Circadian clocks and exercise; Does time of training matter?

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19th November 2025

Red Roses Research Academic Meeting

Where opportunity creates success

Outline of today's talk

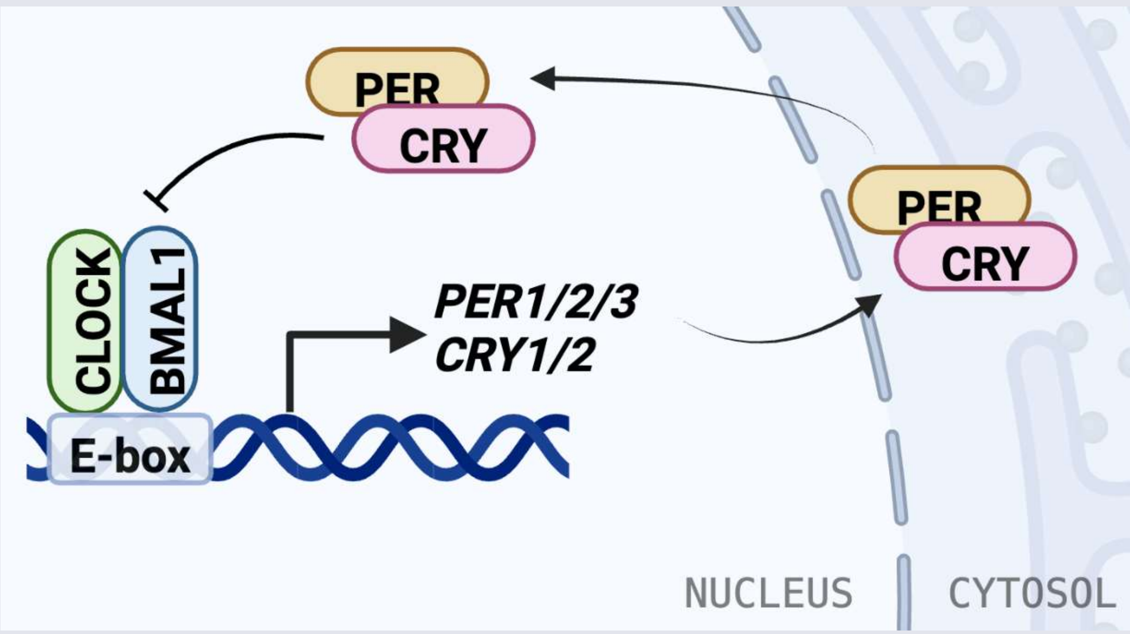
Two short stories

1. Very brief primer on circadian clocks in skeletal muscle and how time of exercise can modify the clock settings or phase

2. Can time of run training elicit different performance outcomes and improve health?

The molecular clock

The circadian clock is a ~24 h transcription: translation feedback loop found in all cells



Simplified cartoon

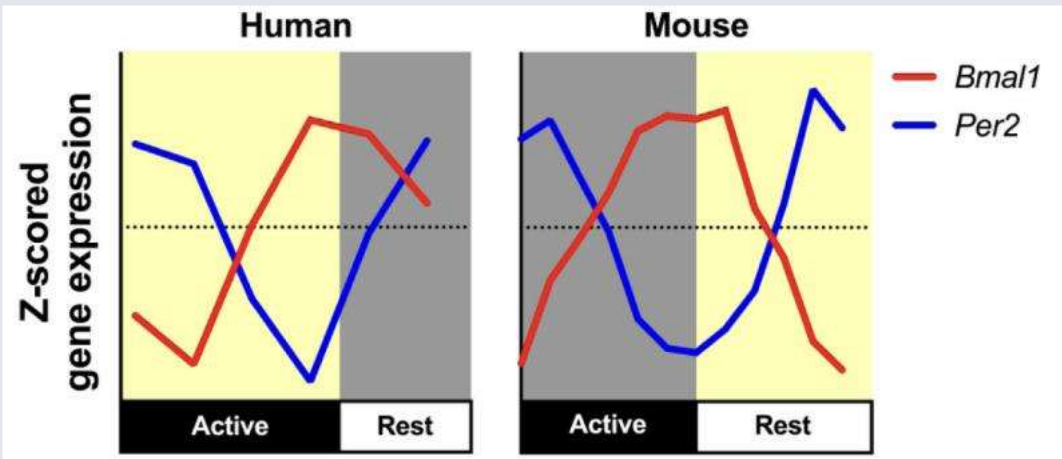
Positive Limb
BMAL1:CLOCK

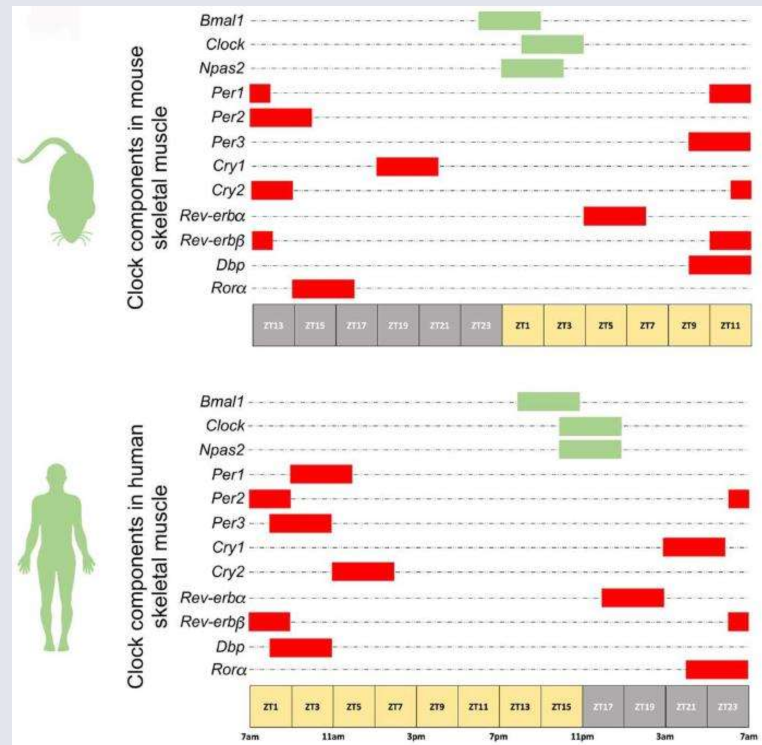
Negative Limb
PER1/2
CRY1/2

Clock is self-sustaining

The mouse and human muscle clock mechanisms are the same

Think about the day as **rest: active** periods and not light: dark



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Ticking for Metabolic Health: The Skeletal-Muscle Clocks

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Why does timing of the muscle clock matter?

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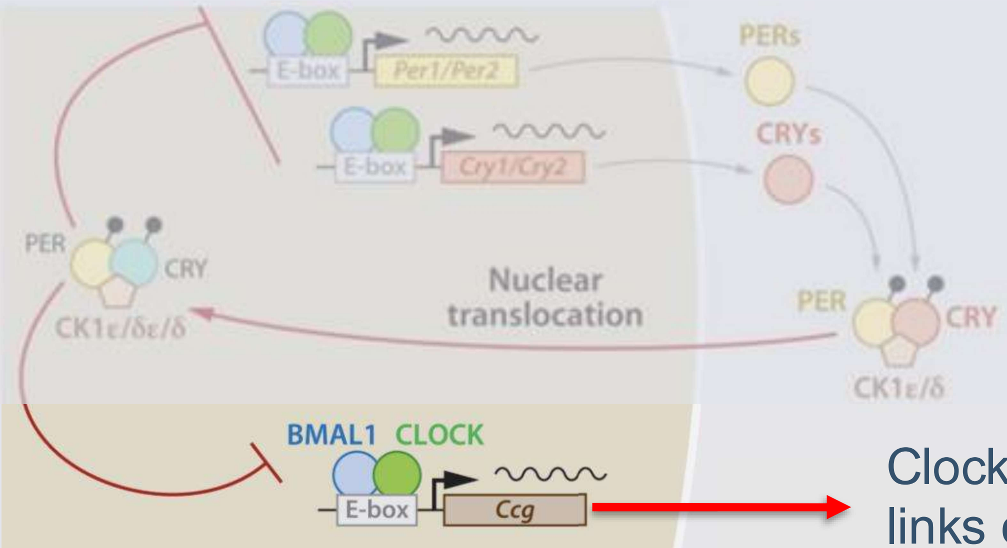
Timing of <u>clock output</u>



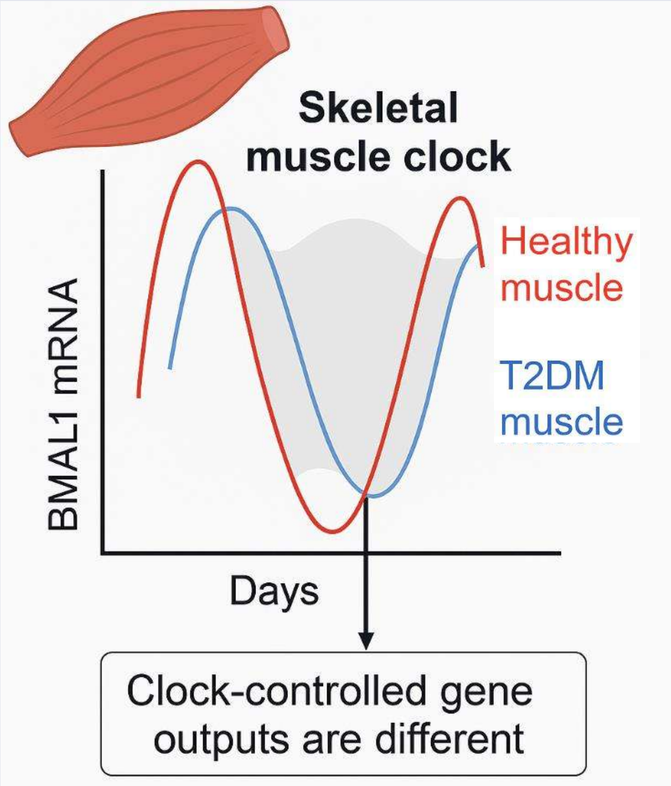
Adapted from Mohawk et al, (2012). *Annu. Rev. Neurosci.*

Core clock mechanism
daily timer

Clock output > 1000 genes:
links clock timing to physiology

Clock output is a temporal program of gene expression that contributes to time-of-day cell physiology

Mal-adaptive/ Misalignment in metabolic associated diseases e.g., T2DM



Healthy muscle

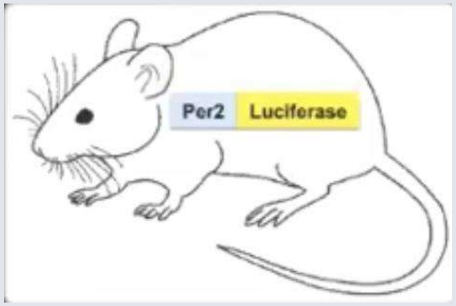
- Robust, predictable rhythm
- Rhythmic mitochondrial genes (NDUFB7, COX10, MFN2)
- Mito OCR oscillations
- Insulin sensitive
- Metabolic flexibility

T2DM muscle

- Reduced rhythmicity & amplitude (BMAL1/CLOCK, PER/CRY)
- Loss of OCR rhythmicity
- Insulin in-sensitive
- Metabolic in-flexibility

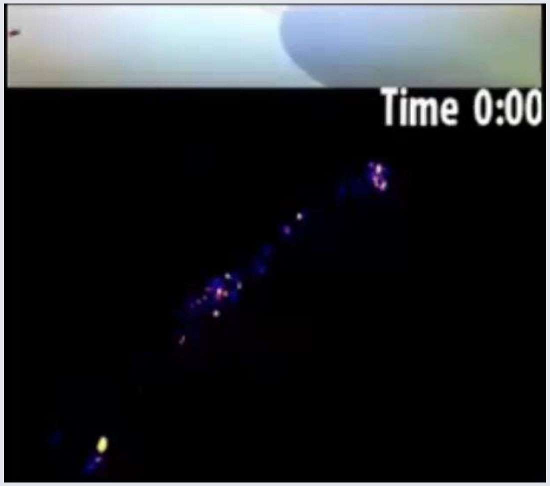
Adapted from Gabriel et al, (2021). *Sci Adv*.; Hesketh, (2025). *Obesities*

So, how do we measure circadian phase?
…isn't the brain needed?



Circadian reporter mouse
Yoo et al, (2004). *PNAS*

Tissue explants +
Luciferin



PERIOD2::LUCIFERASE
FDB Moyfibre
DMEM + 1mM Luciferin
Imaged via 20x objective

Does the muscle clock adjust to scheduled running?

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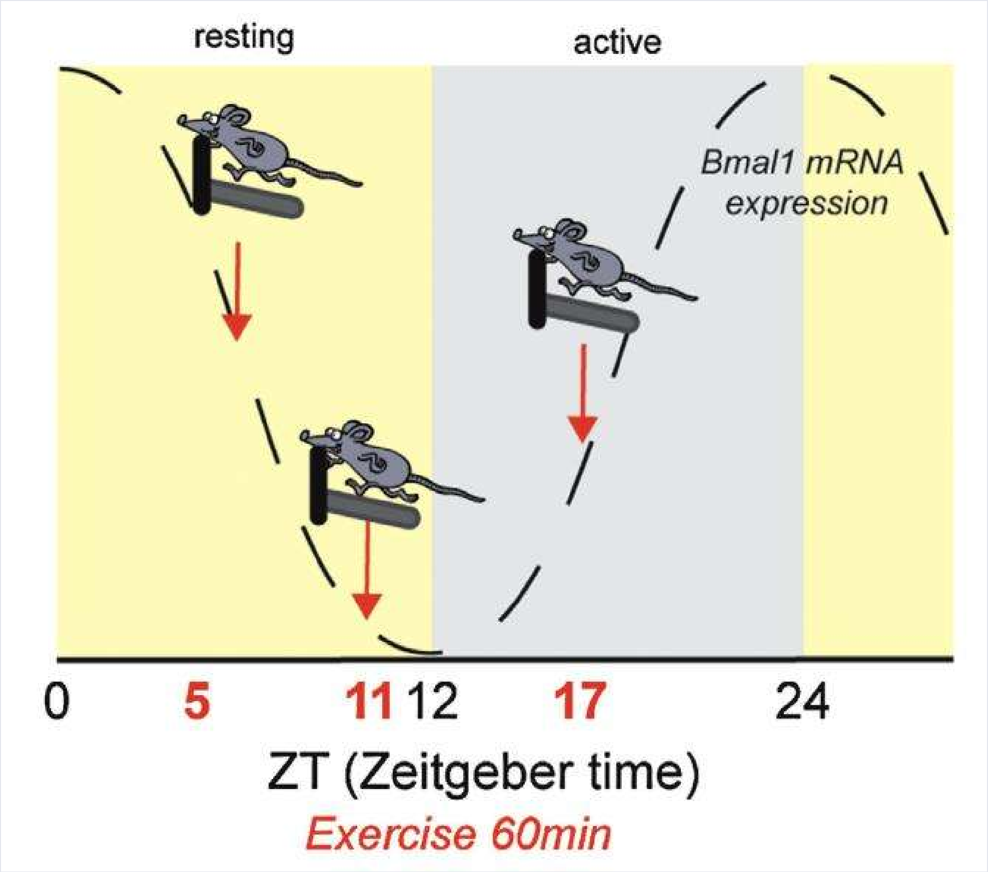

Kemler et al, (2020). *J Physiol*

An acute bout of running does shift the muscle clock

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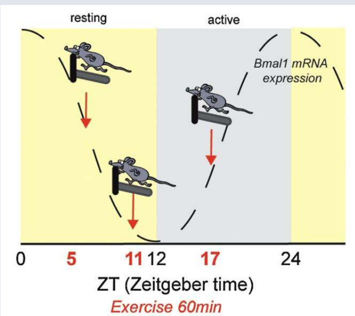
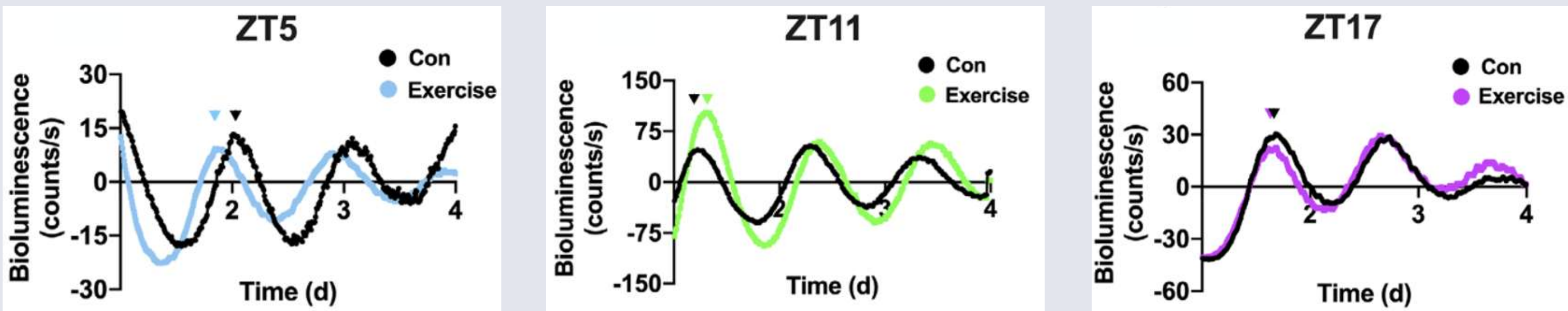


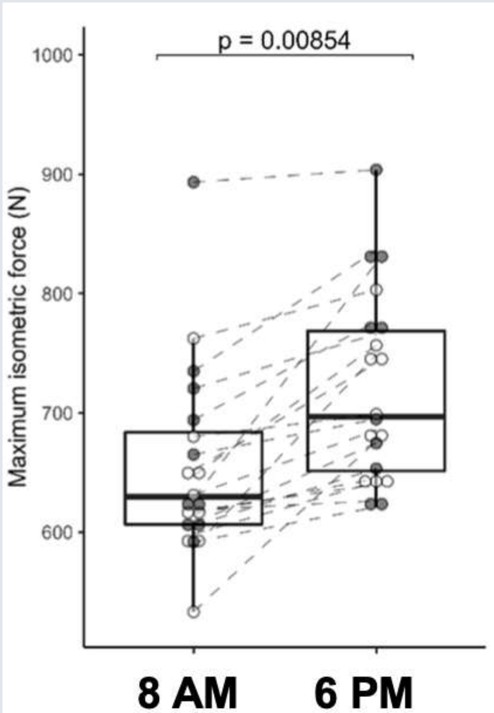
Depending on time of running: ~90 min phase advance, phase delay, and no change.



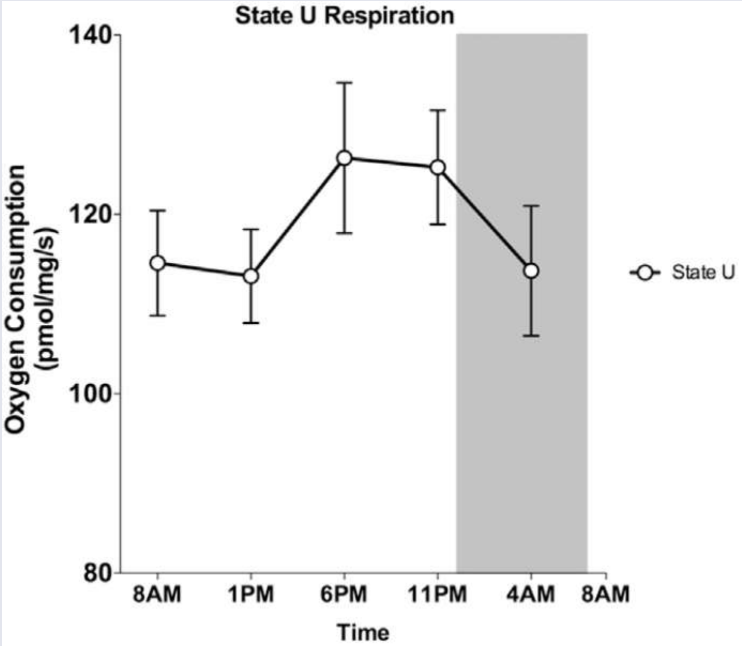
Evidence that time of exercise can function as a muscle clock 'time setter' or zeitgeber.

Two examples of time-of-day muscle physiology

Over 20 years of observations – Muscle strength and mitochondrial function are higher in the afternoon compared to morning



Ab Malik et al, (2020). *Proteomes*



Van Moorsel et al, (2016). *Mol Metab*

It is well established that pre-clinical models exhibit significantly higher running time in the afternoon. Can we quantify endurance performance?

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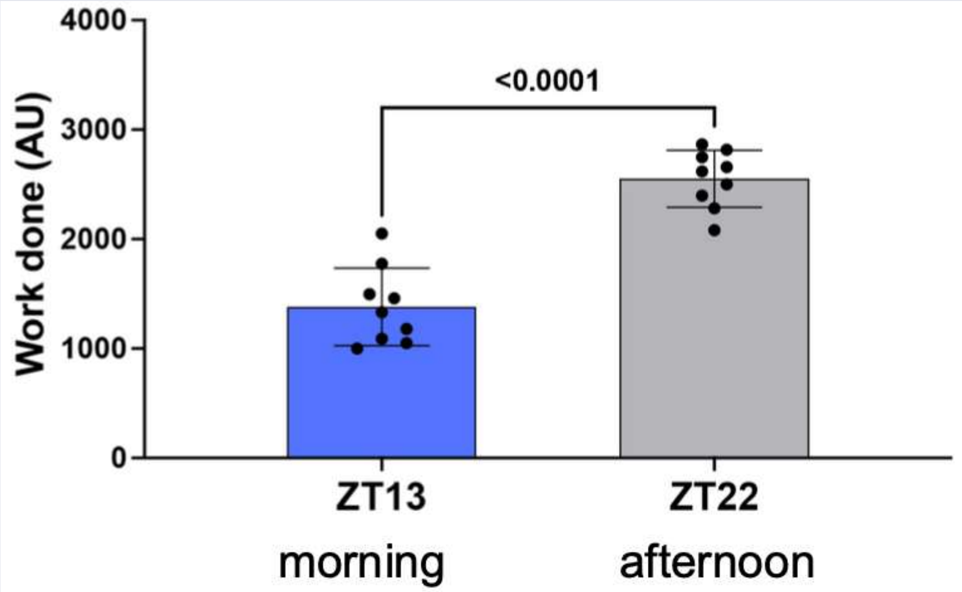
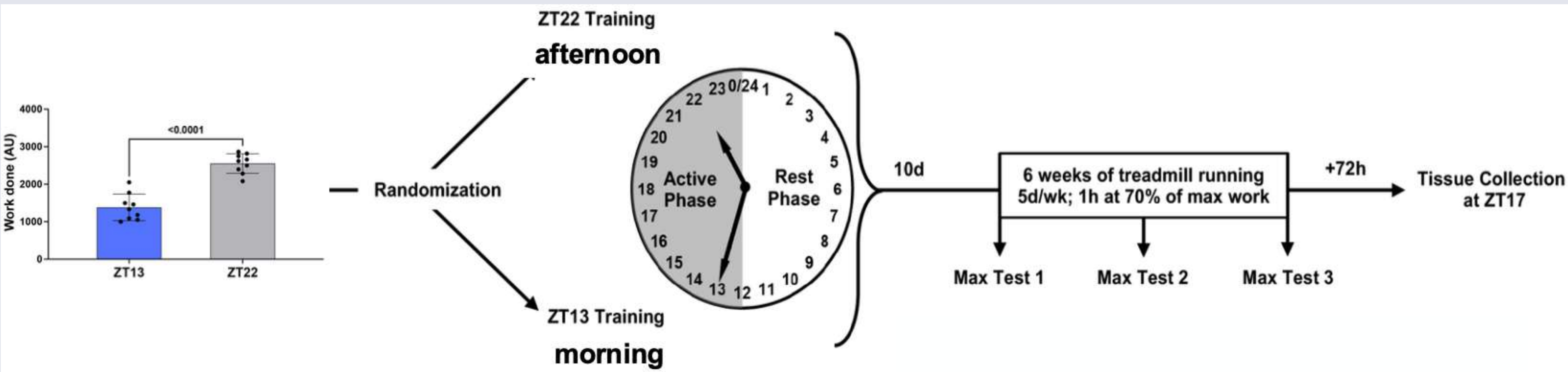
Work done = speed x grade x time x body weight



We asked: if muscle clocks are 'movable', can we eliminate this performance difference with time of training?

Hesketh et al, (2025). *Am J App Physiol*

Does time-of-day training
a) improve performance but maintain the time-of-day difference, or
b) diminish the difference, which implies differential adaptations for TOD?



Standardised treadmill training

- Duration, 1 hr/day 5 d/week
- Intensity 70% max work
- Intensity adjusted at 3 weeks
- <u>Mice run at same relative workload</u>

We documented that the same relative training elicited the same blood lactate responses in the morning and afternoon

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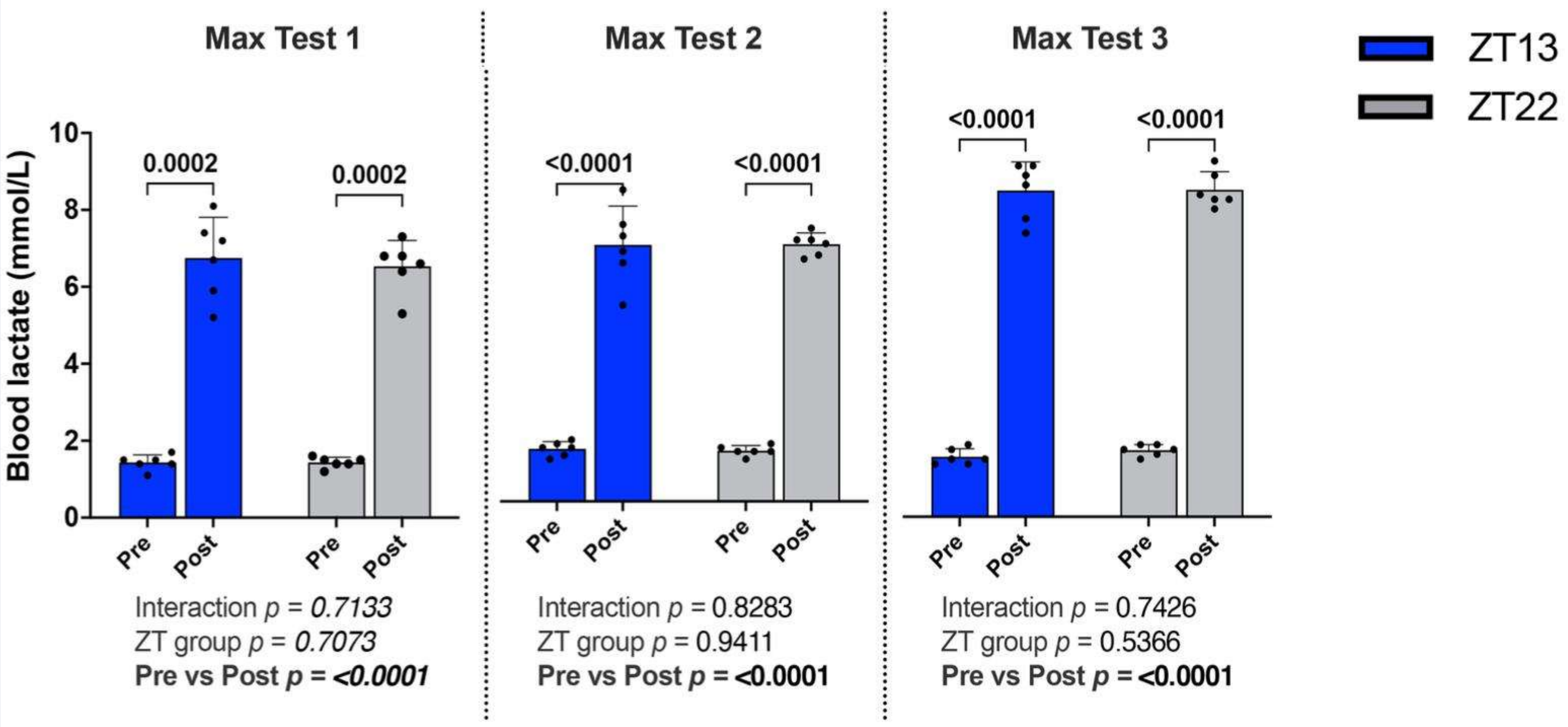
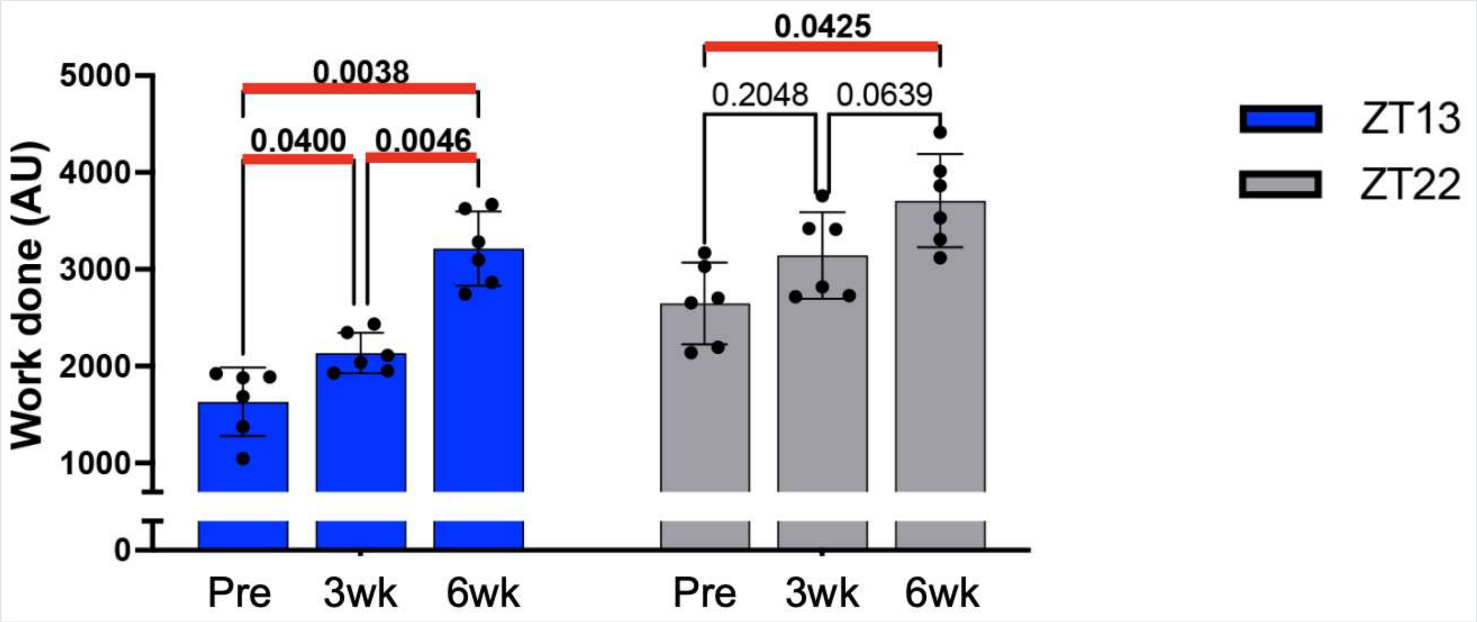


As expected, all mice improved performance with training

However, morning runners significantly improved at each stage while afternoon runners only showed a statistical difference comparing start to finish



Morning Afternoon

Morning and afternoon performances were all different at 3 weeks

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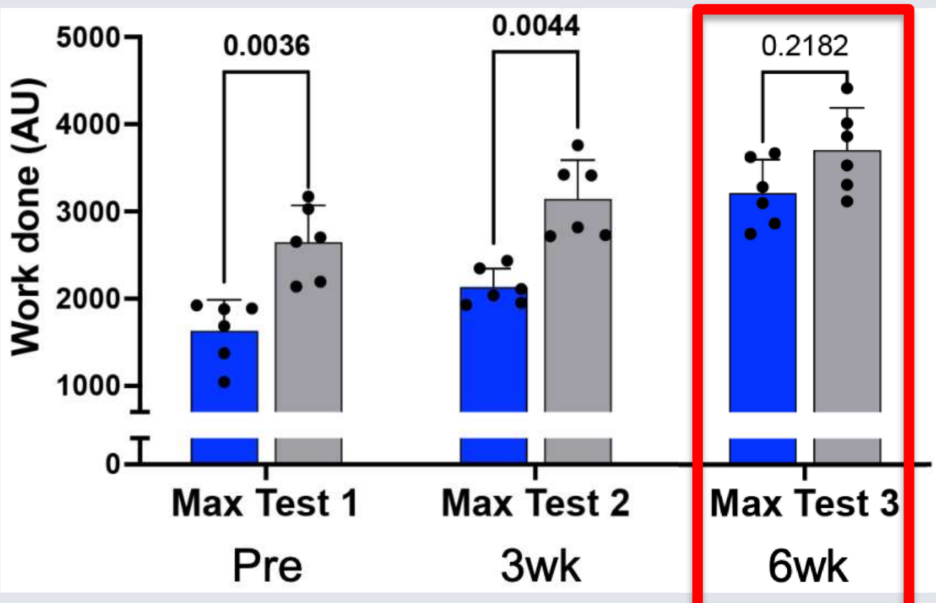
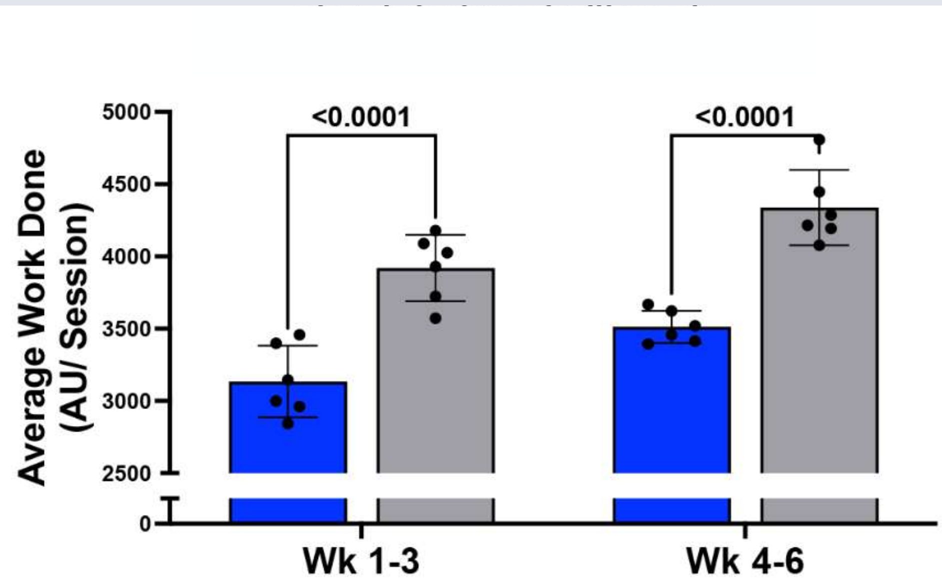
However, by 6 weeks of TOD training there is no difference between the groups

Between Group Comparison



Morning runners caught up even though they did significantly less absolute treadmill work



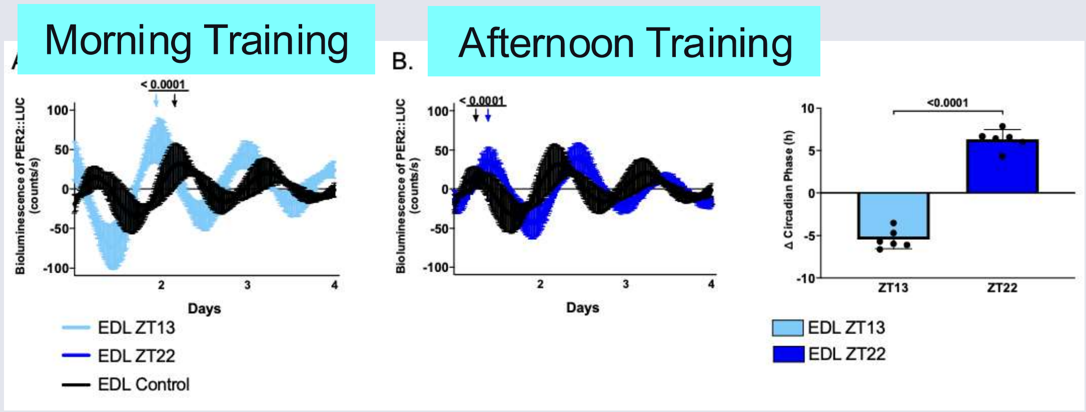
How or Why?

What is different in the early vs late runners?

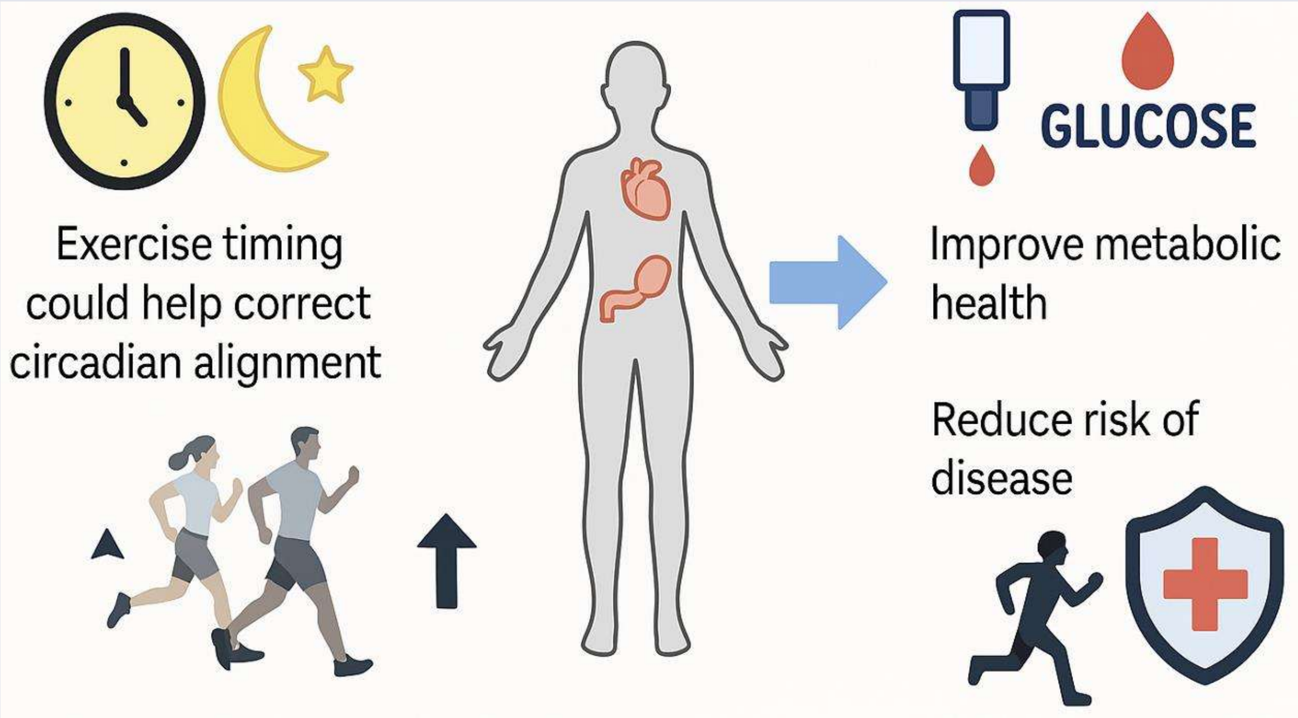
PER2::LUC morning vs evening TOD run training in skeletal muscle

~3-5 h phase advance for morning (ZT13) training

~3-5 h phase delay for afternoon (ZT22) training



Clinical implication



- Exercise timing to correct misalignment e.g., shift workers, jet lag etc.
- Shift to improve metabolic health, or reduce disease risk
- Personalised chronotherapy based on different phase = different physiological effects.

Time-of-day training take home points
Much work to be done…

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- For acute performance, afternoon running elicits a greater maximum treadmill performance compared to morning in mice: consistent with human data.

- Both morning and afternoon training lead to performance adaptations as expected: consistent with human studies.

- The new finding is that morning training led to enhanced performance adaptation compared to afternoon training: not apparent until 6 weeks
    - **6 weeks of morning training results in phase advance**
    - **6 weeks of afternoon training results in phase delay**

- New finding, highlights potential of personalised, chronobiology-informed training programs to improve adaptations to exercise and/or be used to prevent or treat metabolic conditions/diseases.

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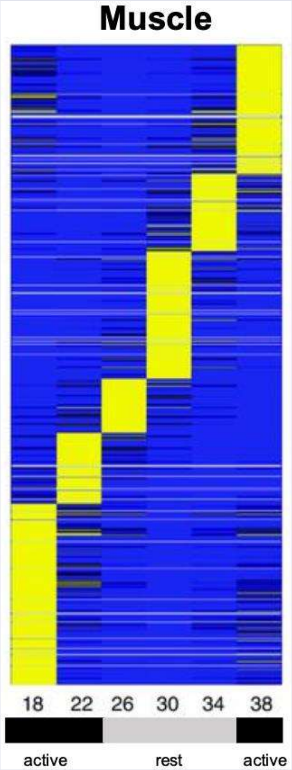
[redacted]

Clock output in skeletal muscle includes > 1200 genes - Similar between mouse and human



Clock output contributes to following categories:

Transcription, e.g. MyoD1

Ubiquitin conjugation, e.g. Atrogin

Alterative splicing, e.g. Rbm20

Nutrient signaling, e.g. Tbc1d1

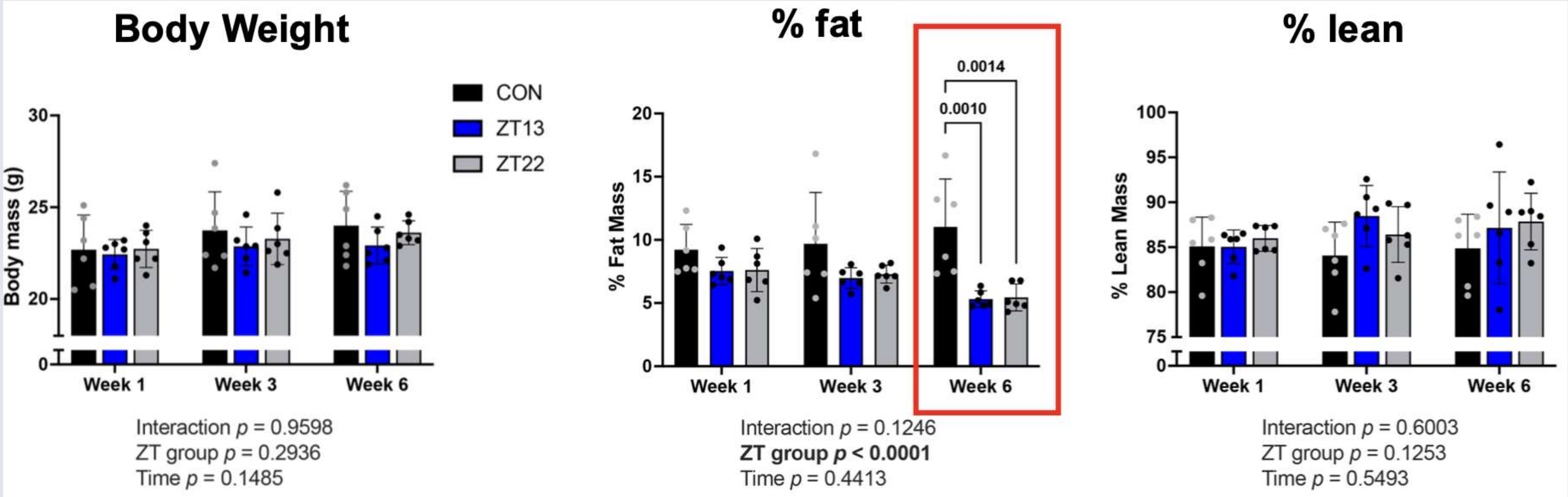
Mitochondria, e.g. Opa1, Pdk4

Glucose metabolism, e.g. Glut4

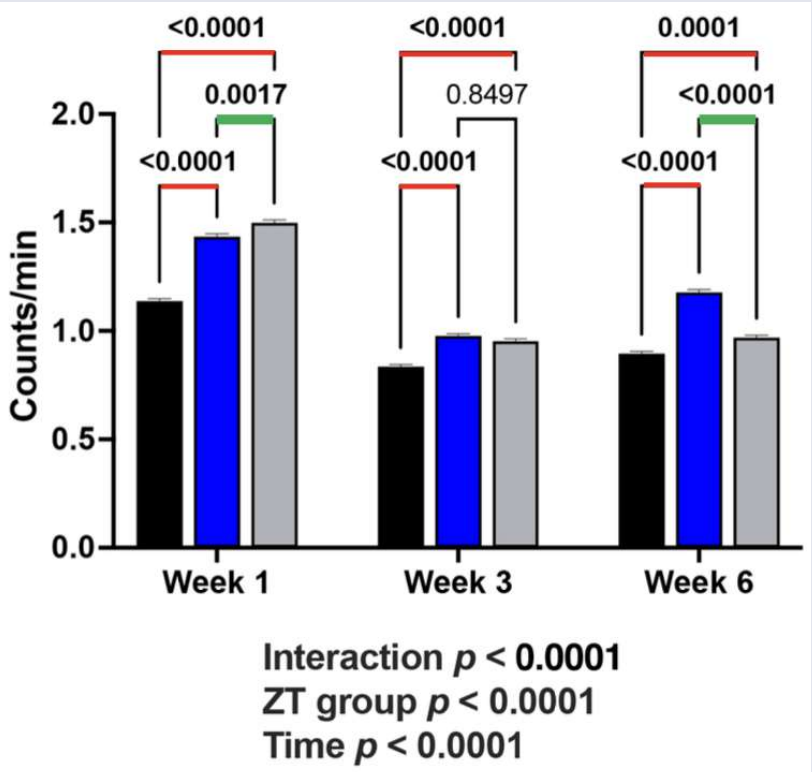
Z-disc structure, e.g. Tcap

Many genes with links to muscle metabolism and exercise

No difference in body weight or body composition between runners.
Runners vs. Sed controls: decline in body fat % by 6 weeks.

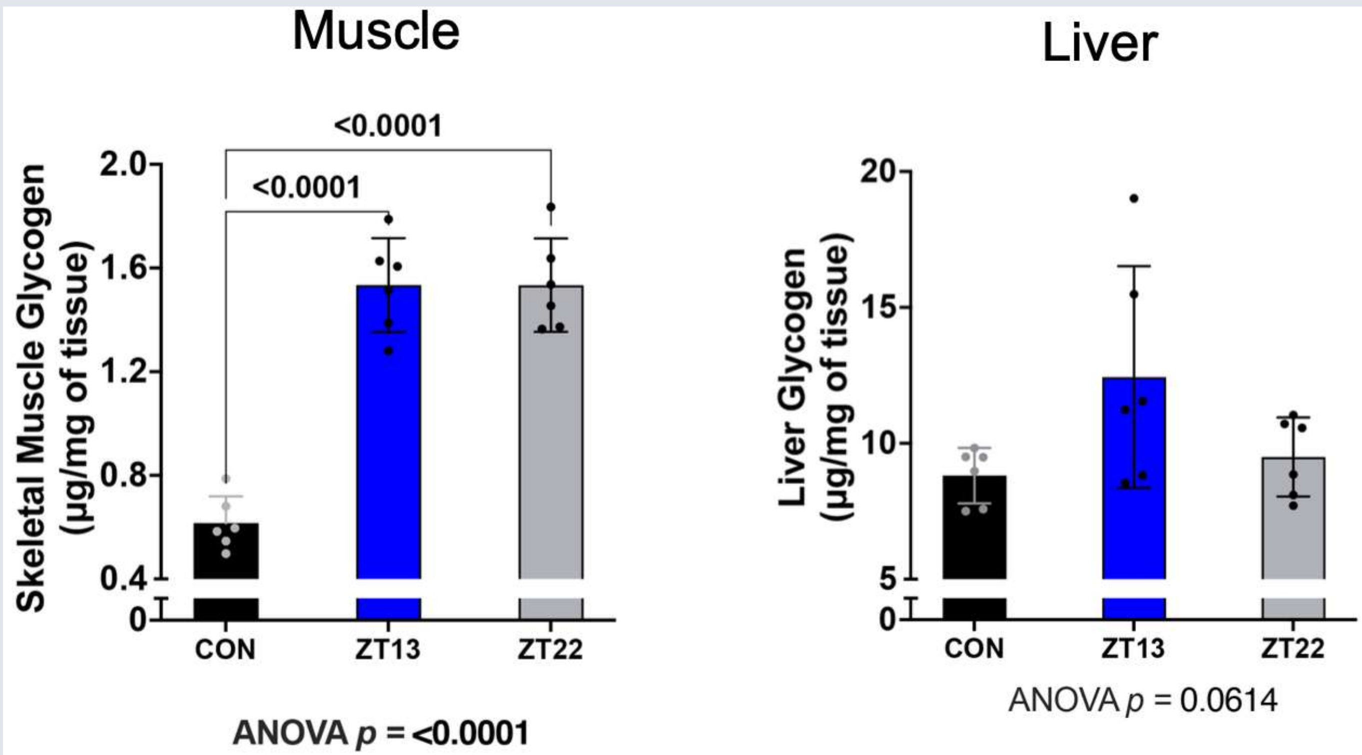


Treadmill training was associated with increased home cage activity levels for all runners.
At week 1, afternoon runners were more active but by week 6, morning runners were more active.



Interesting to note that 1 h of training did change mouse activity behavior

Glycogen storage: Muscle increased the same in both training groups.
We did not detect a change in liver glycogen, but maybe under-powered.



We did determine muscle citrate synthase activity was significantly higher in the morning vs. afternoon training or sed groups. No changes in COX IV or VDAC (mito content).

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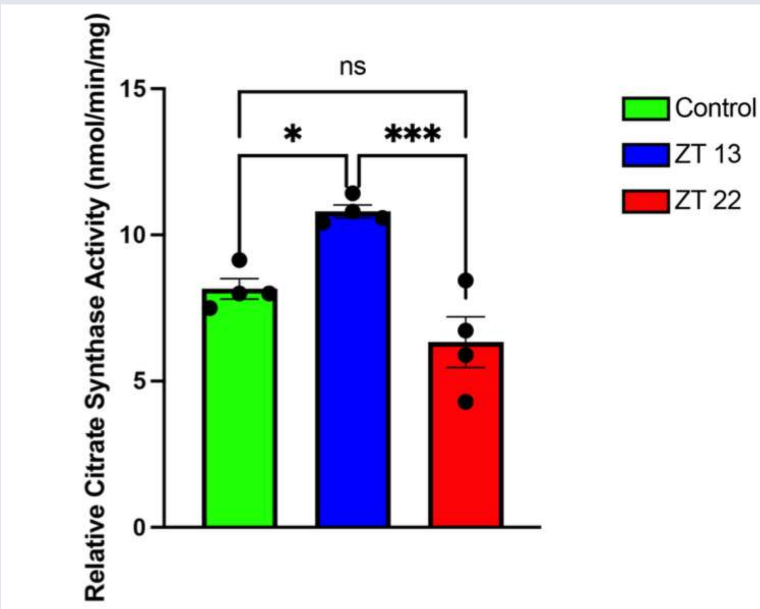
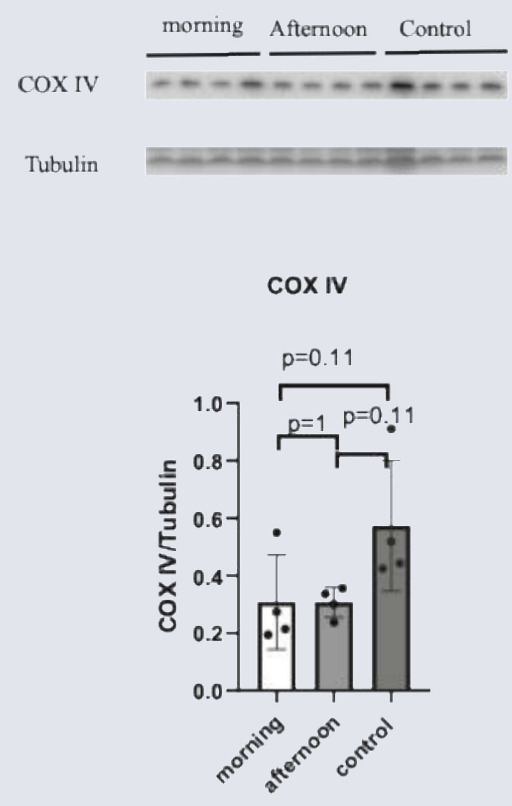
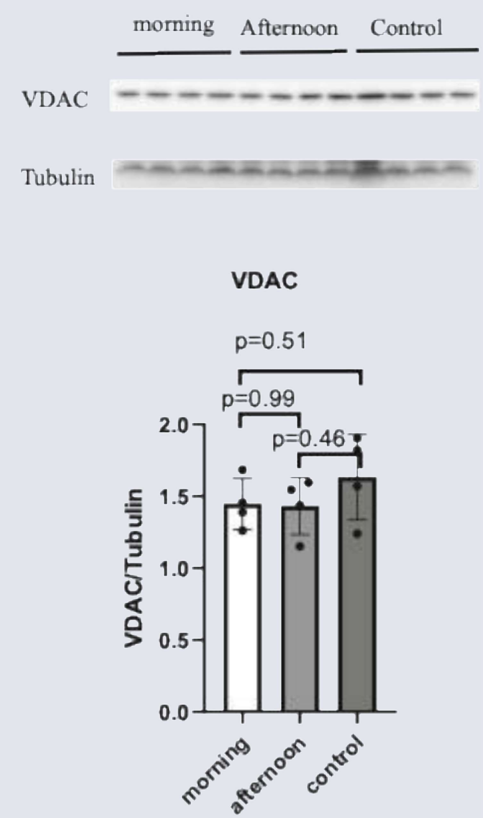
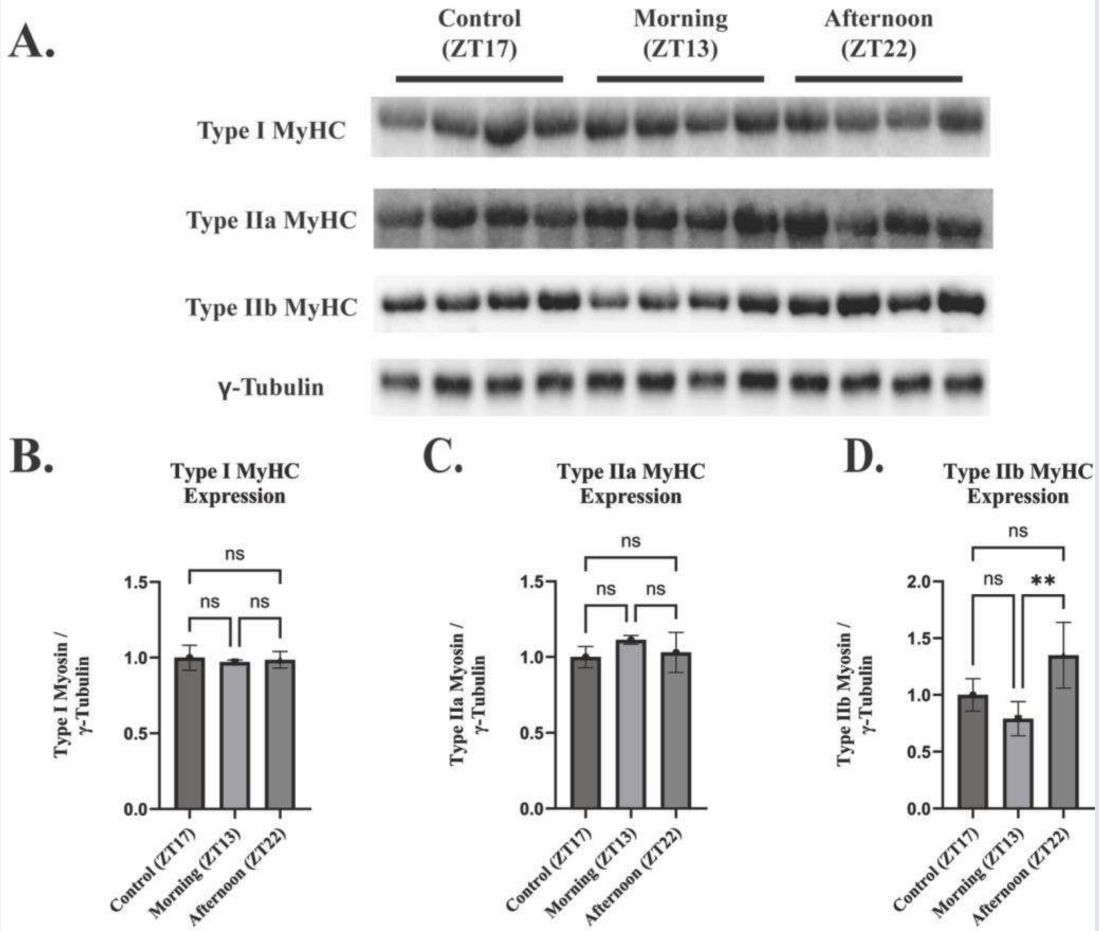


Enzyme activity is changed but not amount of CS protein





No change across MyHC profile with TOD treadmill training (GAS-data not shown), but decreases in IIb expression in morning runners from Quad



What happens when we train for longer durations?

- 4 weeks of treadmill or voluntary wheel run training can cause a shift in the muscle and lung clocks.

- Taken 48 h after last bout; shift is 3-4 h.

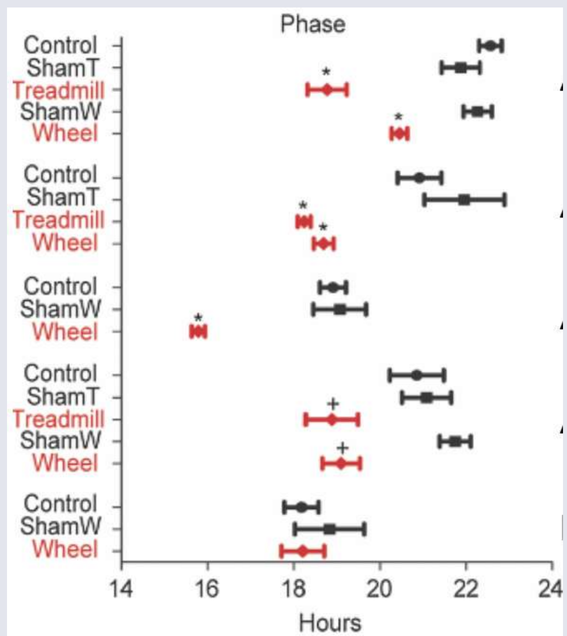
Soleus — Advance 3-4 h

EDL — Advance 4 h

FDB — Advance 4 h

Lung — Advance 3 h

SCN — No change



Wolff & Esser, (2012). *Med Sci Sports Exerc*

PER2::LUC morning vs evening TOD run training on lung and WAT

No phase change for morning (ZT13) training

~3-5 h phase delay for afternoon (ZT22) training