Physiology and Endocrinology Symposium: The Next Generation of Metabolic Endocrinology

229 Novel insights in to the biology of the emerging metabolic regulator FGF21. A. C. Adams* and A. Kharitonenkov, *Eli Lilly & Co., Indianapolis, IN.*

Fibroblast growth factor 21 (FGF21) is a multifaceted metabolic regulator which has potential applications in the treatment of diseases such as obesity, diabetes and fatty liver. When administered in vivo either peripherally or centrally FGF21 has a plethora of beneficial activities. To date the mechanism and site of action underlying these effects remains unknown with recent studies suggesting some of its effects may be mediated indirect and occur via modulation of other factors such as adiponectin and leptin. Using tissue specific knock out mouse models lacking either the FGF receptor (FGFR1) or the critical co factor β-Klotho (KLB) we sought to determine the tissue upon which FGF21 acts and receptor complex responsible for FGF21 its mediating in vivo efficacy. Importantly, when KLB is ablated from all tissues FGF21 action is completely abrogated. To determine the precise tissue of action we created 2 tissue specific lines harboring deletion of FGFR1 in neurons and in adipose tissue, respectively. Surprisingly, in animals with neuronal FGFR1 loss there was no change in the metabolic activity of FGF21 suggesting direct central FGF21 action is not required for its physiological endpoints. In contrast, when we examined the adipose FGFR1 mutants we found significant attenuation of metabolic efficacy. Importantly, the action of FGF21 via adipose tissue results in alterations in adipokine secretion and systemic sensitivity to these factors. Therefore, while FGF21 itself may not directly act on the CNS, leptin and other adipokines are likely be mediators of FGF21's secondary central effects downstream of direct adipose tissue engagement. Further studies are required to delineate the precise mechanistic basis underlying central FGF21 mediated physiology.

Key Words: FGF21, metabolism, diabetes

230 Biology of the novel hormone fibroblast growth factor-21 in the transition dairy cow. Y. R. Boisclair*, S. L. Giesy, and L. S. Caixeta, *Cornell University, Ithaca, NY.*

The modern dairy cow reaches near maximal productivity soon after parturition despite a substantial nutritional deficit. This ability is underpinned by coordinated adaptations that are most obvious in liver, adipose tissue and skeletal muscle and affect the metabolism of all major organic nutrients. Over the last 2–3 decades, most research in dairy cattle has focused on the roles played by growth hormone, insulin-like growth factor-I and insulin in driving these adaptations. The last 10–15 years, however, have witnessed the discovery of novel hormones with potent metabolic actions. Among those, the hormone fibroblast growth factor-21 (FGF21) appears relevant on the basis of its ability in laboratory animals to regulate mobilization of lipids from adipose tissue and their oxidation in liver, 2 processes that are crucial to the efficient utilization of lipid reserves in early lactating dairy cows. These observations led us to examine the regulation of the FGF21 system in high yielding dairy cows over the last 4 weeks of pregnancy (LP) and the first 8 weeks of lactation (EL). Plasma FGF21 was nearly undetectable in LP, peaked on the day of parturition and then stabilized at lower, chronically elevated concentrations during the energy deficit of EL. Gene expression studies showed that liver was the major source of plasma FGF21 in EL with little or no contribution by adipose tissue, skeletal muscle and mammary gland. Finally, we identified liver and adipose tissue as the only 2 major tissues with meaningful expression of β-Klotho, a co-receptor that is absolutely essential for FGF21 signaling. These results suggest that FGF21 may be a key metabolic hormone in early lactating dairy cows and provide impetus to identify factors triggering its production as well as FGF21-dependent liver and adipose tissue responses.

Key Words: periparturient period, liver, adipose tissue

231 Role of adiponectin and visfatin in chicken growth and reproduction. R. Ramachandran*, S. Krzysik-Walker, O. Ocon-Grove, R. Vasilatos-Younken, G. Hendricks III, and J. A. Hadley, *Department of Animal Science, Pennsylvania State University, University Park.*

Adiponectin and visfatin are 2 endocrine factors that affect metabolism in domestic animals. Adiponectin, a 30 kDa adipokine hormone, improves carbohydrate and lipid metabolism in humans and rodent animal models by activating 2 distinct transmembrane receptors (AdipoR1 and AdipoR2) that are widely expressed in various tissues in the chicken. We have cloned and characterized the chicken genes that encode for adiponectin, AdipoR1, and AdipoR2. While adipose tissue is the primary site of adiponectin expression in the chicken, we found that adiponectin and its receptors are ubiquitously expressed in other tissues. Adiponectin undergoes multimerization during biosynthesis in the adipose tissue and circulates as a unique heavy molecular weight isoform that is larger than 669 kDa mass. Plasma adiponectin levels were found to be significantly lower in 8-wk-old compared with 4-wk-old male chickens and inversely related to abdominal fat pad mass. In vitro studies using hepatocytes and ovarian follicular cells revealed that recombinant chicken adiponectin (rcADN) increased the abundance of phosphorylated adenosine monophosphate-activated protein kinase, phosphorylated acetyl coenzyme A carboxylase, and phosphorylated Erk 1/2, as well as increased glucose uptake. Future studies will focus on regulating the signal transduction pathways of adiponectin for improving growth and reproductive efficiency. Nicotinamide phosphoribosyltransferase (Nampt/visfatin/PBEF) has been identified as a rate-limiting NAD^+ biosynthetic enzyme and an adipokine/myokine found within the cells and circulation. Human and chicken skeletal muscles are reported to have the highest level of Nampt expression among various tissues. We found that plasma levels of visfatin dramatically increases 28-fold in pubertal broiler breeder chickens compared with pre-pubertal chickens. Furthermore, recombinant visfatin was found to alter the expression of key myogenic transcription factors in satellite cells suggesting that visfatin may influence postnatal myogenesis. Future studies will focus on elucidating the functional role of circulating visfatin in growth and reproduction of farm animals.

Key Words: adiponectin, visfatin, chicken

232 Characterization of serum adiponectin during lactation in dairy cows supplemented with conjugated linoleic acids. S. P. Singh*[1], S. Häussler[1], S. Dänicke[2], M. Mielenz[3,1], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, Bonn, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany, [3]Leibniz Institute for Farm Animal Biology (FBN), Department of Nutritional Physiology, Dummerstorf, Germany.*

The adipokine adiponectin (Aq) is known for its insulin sensitizing effects. The metabolic profile of dairy cows may be affected by conjugated linoleic acids (CLA). The objectives of our study were to characterize serum Aq concentrations throughout lactation and to test whether dietary CLA supplementation affects this time course in multiparous (MP) and primiparous (PP) dairy cows. Thirty 3 pregnant German Holstein cows (22 MP and 11 PP) were included in the study from d 21 antepartum (ap) until d 252 postpartum (pp). From d 21 ap until calving, all animals received same diet. From d 1 to d 182 pp, animals received same lactation diet but were either fed CLA (100 g/d Lutrell Pure, BASF, Ludwigshafen, Germany; group CLA: 11 MP and 5 PP cows) or a control fat supplement (100 g/d Silafat; BASF; group CON: 11 MP and 6 PP cows). After d 182 pp, all animals were continuously fed with same lactation diet but without CLA or control fat supplement for further 12 wk. Blood samples were collected for determining serum Aq by ELISA (Mielenz et al., 2013, doi:10.1016/j.domaniend.2012.10.04). Data were analyzed by mixed model (SPSS). Treatment and parity were considered as fixed factors and sampling days as repeated effects. Serum Aq (μg/mL; means ± SEM) decreased from 21 d ap reaching a nadir at calving [18.5 ± 1.6 (mean for all groups together)] and increased gradually thereafter until d 21 pp in PP cows [35.9 ± 3.7 (CON), 25.8 ± 2.7 (CLA)] and until d 49 pp in MP cows [34.6 ± 2.2 (CON), 28.2 ± 2.1 (CLA)], respectively and remained unchanged thereafter. Serum Aq were lower ($P < 0.05$) in CLA than in CON cows; this difference emerged 4 wk earlier in PP cows than MP cows. Circulating Aq tended ($P = 0.09$) to be higher in MP cows of both CLA and CON groups compared with respective PP cows. Our results indicate that major changes in circulating Aq occur peripartum and that dietary CLA decreases serum Aq in both PP and MP dairy cows albeit at different times. In view of the insulin sensitizing actions of Aq described in monogastrics, the CLA effects observed herein point to a CLA-induced decrease in insulin sensitivity.

Key Words: adiponectin, conjugated linoleic acid, dairy cow

233 Daily injection of tumor necrosis factor alpha in the first week of lactation decreases milk production and promotes health disorders in Holstein dairy cows. J. K. Farney*, K. Yuan, L. K. Mamedova, and B. J. Bradford, *Kansas State University, Manhattan.*

Inflammation may contribute to transition disorders in dairy cattle. The objective of this study was to determine the production responses to administration of an inflammatory cytokine, tumor necrosis factor a (TNFα), in the first week of lactation. At calving, 33 Holstein cows (n = 9 primiparous, n = 24 multiparous) were blocked by parity and alternately assigned to either control (CON; 0 μg TNFα/kg BW), low dose (LOW; 1.5 μg TNFα/kg BW), or high dose (HIGH; 3.0 μg TNFα/kg BW) s.c. injections daily for 7 d. Daily DMI, water intake, and health disorder data were recorded; plasma samples were also collected daily for metabolite analyses. Data were analyzed using mixed models with repeated measures over time and significance was declared at $P < 0.05$ and tendencies at $P < 0.10$. Preplanned contrasts evaluating CON vs. TNFα treatments and LOW vs. HIGH were evaluated. Plasma TNFα concentrations tended to be increased in cows receiving TNFα injections ($P = 0.09$, 64% increase), but there were no differences observed between HIGH and LOW treatments. DMI was significantly reduced in cows receiving TNFα injections (18% decrease). Similarly, water intake was decreased 13% with TNFα. Milk production was reduced in TNFα treated cows as evidenced by 15 to 18% decreases in yields of milk, milk fat, milk protein, milk lactose, energy-corrected milk, and solids-corrected milk. Milk fat yield and SCM tended to be further depressed in HIGH cows, but no differences were observed between LOW and HIGH for any other milk variable. No treatment differences were observed for plasma glucose, β-hydroxybutyrate, nonesterified fatty acid, or triglyceride concentrations. Daily injection of TNFα increased total diagnosed health disorders in the first week of lactation, due in part to a tendency for an increased incidence of ketosis in cows injected with TNFα. The results of this study indicate that low grade inflammation induced by daily injection of TNFα negatively affects milk production and increases physiological stress on the transition dairy cow as evidenced by increased health disorders.

Key Words: inflammation, anorexia, metabolism

234 Inflammation and endoplasmic reticulum (ER) stress gene network expression in liver of peripartal Holstein cows fed two levels of dietary energy prepartum. M. J. Khan*[1], E. Trevisi[2], D. E. Graugnard[1], G. Bertoni[2], and J. J. Loor[1], *[1]University of Illinois, Urbana, [2]Universita Cattolica del Sacro Cuore, Piacenza, Italy.*

The peripartal period is characterized by marked changes in inflammatory status that are functionally related with impaired immune and metabolic responses in the cow. We examined blood metabolites and expression of genes related to inflammation and ER stress in cows assigned (6/diet) to a control (high-straw, CON; NEL = 1.34 Mcal/kg) or moderate-energy (OVE; NEL = 1.62 Mcal/kg) diet during the entire dry period. All cows were fed a common lactation diet (NEL = 1.69 Mcal/kg) postpartum. Blood was collected on d (±3) −14, −5, −2, −1, 0, 1, 2, 5, 7, 10, 14 and 21 d relative to parturition. A percutaneous liver tissue biopsy was harvested at −14, 7, 14, and 30 d relative to parturition for transcript profiling via quantitative PCR. Estimated prepartal energy balance (EBAL) OVE was greater ($P < 0.05$) and averaged 159% of requirements compared with 102% in CON. However, EBAL during the first week postpartum was lower in OVE (83% vs. 89% of requirements). After parturition the concentration of ceruloplasmin, creatinine, bilirubin and reactive oxygen metabolites (ROM) was greater (Diet × Time; $P < 0.05$) in OVE. Around calving the expression of ER and oxidative stress indicator genes XBP1, PERK, GRP94 and HSP40 was lower in OVE than CON but TRB3, HSPA1A, HSPA1B and CREB3L3 had greater (Diet × Time; $P < 0.05$) expression in OVE. Expression postpartum of the inflammatory genes NFKB1, RELA, CHUK, MYD88, TNF, SAA3, and PTX3 increased (Diet × Time; $P < 0.05$) in OVE. Genes associated with cell growth (mTOR, RPTOR, AKT3, TP53) also had greater (Diet × Time; $P < 0.05$) expression in OVE after parturition. Overall, results indicated that negative EBAL induced by prepartal OVE was associated with hepatic pro-inflammatory and pro-stress upregulation.

Key Words: transition cow, nutrition, transcriptomics