a wider distribution in soft and hard tissues and many other biological fluids.

In previous work we have shown that a number of casein phosphopeptides can sequester amorphous calcium phosphate [3,4] to form a thermodynamically stable [5] nanocluster of radius 4nm. Calcium phosphate nanoclusters occur naturally as substructures in the colloidal casein particles of milk, known as casein micelles [6]. They allow a high concentration of an otherwise highly insoluble calcium salt to be achieved without danger of precipitation of a solid phase. Accordingly, we have suggested that the casein micelle is a solution to the problem of pathological calcification in the mammary gland.

We will consider the structural and thermodynamic requirements for the formation of nanoclusters and show that one other non-casein member of the group of SCPPs is able to sequester calcium phosphate and form nanoclusters. These findings suggest that calcium phosphate sequestration by phosphoproteins may be part of a general solution to the problem of the control of biocalcification.

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400 HAMLET, an alpha-lactalbumin folding variant that induces tumor cell apoptosis. C. Svanborg*, *University of Lund*, *Sweden*.

HAMLET (**H**uman **a**-lactalbumin **M**ade **LE**thal to **T**umour cells) is a structurally defined protein-fatty acid complex derived from human milk. The complex kills cancer cells but leaves healthy differentiated cells intact. In the laboratory, HAMLET kills >40 different cancer cell lines, with leukemic cells being the most sensitive. The cells die by an apotosis like mechanism which is paradoxical as most tumour cells carry mutations that prolong their life span by allowing them to avoid apoptotic cell death. Thus, HAMLET appears to identify a death mechanism that is conserved in tumor cells, but lost as cells differentiate and mature. As the protein and lipid that form HAMLET occur naturally in human milk it may be speculated that HAMLET might contribute to the lowered cancer incidence in breast-fed children.

Since the discovery of HAMLET in 1995, we have studied

1. The **molecular characteristics of the compound**

2. **The mode of action on tumour cells.**

3. **The protective potential of HAMLET in tumor models** and human patients

HAMLET was used for brain tumour treatment in a human rat xenograft model. (Ref: ***Cancer Res** 64:2105, 2003*.) Infusion of HAMLET into the tumor was shown to delay tumor growth and prolong survival of immmunodeficient rats, carrying a human glioblastoma tumor. Apoptotic cells were detected in the tumor but not in surrounding healthy brain tissue.

HAMLET was also used for human skin papilloma treatment (Ref: ***N Engl J Med** 350:2663, 2004*.). A placebo controlled trial of topical HAMLET treatment was carried out and completed with a two-year follow-up. HAMLET was shown to reduce the volume of skin papillomas and to promote the resolution of the lesions.

The molecular, functional and therapeutic aspects will be discussed

Nonruminant Nutrition: Stable Isotope Tracer Techniques for Nonruminant Nutrition Research and Their Practical Applications

401 Mass isotopomer distribution analysis (MIDA) for studying intermediary nutrient metabolism. B. J. Bequette*, *University of Maryland*, *College Park*.

Functional genomic and proteomic investigations are beginning to characterize the metabolic controls and relationships between gene function and nutritional physiology. Still missing in this metabolic roadmap is characterization of the activities or fluxes through these integrated pathways that ultimately determines nutrient utilization. In the past 10 years, stable isotope (^{13}C, ^{15}N, ^{2}H) labeling (mass isotopomer) with mass spectrometric analysis has allowed fluxes through metabolic pathways to be measured in vivo and in vitro. MIDA refers to the measurement of the mass distributions in a molecule or molecular fragment that are characteristic of the unique biochemical pathway(s) of the nutrient's metabolism. This review will discuss the use of MIDA and how it can be used to dissect the integrative pathways of macronutrient (protein, carbohydrates, fat) metabolism. For example, in studies with laying hens, fish and chicks, MIDA has been applied to ascertain the nutritional essentiality, and thus metabolic inability for synthesis, of nucleic acids and some "non-essential" amino acids. Another application of MIDA has been the determination of the pathways and cycles of essential amino acid metabolism with regards to their metabolic roles other than for protein synthesis. When applied to the measurements of new gluconeogenesis and tissue utilization of glucose, MIDA has exposed the importance of amino acids as glucogenic substrates, and also highlighted the importance of the interconnectivity of the pathways of metabolism of carbohydrates, volatile fatty acids, amino acids and fatty acids to maintain anaplerosis and cataplerosis (metabolic balance) via the Krebs cycle. As gene and protein expression profiles begin to build the global roadmap of nutrient utilization, it will be necessary to determine the functional and quantifiable significance of these metabolic pathways that make up the roadmap. Here, the use of MIDA, when applied to the study of macronutrient metabolism, can provide the details of the biochemical networks of nutrient utilization.

Key Words: Stable Isotope, Amino Acid, Glucose

402 Measuring splanchnic amino acid metabolism by using stable isotope tracers. B. Stoll* and D. Burrin, *USDA-ARS Children's Nutrition Research Center, Baylor College of Medicine*, *Houston, TX*.

The splanchnic bed is comprised of the liver and the portal drained viscera (PDV). The PDV, which include the stomach, intestines, pancreas, and spleen, represent 4-6% of body weight, yet they account for 20-35% of whole-body protein turnover and energy expenditure. The high nutrient needs of the gut are met first as a result of first-pass metabolism. Consequently, the first-pass metabolism of dietary nutrients by the gut, especially amino acids, has a critical influence on their availability to peripheral tissues and whole body requirements. Moreover, the systemic availability of dietary amino acids is key determinant of lean body growth rate. A complicating factor in the measurement of gut nutrient utilization is that the intestinal mucosa receives nutrients from two sources, the diet and the arterial circulation. However, combining measurements of the net portal balance with enteral and intravenous infusions of stable isoto-

pically labeled amino acids provides an in vivo model that can distinguish the proportion of amino acids that are derived from the diet and arterial input. Using this technique in fed infant pigs, we found that 30-40% of the total amino acid intake is used by gastrointestinal tissues. The relative PDV utilization of individual amino acids from the diet and arterial inputs varies widely and dietary amino acids are the preferred fuel over dietary glucose. Stable isotopically labeled amino acids also enable the determination of the metabolic fate of individual amino acids. These studies have shown that insufficient protein supply or mode of feeding affects PDV amino acid utilization and consequently has a bearing on whole-body growth.

Key Words: Intestine, Swine, Nutrition

403 Mineral bioavailability and metabolism determined using stable isotope tracers. J. R. Turnlund*, *USDA/ARS/Western Human Nutrition Research Center, University of California*, *Davis*.

Definitive data on mineral bioavailability in humans and animals can be obtained by using isotopic tracers. The use of stable isotope tracers to study important issues in mineral nutrition has expanded rapidly in the past two decades, particularly in humans. Stable isotopes have a number of advantages over radioisotopes. There is no exposure to radiation with stable isotopes and some minerals have no radioisotope that can be used satisfactorily as a tracer. Multiple stable isotopes of one mineral and isotopes of multiple minerals can be administered simultaneously or sequentially. The analytical methods of choice for stable isotopes are thermal ionization mass spectrometry (TIMS) and inductively coupled plasma mass spectrometry (ICPMS). TIMS offers the highest precision and accuracy, but is slower, more labor intensive, and more costly than ICPMS. Bioavailability data are critical to establishing reliable dietary mineral requirements and recommendations. Combined with a computer program for compartmental modeling, mineral kinetics can be studied, including mineral turnover, pool sizes, and transfer rates between compartments. Our laboratory conducts studies using stable isotopes of zinc, copper, iron, calcium, magnesium, and molybdenum. We have studied the effect of the amount of dietary intake of minerals on bioavailability and utilization, pregnancy and aging, and interactions between minerals. The work resulted in establishing new dietary recommendations in humans for copper and molybdenum and compartmental models were developed for these minerals. While stable isotopes have been used more extensively to date in humans than in animals, the techniques applied to humans can be used to study a number of issues important to optimizing feeding strategies for animal production.

Key Words: Stable Isotopes, Mineral Bioavailability, Mineral Metabolism

404 Measuring nitrogen-containing polymer synthesis rates by using stable isotope tracers. M. Z. Fan*[1], L. I. Chiba[2], P. D. Matzat[3], and Y. L. Yin[4], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*Auburn University*, *Auburn, AL*, [3]*Elanco Animal Health*, *Greenfield, IN*, [4]*The Institute of Subtropical Agricultural Research, the Chinese Academy of Sciences*, *Changsha, Hunan, China*.

The major nitrogen (N)-containing polymer compounds in the body include DNA, RNA, and proteins. The gastrointestinal endogenous secretions as well as the portal-drained visceral and the peripheral immune responses are of basic physiological functions. Elevated endogenous secretions and immune activities, as affected by developmental stages, diets and environmental factors, decrease the efficiency and availability of the major dietary nutrients for peripheral muscle synthesis and deposition. Measurements of in vivo fractional DNA (cell proliferation), RNA (transcriptional efficiency/mRNA stability) and protein (translational efficiency/metabolism) synthesis rates associated with the visceral organs, peripheral immune cells and skeletal muscles should, in principle, be the sensitive biochemical and cellular endpoints for studying factors affecting monogatric animal nutrition and metabolism. The selection of precursor stable isotope tracers, routes of tracer delivery and the gas chromatography-mass spectrometric (GC-MS) analyses of tracer enrichments are the major methodological considerations. Oral feeding the heavy water (^2H_2O) and intravenously continuous infusion of [U-^{13}C]glucose and [^{15}N]glycine for labeling the ribose and deoxyribose sugar moieties, de novo base synthesis, and non-essential amino acids have been established to measure in vivo fractional DNA, RNA, and protein synthesis rates. Flooding doses of tracer phenylalanine (Phe), e.g., L-[ring-$^2H_5$]Phe, via i.v. and i.p. routes, are reliable and cost-effective for measuring fractional protein synthesis rates especially for the visceral organs in suckling and weanling pigs. Therefore, measuring the major N-containing polymer fractional synthesis rates in the visceral organs and the peripheral immune cells through oral feeding ^2H_2O and/or ip flooding doses of tracer Phe are the emerging powerful tools for studying monogastric animal nutrition and metabolism under controlled experimental and field conditions.

Key Words: Stable Isotope Tracers, Fractional Synthesis Rates, Pigs

405 Factors affecting in vivo fatty acid and triglyceride synthesis rates measured by stable isotope tracers. E. Murphy*, *University of California*, *San Francisco*.

Synthesis of fatty acids (de novo lipogenesis) and triglyceride synthesis are important factors in body fat accumulation. Recently, new stable isotope methods using heavy water (^2H_2O) have made possible the safe, and relatively easy, measure of both of these processes in vivo in animals and humans. New methods also provide information on the preferential use of specific triglyceride synthesis pathways under different physiological settings. Data suggest that numerous dietary factors may affect de novo lipogenesis including nutrient composition, fructose intake, caloric content and fatty acid composition. Significant differences in de novo lipogenesis have also been seen across species. Rates of triglyceride synthesis have been shown to differ significantly between different adipose depots with metabolically active depots (e.g., visceral fat) having much more rapid triglyceride turnover than subcutaneous depots. Dietary fat, leptin deficiency and treatment with insulin sensitizers such as the PPAR-γ agonist rosiglitazone have all been shown to influence triglyceride synthesis rates. Application of these new techniques to nonruminant animals other than rodents will undoubtedly enhance our understanding adipose biology.

Key Words: Lipogenesis, Stable Isotope, Triglyceride Synthesis

Physiology and Endocrinology IV

406 Effect of postpartum nutrition of primiparous beef cows on concentration of insulin in follicular fluid and abundance of mRNA for binding proteins (IGFBP) -4 and -5 and aromatase in granulosa cells of dominant follicles. I. Rubio*, R. P. Wettemann, F. J. White, P. Y. Aad, and L. J. Spicer, *Oklahoma Agricultural Experiment Station*, *Stillwater, OK*.

Greater nutrient intake increased concentrations of IGF-I and IGFBP-4 and -5 in follicular fluid (FF) of postpartum anovulatory primiparous cows. This experiment evaluated the effect of nutrient intake on insulin in FF, and abundance of mRNA for IGFBP-4 and -5 and aromatase in dominant follicles (DF) at 56 ± 9 d postpartum in the same anovulatory Angus x Hereford cows. Body condition score (BCS) at calving was 4.8 ± 0.2. Cows (n=28) were blocked based on BCS and randomly assigned to one of two nutritional treatments at calving; moderate (M), 2.3 kg/d of a 40% CP supplement and ad libitum hay, or high (H), ad libitum access to a 12% CP-50% concentrate diet and hay. Growth of DF was evaluated daily by ultrasonography for 5 d before aspiration. When growth of DF plateaued, FF was obtained by transvaginal ultrasound-guided aspiration. Data were analyzed using the MIXED procedure of SAS and Pearson correlations coefficients. Concentrations of insulin in FF were greater ($P < 0.05$) for H (1.59 ± 0.22) than M (0.97 ± 0.17 ng/ml) and H cows had greater ($P < 0.01$) insulin in plasma (1.61 ± 0.17) than M (0.97 ± 0.17 ng/ml). Concentrations of IGF-I in FF were greater ($P < 0.01$) for H than M cows. Abundance of