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Meta-analysis of targeted metabolomic studies in equids with insulin dysregulation

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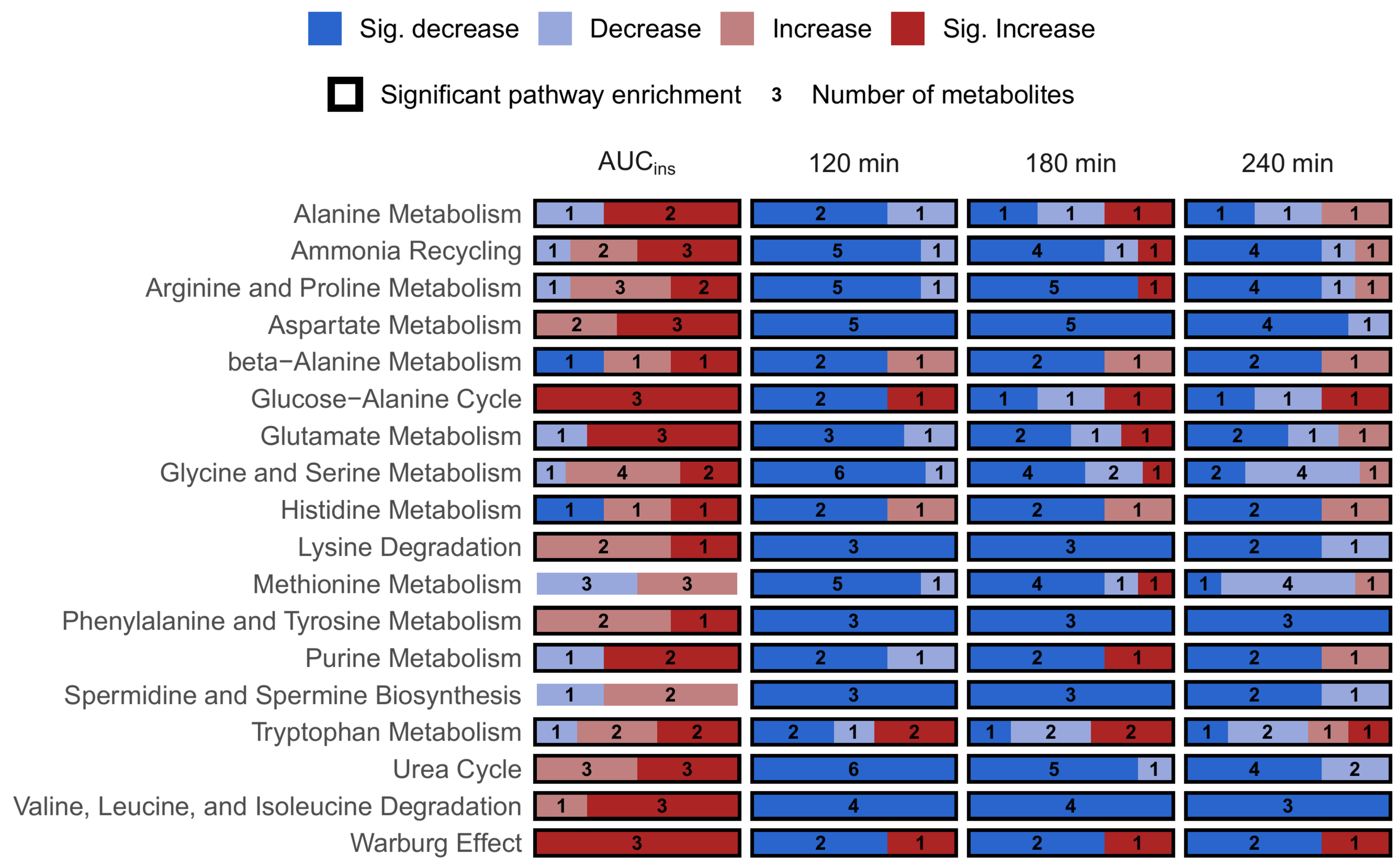
Introduction

- Insulin dysregulation (ID) is associated with hyperinsulinemic episodes [1]. Since insulin has a profound effect on the metabolism, metabolomic studies are useful in investigating the metabolic effects associated with ID [2].
- Metabolomic studies rely on various, but generally costly, methods. They therefore often only include few individuals.
- Meta-analyses can be used to increase the statistical power of experiments with small samples sizes and evaluate the reliability and reproducibility of the results.
- ➢ The objective of this study was to perform a meta-analysis of targeted metabolomic experiments in equids with and without ID undergoing an oral glucose test (OGT).

Methods

- On the 10th of September 2025, PubMed was searched with following keywords: *(metabolomic OR metabolome) (horse OR pony OR ponies) (“insulin dysregulation” OR “insulin resistance” OR “equine metabolic syndrome” OR laminitis OR endocrine) NOT (“non-targeted” OR untargeted OR NMR OR “nuclear magnetic” OR Raman)*
- Six datasets available to the authors were considered in addition to the records found on PubMed. A total of 38 records were screened for inclusion.
- The six retained datasets (**Table 1**) were normalized by Box-Cox power transformation, scaled, and merged for analysis with linear models accounting for repeated measures and batch effect where necessary.

Results

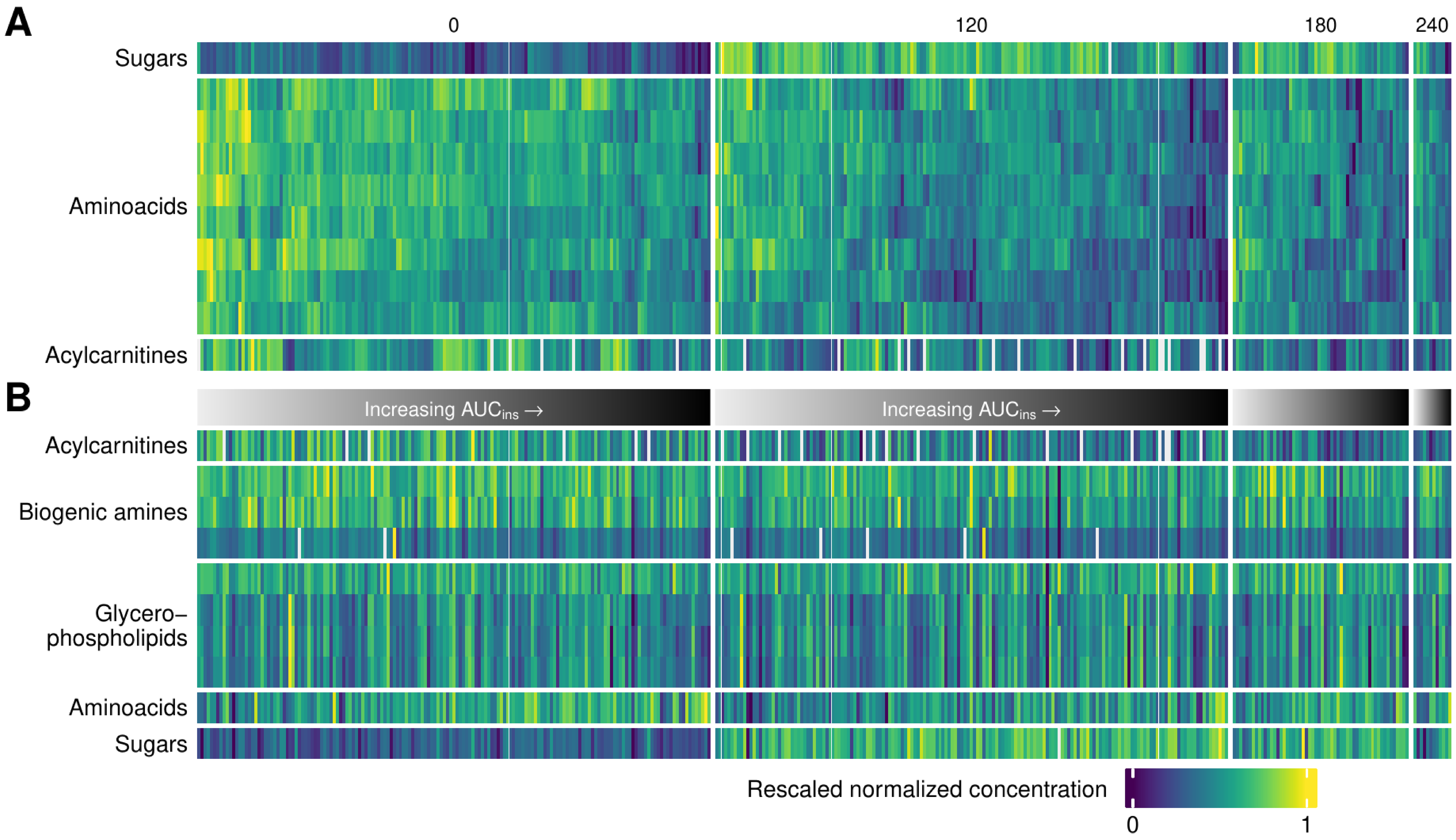


▼ **Table 1 |** Detail of the studies included in the analysis.

Study descriptor	n	Experimental conditions
Kenéz et al., 2018 [3]	20	Basal samples of fasted horses
	20	120 min OGT samples
	20	180 min OGT samples
dlr-trh (unpublished)	12	Basal samples of fasted horses
	12	10 min TRH-stimulation-test samples
Delarocque et al., 2020 [4]	36	Basal samples of fasted horses
	36	120 min OGT samples
	36	180 min OGT samples
dlr-pasture (unpublished)	12	0 min after 1 hour on pasture
	12	240 min after 1 hour on pasture
Delarocque et al., 2021a [5]	95	Basal samples of fasted horses
	95	120 min OGT samples
Delarocque et al., 2021b [6]	10	Basal samples of fasted ponies
	20	90 min samples of dietary challenge
frers-ogt (manuscript under preparation)	12	Basal samples of fasted horses
	12	120 min OGT samples
	12	240 min OGT samples

◀ **Figure 1 |** Metabolite set enrichment analysis reveals consistent changes over time, characterized by a decrease in metabolite concentrations across most pathways. In contrast, higher insulin responses are associated with higher metabolite concentrations, suggesting a blunted insulin response in certain highly metabolically active organs.

▼ **Figure 2 |** Heatmaps of the top 10 metabolites associated with time (**A**) and total insulin response (AUC_{ins}) (**B**) during the OGT. Overall, the OGT results in a marked influx of amino acids. However, individuals with ID show a less pronounced influx of hexoses (H1), alanine, and serine. Two unshown metabolites positively associated with time are tryptophan and kynurenine (adj. p-value ≤ 0.003). Their ratio (Kyn:Trp) increases over time, indicating possible inflammation resulting from glucose administration via activation of the indole-amine 2,3-dioxygenase in the kynurenine pathway. Trans-4-hydroxyproline (t4-OH-Pro) is necessary for vitamin C synthesis. Thus, the decreased t4-OH-Pro concentrations in horses with high AUC_{ins} suggests a lower antioxidative capacity.



A

Metabolite	120 min	180 min	240 min	Adj. p−value
H1	**1.493**	**1.324**	0.491	< 0.001
Met	**−0.704**	**−0.927**	**−1.173**	< 0.001
Pro	**−0.752**	−0.643	**−0.968**	< 0.001
Phe	**−0.604**	**−0.689**	**−0.798**	< 0.001
Ile	**−0.895**	**−1.129**	**−1.035**	< 0.001
Lys	−0.646	−0.672	**−1.083**	< 0.001
Orn	**−0.760**	**−0.858**	**−1.074**	< 0.001
Glu	**−0.674**	−0.639	**−0.859**	< 0.001
Cit	**−0.877**	**−0.902**	−0.746	< 0.001
C2	**−0.741**	**−0.959**	**−1.118**	< 0.001

B

Metabolite	logFC	Adj. p−value
C2	**−0.221**	0.010
t4−OH−Pro	**−0.189**	0.010
ADMA	**−0.200**	0.010
SDMA	**−0.229**	0.006
PC aa C32:0	**−0.268**	0.005
PC ae C38:6	**−0.205**	0.010
PC ae C36:5	**−0.252**	0.001
PC ae C36:4	**−0.200**	0.010
Ala	**0.225**	0.006
H1	**0.224**	0.001

Conclusions

The present results corroborate previous evidence that:

- ID is associated with a distinct metabolic signature beyond insulin levels [3-6]
- ID is likely associated with a reduced anti-oxidative capacity [3, 4]
- Glucose administration induces low-level inflammation [3, 4]
- Horses with ID have a blunted response to insulin in some highly metabollicaly active organs [5]

Inflammation, oxidative stress, and insulin resistance all constitute possible therapeutic targets.

References

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