Physiology and Endocrinology

205 Methionine and arginine supplementation alters mechanistic target of rapamycin (mTOR) and insulin signaling in bovine subcutaneous adipose explants challenged with C2-ceramide. Y. Liang1, N. Ma1, D. N. Coleman1, F. Liu1, Y. Li1, H. Y. Ding1, F. F. Cardoso1, F. C. Cardoso1, and J. J. Loor1, 1Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL, 2College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China, 3Department of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China, 4Department of Veterinary Medicine, College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.

Periparturient cows are exposed to increased circulating levels of ceramide which contributes to insulin resistance. Both Met and Arg promote protein synthesis via the mechanistic target of rapamycin (mTOR) and synthesis of polyamines and glutathione (antioxidants). Our previous work underscored the potential for Met supply to enhance insulin sensitivity in s.c. adipose tissue (SAT). The objective of this study was to investigate effects of increased Met and Arg supply alone or in combination on protein abundance of mTOR and insulin signaling pathways in adipose explants during ceramide stimulation. SAT from the tail-head of 4 Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) was incubated in duplicate for 4 h with one of the following media: ideal profile of essential AA as the control (IPAA; Lys:Met 2.9:1, Lys:Arg 2:1), increased Met (incMet; Lys:Met 2.5:1), increased Arg (incArg; Lys:Arg 1:1), or incMet plus incArg (Lys:Met 2.5:1 Lys:Arg 1:1) with or without exogenous C2:0-ceramide (100 μM). Total protein extracted from tissue explants was used for Western blotting. Data were analyzed as a 2 × 2 factorial using the MIXED procedure of SAS 9.4. There was a triple interaction between Met, Arg and ceramide for phosphorylated (p) protein kinase B (AKT) and p-mTOR (P < 0.05). Ceramide stimulation downregulated overall abundance of p-mTOR and p-AKT (P < 0.05). Without ceramide stimulation, enhanced Met and Arg alone or in combination led to lower p-mTOR (P < 0.05). However, compared with IPAA challenged with ceramide, increased Met or Arg led to greater p-AKT and p-mTOR, with a more pronounced response due to Arg and Met individually (P < 0.05). Compared with IPAA challenged with ceramide, enhanced Met or Arg supply resulted in greater activation of mTOR (p-mTOR/total mTOR) and AKT (p-AKT/total AKT), with a more pronounced response due to Arg. Overall, data suggest that greater Met or Arg supply might help preserve SAT functionality during the periparturient period when systemic ceramide concentrations increase.

Key Words: branched-chain amino acid, mechanistic target of rapamycin, subcutaneous adipose tissue

206 Branched-chain amino acid supplementation alters mechanistic target of rapamycin (mTOR) and insulin signaling pathway components in bovine subcutaneous adipose explants. Y. Liang1, N. Ma1, D. N. Coleman1, F. Liu1, Y. Li1, H. Y. Ding1, F. F. Cardoso1, F. C. Cardoso1, and J. J. Loor1, 1Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL, 2College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China, 3Department of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China, 4Department of Veterinary Medicine, College of Animal Science and Technology, Anhui Agricultural University, Hefei, China.

Branched-chain amino acids (BCAA) are import regulators of mechanistic target of rapamycin (mTOR). In humans and rodents, increased circulating BCAA levels are positively associated with insulin resistance. The objective of this study was to investigate changes in protein abundance of mTOR and insulin signaling pathway components and AA metabolism in bovine s.c. adipose explants in response to increased supply of Leu, Ile or Val. Four Holstein cows (parity 4 ± 1.4, DIM 248 ± 38 before slaughter; mean ± SD) were used in this study. All cows were fed a common lactation diet before slaughter. Subcutaneous adipose tissue (SAT) from the tail-head was incubated with basal media containing an “ideal” mix of essential AA (IPAA; 2.9:1 Lys:Met; 1.8:1 Lys:Thr; 2.38:1 Lys:His; 1.23:1 Lys:Val; 1.45:1 Lys:Ile; 0.85:1 Lys:Leu; 2.08:1 Lys:Arg) or IPAA supplemented with Ile, Val, and Leu to achieve a Lys:Ile of 1.29:1, Lys:Val 1.12:1, or Lys:Leu 0.78:1 for 4 h. Total protein extracted from the explants was used for Western blotting. The statistical model in SAS 9.4 included the fixed effect of Lys:Ile, Lys:Val and Lys:Leu ratios. Compared with IPAA, enhanced Leu and Ile supplementation led to greater activation of protein kinase B (AKT; p-AKT/total AKT) and mTOR (p-mTOR/total mTOR) (P < 0.05). However, compared with IPAA, Val supplementation led to lower p-mTOR (P < 0.05). Despite protein abundance of solute carrier family 38 member 1 (SLC38A1) and branched-chain ketoacid dehydrogenase kinase (BCKDK) being greater compared with IPAA, supplementation of BCAA also led to greater activation of eukaryotic elongation factor 2 (EEF2; p-EEF2/total EEF2) (P < 0.05). Furthermore, compared with increased Leu or Ile, enhanced Val supplementation led to greater abundance of SLC38A1 and BCKDK (P < 0.05). Overall, data suggest that enhanced supply of BCAA activates mTOR and insulin signaling in SAT partly due to increased transport into tissue and lower catabolism.

Key Words: branched-chain amino acid, mechanistic target of rapamycin, subcutaneous adipose tissue

207 Body condition alters lipidic profiles in subcutaneous adipose tissue of Holstein cows during the periparturient period. Y. Liang2, A. Elomily3, K. Wilachai3, A. Alharthi4, V. Lopreiato5, R. Bucktrout6, I. Martinez-Cortes6, and J. J. Loor1, 1Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL, 2Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, 3Program of Animal Science, Faculty of Agricultural of Technology, Rajabhat Maha Sarakham University, Thailand, 4Animal Production Department, King Saud University, Kingdom of Saudi Arabia, 5Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Universita’ Cattolica del Sacro Cuore, Piacenza, Italy, 6‘Agricultural and Animal Production Department, UAM-Xochimilco, Mexico City, Mexico.

The periparturient period is characterized by excessive adipose tissue (AT) lipid mobilization and increased incidence of metabolic disorders in dairy cows. The aim of this study was to characterize the lipid profiles in s.c. adipose tissue (SAT) between peripartal cows with different prepartal body condition score (HBCS vs. LBSCS). SAT from a subset of 10 healthy multiparous Holstein cows from a larger cohort classified as low BCS (LBCS; 3.0 ≤ BCS < 3.25; n = 5) or high BCS (HBCS; 3.5 ≤ BCS ≤ 4.0; n = 5) at 4 wk prepartum were used. Biopsies obtained on d −15, 7, and 30 relative to parturition were used for lipidomic analysis via liquid chromatography and tandem mass spectrometry. The multivariate statistical analysis of the lipodmic data was performed with the web-based metabolomic data processing tool MetaboAnalyst 4.0. Briefly, as quality control, variables containing more than 50% missing values were not considered for the statistical analysis. The raw data were transformed using the generalized log- transformation and then Pareto scaled to correct for heteroscedasticity. According to the VIP scores using the plots of partial least squares discriminant analysis (PLS-DA) model, there were 15 metabolites with a VIP > 2. Ceramide (d18:1/32:2) and diglyceride (DG) (18:0/22:4) were less abundant in HBCS cows; while those cows had greater triglycerides (TG) (4:0/14:1/16:1; 49:2). Analysis of variance-simultaneous component analysis was performed to ascertain variation in lipid profiles between pariparous Holstein cows from a larger cohort classified as low BCS (LBCS; 3.0 ≤ BCS < 3.25; n = 5) or high BCS (HBCS; 3.5 ≤ BCS ≤ 4.0; n = 5) at 4 wk prepartum were used. Biopsies obtained on d −15, 7, and 30 relative to parturition were used for lipidomic analysis via liquid chromatography and tandem mass spectrometry. The multivariate statistical analysis of the lipodmic data was performed with the web-based metabolomic data processing tool MetaboAnalyst 4.0. Briefly, as quality control, variables containing more than 50% missing values were not considered for the statistical analysis. The raw data were transformed using the generalized log- transformation and then Pareto scaled to correct for heteroscedasticity. According to the VIP scores using the plots of partial least squares discriminant analysis (PLS-DA) model, there were 15 metabolites with a VIP > 2. Ceramide (d18:1/32:2) and diglyceride (DG) (18:0/22:4) were less abundant in HBCS cows; while those cows had greater triglycerides (TG) (4:0/14:1/16:1; 49:2). Analysis of variance-simultaneous component analysis was performed to ascertain variation in lipid profiles between groups, time and their interaction using MetaboAnalyst 4.0. Sixteen metabolites including 3 ceramides (d18:1/32:2; d50:4; d18:1/18:0), 2 ω-hydroxy fatty acids, 8 DG and 3 TG increased after calving and reached their
peaks at d7 postpartum; in contrast, phosphatidylcholine (39:3) decreased after parturition. Time-course profiles of the top 50 lipid species including TG, PC, DG, phosphatidylglycerol, phosphatidylserine, phosphatidylethanolamine, lysophosphatidylethanolamine, lysophosphatidylcholine, and sphingomyelin were identified by a multivariate empirical Bayes approach (MEBA). Triglyceride (15:0/14:0/14:0) had the highest statistical value. Overall, the data indicated that BCS is associated with unique lipid profiles in dairy cow SAT during the peripartal period. Further studies are needed to understand the physiological relevance of these differences.

**Key Words:** body condition score, lipidomics, adipose tissue

### 208 Influence of cobalt source, folic acid and rumen-protected Methionine supplementation on performance, metabolism, and liver tissue 1-carbon metabolism biomarkers in peripartal Holstein cows.

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We investigated effects of supplementing an experimental source of Folic acid (FOA; Zinpro Corp.), 2 Co sources [Co glucohosphate (Copro) or Co pectin (CoPectin), Zinpro Corp.] and rumen-protected methionine (RPM) on performance and metabolism of multiparous Holstein cows from −30 relative to parturition through 30 d of lactation. Multiparous Holstein cows blocked according to the expected calving date, cows within each block were balanced for parity, previous lactation milk yield, and BCS before the close-up. Seventy-two cows (17 to 19 per treatment) were individually fed a basal diet [1.37 Mcal/kg of dry matter (DM), 14.5% crude protein] supplemented with Copro, FOA+Copro, CoPectin, or FOA+CoPectin+RPM. In each FOA group cows received 30 g FOA.

Cobalt treatments delivered 1 ppm Co/kg DM. The RPM was fed at 0.09% of DMI to achieve a ratio of 2.8:1 Lys:Met in the MP. Blood samples and liver tissue were collected at −10, 7, 15, and 30 d relative to parturition. The statistical model used in SAS 9.4 included the random effect of block, animal within block, and fixed effect of treatment, time and interactions. Preplanned contrasts were FOA vs. no FOA, CoPectin vs. Copro, and RPM vs. no RPM. No differences were observed for DMI (13.1 ± 0.5 kg/d prepartum, 14.9 ± 0.8 kg/d postpartum) or milk yield (40.1 ± 1.8 kg/d). Cows fed FOA had lower (P = 0.05) milk fat and total solids %; whereas RPM led to greater (P = 0.01) milk protein %. Plasma cholesterol, fatty acids, and bilirubin were lower (P ≤ 0.05) and retinol and tocopherol greater (P ≤ 0.05) in cows fed FOA. In contrast, CoPectin led to lower (P ≤ 0.02) hydroxybutyrate and nitric oxide, and tended to lower (P = 0.06) meleperoxidase activity and increase antioxidant capacity (FRAP). Among 13 genes in the 1-carbon metabolism pathway measured, abundance of the vitamin B12-dependent enzyme methionine synthase (MTR) was the only one affected, being greater in cows fed CoPectin. Despite similar performance, data revealed alterations in plasma and liver tissue biomarkers when feeding different Co sources with or without FOA and rumen-protected methionine.

**Key Words:** prepartum, postpartum, vitamin B12

### 209 Rumen-protected methionine supply to cows during heat stress alters liver tissue protein abundance of mechanistic target of rapamycin.

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The objective was to investigate effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on protein abundance of mechanistic target of rapamycin (mTOR), insulin, and antioxidant signaling in liver tissue. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental (Env) treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases. During phase 1 (9 d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9 d), group 1 was exposed to HS using electric heat blankets. Group 2 remained in TN but was paired-fed to HS counterparts. After 14 d washout and 7 d adaptation period, the study was repeated (period 2) and environmental treatments were inverted relative to phase 2, but dietary treatments were the same. Liver biopsies were performed at the end of each period. Tissue was incubated in Dulbecco’s modified eagle medium for 2 h and protein extracted. Data were analyzed using PROC MIXED in SAS. An Env × diet was detected for CUL3, an inhibitor of nuclear factor erythroid 2 like 2 (NFE2L2), due to upregulation in CON cows during HS vs. TN. Abundance of CUL3 did not differ due to RPM during HS or TN and tended to be lower than CON, suggesting that RPM alleviated HS-induced oxidative stress. An Env × diet was detected for phosphorylated (p) mTOR (P = 0.001); compared with CON, RPM led to greater abundance under TN conditions. However, p-mTOR decreased with RPM but increased with CON in HS. These effects suggested a potential shift in protein synthesis with RPM supply during HS. There was also an interaction for p-AKT (P < 0.001); HS cows on the CON diet had greater abundance than all other treatment responses. This response in CON cows was similar to CUL3, suggesting altered insulin signaling. Overall, preliminary evaluation indicates that RPM supplementation may help cows maintain hepatic homeostasis in mTOR, insulin, and antioxidant signaling in liver during a HS challenge.

**Key Words:** amino acid, lactation

### 210 Heat stress and rumen-protected methionine alter whole-blood mRNA abundance of transsulfuration and antioxidant pathway genes.

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The objective was to investigate the effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on whole blood mRNA abundance of genes related to immune and antioxidant responses and the transsulfuration pathway. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental (Env) treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases per period. During phase 1 (9 d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9 d), group 1 (n = 16) was exposed to HS using electric heat blankets. Group 2 (n = 16) remained in TN but was pair-fed to HS counterparts. After a 14 d washout and 7 d adaptation period, the study was repeated (period 2) and environmental treatments were inverted relative to phase 2 in period 1, while the dietary treatments were the same. Blood was collected from the coccygeal vein approximately 4 h postfeeding on d 8 of phase 2. Data were analyzed using PROC MIXED in SAS. An Env × diet interaction was observed for mRNA abundance of cystathionine β synthase (CBS; P = 0.03); with RPM cows having greater CBS during TN compared with HS. The same Env × diet interaction was detected for the mRNA abundance of superoxide dismutase (SOD) 1 (P = 0.01)
and cysteine dioxygenase type 1 (CDO1; \(P = 0.02\)). A tendency for an interaction was observed for the expression of toll-like receptor 2 (\(P = 0.09\)) due to an increase in abundance in CON cows during HS compared with TN. Regardless of diet, mRNA abundance of CDO1 and cystathionine gamma-lyase was downregulated by HS \((P < 0.05)\) and glutathione synthase tended to be downregulated \((P = 0.08)\). Overall, preliminary evaluation suggests that supplying RPM may enhance the antioxidant responses in whole blood. Results also highlight the negative effect of HS on whole blood transsulfuration pathway and antioxidant responses.

**Key Words:** amino acid, immune response