup>) populations of rats (Figure 2 and Table 1).

### CK and CK-MB

Cardiac CK and CK-MB activity in population of knockout genotype rats (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) was significantly lower compared to population of rats with the typical genotype (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) (Figure 3A, B and Table 1). On the other hand, there was a statistically significant increase in CK activity in the trained population (*BDNF*<sup>+/+T</sup> and *BDNF*<sup>+/-T</sup>) compared to the control population of rats (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/-</sup>) (Figure 3A and Table 1), while no differences in CK-MB activity were found between the aforementioned rat populations (Figure 3B and Table 1).

### Lipids

CHOL and LDL concentrations were significantly lower in the population of rats with the knockout genotype (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) compared to the population of rats with the normal genotype (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) (Figure 4A, B and Table 1). Furthermore, interaction analysis for LDL showed significantly lower concentrations in the control group of rats with the knockout genotype (*BDNF*<sup>+/-</sup>), compared to the control and trained groups of rats with the normal genotype (*BDNF*<sup>+/+</sup>, *BDNF*<sup>+/+T</sup>) (Figure 4B and Table 1). On the other hand, there were no statistically significant differences in TGs levels between rat populations with different BDNF genotypes (Figure 4C and Table 1). Moreover, comparison of cardiac CHOL, LDL and TG levels between control (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/-</sup>) and trained (*BDNF*<sup>+/+T</sup> and *BDNF*<sup>+/-T</sup>) rat populations showed no significant changes (Figure 4A–C and Table 1), as well.



**Figure 1** The values of BDNF (A) and Trk-B (B) concentrations in the heart of studied rats along with their mean values (bold line) and standard deviation ( $\pm$ SD) (upper and lower whiskers). *BDNF*<sup>+/+</sup> - control group with normal genotype; *BDNF*<sup>+/-</sup> - control group with BDNF knockout genotype; *BDNF*<sup>+/+T</sup> - trained group with normal genotype, *BDNF*<sup>+/-T</sup> - trained group with BDNF knockout genotype. Comparisons between groups were made by two-way ANOVA. G - genotype (*BDNF*<sup>+/+</sup> genotype vs *BDNF*<sup>+/-</sup> genotype); T - training (control vs training); G x T, the interaction between genotype and training. Non-significant results are denoted by ns. Detailed results of performed tests are given in Table 1.

**Table 1** Interactions Between Studied Biomarkers

Studied Biomarkers		Genotype	Training	Genotype/Training
<b>BDNF/Trk-B</b>				
BDNF	F <sub>1,37</sub>	0.76	1.17	1.61
	p	0.3878	0.2873	0.2124
	$\eta p^2$	0.02	0.03	0.04
Trk-B	F <sub>1,32</sub>	6.6	0.02	0
	p	0.0143	0.8994	0.9675
	$\eta p^2$	0.15	0	0
<b>Interleukin</b>				
IL-1	F <sub>1,34</sub>	0.42	0.54	1.34
	p	0.52	0.4649	0.2544
	$\eta p^2$	0.01	0.01	0.03
<b>Creatine Kinase</b>				
CK	F <sub>1,37</sub>	16.69	5.76	0.32
	p	0.0002	0.0215	0.5773
	$\eta p^2$	0.31	0.13	0.01
CK-MB	F <sub>1,33</sub>	8.13	0.24	0.98
	p	0.0071	0.6296	0.3296
	$\eta p^2$	0.18	0.01	0.03
<b>Lipids</b>				
CHOL	F <sub>1,33</sub>	27.61	0.56	2.2
	p	<0.0001	0.4604	0.1467
	$\eta p^2$	0.43	0.01	0.06
LDL	F <sub>1,35</sub>	13.47	0.08	4.23
	p	0.0008	0.7838	0.0468
	$\eta p^2$	0.27	0	0.10
TG	F <sub>1,35</sub>	3.09	0.28	1.41
	p	0.0873	0.6001	0.2418
	$\eta p^2$	0.08	0.01	0.04
<b>Enzymes</b>				
LDH	F <sub>1,37</sub>	0.69	1.48	0
	p	0.4107	0.2312	0.9964
	$\eta p^2$	0.02	0.04	0

(Continued)

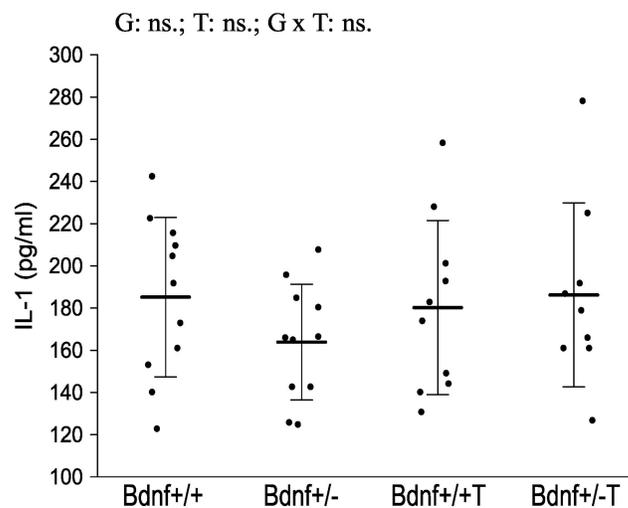
**Table 1** (Continued).

Studied Biomarkers		Genotype	Training	Genotype/Training
ALAT	F <sub>1,36</sub>	34.74	0.38	0.42
	p	<0.0001	0.5396	0.5218
	$\eta p^2$	0.48	0.01	0.01
ASAT	F <sub>1,36</sub>	22.93	1.04	0.24
	p	<0.0001	0.3149	0.6291
	$\eta p^2$	0.38	0.03	0.01
GGT	F <sub>1,35</sub>	53.2	0.71	1.22
	p	<0.0001	0.4104	0.2770
	$\eta p^2$	0.59	0.02	0.03

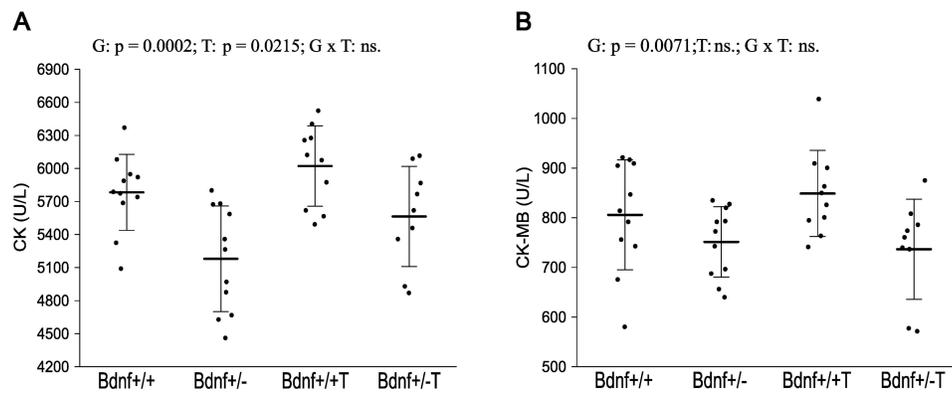
**Notes:** Results of significance (p), effect size ( $\eta p^2$ ) and test power (F) for particular effects and interactions regarding studied biomarkers such as: BDNF and Trk-B (Figure 1A and B), IL-1 (Figure 2), CK and CK-MB (Figure 3A and B), lipids – CHOL, LDL and TG (Figure 4A–C), enzymes – ALAT, ASAT, GGT, LDH (Figure 5A–D). BDNF/Trk-B, Interleukin, Creatine Kinase, Lipids, Enzymes: Groups of tested biomarkers. Two-way analysis of variance (ANOVA) was performed. Genotype (*BDNF*<sup>+/+</sup> genotype vs *BDNF*<sup>+/-</sup> genotype); Training (control group vs trained group); Genotype/Training (the interaction between genotype and training).

## Enzymes

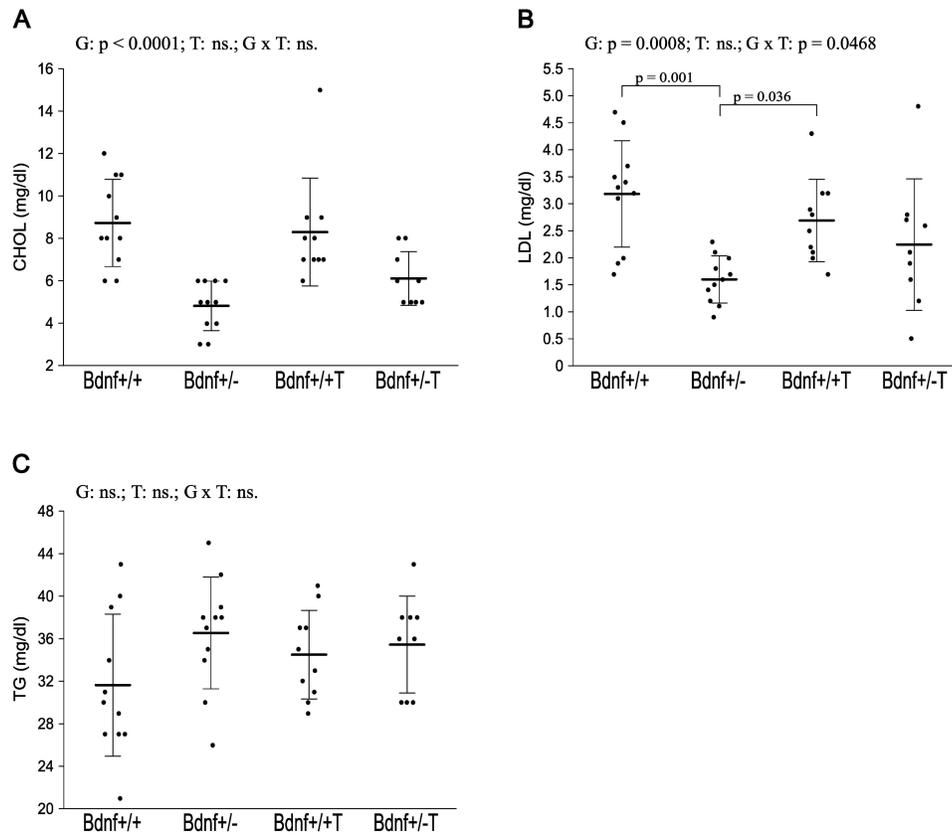
The activities of most of the enzymes tested (ALAT, ASAT and GGT) were significantly lower in the population of rats with the knockout genotype (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-</sup>T) compared to the control population of rats with the normal genotype (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+</sup>T) (Figure 5A–C, Table 1), with the exception of LDH, whose activity was comparable in both populations (Figure 5D and Table 1). On the other hand, there were no significant differences in ALAT, ASAT, GGT and LDH activities when comparing the control population (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/-</sup>) with the trained ones (*BDNF*<sup>+/+</sup>T and *BDNF*<sup>+/-</sup>T) (Figure 5A–D and Table 1).



**Figure 2** The values of IL-1 concentrations in the heart of studied rats along with their mean values (bold line) and standard deviation ( $\pm$ SD) (upper and lower whiskers). *BDNF*<sup>+/+</sup> - control group with normal genotype; *BDNF*<sup>+/-</sup> - control group with BDNF knockout genotype; *BDNF*<sup>+/+</sup>T - trained group with normal genotype, *BDNF*<sup>+/-</sup>T - trained group with BDNF knockout genotype. Comparisons between groups were made by two-way ANOVA. G - genotype (*BDNF*<sup>+/+</sup> genotype vs *BDNF*<sup>+/-</sup> genotype); T - training (control vs training); G x T, the interaction between genotype and training. Non-significant results are denoted by ns. Detailed results of performed tests are given in Table 1.



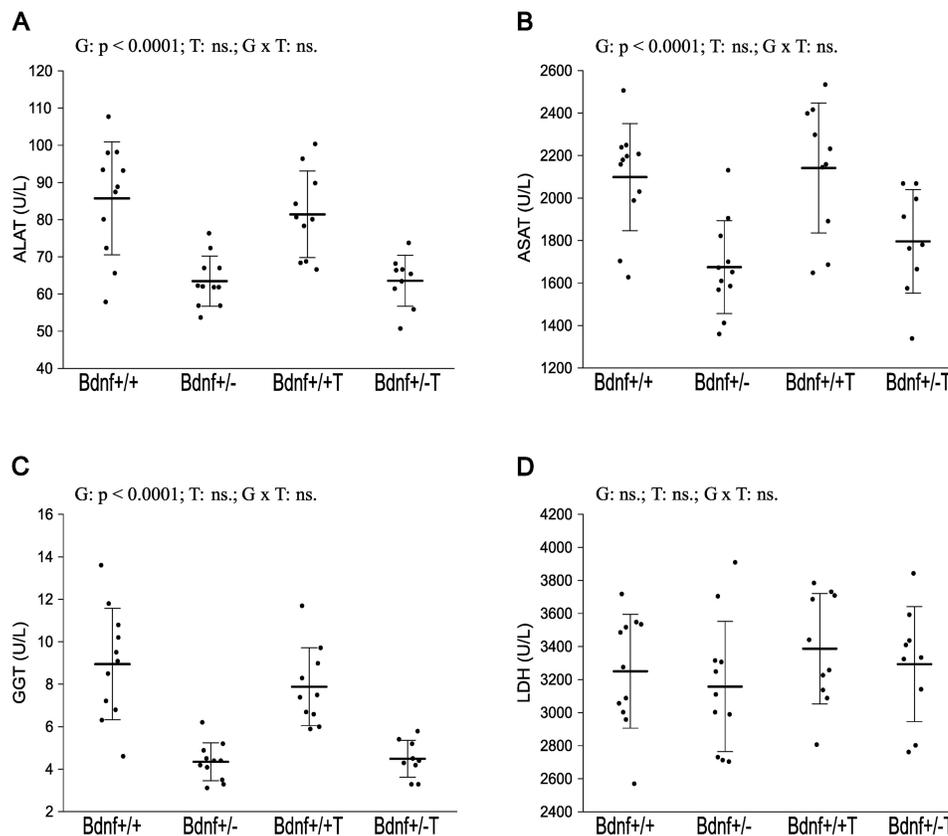
**Figure 3** The values of CK (A) and CK-MB (B) activities in the heart of studied rats along with their mean values (bold line) and standard deviation ( $\pm$ SD) (upper and lower whiskers). BDNF<sup>+/+</sup> - control group with normal genotype; BDNF<sup>+/-</sup> - control group with BDNF knockout genotype; BDNF<sup>+/+T</sup> - trained group with normal genotype, BDNF<sup>+/-T</sup> - trained group with BDNF knockout genotype. Comparisons between groups were made by two-way ANOVA. G - genotype (BDNF<sup>+/+</sup> genotype vs BDNF<sup>+/-</sup> genotype); T - training (control vs training); G x T, the interaction between genotype and training. Significant results are denoted by p, while non-significant results are denoted by ns. Detailed results of performed tests are given in Table 1.



**Figure 4** The values of CHOL (A), LDL (B) and TG (C) concentrations in the heart of studied rats along with their mean values (bold line) and standard deviation ( $\pm$ SD) (upper and lower whiskers). BDNF<sup>+/+</sup> - control group with normal genotype; BDNF<sup>+/-</sup> - control group with BDNF knockout genotype; BDNF<sup>+/+T</sup> - trained group with normal genotype, BDNF<sup>+/-T</sup> - trained group with BDNF knockout genotype. Comparisons between groups were made by two-way ANOVA. G - genotype (BDNF<sup>+/+</sup> genotype vs BDNF<sup>+/-</sup> genotype); T - training (control vs training); G x T, the interaction between genotype and training. Significant results are denoted by p, while non-significant results are denoted by ns. Tukey's post-hoc test was conducted to assess the statistical significance of the interaction between genotype and training. Difference of LDL level between BDNF<sup>+/+</sup> group and BDNF<sup>+/-</sup> group ( $p=0.001$ ) and between BDNF<sup>+/-</sup> group and BDNF<sup>+/+T</sup> group ( $p=0.036$ ). Detailed results of performed tests are given in Table 1.

## Discussion

Our study showed that BDNF-deficient rats (BDNF<sup>+/-</sup> and BDNF<sup>+/-T</sup>) exhibit lower myocardial concentrations of CK and CK-MB compared to control rats (BDNF<sup>+/+</sup> and BDNF<sup>+/+T</sup>). This reduction suggests cardiac dysfunction, as these



**Figure 5** The values of ALAT (**A**), ASAT (**B**), GGT (**C**) and LDH (**D**) activities in the heart of studied rats along with their mean values (bold line) and standard deviation ( $\pm$ SD) (upper and lower whiskers). BDNF+/+ - control group with normal genotype; BDNF+/- - control group with BDNF knockout genotype; BDNF+/+T - trained group with normal genotype, BDNF+/-T - trained group with BDNF knockout genotype. Comparisons between groups were made by two-way ANOVA. G - genotype (BDNF+/+ genotype vs BDNF+/- genotype); T - training (control vs training); G x T, the interaction between genotype and training. Significant results are denoted by p, while non-significant results are denoted by ns. Detailed results of performed tests are given in Table 1.

enzymes tend to decrease in failing hearts<sup>32,33</sup> and may leak into the bloodstream due to myocardial ischaemia, infarction or necrosis.<sup>34-36</sup> In addition, BDNF+/- rats showed significantly lower levels of CHOL and LDL in the heart, suggesting increased lipoprotein permeability and transport into the bloodstream. This process contributes to the progression of atherosclerosis and plaque rupture, leading to tissue hypoxia.<sup>37-40</sup> Significantly lower metabolic enzyme activities (ALAT, ASAT and GGT) were also observed in the myocardium of these rats.<sup>35,41</sup> Sammeturi et al<sup>35</sup> reported results consistent with ours, showing decreased ALAT, ASAT and GGT activity in the heart, along with their increased serum levels in rats with isoproterenol-induced myocardial infarction. Abdulkareem et al<sup>41</sup> suggested that organ damage leads to the release of enzymes from tissues into the bloodstream, resulting in their decreased activity in tissues and increased activity in peripheral blood. Hence, the decrease in cardiac enzymes that we demonstrated may contribute to the increased levels of these enzymes in the peripheral blood. Elevated ALAT and ASAT blood levels may indicate liver or heart damage,<sup>42,43</sup> while elevated serum GGT levels are associated with coronary artery disease.<sup>44</sup> Elevated blood levels of these enzymes are always associated with cardiac damage and cardiovascular disease.<sup>42-46</sup> It should be noted that the reductions in lipoprotein concentrations (LDL, CHOL) and enzyme activities (ALAT, ASAT, GGT) in the hearts of the studied rats were almost identical to those observed in their livers.<sup>47</sup> These similarities suggest a strong interaction between heart and liver function in BDNF knockout rats. Meller and Bernardi<sup>48</sup> reported that such a heart-hepatic axis has been identified in clinical practice in patients with coexisting heart and liver disease.

Additionally, a significant increase in Trk-B levels was observed in the myocardium of BDNF+/- and BDNF+/-T rats. This finding is also consistent with previous studies reporting elevated Trk-B levels in failing myocytes from BDNF knockout mice<sup>8</sup> and in myocardium under experimentally induced cardiac hypoxia.<sup>49</sup> Therefore, all the above observations suggest that BDNF+/- rats exhibit numerous disorders in the myocardium.

Results presented have demonstrated that myocardial BDNF levels in the knockout rats (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) were not statistically different from those with normal genotype (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>). In our previous study we found a significant decrease in BDNF levels in both the blood serum<sup>31</sup> and liver<sup>47</sup> of these rats. However, we did not observe any changes in BDNF levels in fast-twitch muscles (tibialis, medial gastrocnemius) or slow-twitch muscles (soleus)<sup>31</sup> in BDNF-deficient rats population. Therefore, such results can suggest that the knockout procedure does not alter BDNF concentrations in the hind limb muscles and heart of BDNF knockout rats.

Therefore, according to obtained results both wild-type (*BDNF*<sup>+/+</sup> and *BDNF*<sup>+/+T</sup>) and knockout rats (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) exhibit the same BDNF levels in the myocardium. This may additionally indicate that cardiac BDNF is not directly involved in the restoration of myocardial dysfunction induced by BDNF gene knockout, as previously suggested by Okada et al<sup>12</sup> in experiments on various animal and cellular models with BDNF and Trk-B knockouts. On the other hand, the aforementioned authors showed that Trk-B present in the myocardium can be effectively activated by BDNF from the bloodstream, resulting in improved cardiac function after myocardial infarction. Therefore, it cannot be ruled out that reduced BDNF levels in peripheral blood<sup>31</sup> may be factors responsible for the increase in myocardial Trk-B levels in BDNF knockout rats, in addition to the previously mentioned CHOL leakage from cardiac tissues.

Reduction in CHOL levels may alter the thickness of myocardial fiber membranes and affect Trk-B receptor orientation due to reduced signaling efficiency.<sup>50</sup> This disruption in membrane integrity could impair Trk-B receptor function, triggering a compensatory increase in Trk-B expression, which is necessary to maintain adequate signaling pathways in the myocardium. In addition, the increase in Trk-B levels in the heart may also be a compensatory factor for the reduced amount of BDNF in the blood,<sup>31</sup> which, according to Harris et al,<sup>51</sup> and Garner et al,<sup>52</sup> is due to reduced BDNF levels in the hippocampus, amygdala and frontal cortex in knockout rats. Therefore, it seems likely that the increase in cardiac Trk-B in BDNF-deficient rats (*BDNF*<sup>+/-</sup> and *BDNF*<sup>+/-T</sup>) may complement the BDNF deficiency required to stabilize the BDNF/Trk-B axis. This is confirmed by unaltered levels of IL-1, a key pro-inflammatory cytokine indicating systemic and local inflammation, including inflammation in the heart,<sup>53–55</sup> demonstrated in *BDNF*<sup>+/+T</sup> and *BDNF*<sup>+/-T</sup> rats. Hence, the lack of inflammation in the myocardium of knockout animals can be attributed to increased levels of Trk-B, which regulates the stability of the BDNF/Trk-B axis, responsible for attenuating myocardial ischaemic injury and inhibiting cardiomyocyte apoptosis.<sup>56</sup>

For our study, 5-week moderate-intensity endurance training on a treadmill was chosen because prolonged and regular physical activity has often been recommended as a beneficial strategy for heart health,<sup>57</sup> which optimizes cardiac function<sup>58</sup> and prevents cardiovascular disease.<sup>59</sup> Our previous studies have shown that a 5-week training regime increased levels of BDNF, GDNF and selected myokines (myoglobin and IL-15) in hind limb muscles, altered excitability of fast-type spinal motoneurons, elevated ALAT, ASAT and IL-6 levels, as well as insulin and leptin in the liver of knockout rats.<sup>31,47</sup> Additionally, according to Wang et al,<sup>60</sup> endurance training on a treadmill increases BDNF protein levels in the circulation and heart and improves vascularization and cardiac function in rats.

The only noticeable altering observed after the applied training in this experiment was an increase in cardiac CK activity in both populations of rats with different BDNF genotypes (*BDNF*<sup>+/+ T</sup> and *BDNF*<sup>+/-T</sup>, respectively). According to Zervou et al,<sup>61</sup> such an increase in CK levels may suggest increased production and energy consumption in the myocardium, which improves cardiac function and contributes to more efficient cardiac contractions. For this reason, the creatine/phosphocreatine metabolic pathway catalyzed by CK is emerging as a promising therapeutic direction for the prevention and treatment of ischaemic cardiovascular disease.<sup>62</sup> Hence, a training-induced increase in CK is undoubtedly an important factor in improving myocardial contractile properties. On the other hand, such a result suggests that moderate endurance activity does not substantially affect the tested levels of BDNF, Trk-B and related biomarkers in the myocardium. However, such a result is consistent with the observations of Maroofi et al,<sup>26</sup> who found that moderate-intensity endurance training did not increase BDNF and TrkB-T1 expression in the rat myocardium, nor did it alter systolic cardiac function, in contrast to high-intensity interval training.

In conclusion, our study demonstrates the reduced levels and activities of cardiac biomarkers (CK and CK-MB), metabolic enzymes (ALAT, ASAT, GGT), and lipoproteins (CHOL and LDL), along with elevated Trk-B levels in the myocardium of BDNF-knockout rats. Therefore, the altered levels of the aforementioned biomarkers clearly indicate serious disorder in myocardium of these rats. Furthermore, our results confirmed that the same lipoproteins and metabolic

enzymes are reduced in the heart of BDNF-deficient animals as in their liver<sup>47</sup> suggesting functional interaction between these organs. Therefore, we cannot exclude that the changes observed in heterozygous BDNF-deficient rats suggest the onset of metabolic syndrome symptoms in these animals. According to Todosenko et al,<sup>63</sup> a significant number of genes involved in the development of metabolic syndrome are located near regions of BDNF genes, while a broad spectrum of signaling pathways controlling the development of metabolic syndrome are regulated by BDNF expression. Thus, BDNF knockout rats may have impaired homeostasis of metabolic processes that contribute to metabolic syndrome. Such an association between BDNF content and appetite and obesity (as manifestations of the metabolic syndrome) has already been demonstrated by Kernie et al<sup>64</sup> in mice with BDNF gene ablation. Hence, it seems reasonable to recommend BDNF deficient rats for in-depth studies on various aspects of the metabolic disorder.

Surprisingly, used endurance training evoked a significant increase in myocardial CK content in both populations of trained rats, but it had no effect on the Trk-B, BDNF and related lipids and enzymes in cardiomyocytes. Therefore, moderate-intensity endurance training, which increases CK activity in rat hearts, appears to be responsible for improving cardiac contraction performance in rats regardless of their genotype.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author WM, on reasonable request.

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## Disclosure

The authors declare no conflict of interest.

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