25 Usefulness of in vitro and in vivo experimental models. J. Novakofski*[1], [1] *University of Illinois, Department of Animal Sciences.*

Use of experimental models is the foundation of experimental biology. A critical question is always; "how well does the model reflect in an actual animal?" The real answer of course is that models are in fact models: extremely useful for specific purposes but much less so for others. Because of this, carte blanche denial, or acceptance, of models is equally likely to impede progress. Models should be evaluated with scientific method and usefulness decided on the basis of results, not opinion. Adipose tissue research provides a useful context for examining issues surrounding this problem because regulation of adipose growth and metabolism has important economic implications for livestock production, product quality and product yield. This presentation will compare the usefulness of models for aspects of adipose research. Much of the progress in understanding adipocyte biology has depended on cell culture experiments. Perhaps surprisingly, a large amount of this progress has used a few established cell lines with well known "eccentricities." Comparison of established cell lines with primary adipocyte culture will demonstrate that primary culture has played a minor role despite many advocates. This comparison will be less clear for research on adipose metabolism. Cultures have been less useful for examining metabolism but essential for examining intracellular signaling pathways. Cell cultures, examined in regulatory isolation have even provided a few notable misconceptions, for example the insulin-like effect of growth hormone that is irrelevant in vivo. Gene knockouts, a technical variation of classic ablation models, have proven to be extremely useful in adipose research. Lethality problems have been limited because variations in lipid metabolism are seldom life threatening. Knockouts have revealed many functions, redundant pathways and unrecognized regulation. In contrast, "gene chip" experiments have been disappointing. While not a model per se, but a technology to examine models, gene chips emphasize the importance of model selection. Even expected changes may be difficult to see in experimental models because current chips lack adequate sensitivity relative to normal variations in lipid metabolism.

Key Words: Adipose tissue, Primary cell culture, Established cell lines

26 Role of fatty acids in adipocyte growth and development. M.J. Azain*[1], [1] *University of Georgia.*

The most common association of fatty acids with adipose tissue is related to their storage as triglycerides in mature adipocytes and the consequences of excess accumulation in obesity. There is considerable evidence from cell culture experiments and studies in rodents that fatty acids can also regulate adipocyte development. In this role, fatty acids have hormone like effects and can be shown to regulate gene expression in pre-adipocytes that can have effects on both proliferation and differentiation. Long chain, saturated and polyunsaturated fatty acids have been shown to regulate transcriptional factors, such as CAAT / enhancer binding protein (C/EBP), peroxisome proliferator activated receptor (PPAR), and other adipose-specific genes, very early in adipocyte development. These effects have the potential to affect fat cell number at maturity. Specifically, there is evidence that the fatty acids in fish oil, such as docosahexaenoic (DHA) and eicosopentaenoic (EPA) acids, and fatty acids in the conjugated linoleic acid (CLA) series, reduce pre-adipocyte proliferation in cell lines and reduce adiposity in rodents. Conversely, diets high in saturated fatty acids have been shown to increase adipose tissue mass. There is little direct evidence of the ability of fatty acids to manipulate adipocyte development in non-rodent species. However, it should be noted that only recently has the importance of essential fatty acids, such as arachidonate and DHA, for brain development been documented. This has led to introduction of human infant formula supplemented with these fatty acids. The genetic, nutritional, and pharmacological manipulation of adipose tissue in meat animals has been a long term interest of animal scientists. An understanding of the ability of fatty acids to regulate such factors as adipocyte number, particularly in meat animals, would be of great interest. The evidence for regulatory roles of fatty acids in development from rodent and in vitro studies and their potential application to meat animals will be reviewed.

Key Words: Dietary fat, Fish oil, Conjugated linoleic acid

27 Adipose tissue angiogenesis. G. J. Hausman, *USDA-ARS.*

A review of adipose tissue angiogenesis includes discussion of the morphological and cytochemical development of adipose tissue vasculature and the concept of primitive fat organs. Spatial and temporal relationships between vascular and fat cell development in the fetus are also discussed including depot and genetic dependent arteriolar differentiation. The relationship between connective tissue deposition and elaboration of adipose tissue vasculature is discussed with respect to regulating adipocyte development in a depot dependent manner. In vitro studies indicate that depot dependent vascular traits may be attributable to intrinsic growth characteristics of adipose tissue endothelial cells. These studies indicate that adipogenesis may be regulated by factors that drive angiogenesis. Fundamental aspects of angiogenesis including basement membrane breakdown, vasculogenesis, angiogenic remodelling, vessel stabilization and vascular permeability are reviewed. Critical angiogenic factors including vascular endothelial growth factor (VEGF), VEGF receptors, angiopoietins, metalloproteinase enzymes and the plasminogen enzymatic system are also discussed. VEGF is the most critical factor since it initiates the formation of immature vessels and disruption of a single VEGF allele leads to embryonic lethality in mice. Expression of VEGF is influenced by hypoxia, insulin, growth factors and several cytokines. Angiogenic factors known to be secreted and / or produced by adipocytes or preadipocytes are discussed. VEGF expression and secretion by adipocytes is regulated by insulin and hypoxia and is associated with adipose tissue accretion. VEGF accounts for most of the angiogenic activity of adipose tissue. The proposed role of leptin as an angiogenic factor is reviewed with respect to efficacy on various aspects of angiogenesis relative to other angiogenic factors. Potential links between VEGF and leptin gene expression have not been examined but both genes are induced by hypoxia. Finally, several studies including a study of mice treated with anti-angiogenic factors indicate that adipose tissue accretion can be controlled through the vasculature per se.

Key Words: Angiogenesis, Adipose tissue, Leptin

28 The adipocyte as an endocrine cell. J. L. Miner* and K. M. Hargrave, *University of Nebraska.*

It has been hypothesized since at least since the 1940s that communication between adipose and other tissues may be bidirectional. Despite this expectation, early progress in our understanding of adipose tissue function was largely limited to its role in metabolism and storage of fatty acids, its development, and its response to endocrine and neural cues. However, the last decade has witnessed identification of several molecules that are secreted from adipocytes apparently for the purpose of signaling to other tissues. Cloning of the mouse obesity gene in 1994 is perhaps the most famous impetus for recognition that adipocytes are active in regulation of multiple body functions. The product of this gene, leptin, has since been found to inhibit feeding, enhance energy expenditure, and stimulate gonadotropes. Evidence for the roles other adipocyte-derived signals is being generated. Resistin is a protein that can cause whole body insulin resistance. Its expression is correlated with body fat and is inhibited by thiazolidinediones, perhaps mediating the association of diabetes with obesity, and the effectiveness of these drugs. It and a related molecule, RELMa, also can inhibit differentiation of preadipocytes. Tumor necrosis factor-a is secreted from adipocytes and antagonizes insulin action. Adiponectin/Acrp30 secretion from adipocytes is diminished in obese states. This protein can enhance use of fatty acids in lean tissues, and reduce both blood glucose and body weight. Secretion of complement proteins has been observed in adipocytes and these interact to generate a signal called acylation stimulating protein which can promote triacylglycerol synthesis. Similarly, adipocytes secrete renin-angiotensin system components and adenosine, both of which are anabolic in adipose tissue. It is unlikely that all of the adipocyte's endocrine signals have been identified. Certainly, much is yet to be learned about how these signals function. However, it is clear that these new discoveries comprise a useful model for our study of growth and development in livestock.

Key Words: Adipose tissue, Secretion, Hormone

29 Metabolism and development of bovine brown adipose tissue. S. B. Smith* and G. E. Carstens, *Texas A&M University, College Station, TX.*

Angus newborn calves have a greater ability to generate heat by nonshivering thermogenesis than Brahman newborn calves. We have worked to document the biochemical basis for this phenomenon. Brahman perirenal brown adipose tissue (BAT) contains two-to-three times as many -receptors as Angus BAT, although the dissociation constant is not different between breed types. Mitochondrial uncoupling protein (UCP1) mRNA concentration is greater in newborn Brahman BAT than in Angus BAT, whereas lipogenesis from acetate is greater in Angus BAT than in Brahman BAT. We obtained fetuses of each breed type at 96, 48, 24, 14, and 6 d before expected parturition, and at parturition. Glycerolipid synthesis from palmitate declined by 85% during the last trimester, but still contributed 98% to total lipid synthesis at birth. The concentration of UCP-1 mRNA tripled during gestation in both breed types. Uncoupling protein-1 mRNA initially was elevated in tailhead s.c. adipose tissue, but was barely detectable by birth, and tended to be higher overall in Angus than in Brahman s.c. adipose tissue. In a third experiment, male Angus and Brahman calves were assigned to one of three groups; (1) newborn; (2) 48 h of warm exposure starting at 15 h of age; and (3) 48 h of cold exposure starting at 15 h of age. The calves in the newborn treatment were euthanized at 15 h of age. The warm-treatment calves (22C) and cold-treatment calves (4C) were maintained euthanized after 48 h at each temperature. Brahman BAT adipocytes were smaller than Angus BAT adipocytes initially and shrank with cold exposure, whereas Angus BAT adipocytes did not. The production of CO_2 from palmitate and acetate increased with cold exposure. Lipogenesis from acetate and palmitate was greater in BAT from calves held at warm or cold temperatures than in BAT from newborn calves. Also, BAT from Angus calves had greater rates of lipogenesis from palmitate than BAT from Brahman calves. The data indicate that BAT from Brahman calves mobilizes stored lipids more rapidly than BAT from Angus calves, but resynthesizes lipids more slowly. Thus, BAT from Brahman calves may be exhausted of lipid shortly after birth during times of cold exposure, leading to reduced thermogenesis during times of extended cold stress.

Key Words: Brown fat, Bovidae, Lipid metabolism

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30 Altered growing conditions can inhibit nisin production in lactic cultures by disrupting the signal transduction pathway. H. Li* and D. O'Sullivan, *University of Minnesota.*

A signal transduction pathway controls the production of the nisin bacteriocin by Lactococcus lactis. In this system, external nisin can signal the nisin genes to be switched on in a dose dependent fashion, involving the membrane bound kinase (NisK) and the intracellular regulator, NisR. Phosphorylated NisR initiates transcription of the genes involved in nisin production. However, we have found that nisin production can be switched off under certain conditions. These conditions are: 1) growth of L. lactis at it maximum growth temperature of 40(C; 2) transfer of the nisin gene cluster into a dairy Enterococcus strain; 3) electroporation of plasmids into certain nisin producing L. lactis strains. In these three cases, Northern and RT-PCR analysis confirmed that the nisA gene was not expressed, but the immunity genes were. This suggested the lack of nisin production under these conditions was possibly due to a blockage in the signal transduction pathway. To address this hypothesis, gel shift experiments were conducted with a nisA promoter fragment using crude cell extracts from cultures growing under these conditions. A shift was observed for cell extracts from the positive control, L. lactis ATCC 11454 grown at 30°C, but not for crude extract from L. lactis ATCC 11454 grown at 40°C or the dairy Enterococcus strain containing the nisin gene cluster. Furthermore, a protein with the same size as NisR (26 kDa) was isolated by nisA competitive heparin-affinity column chromatography from crude extracts from L. lactis ATCC 11454 grown at 30°C, but not from cell extracts under the non-nisin producing conditions. This suggested the lack of an activated, phosphorylated NisR, under these conditions. In addition, RT-PCR and Northern hybridization confirmed the presence of the nisRK transcript, indicating that NisRK was most probably produced. Therefore, the nisin gene expression was likely blocked from the reduced inability of external nisin to initiate the signal transduction pathway during these conditions. This is the first evidence of a specific mechanism for inhibition of nisin production in lactic cultures. This novel finding should enable culture users to more reliably predict nisin production kinetics of cultures during specific culture uses.

31 Invasion of *Mycobacterium avium sub sp paratuberculosis*in bovine epithelial cells and bovine mammary epithelial cells. D. Patel*[1], L. Goddik[1], and L. Bermudez[2], [1]*Food Science and Technology, Oregon State University, Corvallis, OR 97331-6602,* [2]*Department of Biomedical sciences, College of Vet Med, Oregon state Univ, Corvallis OR 97331-4804.*

Main objective of our experiment was to investigate invasion characteristics of *M*ycobacterium avium sub sp. paratuberculosis (MAP)against bovine epithelial cells and bovine mammary epithelial cells as targets. Johne's disease is a chronic infectious disease of ruminants caused by a bacterium *M*ycobacterium avium sub sp. paratuberculosis (MAP). It is estimated that about 30 % of dairy herds are infected with Johne's disease in the US. It is known that MAP infects the host by the oral route and young calves are infected at early age. Intestinal epithelial cells thus become primary site of infection. For current experiment we hypothesized : 1. Mammary epithelial cells can be a reservoir for MAP and therefore invasion could take place by apical or basolateral surface ; 2. MAP can enter intestinal epithelial cells. To test the above hypothesis we evaluated invasion employing immortalized epithelial cell lines, namely Bovine epithelial cell(MDBK purchased from ATCC) and Mammary epithelial cell (MACT, given by Dr. Sheffield, Univ of Wisconsin). MAP strain ATCC 19698 was used in our study. Invasion assay protocol was standardized in our lab. Based on the statistical analysis of data we found that MAP invades MAC-T and MDBK cells successfully, albeit poorly. MAP showed markedly higher rate of invasion in case of MDBK compared to MAC-T. Exposure of basolateral surface did not have marked influence on invasion in case of mammary epithelial cells, suggesting that the apical surface is the main route of entry however, exposure of basolateral surface of bovine epithelial cells significantly increased the uptake of bacteria., a puzzle that we cannot explain without further studies. MAP is a pathogen that is extremely resistant to wide spectrum of antibiotics. Its control lies in breaking its transmission cycle by inhibiting molecular interaction with epithelial cell surfaces. In this endeavor, in-vitro invasion assay described here can serve as a useful model in screening interesting MAP mutants with reduced or altered invasion efficiency. Main conclusion of our study is- MAP can successfully invade bovine epithelial cells and mammary epithelial cells. Exposure of basolateral surface significantly increases invasion rate in case of bovine epithelial cells.

Key Words: Mycobacterium avium sub sp paratuberculosis, Invasion assay, Bovine mammary epithelial cells

32 Epidemiology and ecology of *Listeria monocytogenes* at the pre-harvest food level. K. K. Nightingale*, E. D. Fortes, C. R. Nightingale, Z. Her, Y. H. Schukken, Y. T. Grohn, and M. Wiedmann, [1]*Cornell University.*

Listeria monocytogenes is a human foodborne pathogen and causes severe systemic infections in animals. *L. monocytogenes* is responsible for a significant portion of dairy product Class I recals. Raw milk may harbor *L. monocytogenes* and pasteurized dairy products may be contaminated if the pathogen become established in processing plant environments. A case-control study involving 22 case farms (13 dairy cattle, 1 beef cattle, 4 goat, and 2 sheep) and 22 pair-matched controls was conducted to probe the epidemiology and ecology of *L. monocytogenes*. A total of 1652 fecal (n=424), feed (n=420), and environmental samples (soil, n=397; water, n=411) were cultured for *L. monocytogenes*. While prevalence of *L. monocytogenes* was not significantly different (p=0.1492) in bovine case (23.13%) and control (19.58%) farms, the pathogen was more common ($p<0.0001$) in small ruminant (caprine and ovine pooled) case farms (26.41%) than controls (4.40%). The prevalence of *L. monocytogenes* was not significantly different ($p>0.05$) in fecal, soil, feed, and water samples from bovine case and control farms. Small ruminant case farms showed a significantly higher prevalence ($p<0.05$) of *L. monocytogenes* in all sampling categories than small ruminant controls. Molecular subtyping (*Eco*RI ribotyping) of clinical (n=15) and farm isolates (n=310) differentiated 49 unique ribotypes.