Lee Honors College Thesis Defense

Walk-training Increases Expression of GDNF in Pectoralis Muscle But Not Diaphragm From Mouse

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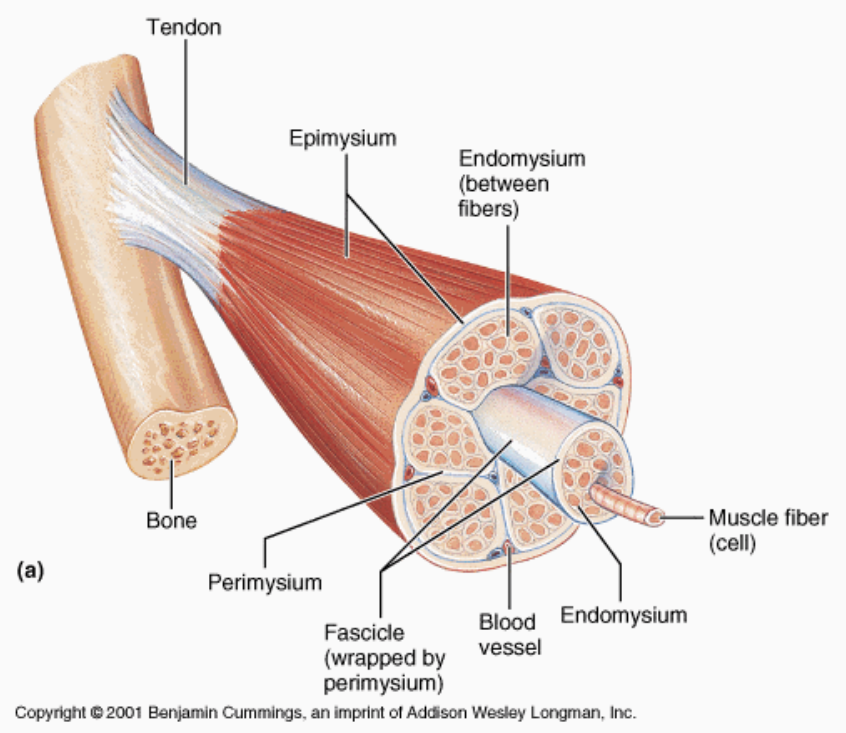
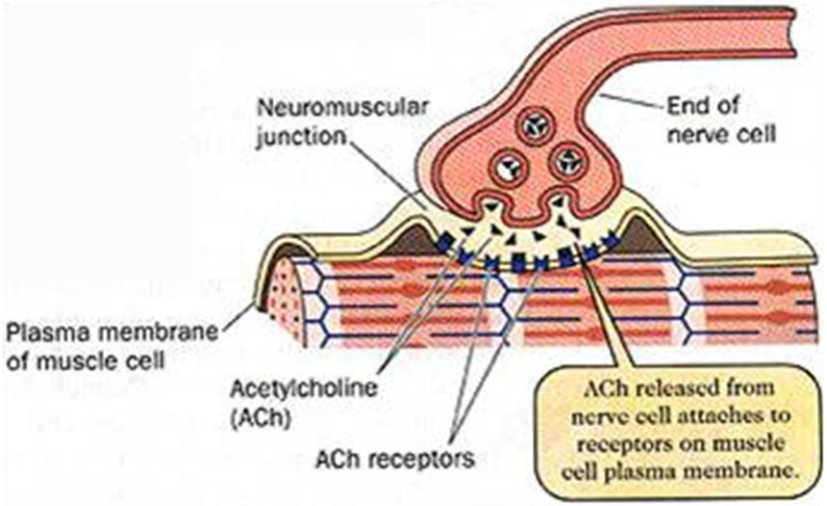
Primary Objectives

- To measure GDNF in mouse muscle using enzyme-linked immunosorbant assay (ELISA) methods previously described in rat.
- To visualize GDNF in mouse muscle using immunocytochemical staining methods previously described in rat.
- To investigate whether exercise increases GDNF content in mouse skeletal muscle.
- To determine if tonically active diaphragm muscle contains more GDNF protein than phasically active pectoralis major muscle.

Background Information

Skeletal Muscles & Contraction

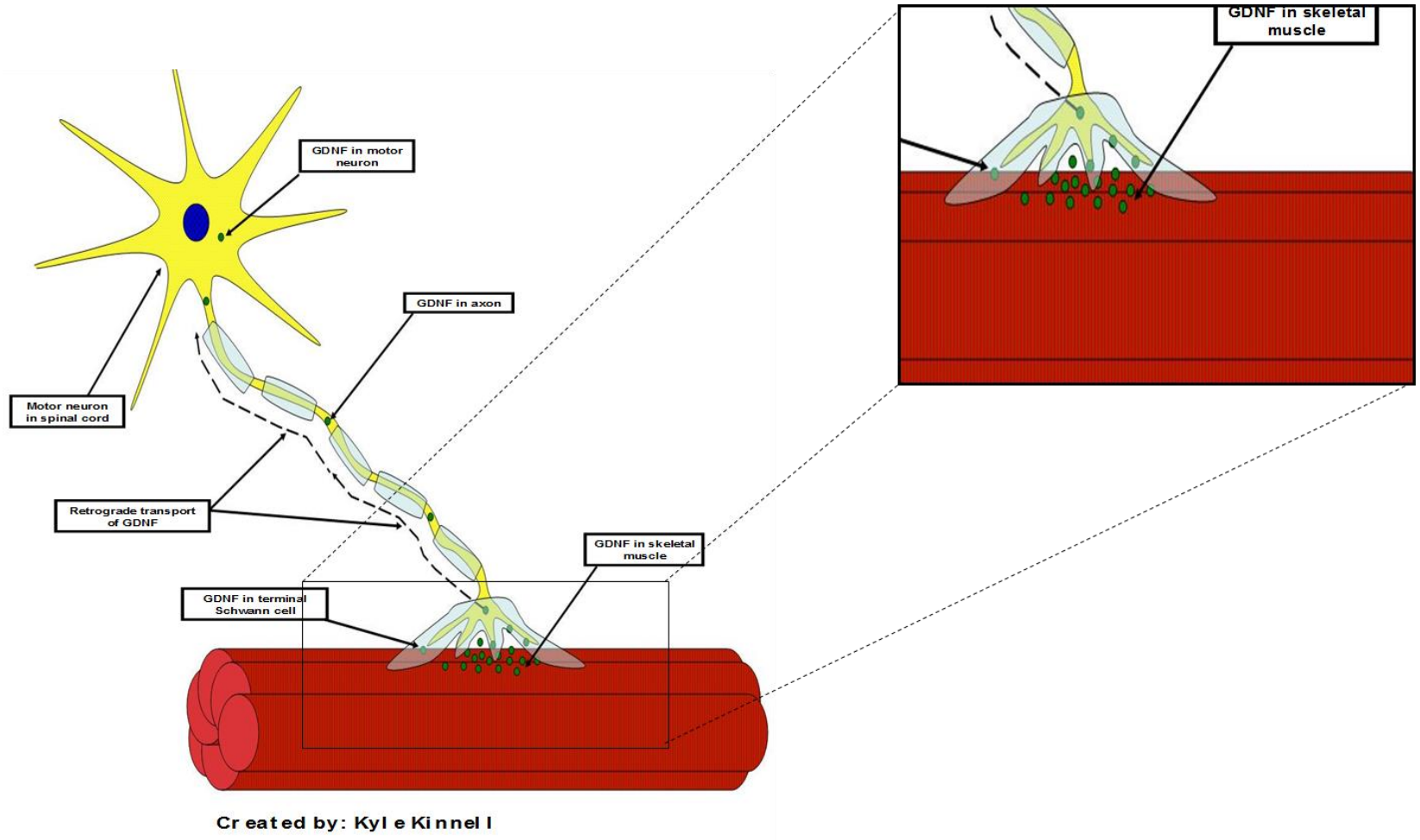




Source:http://www.octc.kctcs.edu/gcaplan/anat/images/Image285.gif

Source: http://www.physioweb.org/muscular/contraction.html

Neuromuscular Junction



GDNF

- A neurotrophic factor→ Extracellular signaling proteins that specialize in acting on the cells of the nervous system.
- Critical in the maintenance and survival of peripheral motor neurons.
- A variety of functions in the neuromuscular system.
  - Muscle Innervation
  - Neurotransmitter Release

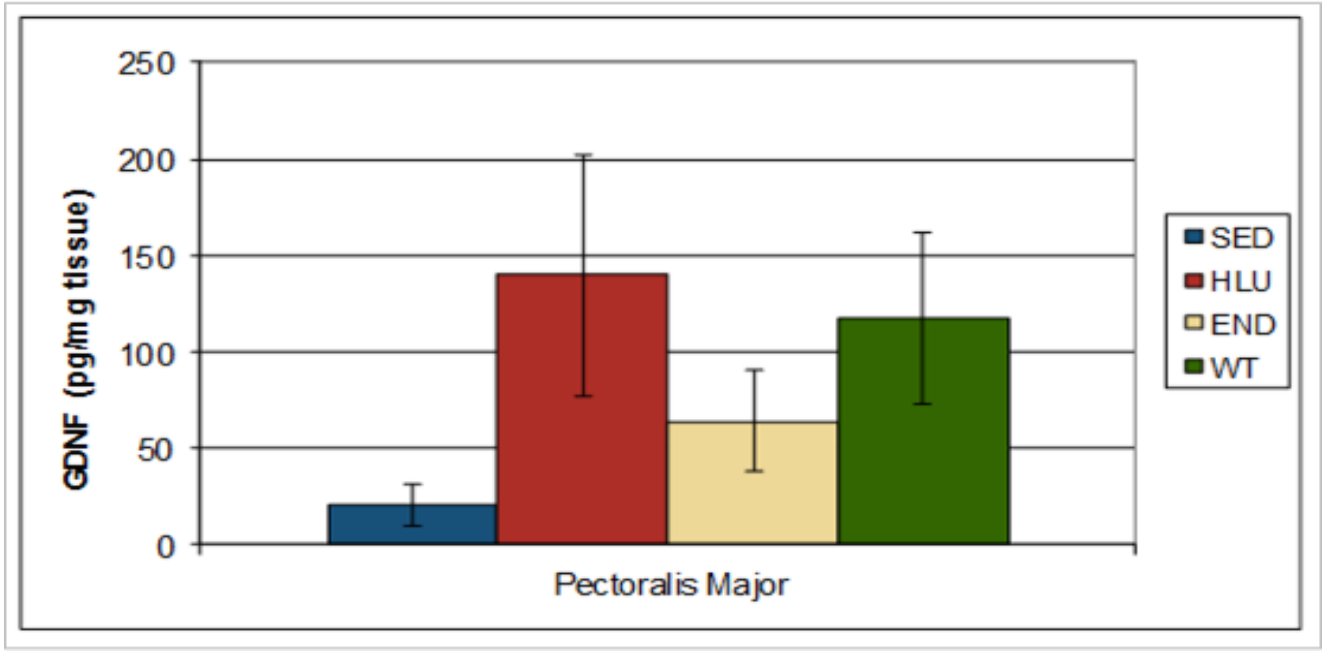
GDNF Mechanism of Action

- Exact mechanism still widely debated.
- Synthesized and secreted by muscle cells.
- Localizes at NMJ.
- Taken up by axon of motor neuron and transported to cell body.
- From there, effects seem limitless.

Problems with GDNF

- Problems can arise with low levels of GDNF.
- Low levels can come from usual aging, inactivity, or disease.
- GDNF could play a vital role in neuromuscular diseases.
  - Amyotrophic lateral sclerosis (ALS)
  - Parkinson's Disease (PD)

But, not to worry…



Previous studies have shown that exercise increases GDNF in rat muscle.

My Goals

- To expand research by exploring new options.
- To confirm changes in GDNF with exercise.
- To research my own hypothesis.

Hypothesis

Muscles showing higher levels of contractile activity will produce higher levels of GDNF protein.

Methodology

Subjects

- 13 young adult mice

Treatment Groups:

- 7 control mice
  - No access to a running wheel
- 6 exercise mice
  - Walk training
  - 1 week training period followed by 2 weeks of exercise
  - 30 minutes/day, 5 days/week
  - 8 meters/minute

*All animal experiments were performed in accordance with the "Guide for the Care and Usage of Laboratory Animals" (National Research Council) and all protocols have been approved by the Institutional Animal Care and Usage Committee at Western Michigan University.

Tissue Processing

- Mice euthanized within 72 hours after last exercise session.
- Tissues Selected:
  - Diaphragm (DIA)→ involuntary slow twitch muscle
  - Pectoralis Major (PEC)→ voluntary slow twitch muscle
- Preparation for GDNF protein quantification and immunohistochemical staining.

GDNF Protein Quantification

- Enzyme-linked immunosorbant assay (ELISA).
- Protein content expressed as:

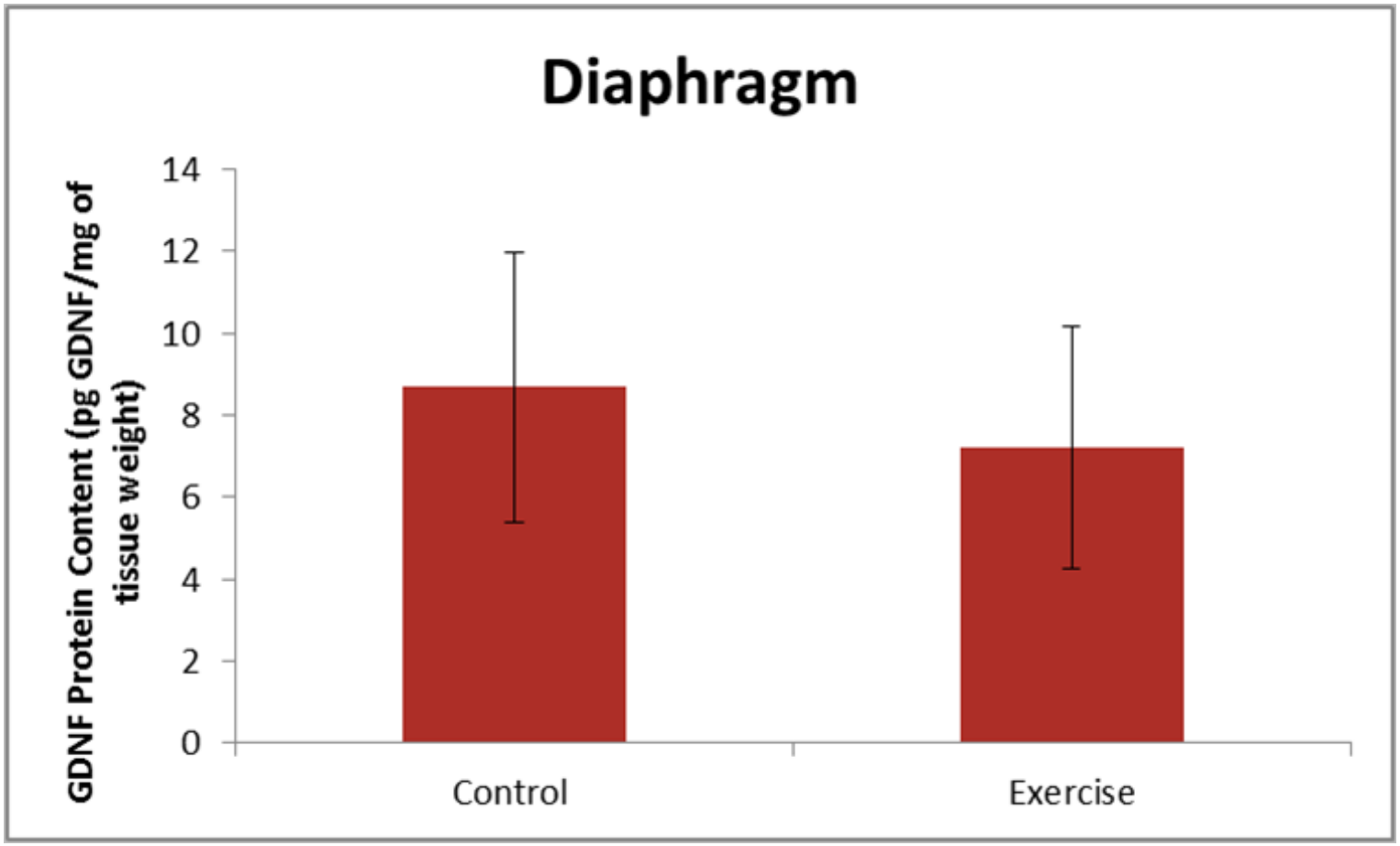
  pg GDNF/ mg Tissue Weight
- Reported as a mean with a standard error of mean (SEM).
- Statistical t-test analysis, with p values ≤ 0.05 considered statistically significant.

Immunocytochemistry

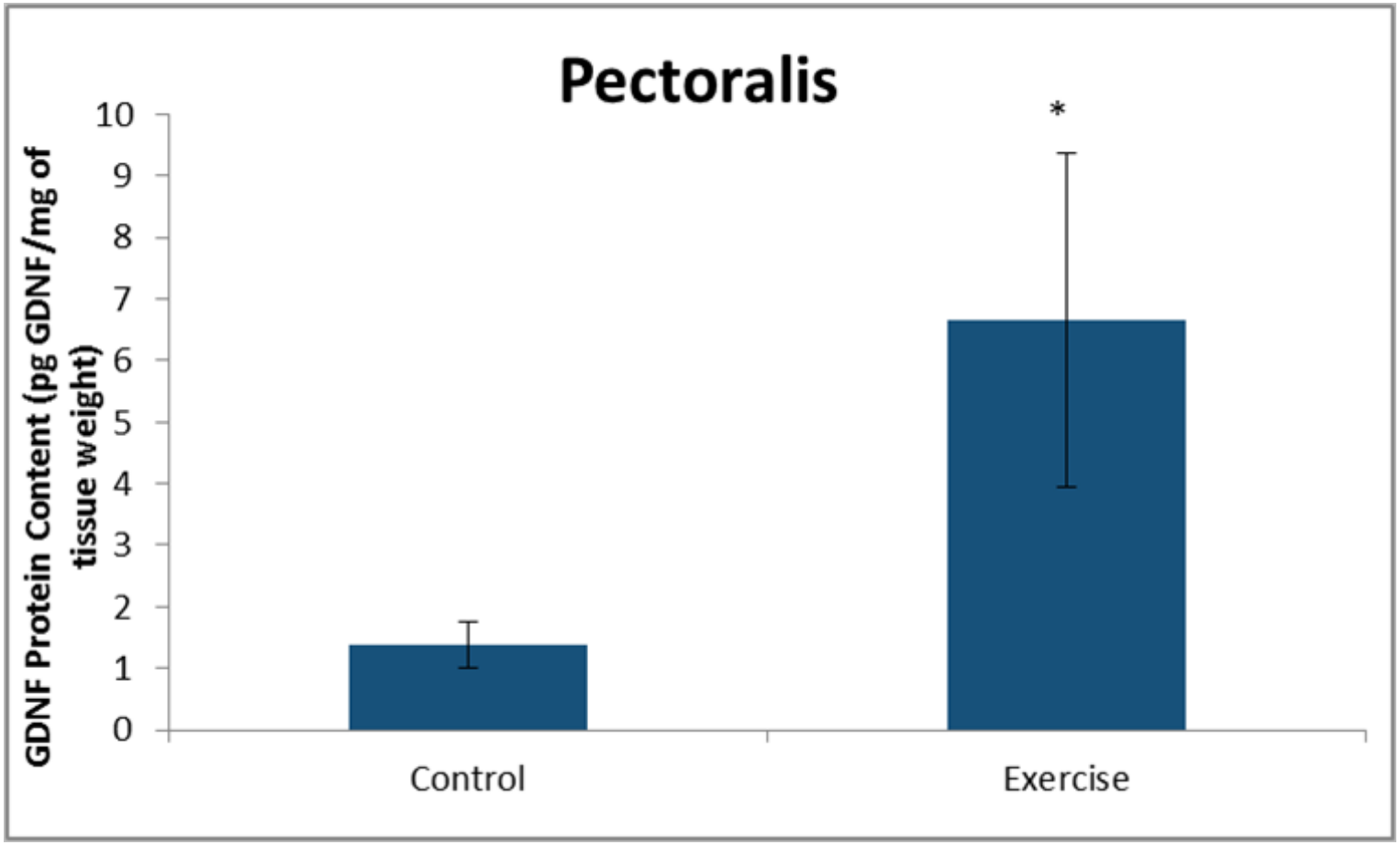
- Tissues were fixed, cut and mounted on slides.
- Stained with primary and secondary antibodies conjugated to AlexaFluor:
  - **GNDF:** Rabbit anti GDNF
  - **Ach Receptors:** α-Bungarotoxin
  - **Axon of Motor Neuron:** Mouse neurofilament
- Visualized on Zeiss Axiovert 100M confocal laser scanning microscope.

Results

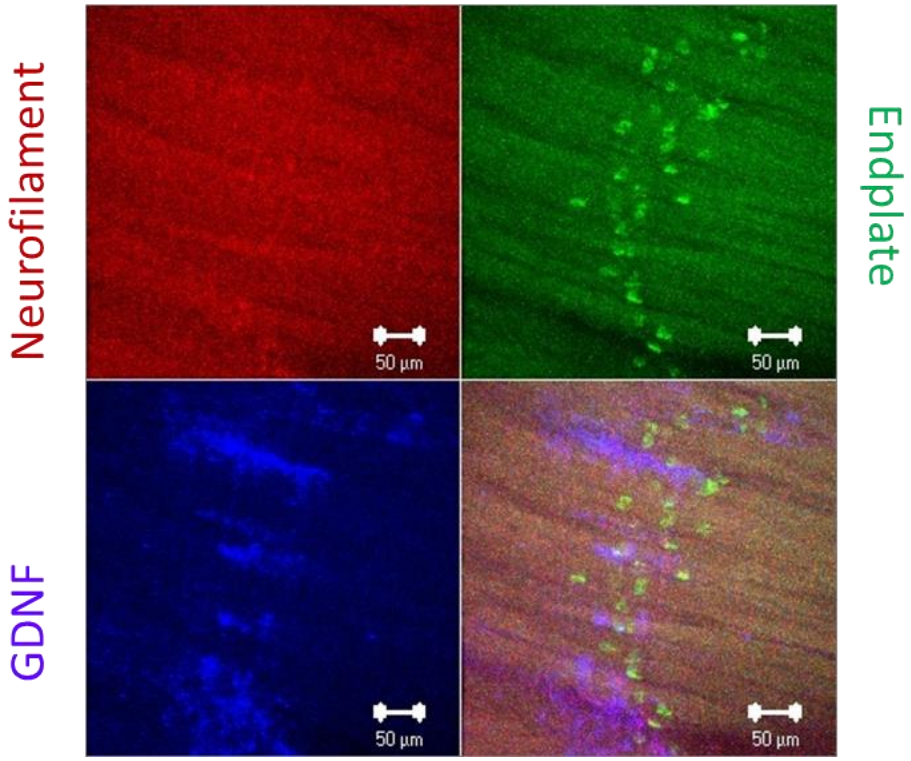
Exercise had no effect on GDNF content in the diaphragm



Exercise increases GDNF protein content in the pectoralis major

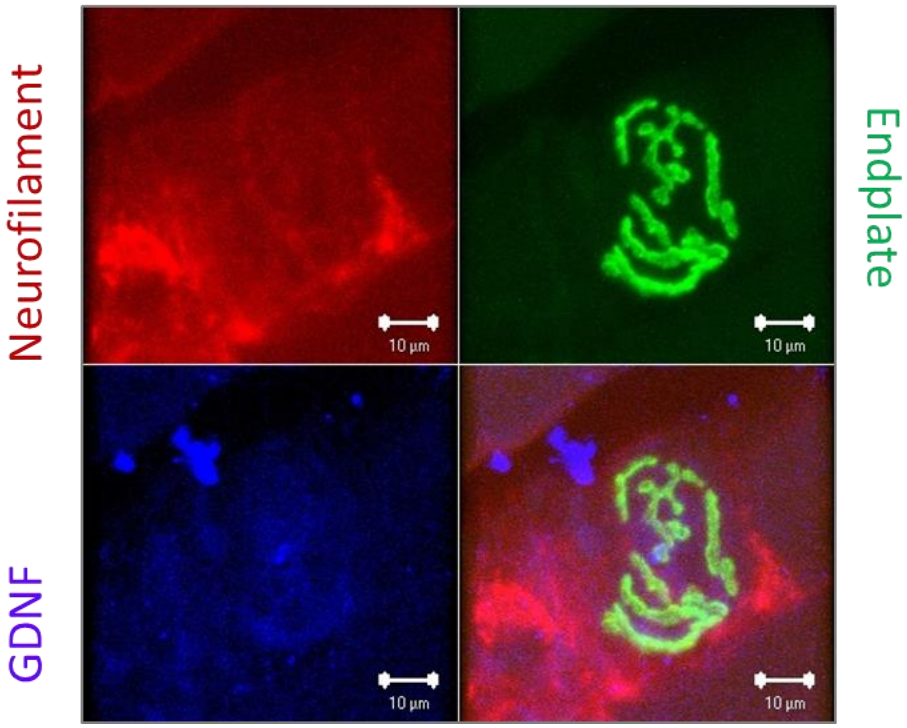


GDNF staining is localized to the endplate region in muscle



EXERCISED DIAPHRAGM AT 20X MAGNIFICATION

GDNF staining is localized to the endplate region in muscle



EXERCISED PECTORALIS AT HIGH MAGNIFICATION

Discussion

Use of Rat Methods in Mouse

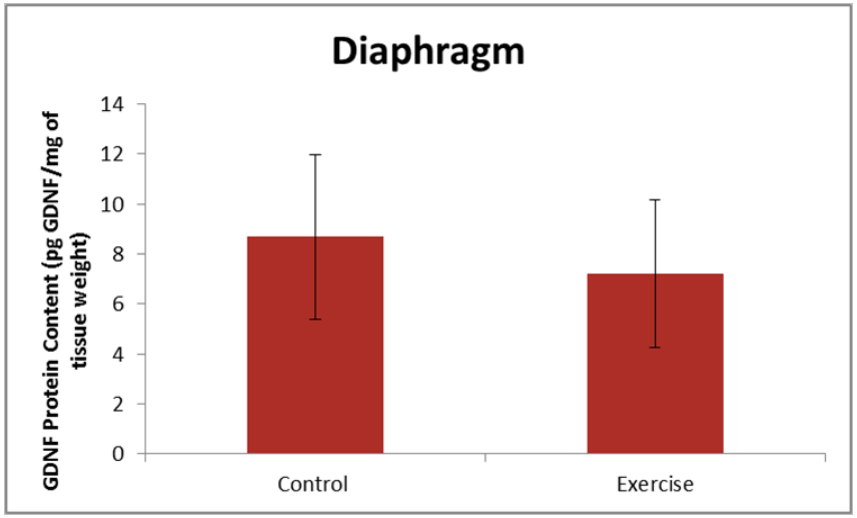
- Able to measure GDNF in mouse muscle using ELISA.
- Able to detect GDNF in mouse muscle using immunohistochemical methods.
- Allows for expansion of research in new directions.
  - Use of many genetic models available in mice.

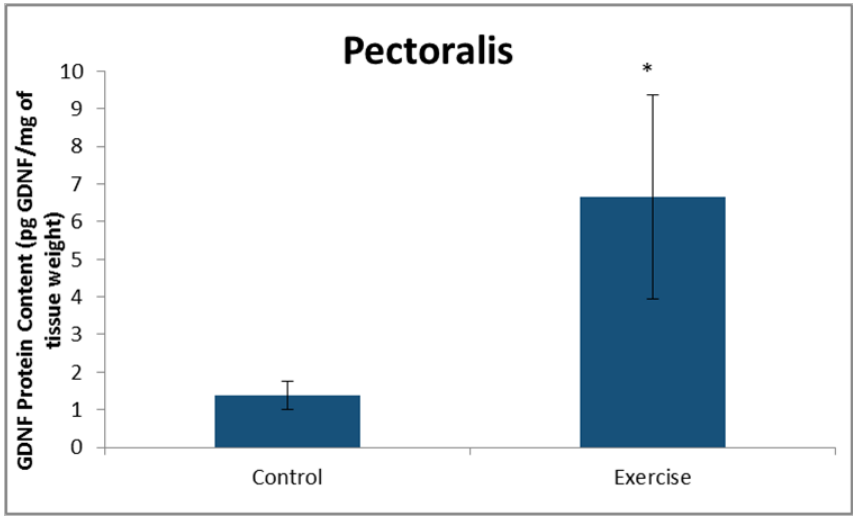
Use of Exercise

- GDNF in mouse skeletal muscle increases with exercise.
  - Supports previous studies in rats.
- DIA (involuntary) showed no significant increase in GDNF after exercise.
- PEC (voluntary) showed significant increase in GDNF after exercise.

Use of Exercise cont.

- With no treatment, DIA produces more GDNF than PEC.
- With exercise, PEC increases to around the same level.
- GDNF production is affected by exercise in voluntary, but not involuntary slow-twitch muscles.





GDNF Localization

- Staining shows localization around the endplate.
  - Supports previous theories.
- Did not have good results in neurofilament staining.
  - Likely that antibody used to identify rat neurofilament does not recognize mouse neurofilament.

So why do I think this is happening?

- GDNF system is regulated by a feedback loop.
  - Increase in muscle growth (hypertrophy).
  - GDNF secretion stimulated to support innervation by motor neurons.
  - Once the muscle is fully innervated, cholinergic neurons start regulating the production of GNDF
    - ACh receptors on the skeletal muscles.
- Equilibrium of GDNF secretion is reached.
  - Cholinergic motor neurons and ACh receptors.

Conclusion

- Expansion of research using mouse models.
  - Various genetic models.
  - Contribute to a more complete understanding of normal expression of GDNF in skeletal muscles.
- Beneficial effects of exercise on motor neurons.
- Use of exercise as possible preventative and therapeutic measures.

Acknowledgements

- Dr. John Spitsbergen
- Dr. Cindy Linn and Amy Gyorkos
- The Spitsbergen Lab members
- Department of Biological Sciences faculty
- Lee Honors College and Dr. Koretsky
- Office of Vice President for Research
- College of Arts and Sciences
- Biological Sciences Imaging Facility