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Rosa Castellano-Perez, Marie-Hélène Perruchot, Frederique Mayeur, Sophie Tesseraud, Yves Mercier, Florence Gondret

► To cite this version:

Rosa Castellano-Perez, Marie-Hélène Perruchot, Frederique Mayeur, Sophie Tesseraud, Yves Mercier, et al.. Effects of sulfur amino acids on porcine preadipocyte differentiation in culture. 65. Annual Meeting of the European Federation of Animal Science (EAAP), Aug 2014, Copenhagen, Denmark. Wageningen Academic Publishers, Annual Meeting of the European Association for Animal Production, 20, 2014, Annual Meeting of the European Association for Animal Production. hal-01210671

HAL Id: hal-01210671

<https://hal.science/hal-01210671>

Submitted on 2 Jun 2020

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Book of Abstracts of the 65th Annual Meeting of the European Federation of Animal Science



**Book of abstracts No. 20 (2014)
Copenhagen, Denmark
25 - 29 August 2014**

Effects of sulfur amino acids on porcine preadipocyte differentiation in culture*R. Castellano¹, M.H. Perruchot¹, F. Mayeur¹, S. Tesseraud², Y. Mercier³ and F. Gondret¹**¹INRA, UMR1348 Pegase, Domaine de la Prise, 35590 Saint Gilles, France, ²INRA, UR83 Recherches Avicoles, 37380 Nouzilly, France, ³ADISSEO, France SAS, 92160 Antony, France; rosa.castellano@rennes.inra.fr*

Adipogenesis is characterized by two temporally distinct processes: (1) proliferation during which pre-adipocytes replicate and increase in number and (2) differentiation during which pre-adipocytes change in morphology and accumulate lipids. Deregulation of the intracellular glutathione, the most important non-enzymatic intracellular anti-oxidant, has recently been shown to affect murine preadipocyte differentiation. Methionine (Met) is the essential precursor of Cysteine (Cys), which is the rate-limiting factor in glutathione synthesis. Here, we investigated whether low levels of Met+Cys may alter porcine adipogenesis. Preadipocytes were isolated from a subcutaneous fat depot of growing piglets and cultured with different doses of Met (0, 40, 70 and 100 μ M (adequate physiological dose) in a media deprived of Cys. Very low levels of Met (0 or 40 μ M) in the culture media dramatically lowered preadipocyte proliferation and reduced lipid accumulation in differentiating cells. Preadipocyte proliferation was similar at doses of 70 μ M and 100 μ M of Met, but lipid droplets in differentiating cells after 13, 21 and 24 days (d) of culture were more numerous at a dose of 70 μ M than at a dose of 100 μ M. At d13 of differentiation, the expression levels of CEBPalpha PPARgamma, two genes regulating the transcription of adipocyte-specific genes, and of LPL and HSL, two enzymes of lipid metabolism, were higher at a dose of 70 μ M than at a dose of 100 μ M. At d24 of differentiation, expression levels of these genes were much higher at a dose of 100 μ M. These results suggest a delay between time-related changes in gene expressions and lipid accumulation in cells. They show that very strong deficiency in Met decreases both proliferation and differentiation, whereas a mild deficiency of Met alters only differentiation of porcine preadipocytes.

Expression of microRNA-196a and -885 in bovine skeletal muscles*S. Muroya, M. Oe, I. Nakajima and K. Ojima**NARO Inst. Livestock and Grassland Science, Animal Products Research Division, Ikenodai 2, Tsukuba, Ibaraki, 305-0901, Japan; muros@affrc.go.jp*

MicroRNAs (miRNA) are non-coding small RNAs that attenuate their target gene expression in various biological processes. Using 5 cattle, we analyzed the expression of miR-196a, myosin heavy chain (MyHC) isoforms, and the predicted target genes of miR-196a, collagen α 1 chain of the type I (Col1a1) and III (Col3a1) in the masseter (MS), diaphragm (DP), semispinalis (SP), semitendinosus (ST), and longissimus dorsi (LD) muscles to investigate effect of miRNA on muscle type specification. According to the quantitative RT-PCR results, miR-196a is exclusively expressed in MyHC-2x isoform-abundant ST and LD muscles compared to the others ($P < 0.001$). On the other hand, expression of miR-885, a ST-enriched miRNA compared to MS in our previous study, was not significantly different among the muscles tested ($P = 0.178$). Especially, the miR-196a expression was correlated with those of MyHC-slow and -2x isoforms ($r = -0.530$ and $r = 0.976$, respectively). The Col1a1 and Col3a1 expression were correlated with the miR-196a expression ($r = -0.311$ and $r = 0.354$, respectively), and showed a higher level in the slow type than in the fast type muscles with a high correlation to the MyHC-slow expression ($r = 0.806$ and $r = 0.801$, respectively). These results indicate that the miR-196a expression is associated with the muscle type with relation to MyHC isoform expression in the bovine skeletal muscles, suggesting a close relationship in transcriptional regulation between MyHC-2x isoform and miR-196a in those muscles. These results also indicate that Col1a1 and Col3a1 expression is highly dependent on MyHC isoform type rather than the post-transcriptional regulator miR-196a.