Humoral Biomarker Detection of Skeletal Muscle Myopathy Using Proteomics

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Aims

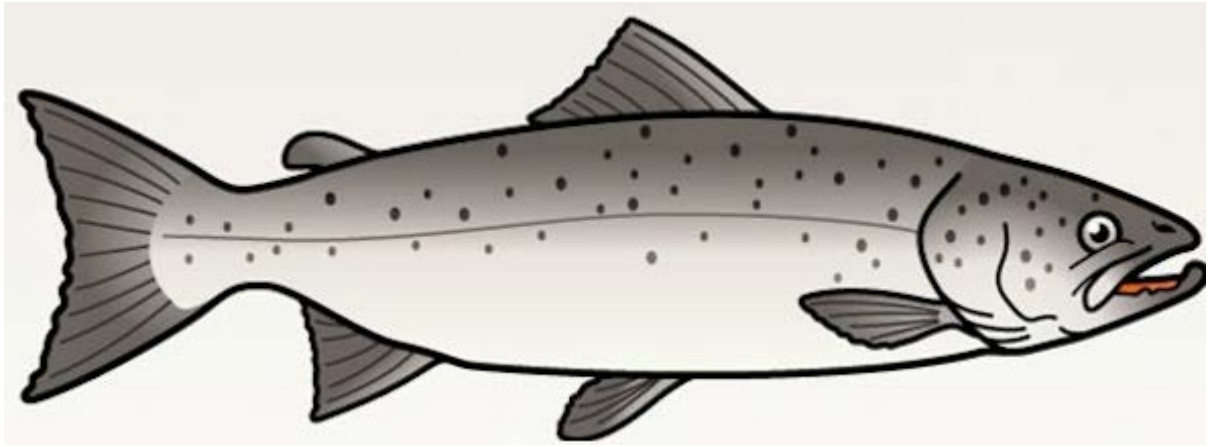
Background

Established Markers and functional feed

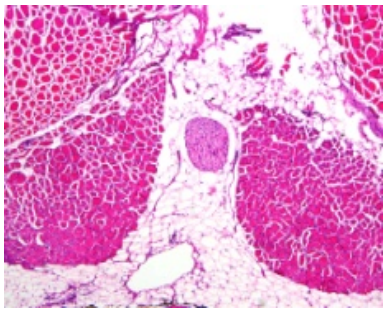
Potential biomarker identification

Validation

Relating to pathology

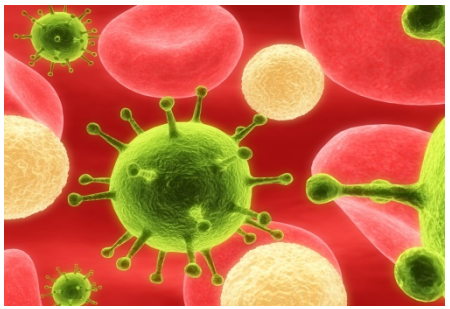


Fish sampled randomly



Destructive

Non-destructive



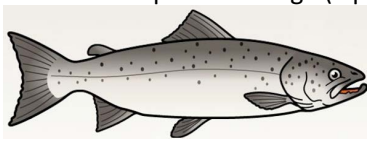
Proactive

Problems

Demand for non destructive markers of myopathies

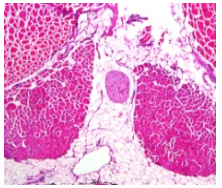
Summary

9 fish from each being sampled at 0, 2, 3, 4, 5, 6, 8, 10 and 12 weeks post challenge (wpc)



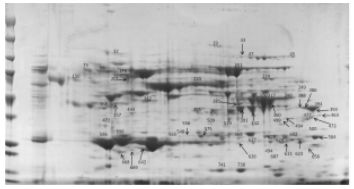
Sera

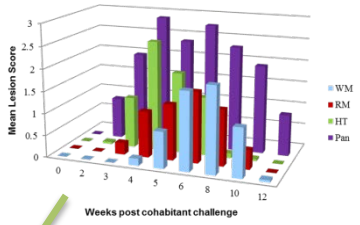
McLoughlin et al (2004)



Established Markers

Braceland et al (2013) JOP





Diet A | Diet B | Diet C | Diet D

3 Tanks per diet
120 Fish (per tank)

24 Fish added to each tank
(= total tank population now 144)

42 Days
X 8 Tanks
350 Fish (per tank)

288 Fish

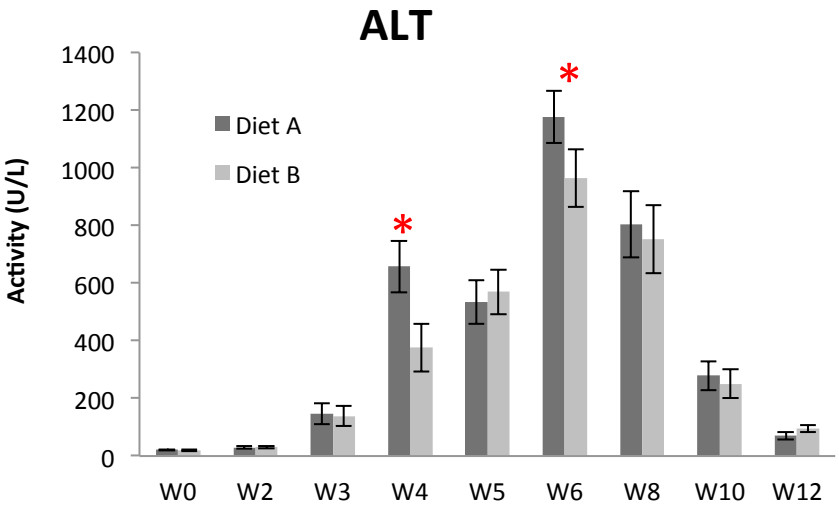
SAV3 infected CHSE cell culture supernatant at ca. 10^5 TCID per fish injected into intraperitoneal cavity.

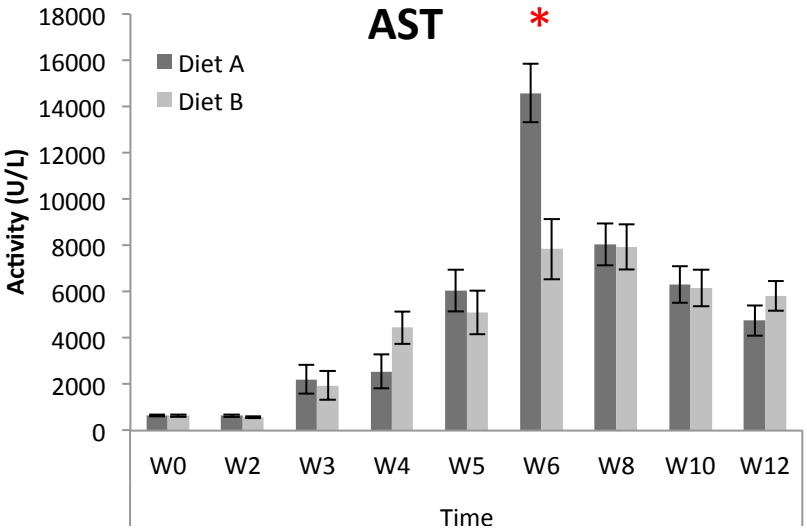
Protein identification by peptide MS/MS

Significant Correlation of spot profiles and histopathological scores of specific tissues, by GLM analysis

Validation

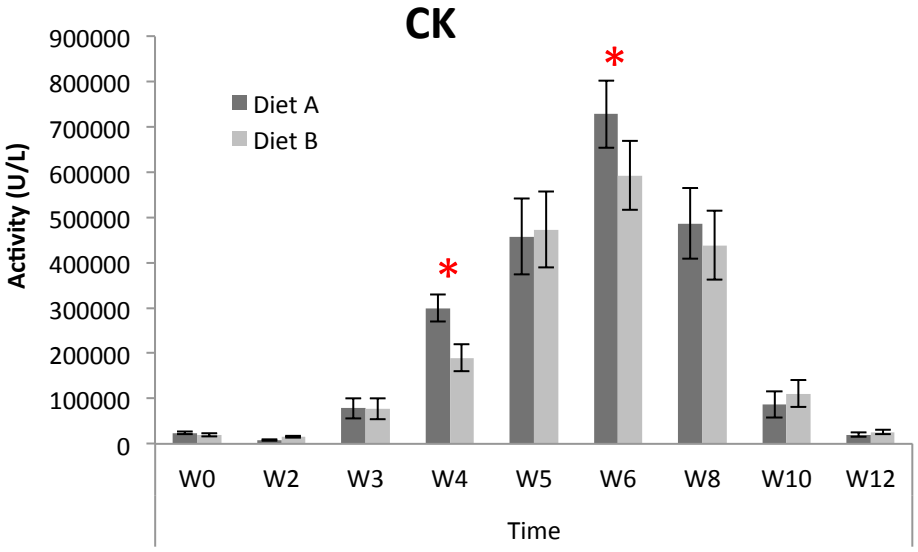
M. Braceland, R. Bickerdike, J. Tinsley, D. Cockerill, M.F. Mcloughlin, D.A. Graham, R.J. Burchmore, W. Weir, C. Wallace, P.D. Eckersall, The serum proteome of Atlantic salmon, Salmo salar, during pancreas disease (PD) following infection with salmonid alphavirus subtype 3 (SAV3), Journal of Proteomics, Volume 94, 6 December 2013, Pages 423-436





Diet A = Control

Diet B = Primo (Plus 3)



Control vs Primo (Plus 3) differences in histopathological scores (by week):

- Heart: W6pc
- White Muscle: W6pc
- Red Muscle: W4pc, W6pc
- Pancreas: W4pc, W6pc

Significance = $p < 0.05$

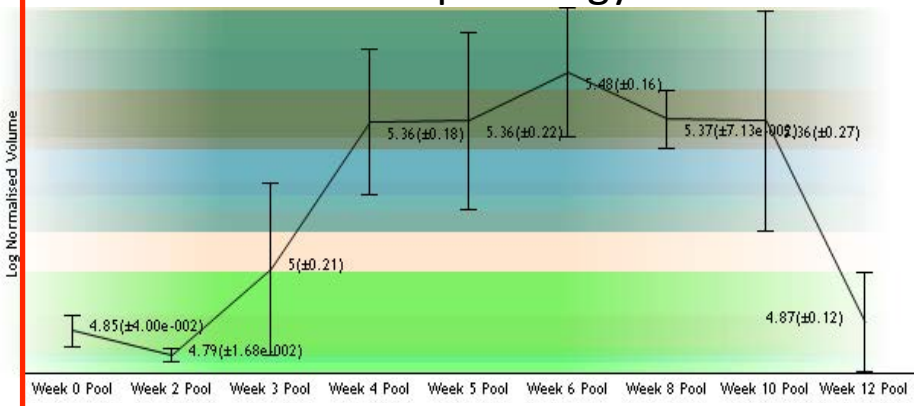
Why? Issues with Enzyme Activity Assays

- Total enzyme assay thus lacks tissue specificity
- Variation
  - Baseline activity high make it difficult to identify subtle differences
  - E.g Dog(6.25U/L), Cat (19.5 U/L), Cow (7.4 U/L), Goat (4.5 U/L), Pig (8.9 U/L
  - Salmon (1000 – 20,000 U/L)
  - Similar differences in ALT and AST activities
- Disease comparison

Proteomics

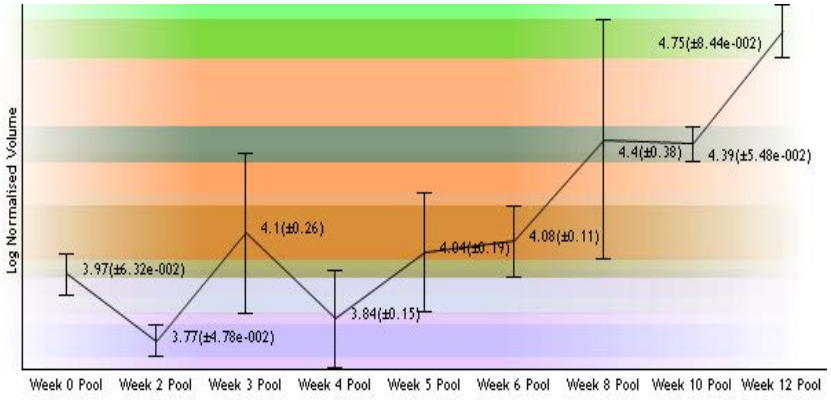
Proteomic results split into 2 categories

Those which alter in serum abundance due to pathology



Creatine kinase (Spot 391)

Those which alter as part of humoral immune response

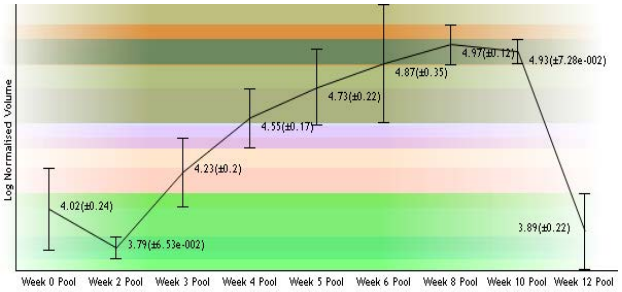


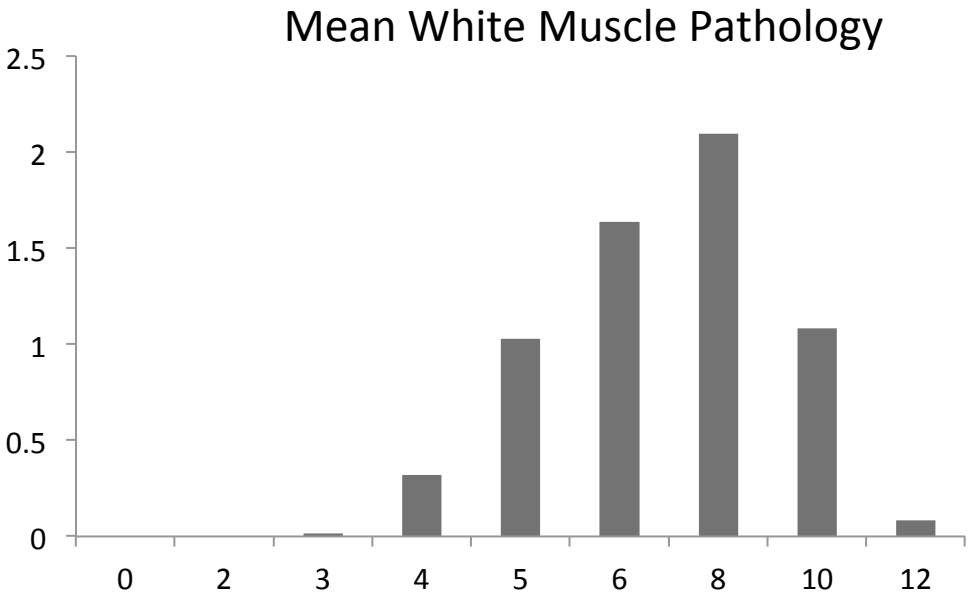
Hemopexin-like protein (Spot 150)

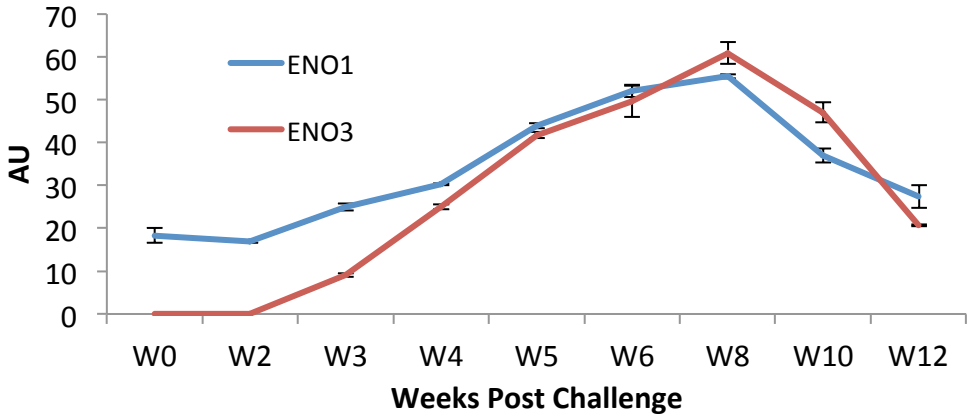
Enolase

- Isoform distribution (mammals):

Enolase 1	Ubiquitous
Enolase 2	Neuron specific
Enolase 3	Mainly muscle


Log Normalised Volume
4.02(±0.24)
3.79(±6.53e-002)
4.23(±0.2)
4.55(±0.17)
4.73(±0.22)
4.87(±0.35)
4.97(±0.12)
4.93(±7.28e-002)
3.89(±0.22)
Week 0 Pool
Week 2 Pool
Week 3 Pool
Week 4 Pool
Week 5 Pool
Week 6 Pool
Week 8 Pool
Week 10 Pool
Week 12 Pool


Mean White Muscle Pathology
2.5
2
1.5
1
0.5
0
0
2
3
4
5
6
8
10
12


70
60
50
40
30
20
10
0
AU
ENO1
ENO3
W0
W2
W3
W4
W5
W6
W8
W10
W12
Weeks Post Challenge

Assessing Sensitivity of Scoring System (ENO3)

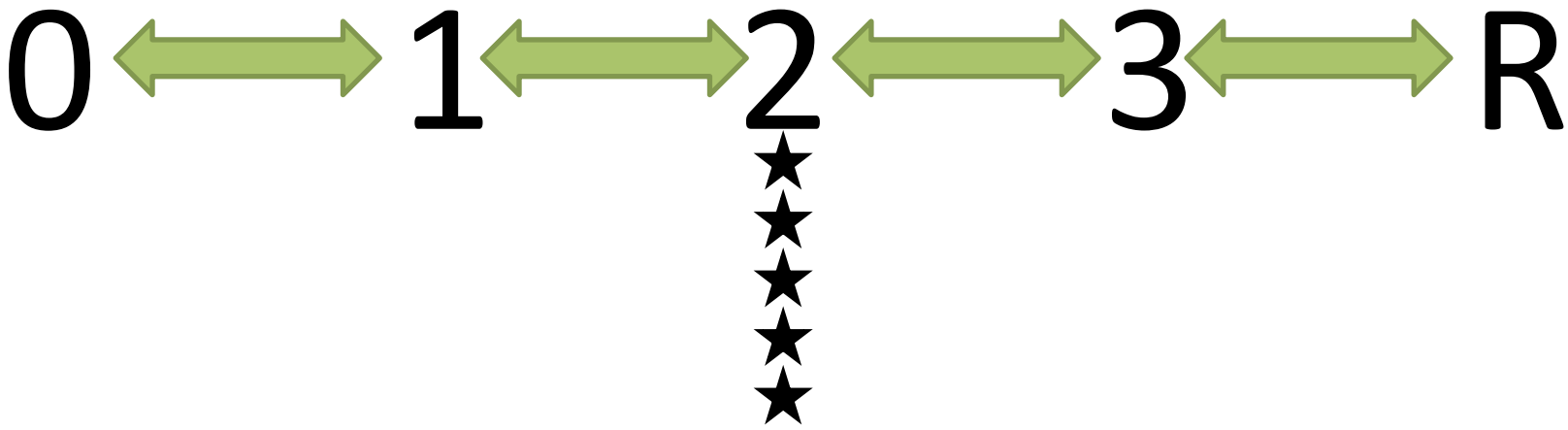
Categorical vs. Continuous

McLoughlin et al (2004)

Assessing Sensitivity of Scoring System

Categorical vs. Continuous

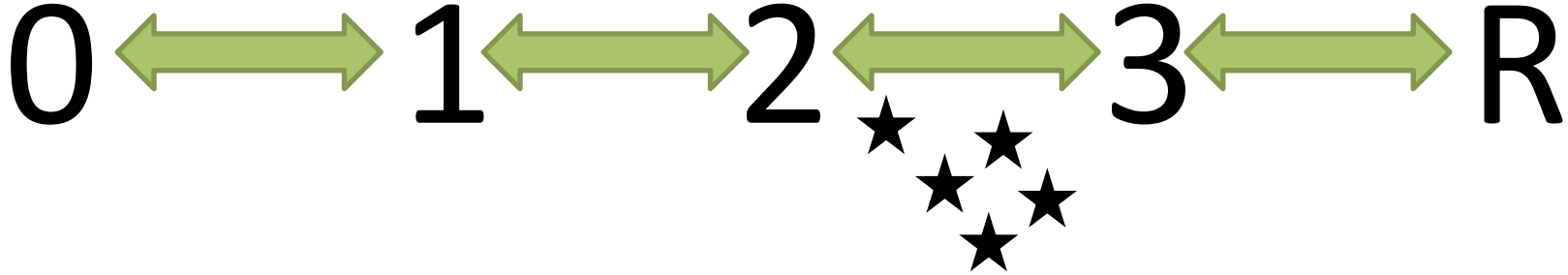
McLoughlin et al (2004)



Assessing Sensitivity of Scoring System

Categorical vs. Continuous

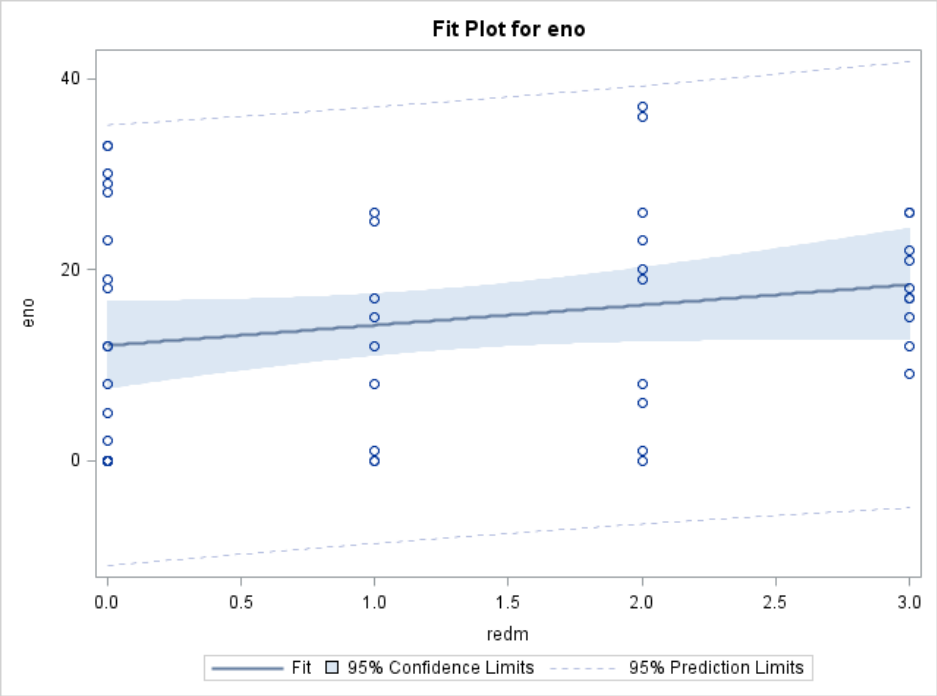
McLoughlin et al (2004)



GLM Analysis

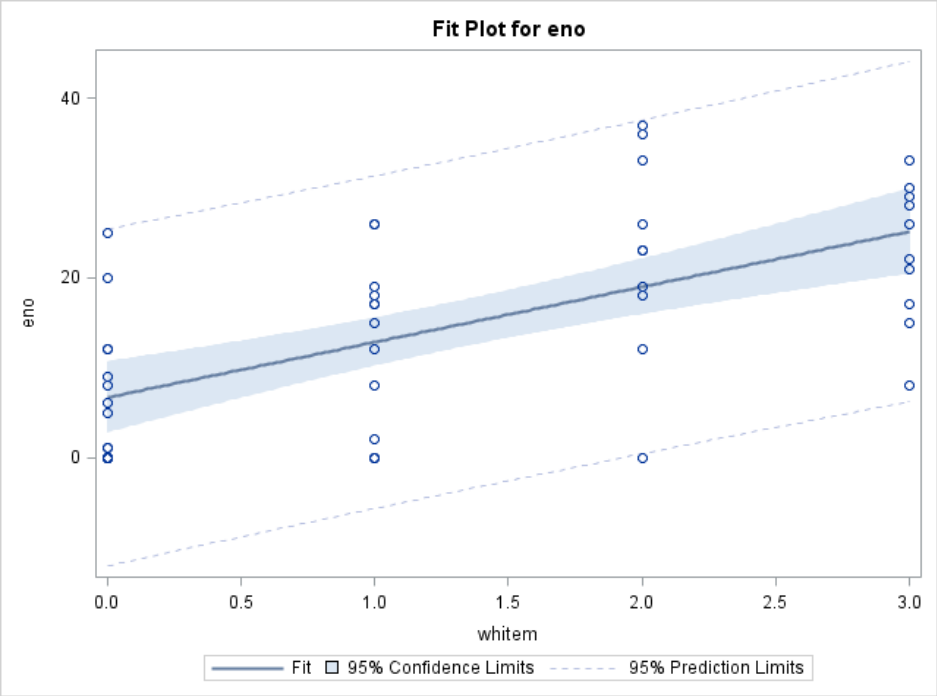
50 fish → White muscle → Red Muscle → Enolase Conc.

Red Muscle

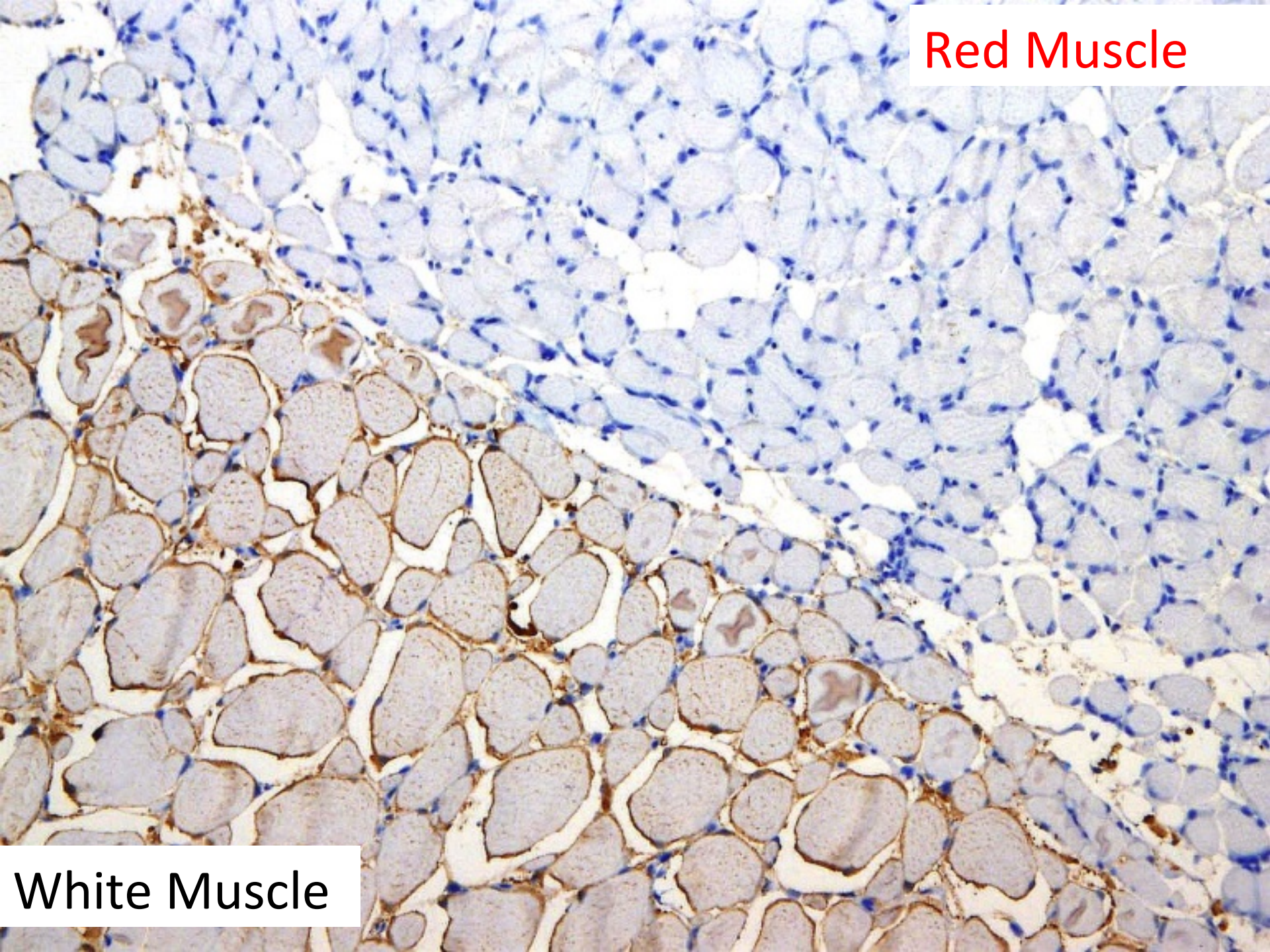


P = 0.1269
5% of variance

White Muscle



P = <0.0001
38% of variance


Red Muscle
White Muscle

Conclusions and Future Work

- ✓ Classical Markers useful in health assessment
- ✓ Enolase is a marker of myopathy of white muscle
  - ✓ May Influence health management?
- ✓ Possible marker of flesh quality issues

Acknowledgements

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