Changes in net hepatic flux of nutrients by deacetylation of p-aminohippuric acid in dairy cows.

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Work associated to the REDNEX European project

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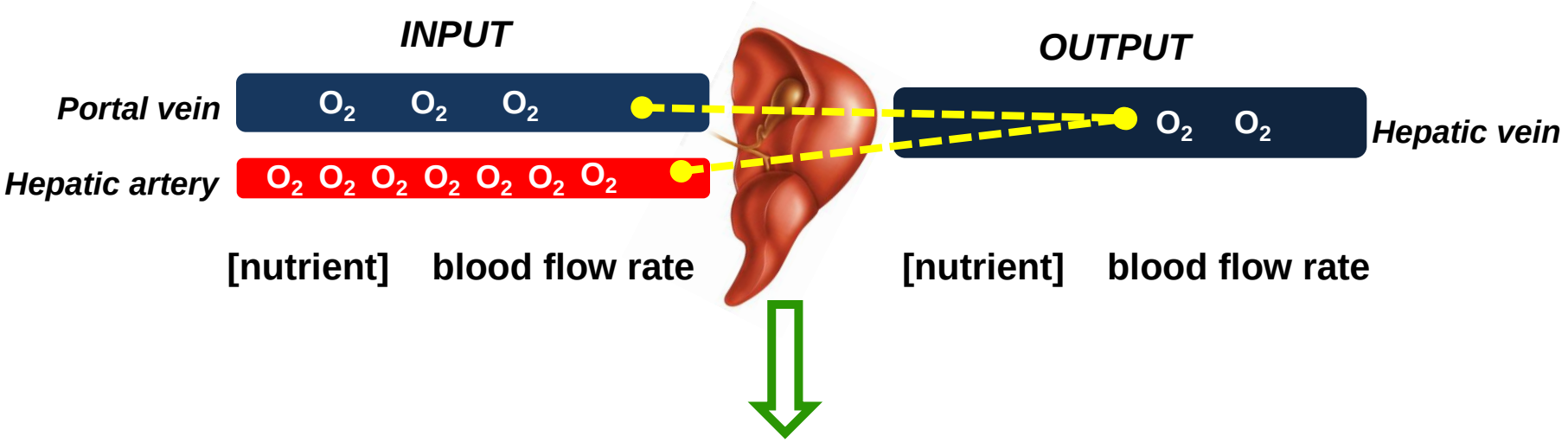
Study of the in vivo metabolism of nutrients in the liver

Arterio-venous difference method

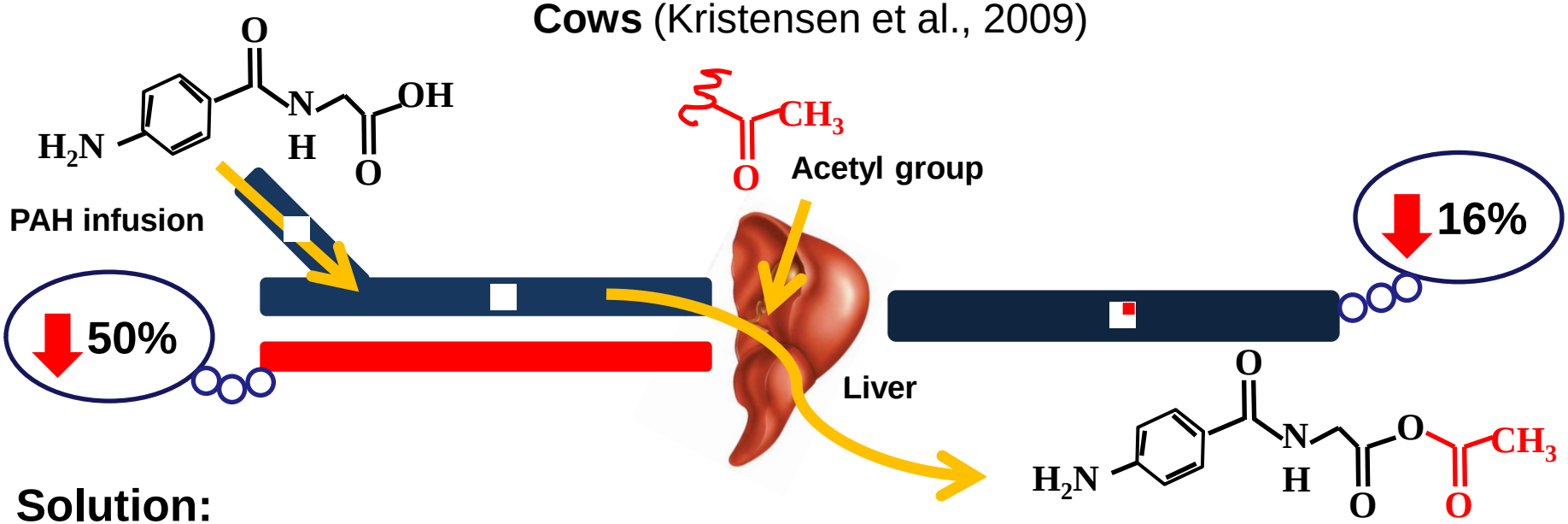


Net uptake or release = output – input

Dilution method with *p-amino*hippuric acid (PAH)

Ideal marker: homogenization in blood, no loss and an easy determination.

PAH hepatic acetylation: **Sheep** (Katz and Bergman, 1969) (Eisemann et al., 1987)
Cows (Kristensen et al., 2009)



Solution:

Inclusion of a deacetylation step

1st objective: PAH validation process

The accuracy profile (NF V03-110, 2010)

Accuracy		**Closeness of agreement between the accepted reference value and each value measured**
Trueness		**Closeness of agreement between the accepted reference value and an average value from several repetitions**
Precision:	**Repeatability**	**Same analytical procedure, same operator and equipment during a short interval of time**
	Intermediate precision	**Same analytical procedure, different operator or equipment during a more prolonged interval of time**

1st objective: PAH validation process

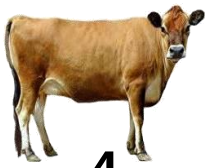
The accuracy profile (NF V03-110, 2010)

PAH concentration levels:
0, 2.5, 5, 10, 15, 20, 25 and 30 mg/l

3 repetitions per level for 5 days

With and Without the
PAH deacetylation step

2nd objective: effect of the PAH deacetylation on the net hepatic flux



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PAH infused into ruminal and mesenteric veins: 38 mmol/h, 6h

Blood collected from portal and hepatic vein and mesenteric artery (hourly samples over the last 5 hours).

Plasma PAH was measured by spectrophotometer (Hamburger et al., 1948)

With
Without **the PAH deacetylation step**

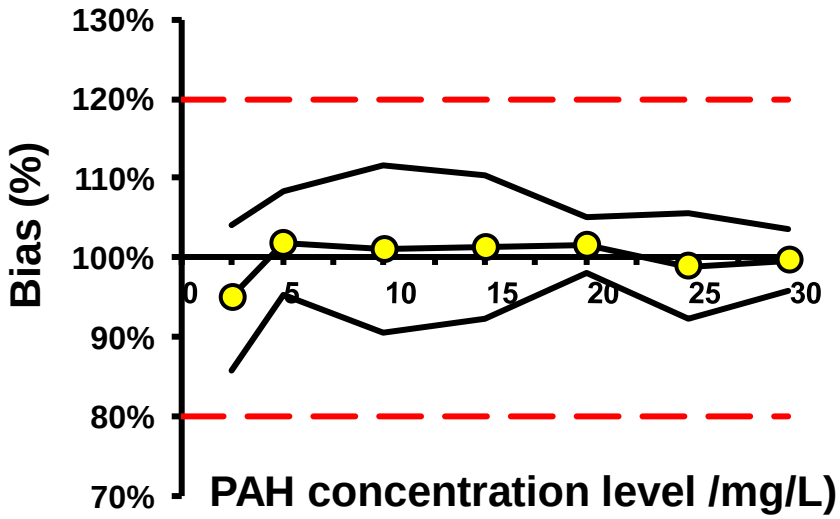
Heating cycle with HCl 5N at 90°C for 60 min (Isserty et al., 1998)

Plasma flow x **Nutrient concentrations** = **Net hepatic flux of nutrient**

Statistical analyses **Paired-t test**

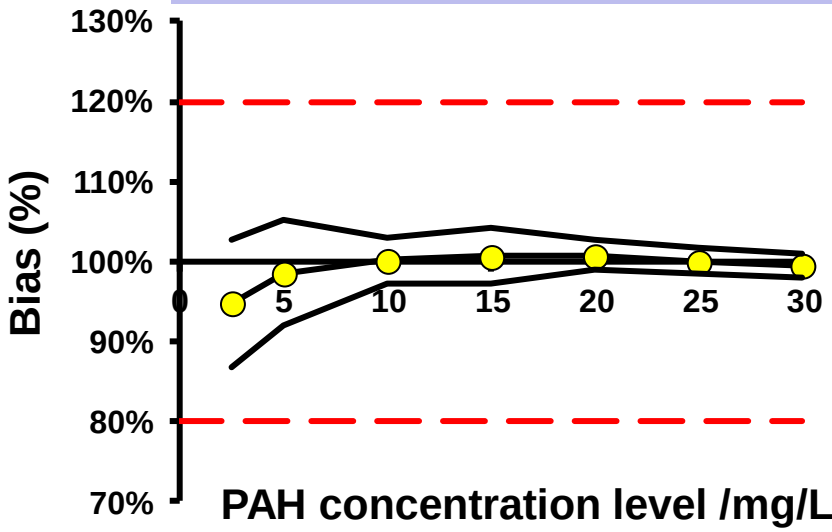
1st objective: PAH validation process

With PAH deacetylation



Trueness:	99.9%
SD Repeatability:	0.12
SD Intermediate precision:	0.33

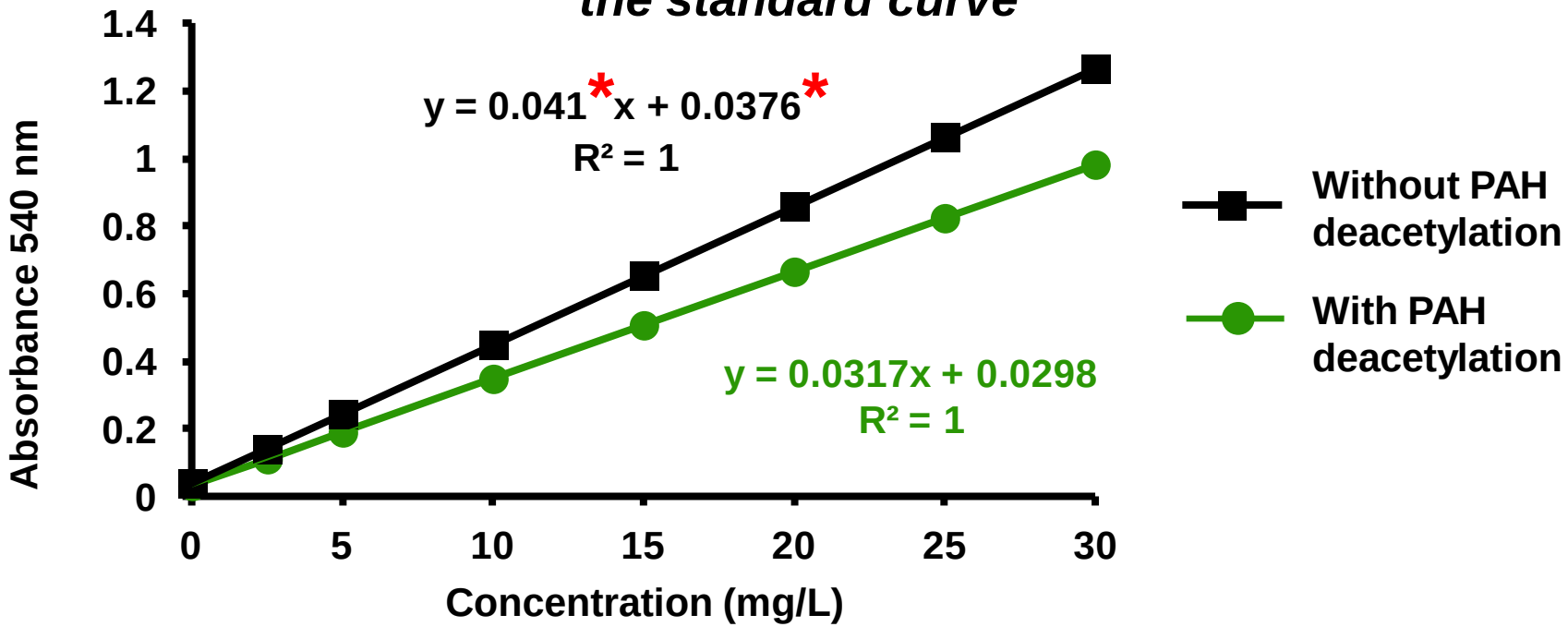
Without PAH deacetylation



Trueness:	99.2%
SD Repeatability:	0.16
SD Intermediate precision:	0.16

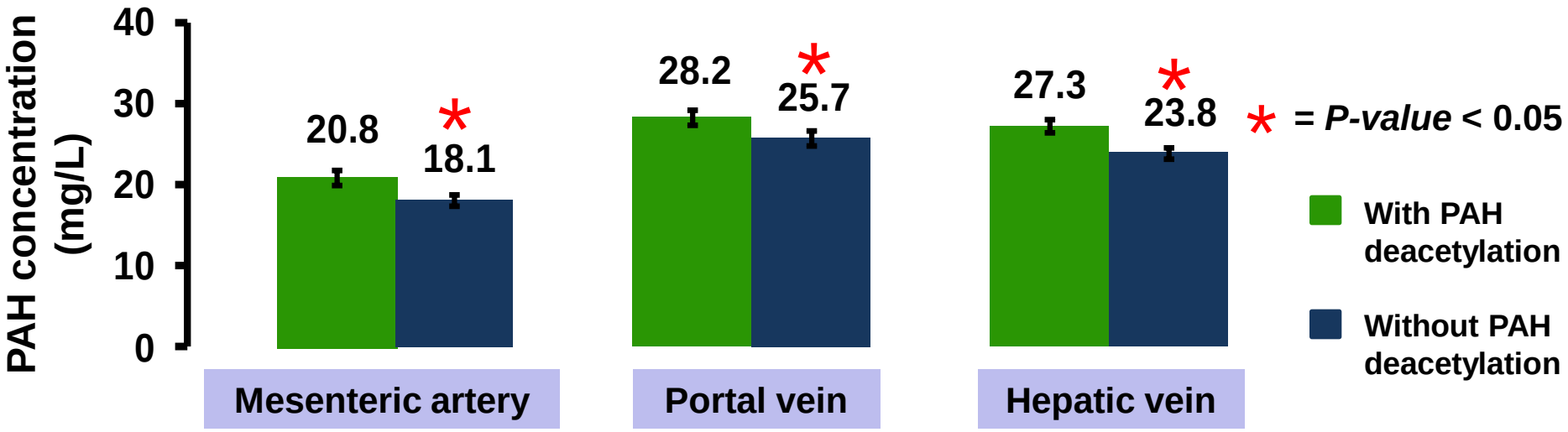
1st objective: PAH validation process

Effect of the inclusion of the PAH deacetylation step on the standard curve



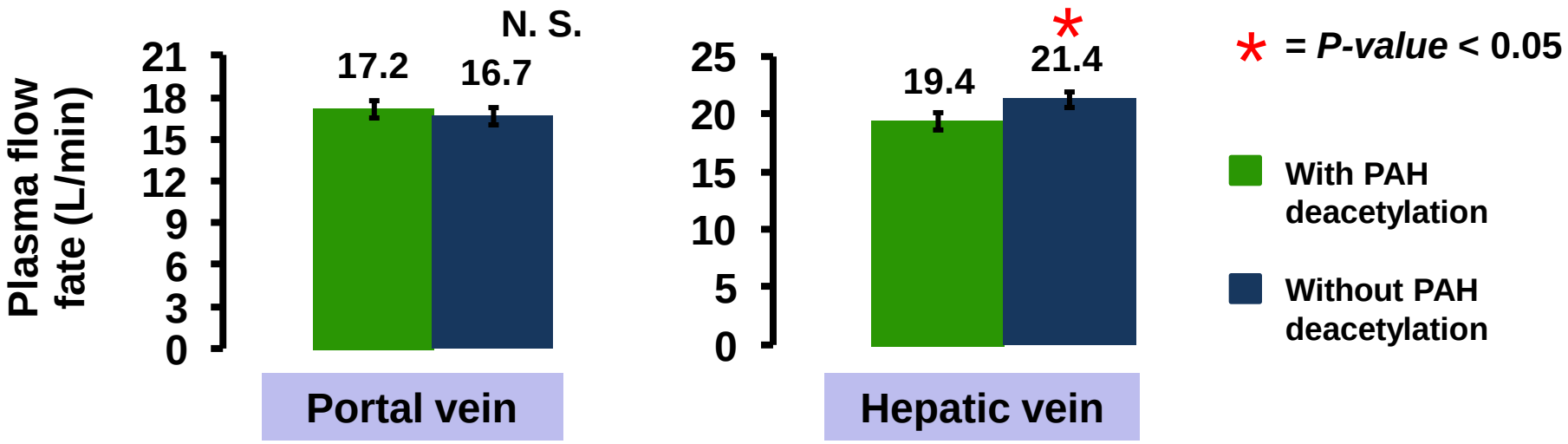
Slopes and y-intercepts were significantly different between methods.

2nd objective: effect of the PAH deacetylation on the plasma PAH concentration



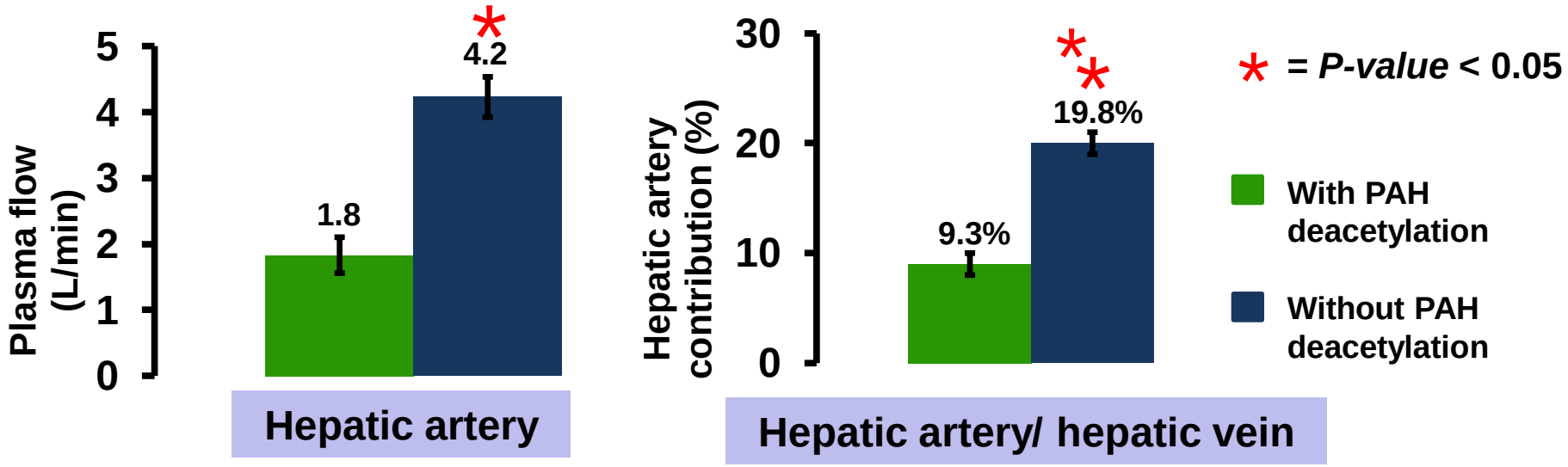
All PAH concentrations in plasma were significantly higher (10-15%) when the PAH deacetylation step was included in the analysis.

2nd objective: effect of the PAH deacetylation on the plasma flow



Method affected hepatic plasma flow rate (more than 10%) with no significant influence on portal vein plasma flow rate.

2nd objective: effect of the PAH deacetylation on the plasma flow



Hepatic artery plasma flow rate and its contribution to total hepatic flow rate changed by more than 50% between methods.

2nd objective: effect of the PAH deacetylation on the net hepatic flux

	Change (%)	*P*-value
Net hepatic flux (mmol/min)		
Acetate	70.6	**
β-Hydroxybutyrate	20.0	**
Oxygen	21.3	**
Total amino acids	40.4	**
Butyrate	5.0	**
Glucose	8.9	*
Propionate	2.9	*
Ammonia	1.8	N.S.
Urea	7.3	N.S.
Lactate	0.7	N.S.

* = *P-value* < 0.05

Modified 0-20%

Modified >20%

The net hepatic flux of nutrients was affected by the PAH deacetylation.

The extent of this effect may be related to the A-V concentration differences

Conclusions

This work confirms the results obtained by Kristensen et al. (2009) that cattle liver acetylates PAH and quantifies the changes on the net hepatic nutrient fluxes when the laboratory procedure includes the PAH deacetylation step.

15% of PAH in hepatic vein is acetylated

With the PAH deacetylation step, portal blood flow was not modified, while hepatic venous and arterial blood flows were reduced by 10 and 57%, respectively.

The PAH analysis method affected the net hepatic flux of nutrients, with changes greater than 20%.

Thank you very much for your attention

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