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Effect of Varying Exercise Intensities on GDNF Expression and Neuromuscular Junction Morphology

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ABSTRACT

Glial cell-line derived neurotrophic factor (GDNF) supports and maintains the neuromuscular system during development and through adulthood by promoting neuroplasticity. The aim of this study was to determine if different intensities of exercise can promote changes in GDNF expression and neuromuscular junction (NMJ) morphology in slow- and fast-twitch muscles. Rats were randomly assigned to a low intensity run-training (run group), higher intensity swim-training (swim group), or control group. GDNF protein content increased in both soleus (SOL) and extensor digitorum longus (EDL) following run-training and in EDL following swim-training determined by enzyme-linked immunosorbent assay. NMJ morphology was analyzed by measuring α-bungarotoxin labeled post-synaptic end plates. GDNF and total end plate area were positively correlated. End plate area decreased in EDL of run group and increased in SOL of swim group. Run group was able to alter plasticity by significantly increasing the number of end plates in EDL. The higher intensity swim-training elicited more dispersed synapses in SOL and less dispersion in EDL. The results indicate that GDNF expression may be altered depending on exercise intensity and fiber phenotype potentially inducing NMJ plasticity.

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INTRODUCTION

GDNF has been shown to

- be the most potent trophic factor to rescue motoneurons
- play a significant role in postnatal remodeling and maintenance of mature neuromuscular junction (NMJ) structures
- cause hyperinnervation and multiple end plate formation at mature NMJ structures
- be expressed at higher levels in slow-twitch muscle following low-intensity exercise

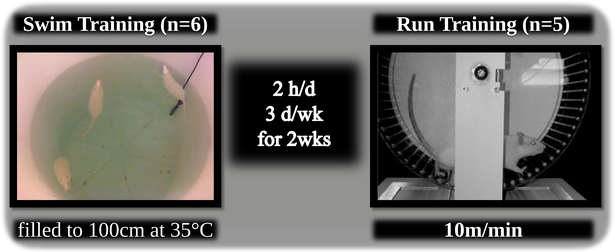
PURPOSE

The purpose of the study is to determine if different intensities of exercise can promote changes in GDNF expression and NMJ morphology in slow- and fast-twitch muscles.

METHODOLOGY

Subjects

- 6 month-old Sprague Dawley rats
  - Randomly assigned to control, swim-training and run-training groups



Skeletal Muscle

- Soleus (SOL; slow twitch)
- Extensor Digitorum Longus (EDL; fast twitch)

Visualization of GDNF & NMJ

- Longitudinal sections cut on cryostat (60µm)
- Alpha-bungarotoxin & antibodies raised against GDNF
- NMJ morphology
  - Measured 50 random end plates from both EDL and SOL (3 animals/group) using confocal microscope
  - Measured total area, stained area, total perimeter, stained perimeter using ImageJ software
- End plate number
  - Counted all end plates in a 10X field of vision at 5 random locations for both EDL & SOL (3 animals/group) using confocal microscope

Visualization of Skeletal Muscle Fibers

- Average Cross Sectional Area (CSA)
  - Transverse sections cut on cryostat (20µm)
  - Antibodies raised against MHC (I, IIa, IIx, IIb)
  - CSA measured for 125-150 random EDL and SOL fibers (3 animals/group) using widefield microscopy

Quantification of GDNF protein content

- ELISA

Statistics

- A one-way ANOVA and PostHoc tests used for statistical significance among different groups ($P<0.05$).
- Linear regression analysis used to evaluate association between variables ($P<0.01$).

RESULTS

Training Alters Muscle Fiber CSA in Recruited Muscles

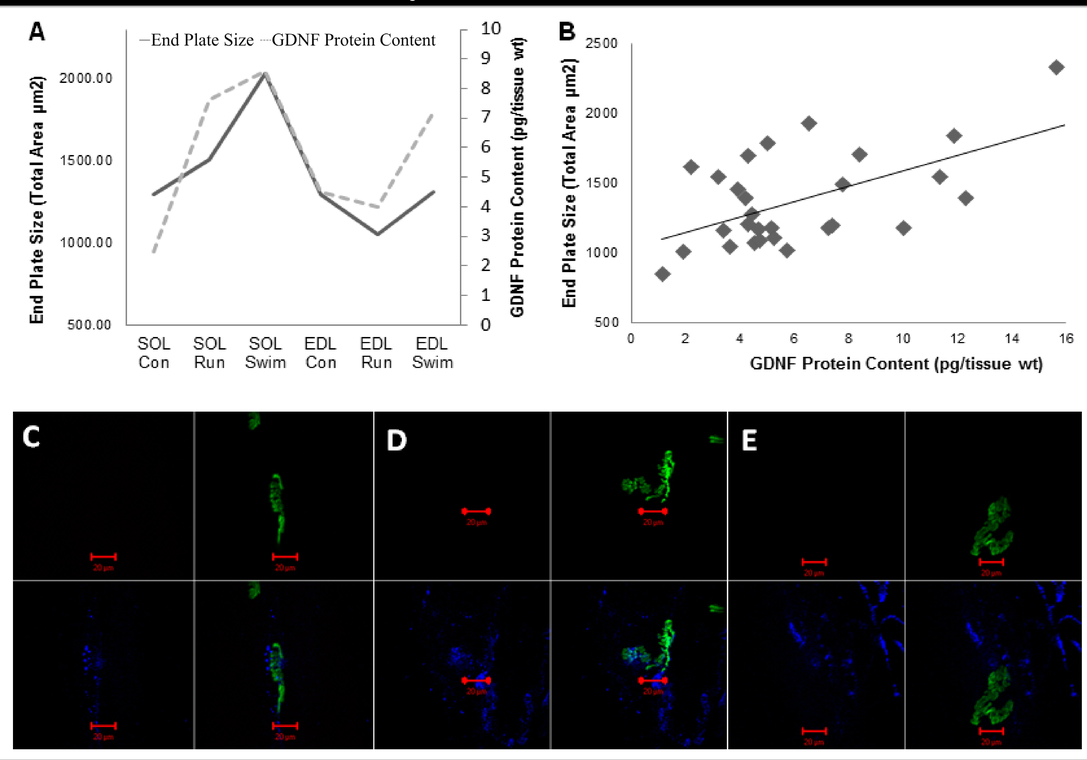
	Soleus	EDL
Control	3831 ± 180	2574 ± 98
Run	2109 ± 65*	2352 ± 103
Swim	3243 ± 101*#	2679 ± 104#

Training Alters GDNF Protein Content in Recruited Muscles

	Soleus	EDL
Control	2.5 ± 2.2	4.5 ± 2.4
Run	7.6 ± 6.4*	4.0 ± 1.4
Swim	8.6 ± 4.5*	7.2 ± 3.0#

*Significant difference between the exercise group and control group; #Significant difference from Run Group; Values are means ± S.E.M.; Protein content (pg GDNF/tissue weight)

GDNF Protein Content Positively Correlates with End Plate Total Area Measurements



Correlation of GDNF levels in skeletal muscle and total end plate area. End plates were visualized with α-bungarotoxin and measurements were taken using ImageJ software. Tissues were processed for GDNF protein content using ELISA and visualized by staining with anti-GDNF antibodies (blue). End plate total area was measured by encircling the entire end plate including the stained ACh receptor clusters and the area in between. (A) Average measurements showing overall trend for all groups comparing GDNF protein content (dashed line) and end plate total area (solid line). (B) Levels of GDNF (pg GDNF/tissue wt.) were positively correlated with end plate measurements for total area ($r=.611$, $P<0.01$, $n=30$). (C-E) Visualization of GDNF (blue) in and around SOL end plates in control- (C), run- (D), and swim groups (E).

CONCLUSIONS

- Swim- and run-training can alter GDNF protein content at the NMJ
- Higher intensity exercise can increase GDNF protein content in fast twitch muscle fibers
- GDNF may play a role in altering the morphology at the NMJ