Physiology and Endocrinology Symposium: Insulin revisited

799 Insulin receptor signaling in normal and insulin-resistant states. Brian O'Neill*[1,2], [1]*Joslin Diabetes Center, Boston, MA,* [2]*Harvard Medical School, Boston, MA.*

As type 2 diabetes has reached pandemic levels affecting nearly 370 million people worldwide, it is critical to understand the cellular processes that influence this disease. Insulin resistance, or the inability of normal levels of insulin to achieve the normal effect, is a hallmark of type 2 diabetes and metabolic syndrome. When going from the fasted to the fed state, insulin binds to the ubiquitously expressed insulin receptor to activate downstream signaling, which regulates many cellular actions such as glucose, lipid, and protein metabolism. At the level of the organism, insulin's effect on glucose and lipid metabolism occurs primarily through actions on liver, skeletal muscle, and adipose tissue. The various proteins and isoforms that positively and negatively modulate the insulin-signaling cascade ensure a proper response to feeding. However, these regulators of insulin signaling can be disrupted in a variety of ways in response to disease states such as obesity, inflammation, or even during the aging process and contribute to insulin resistance. Understanding the processes by which insulin signaling is affected in response to these disease states is critical to the discovery of new treatments to prevent diabetes, metabolic syndrome, and their complications.

Key Words: diabetes, insulin resistance

800 Roles for insulin-supported skeletal muscle growth. Robert P. Rhoads*[1] and Lance H. Baumgard[2], [1]*Virginia Tech, Blacksburg, VA,* [2]*Iowa State University, Ames, IA.*

Basic principles governing skeletal muscle growth and development, from a cellular point of view, have been realized for several decades. Skeletal muscle is marked by the capacity for rapid hypertrophy and increases in protein content. Ultimately, skeletal muscle growth is controlled by 2 basic means; myonuclear accumulation stemming from myoblast proliferation and the protein synthesis and degradation balance. Each process underlies the rapid changes in lean tissue accretion evident during fetal and neonatal growth and are particularly sensitive to nutritional manipulation. Although multiple signals converge to alter skeletal muscle mass, postprandial changes in the anabolic hormone, insulin, link feed intake with enhanced rates of protein synthesis in the neonate. Indeed, a consequence of insulin-deficient states such as diabetes or malnutrition is reduced myoblast activity and a net loss of body protein. A well-characterized mechanism mediating the anabolic effect of insulin involves the phosphatidylinositol 3-kinase (PI3K) mammalian target of rapamycin (mTOR) signaling pathway. Activation of mTOR leads to translation initiation control via the phosphorylation of downstream targets. Modulation of this pathway by insulin, as well as other hormones and nutrients, accounts for enhanced protein synthesis leading to efficient lean tissue accretion and rapid skeletal muscle gain in the growing animal. Dysfunctional insulin activity during fetal and neonatal life stages likely alters growth through cellular and protein synthetic capacities.

Key Words: insulin, skeletal muscle, myoblast

801 The biology of hyperinsulenemia induction of polycystic ovarian syndrome and its complications. Jean-Patrice Baillargeon*[1,2], [1]*Université de Sherbrooke, Sherbrooke, QC, Canada,* [2]*Research Center of Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC, Canada.*

Polycystic ovarian syndrome (PCOS) is a common condition affecting 6 to 10% of women of childbearing age. It is the most frequent endocrine disorder among young women in North America. In addition of being the most frequent cause of female infertility, PCOS is the commonest cause of hyperandrogenism in women, thus leading to esthetical concerns such as excessive hair growth, acne and alopecia. Accordingly, PCOS women are more likely to have impaired quality of life, including depression, anxiety and increased risk of social phobia and suicide attempts. In addition, PCOS is currently considered as a paradigm of cardiometabolic disease, because the prevalence of metabolic syndrome, dyslipidemia and type 2 diabetes (T2D) are much higher in PCOS than in normal age- and BMI-matched women. Indeed, PCOS women demonstrate metabolic insulin resistance (IR) and compensatory hyperinsulinemia, which play a critical role in the syndrome's development. Yet, many questions remain unanswered regarding the mechanisms by which metabolic IR and hyperinsulinemia leads to hyperandrogenemia and PCOS infertility. The existence of women developing PCOS without IR suggests that it is not a requisite for the development of this syndrome. Nevertheless, several evidences suggest that impairment in insulin signaling may be implicated in androgen overproduction at the level of androgen producing tissues. This talk will therefore review potential metabolic mechanisms in the development of PCOS in predisposed women. This talk's objectives are to (1) review briefly the PCOS and some characteristics of insulin resistance in PCOS; (2) describe the mechanisms of insulin action on androgen biosynthesis in PCOS; and (3) discuss the implication of nonesterified fatty acids (NEFAs) in PCOS hyperandrogenemia and its metabolic complications. This talk will discuss the hypothesis that lipotoxicity (the cellular adverse consequences of NEFAs) may cause both the hyperandrogenemia and insulin resistance that characterize PCOS women. Lipotoxicity could therefore explain PCOS symptoms, mainly due to hyperandrogenism, and long-term metabolic consequences.

Key Words: polycystic ovary syndrome, insulin, lipotoxicity

802 Insulin effects on mammary gland extraction and milk synthesis. Wendie S. Cohick*, *Rutgers University, New Brunswick NJ.*

During lactation there is an increased demand for nutrients required for milk protein and lipid synthesis as the mammary gland undergoes functional differentiation from late pregnancy to early lactation. Insulin resistance in insulin-sensitive peripheral tissues channels nutrients to the mammary gland for milk synthesis in early lactation. Glucose uptake by the mammary gland is insulin-independent, with insulin showing little effect on mRNA expression of glucose transporters. In contrast, it has long been known that the lactogenic hormones hydrocortisone, insulin and prolactin are required for maximum expression of milk protein genes in the mammary gland. Recently it has been recognized that the regulation of protein translation may play a central role in determining milk protein production. The mammalian target of rapamycin (mTOR) signal transduction pathway has been identified as a master regulator of protein translation. Data indicating that hormones (i.e., insulin and IGF-I), nutrients (i.e., amino acids) and intracellular energy status interact

to regulate the mTOR signaling pathway and thus protein synthesis in the mammary gland will be presented.

Key Words: insulin, milk synthesis, differentiation

803 Effects of insulin and heat stress on mTOR signaling cascade in bovine mammary epithelial cells. Kimberly R. Kassube*, Jeffrey D. Kaufman, and Agustin G. Ríus, *The University of Tennessee, Knoxville, TN.*

Insulin increases protein synthesis by activating the signaling pathway that regulates protein translation in mammary tissue. Lactating cows exposed to heat stress (HS) have increased basal levels of insulin but exhibit reduction in milk protein synthesis. The activity of mammalian target of rapamycin (mTOR) signaling cascade is mediated upon phosphorylation and dephosphorylation of protein kinase B (Akt), P70 S6 kinase (S6K1), ribosomal protein S6 (rpS6), and eukaryotic elongation factor 2 (eEF2). The objective of this study was to determine the effects of insulin and HS on phosphorylating activity in Akt, S6K1, rpS6, and eEF2 factors in immortalized bovine mammary cell line (MAC-T). Cells were cultured in 15 mL of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1 μg/mL of insulin at 37°C and 5% CO_2 before the treatments were imposed. The experimental design consisted of a 2 × 2 factorial arrangement of treatments with 2 temperature environments, 37°C thermoneutral or 41°C HS, and 2 insulin concentrations, 0 μg/mL and 1 μg/mL for 12 h. Cell lysates were separated by gel electrophoresis and transferred onto a polyvinylidene fluoride membrane. Western blotting was conducted to identify total and site-specific phosphorylated forms of Akt (Thr308/Ser473), S6K1 (Thr389), rpS6 (Ser235/236) and eEF2 (Thr56). The relative densities for phosphorylated and total forms of Akt, S6K1, rpS6 and eEF2 were quantified and expressed as phosphorylated to total ratio. ANOVA was conducted with SAS 9.4 using mixed models. Preliminary results indicate a significant HS by insulin interaction for rpS6 ($P < 0.05$). There was an increase in phosphorylated to total ratio from 0.26 ± 0.09 to 0.6 ± 0.09 in response to insulin when cells were exposed to HS. However, there was a reduction of this ratio from 0.38 ± 0.09 to 0.2 ± 0.09 in response to insulin when cells were exposed to thermoneutral conditions. The remaining protein factors were not affected by treatments. These results would indicate that the response of mTOR signaling cascade to insulin was altered in MAC-T cells exposed to HS.

Key Words: heat stress, insulin, protein synthesis

804 Heat stress reduces the phosphorylation activity of mTOR signaling cascade in bovine mammary cells. Jeffrey D. Kaufman*[1], Kimberly R. Kassube[1], Celina Baravalle[2], and Agustin G. Ríus[1], *[1]The University of Tennessee, Knoxville, TN, [2]Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina.*

Heat stress (HS) alters metabolism of amino acids and reduces synthesis of caseins in bovine mammary glands. The mammalian target of rapamycin (mTOR) cascade regulates the initiation of the translation of protein synthesis and is mediated by protein factors that are activated or inhibited upon phosphorylation. It has been reported that essential amino acids increased protein synthesis by activating the mTOR cascade. Our objective was to determine the effect of HS in phosphorylating mTOR protein factors in immortalized bovine mammary cells line (MAC-T). It was hypothesized that the phosphorylation activity of mTOR signaling factors would be altered in MAC-T cells exposed to HS. Cells were cultured in 15 mL of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum at 37°C and 5% CO_2. Cells were subjected to one of 2 treatments: 1) 37°C (control) and 2) 41.5°C (HS) for 12 h. The treatments were repeated 5 times in 5 different days. Cell proteins were harvested and separated by gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Western blotting was conducted to identify total and site-specific phosphorylated forms of protein kinase B (Akt; Thr308/Ser473), P70 S6 kinase (S6K1; Thr389), ribosomal protein S6 (rpS6; Ser235/236), and eukaryotic elongation factor 2 (eEF2; Thr56). Relative densities for phosphorylated and total forms of Akt, S6K1, rpS6 and eEF2 were quantified and expressed as phosphorylated to total ratio. ANOVA was conducted using a mixed model. Compared with control, cells exposed to HS decreased phosphorylation to total ratio of Akt (0.41 vs. 0.29; $P < 0.001$), S6K1 (1.65 vs. 0.97; $P = 0.042$), and rpS6 (1.45 vs. 1.07; $P < 0.001$). However, preliminary results indicated that HS did not affect the ratio of eEF2. These results indicate that HS impaired the translation of proteins by altering the phosphorylation activity of mTOR signaling factors in MAC-T cells.

Key Words: heat stress, mammary cell, translation of protein

805 Proteome of adipose tissue in periparturient dairy cows related to insulin resistance. Maya Zachut*, *Department of Ruminant Science, ARO, Volcani, Bet Dagan, Israel.*

Adipose tissue serves as a major endocrine organ with a profound influence on metabolism by secreting and regulating numerous molecules, hormones and adipokines. Many proteins activate intracellular pathways that promote the development of insulin resistance (IR); however, the role of specific proteins in adipose tissue dysfunction is not well defined. The objective was to identify proteins in adipose that are linked to IR and to cows' metabolic status. Adipose tissue biopsies were obtained from 8 multiparous cows at −17 and +4 d relative to parturition. Proteins were analyzed by intensity based, label-free quantitative shotgun proteomics at Weizmann Institute of Science (Rehovot, Israel). Proteins were extracted and subjected to in-solution tryptic digestion. This was followed by nanoflow liquid chromatography coupled to high-resolution tandem mass spectrometry (nanoLC-MS/MS). Quantitative data were extracted using the Genedata Expressionist data analysis package and proteins identified using the Mascot search engine. Cows were previously divided to those with IR or insulin-sensitive (IS) adipose based on phosphorylation of protein kinase B (Akt) in response to insulin stimulation. Proteomics data, after logarithmic transformation, were analyzed by 2-way ANOVA to measure the effects of time (prepartum vs. postpartum), subgroup (IR vs. IS) and their interaction. Body weight (BW) differences were analyzed with GLM of SAS. It was found that cows with IR adipose lost more BW postpartum compared with IS cows. Proteomic analysis revealed 586 proteins in adipose tissues. Comparing IR to IS adipose showed that 18.9% of proteins were differentially expressed (fold change (FC) > 1.5 and $P < 0.05$). The expression of 106 proteins were increased, whereas only 5 were decreased, in IR adipose compared with IS. The abundance of several proteins related to lipolysis was increased in IR adipose compared with IS: hormone-sensitive lipase (FC = 6.8, $P < 0.03$), perilipin (FC = 1.5, $P < 0.05$), and monoglycerol-lipase (FC = 8.2, $P < 0.0003$). This is in accordance with the elevated lipolysis in IR adipose. These proteins could be used as novel biomarkers to identify IR cows, which may indicate of the metabolic status of the peripartum dairy cow.

Key Words: proteomics, adipose, insulin