Dietary hydroxyl-methionine supply in pigs: associated changes in muscle biological processes

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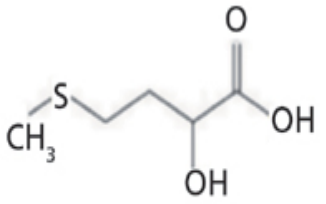
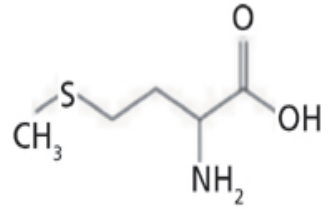
Methionine (Met):

The 3rd limiting AA for growth in pigs fed cereal & soya based diets
=> Added to diet in the form of DL-Met or HMTBA



HMTBA
dl-2-hydroxy-4-methylthio butanoic acid



Methionine

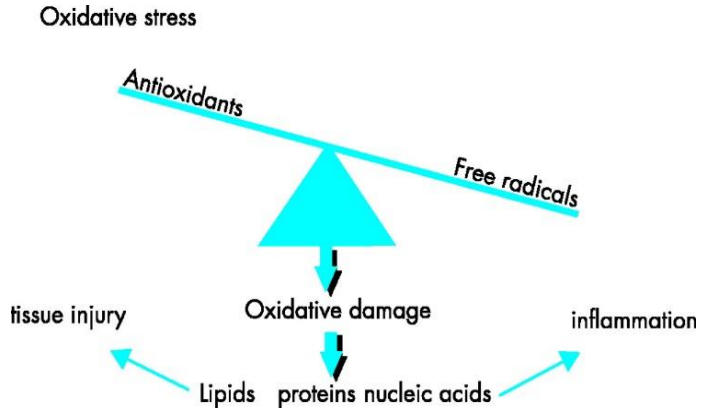
Met: a methyl donor

⇒ precursor (trans-sulfuration pathway) of Cysteine (Cys)

⇒ part of glutathione (**GSH** = Glu – Cys – Gly), the main intracellular non-enzymatic antioxidant

↳ **A functional ingredient** with benefits in the control of oxidative stress, health, and meat quality

(oxidative stress occurs all along animal production chain including the transformation of muscle into meat)



- **Dietary Met short-term deficiency in young pigs**

⇒ ↘ protein synthesis, with skeletal muscle identified as the main altered compartment (compared with carcass, blood, liver and intestine)

⇒ ↘ or ↗ AA concentrations in muscle proteins (= the composition of growth)

⇒ Altered glucose metabolism and ↗ lipid content in the body

⇒ ↘ GSH content and ↗ antioxidant enzyme activities

(Conde-Aguilera et al., 2010; 2016; Castellano et al., 2015)

- **Dietary Met long-term deficiency in growing-finishing pigs**

⇒ Met concentration in muscle protein was unchanged

⇒ Increased glycolytic potential in muscle

⇒ Pork quality traits (pH, drip loss and color) were unchanged

(Conde-Aguilera et al., 2014)

- Few studies addressed **excess in dietary Met** (relative to growth requirements)

⇒ L-Met supplemented diet fed to pigs during the whole growing-finishing period:

↗ glutathione (GSH)

↗ pHu, ↘ drip loss *(Liu et al., 2017)*

⇒ **Diet x 5 in Met (HMTBA)** during the **last 14 d** before slaughter:

No effects on growth rate, BW at slaughter and muscle weight

↗ glutathione (GSH)

↘ intramuscular lipid content

↗ pHu, ↘ drip loss, ↘ lightness *(Lebret et al., 2018)*

=> positive effects on pork quality traits

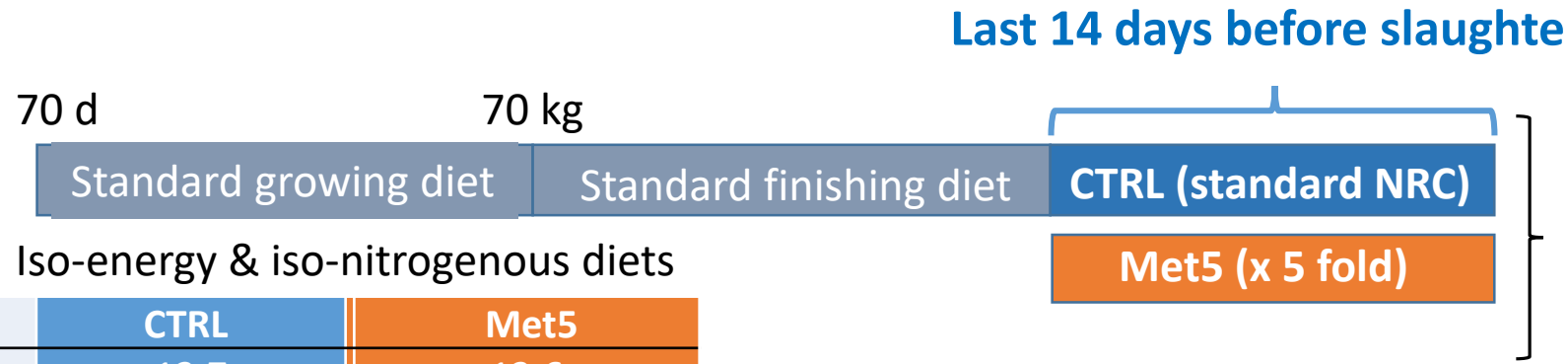
Mechanisms whereby excess dietary Met affected muscle properties and meat quality ?

- **Experimental design**

Diets were formulated with maize, wheat & soybean meal

⇒ 30 pigs fed either:

- **control diet (Met = 0.22%** = growth needs)
- **Met-supplemented diet (1.10% Met** = 5 fold the growth needs)



Iso-energy & iso-nitrogenous diets

Composition		CTRL	Met5
Protein	%	13.7	13.6
Fat	%	5.82	5.79
Cellulosis	%	2.59	2.54
Net energy	MJ/kg	10.38	10.44
dLys	**%**	**0.73**	**0.73**
dMet*	%	**0.22**	**1.10**
dMet+Cys	%	0.45	1.33

***n* = 15 pigs / diet**

*added as DL-HMTBA

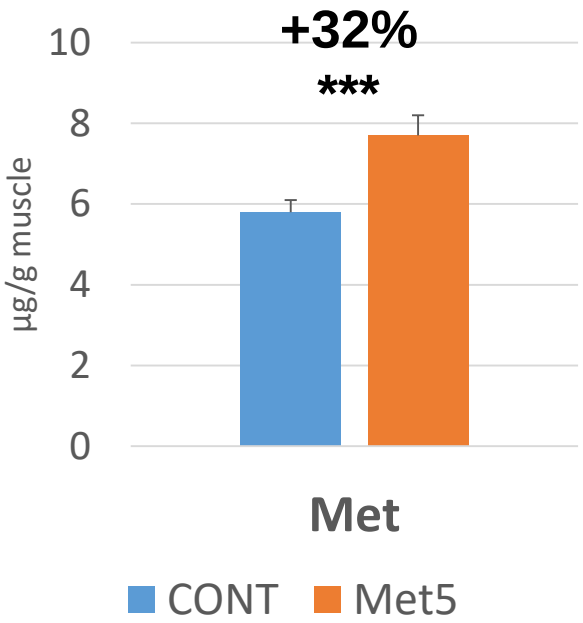
- **Results (1)**

⇒ **Muscle composition at market weight**

	CONT	Met5	*P* diet
BW, kg	125 ± 2	123 ± 1	0.55
Lean meat content, %	58.9 ± 0.6	58.9 ± 0.4	0.91
Longissimus muscle (LL) Weight, g	911 ± 36	879 ± 31	0.47
GSH content, nM/mg proteins	**754 ± 107**	↗ **867 ± 121**	**0.001**
Glycolytic potential, µM eq. lactate/g	151 ± 19	↘ 139 ± 20	*0.06*
Lipid content, %	1.7 ± 0.1	↘ 1.4 ± 0.1	*0.07*
Ultimate pH, units	**5.55 ± 0.02**	↗ **5.66 ± 0.04**	**0.04**

- **Results (2)**

⇒Free AA profile in *LL* muscle



Methionine

Trans-sulfuration

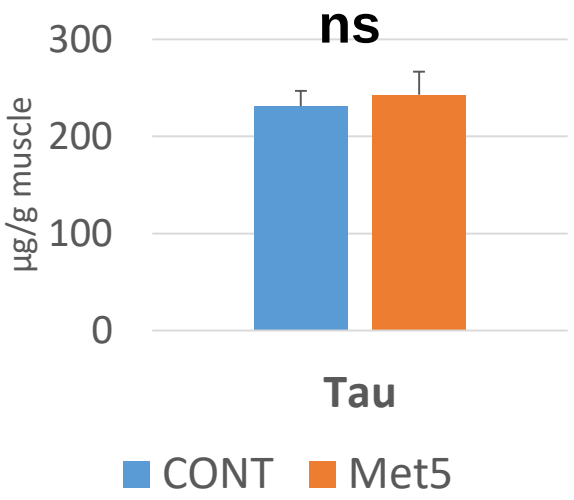
cysteine

homoserine

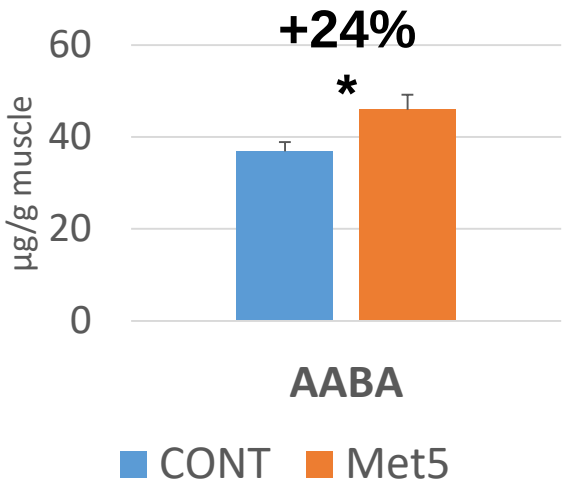
Taurine (Tau)

Glutathione (GSH)

α-aminobutyric acid (AABA)



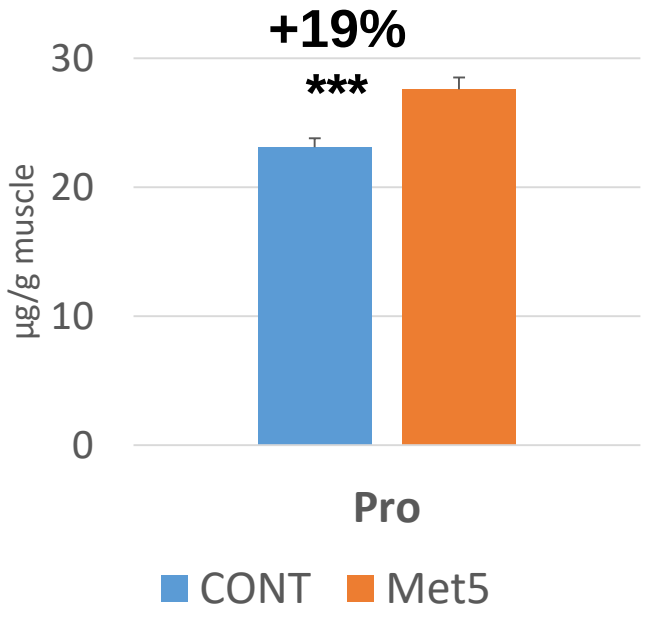
↗ GSH in Met5 pigs

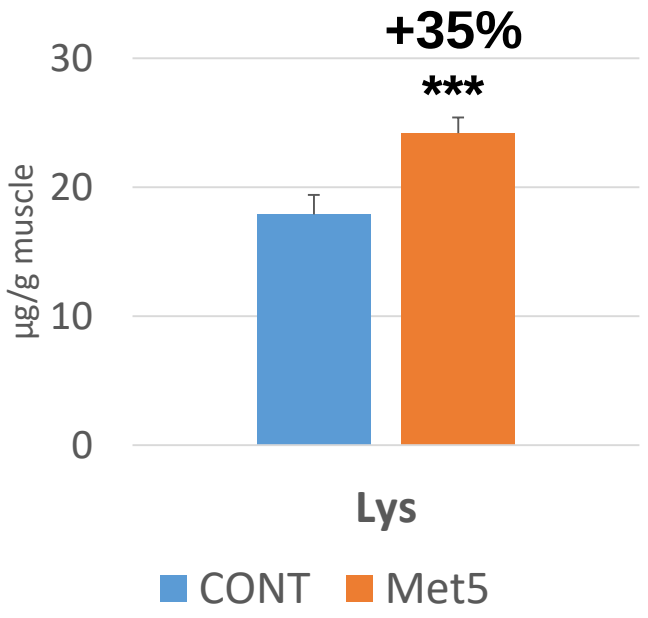


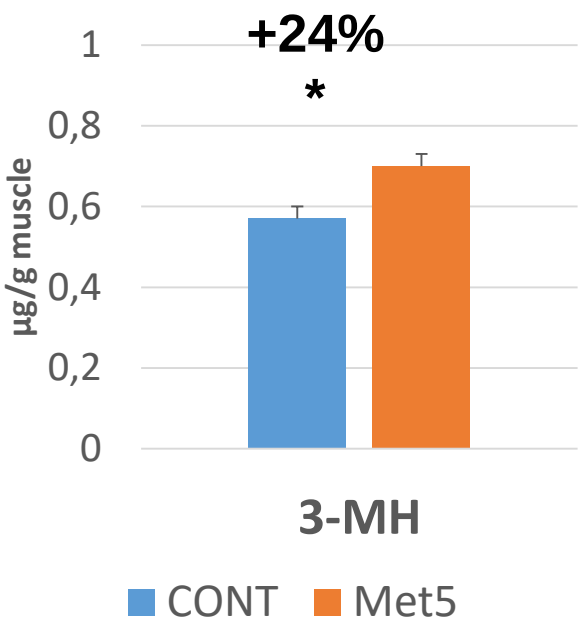
This may influence/reflect the Met content in muscle proteins

- **Results (2)**

⇒Free AA profile in *LL* muscle







Proline

roles in anti-oxidative reactions

3-Methyl-histidine

↗ protein degradation ?

- **Results (3)**

⇒ Protein degradation pathway in LL muscle

Gene expression, mRNA level	CONT	Met5	*P* diet
CTSD (cathepsin D)	0.83 ± 0		
PSMD1 (proteasome)	0.78 ± 0		
CAPN1 (micro calpain)	0.63 ± 0		
CAPN2 (milli calpain)	0.66 ± 0		

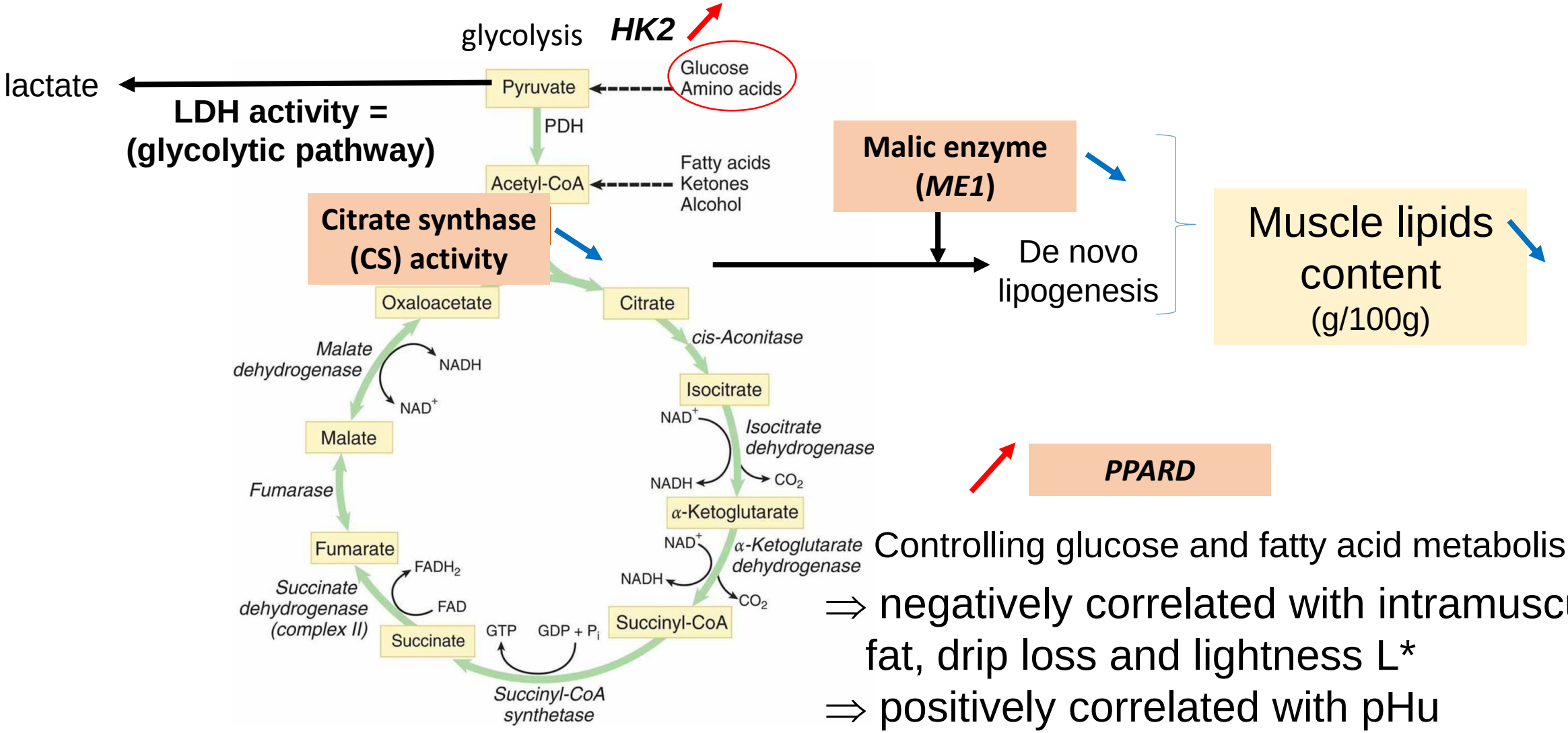
MURF1: cleaves actin and myosin muscle proteins during catabolic situations

OTUD1: a protease that negates the action of ubiquitin ligases

⇒ Autophagy pathway

Gene expression, mRNA level	CONT	Met5	*P* diet
MURF1 (= E3 ubiquitin ligase 1)	**0.79 ± 0.03**	**1.15 ± 0.12**	**0.008**
OTUD1	**0.53 ± 0.10**	**1.08 ± 0.22**	**0.03**
UBE2M (E2 ubiquitin conjugating enzyme)	*0.81 ± 0.02*	*0.74 ± 0.02*	*0.07*
FBXO32 (atrogin)	0.95 ± 0.10	1.33 ± 0.21	0.12

- **Results (4)** **=> Energy metabolism in LL muscle**



- **Conclusions**

Extra Met dietary supply (growth requirements x 5) was associated with:

❖ Changes in free AA concentrations in muscle, with increased Met & Pro.

⇒ This may participate to cope with oxidative stress

❖ 3-MH content was greater (& Lys content), suggesting increased muscle protein degradation

=> Autophagy molecular process (gene expression levels) was also affected by extra Met supply.

❖ Decreased oxidative metabolism and greater expression level of PPARD, a pivot transcription factor

=> These might participate to modulate pork quality traits such as intramuscular fat content, drip loss and color