

# Lincoln University- AKC 2023

Protein biochemistry-Meat Science  
research Laboratories

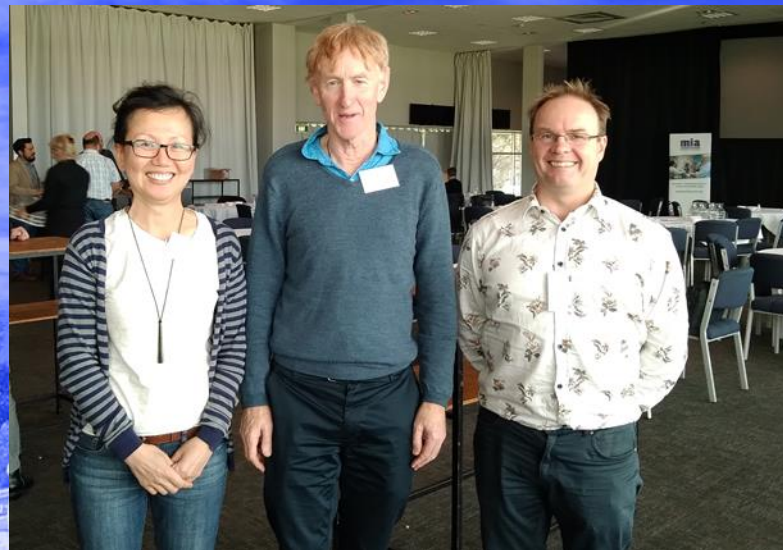
&

Animal Research Farm (JML)



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# Living in Lincoln

Our campus is in the Lincoln township, a thriving semi-rural community of around 8000 people.



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# The effect of farmyard stress on meat quality: *a model* enabling the search for predictive biomarkers of meat pH

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**agresearch**  
āta mātai, mātai whetū

# Experimental capacity at the Animal Research Farm (John Memorial Laboratory, JML, 1.5km from the campus):

- ✓ Facilities for breed and housing in-door or outdoor pasture.
- ✓ Facilities for sheep and meat quality trials: slaughter, dressing and chilling rooms.
- ✓ Competent Farming-research technical staff for science-research field base projects.
- ✓ Dr Hannah Lee: extensive experience managing/co-ordinating *in vivo* sheep trials at JML 2004-presents





# New Zealand Lamb

- Export industry (90%) NZ\$3.5 billion in 2022
- Long distance to markets
  - Top markets China and the EU
  - Mostly chilled

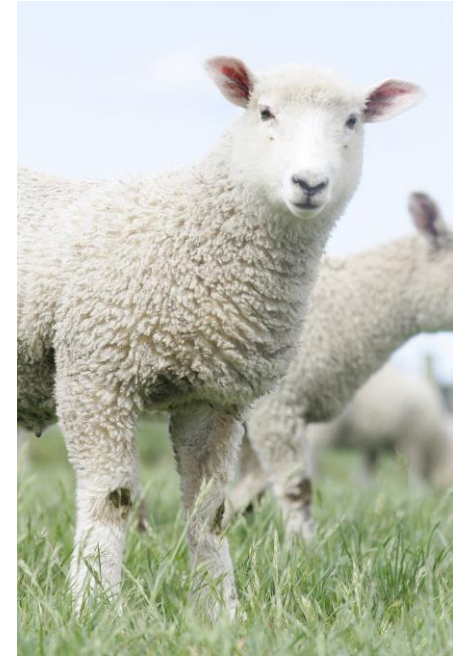


# Importance of Ultimate pH of the meat

- The rate and extent of pH decline a key feature of post-mortem metabolism.
- Influences meat quality particularly colour and water-holding capacity.
- Can measure pH at 24 hours post-mortem
- ✓ Selection decisions made before then about potential chilling regimes and markets
- ✓ Looking for online prediction of ultimate pH

# Exercise Stress Model

- Measured ultimate pH of lamb at processing plants, Level of high pH unpredictable
- Needed a reproducible model for inducing high pH.
- Based on moderate exercise stress: ***Preslaughter farmyard stress in sheep.***





# High pH sheep model





## I. 2021 FIRST Stress model sheep trial: 10 Muscles studied

| Muscle                  | Type*           | Location |
|-------------------------|-----------------|----------|
| Longissimus lumborum LL | Fast Glycolytic | Trunk    |
| Longissimus thoracis LT | Fast Glycolytic | Trunk    |
| Semitendinosus ST       | Fast Glycolytic | Thigh    |
| Supraspinatus SS        | Slow/Oxidative  | Shoulder |
| Infraspinatus IS        | Slow/Oxidative  | Shoulder |
| Semimembranosus SM      | Intermediate    | Thigh    |
| Psoas major PM          | Intermediate    | Trunk    |
| Gluteus Medius GM       | Intermediate    | Thigh    |
| Gracilis (adductor) G   | Intermediate    | Thigh    |
| Sternomandibulous StM   | Not identified  | Neck     |

\*Variation in instrumental meat quality among 15 muscles from 14-month-old sheep and its relationship with fibre typing  
 Animal Production Science, J Ithurralde et al., 2018, 58, 1358–1365.  
<http://dx.doi.org/10.1071/AN16013>



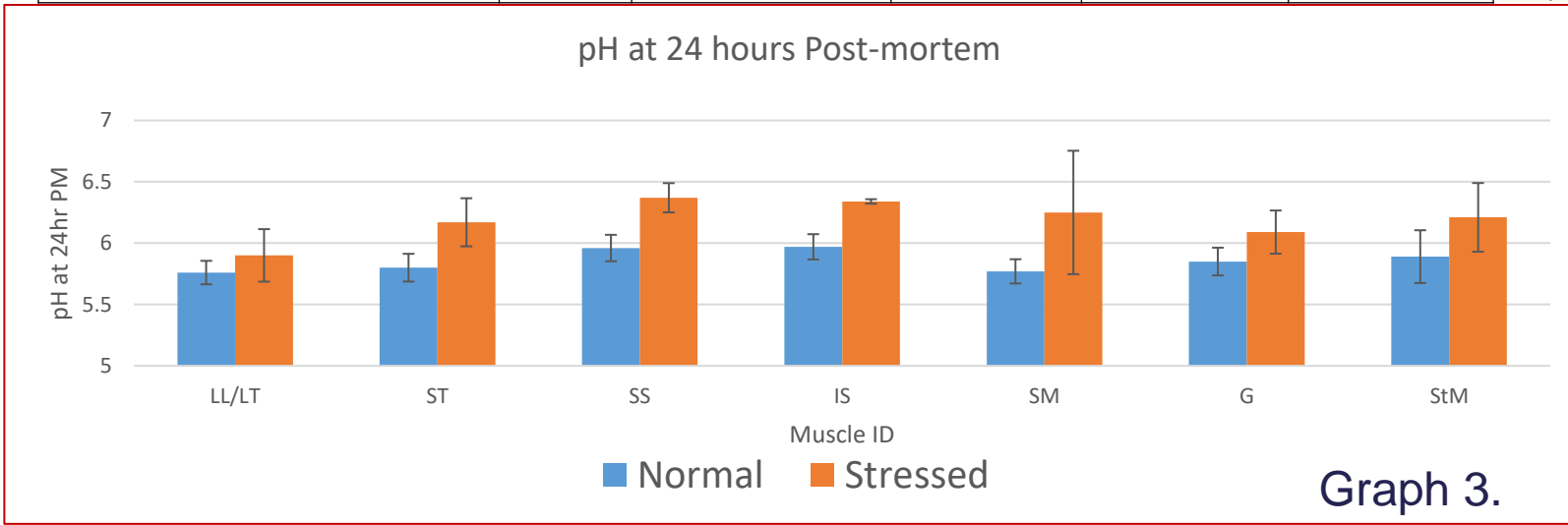
# I. 2021 Sheep Trial Analysis: Stress had an different effect on ultimate pH at 24 hours post-slaughter to various muscles.

Table 1. pH of muscles at 24 hours post-mortem

| Muscle ID                       |       | Muscle Fiber Types | Normal      | Stressed   | p- value |
|---------------------------------|-------|--------------------|-------------|------------|----------|
| Longissimus (lumborum+thoracis) | LL/LT | Fast/Glycolitic    | 5.76±0.096  | 5.92±0.214 | 0.006    |
| Semitendinosus                  | ST    | Fast/Glycolitic    | 5.8±0.113   | 6.17±0.196 | 0.000    |
| Supraspinatus                   | SS    | Slow/Oxidative     | 5.96±0.108  | 6.37±0.119 | 0.000    |
| Infraspinatus                   | IS    | Slow/Oxidative     | 5.97±0.103  | 6.34±0.018 | 0.000    |
| Semimembranosus                 | SM    | Intermediate       | 5.77±0.099  | 6.25±0.503 | 0.016    |
| Gracilis (adductor)             | G     | Intermediate       | 5.85±0.113  | 6.09±0.176 | 0.002    |
| Sternomandibulous               | StM   | unkown             | 5.89±0.215  | 6.21±0.280 | 0.012    |
| Psoas major                     | PM    | Intermediate       | 5.98±0.264  | 6.26±0.381 | N.S      |
| Gluteus Medius                  | GM    | Intermediate       | 5.79±0.0952 | 5.86±0.216 | N.S      |

\*GM stress group N=9 vs normal N=10.

\*\*Statistics: TWO-sample T-test



Graph 3.



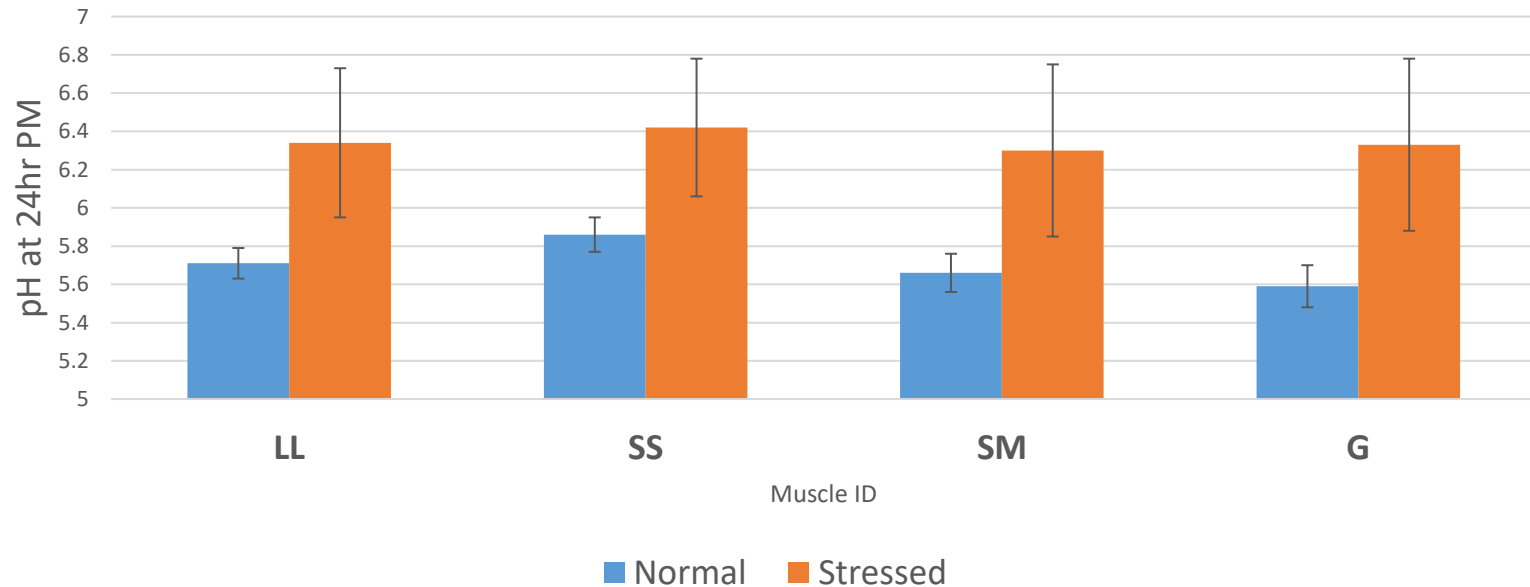


## II. 2022 Sheep Trial Analysis: Stress had an different effect on ultimate pH at 24 hours post-slaughter to various muscles.

pH of muscles at 24 hours post-mortem

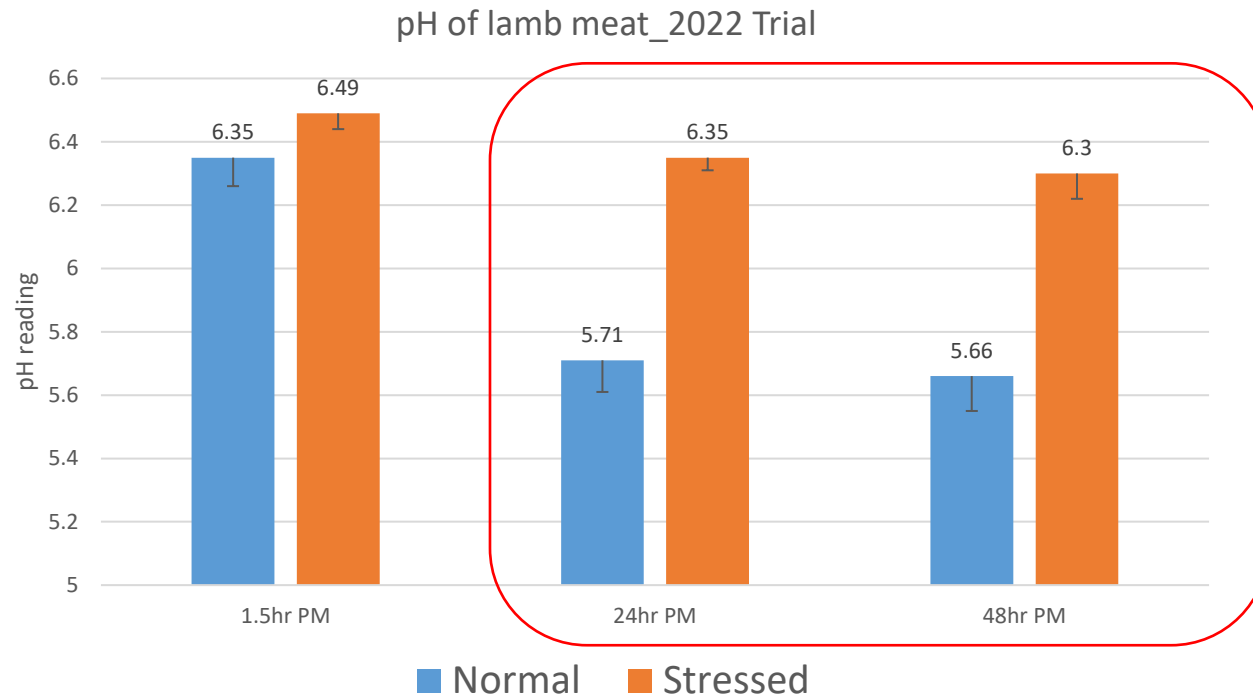
| Muscle ID            |           | Muscle Fiber Types | Normal | SD   | Stressed | SD   | p- value |
|----------------------|-----------|--------------------|--------|------|----------|------|----------|
| Longissimus lumborum | <b>LL</b> | Fast/Glycolitic    | 5.71   | 0.08 | 6.34     | 0.39 | sf       |
| Supraspinatus        | <b>SS</b> | Slow/Oxidative     | 5.86   | 0.09 | 6.42     | 0.36 | sf       |
| Semimembranosus      | <b>SM</b> | Intermediate       | 5.66   | 0.1  | 6.3      | 0.45 | sf       |
| Gracilis (adductor)  | <b>G</b>  | Intermediate       | 5.59   | 0.11 | 6.33     | 0.45 | sf       |

pH at 24 hours PM\_2022 Sheep Trial (9/10month old lamb)



## II. 2022 sheep trial Results: 4 muscles studied. Stress had an effect on pH at 1.5 & 24 & 48 hours post-mortem

pH at 1.5 hours post-mortem: not different between groups, yet starting to show different at 24hr, remain unchanged at 48hr. (N=40)





# 2021/2022 trial: Colour Measurement 48hr postmortem



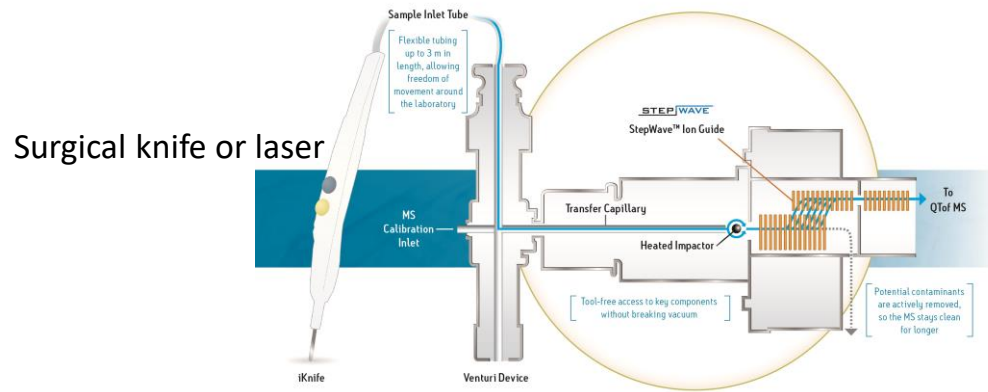
Stressed: Dark, Dried, photo taken at 24hr PM



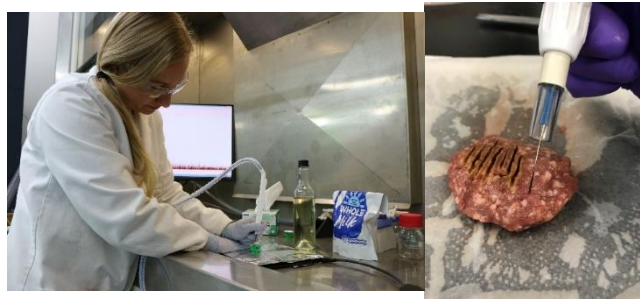
Colour measurement at 48hr PM



# REIMS-based mass spectral fingerprinting – a fast way to get detailed information about meat



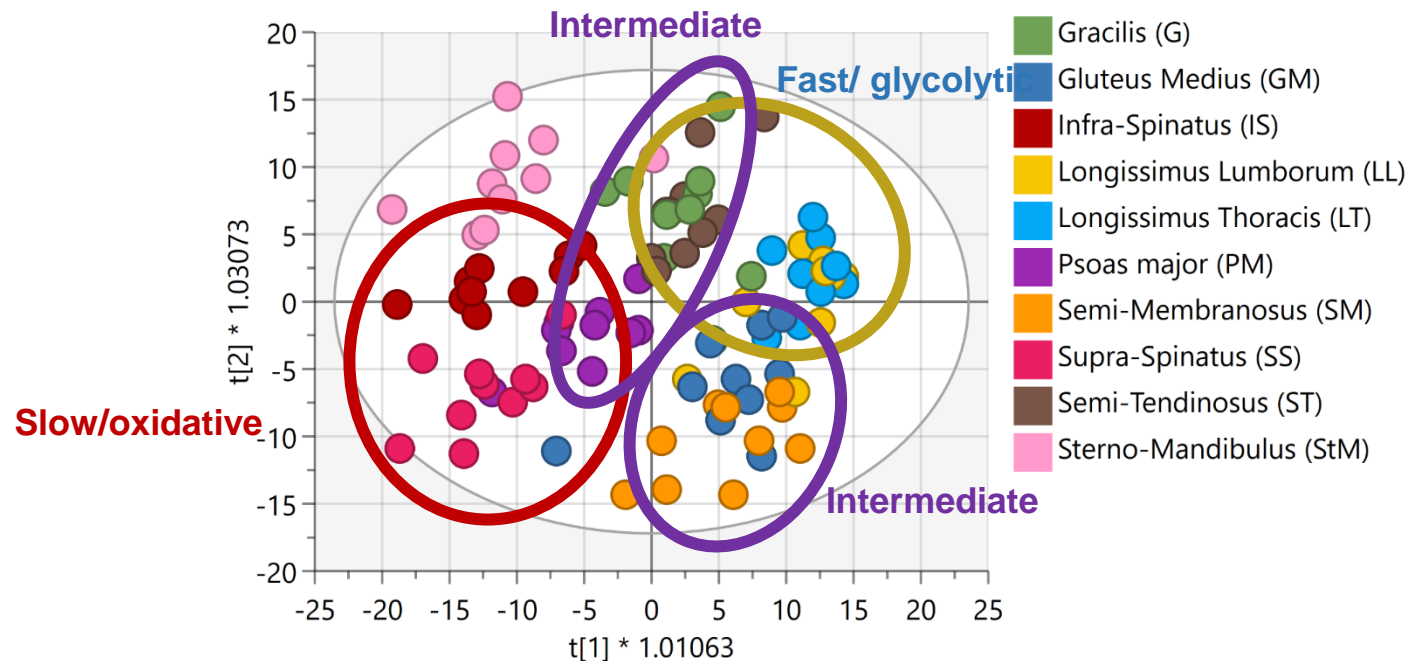
- No sample preparation required
- Measurement takes a few seconds (10 seconds between measurements)
- All molecular features in the sample ‘smoke’ are detected:
  - Can be directly or indirectly related to a wide range of traits
  - Can be stored as detailed information on each sample – possible to retrospectively follow up on problems



REIMS = Rapid Evaporative Ionisation Mass Spectrometry



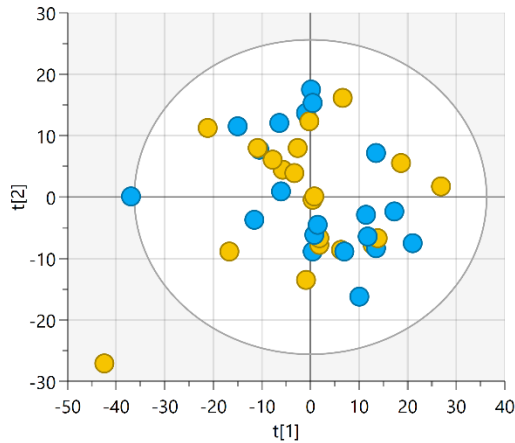
## Results: Muscles have different REIMS fingerprints, cluster based on fibre type



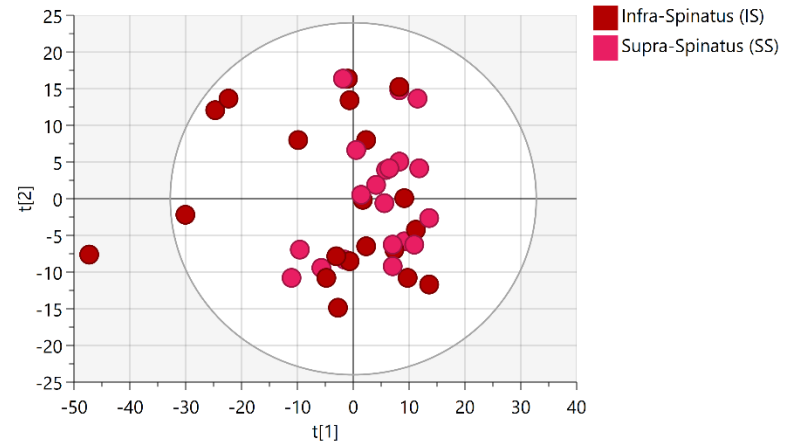
proportionally to R2X, R2X[1] = 0.101, R2X[2] = 0.0539,  
∴ Hotelling's T2 (95%)

Exercised lambs, OPLS-DA plot

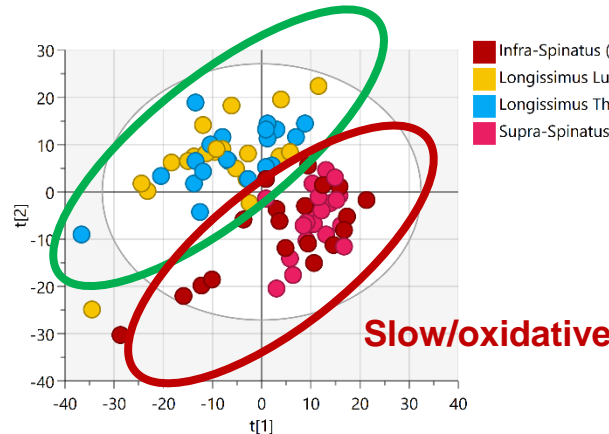
# Results: Similar muscles are not readily distinguishable



= 0.224, R2X[2] = 0.112, Ellipse: t



= 0.0985, Ellipse: Hotelling's T2 (95%)



Fast/ glycolytic

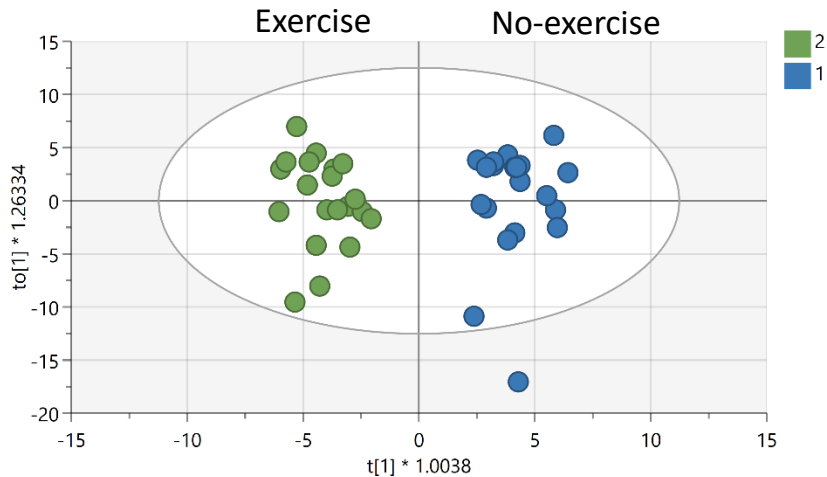
Slow/oxidative

= 0.189, R2X[2] = 0.132, Ellipse: Hotelling's T2 (95%)

**But are readily distinguishable between muscle types**

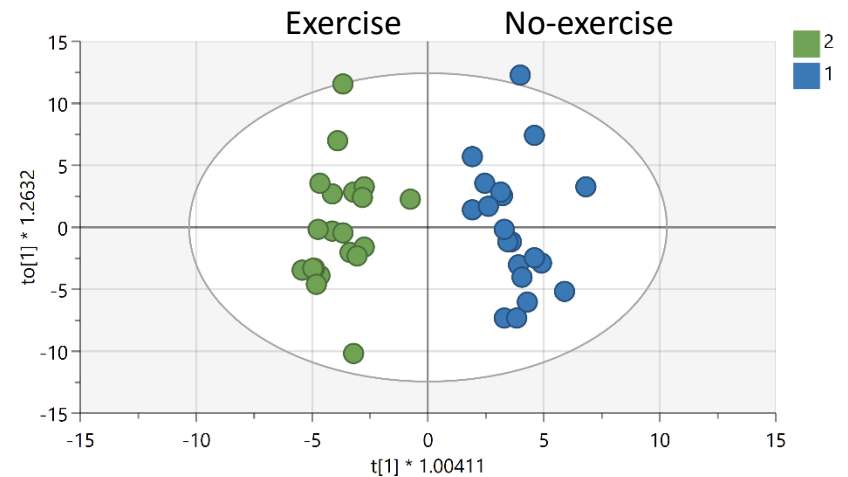
PCA plots of muscles, no feature reduction

# Results: Exercise stress is reflected in muscle fingerprints



Scaled proportionally to R2X, R2X[1] = 0.144, R2Xo[1] = 0.179,  
Ellipse: Hotelling's T2 (95%)

**Slow/oxidative**



Scaled proportionally to R2X, R2X[1] = 0.139, R2Xo[1] = 0.202,  
Ellipse: Hotelling's T2 (95%)

**Fast/glycolytic**

OPLS-DA score plots on feature reduced data, R2X and Q2>0.5





# Conclusion

- The exercise-stress model successfully led to **increased the pH of lamb meat**, and replicated several of the negative impacts of high pH in meat
- REIMS has potential for **detecting markers (metabolite fingerprints)** relevant to meat quality at a speed consistent with lamb processing, able to distinguish between muscles of lambs that have been exercised pre-slaughter compared to those who have not.
- In this work we demonstrate that REIMS is able to distinguish between different muscles, and that this could be related to muscle fibre type.
- In future, biomarkers of high pH in the meat could be used for enhancing animal welfare and confirm the relationship between minimising pre-slaughter stress and improving the consistency of meat quality.



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