Regulation of lipid deposition in farm animals: Parallels between agriculture and human physiology

Werner G Bergen and Terry D Brandebourg

Department of Animal Sciences, Auburn University, Alabama, AL 36849-1554, USA
Corresponding author: Werner G Bergen. Email: bergewg@auburn.edu

Abstract

For many years, clinically oriented scientists and animal scientists have focused on lipid metabolism and fat deposition in various fat depots. While dealing with a common biology across species, the goals of biomedical and food animals lipid metabolism research differ in emphasis. In humans, mechanisms and regulation of fat synthesis, accumulation of fat in regional fat depots, lipid metabolism and dysmetabolism in adipose, liver and cardiac tissues have been investigated. Further, energy balance and weight control have also been extensively explored in humans. Finally, obesity and associated maladies including high cholesterol and atherosclerosis, cardiovascular disease, insulin resistance, hypertension, metabolic syndrome and health outcomes have been widely studied. In food animals, the emphasis has been on regulation of fatty acid synthesis and lipid deposition in fat depots and deposition of intramuscular fat. For humans, understanding the regulation of energy balance and body weight and of prevention or treatment of obesity and associated maladies have been important clinical outcomes. In production of food animals lowering fat content in muscle foods while enhancing intramuscular fat (marbling) have been major targets. In this review, we summarize how our laboratories have addressed the goal of providing lean but yet tasty and juicy muscle food products to consumers. In addition, we here describe efforts in the development of a new porcine model to study regulation of fat metabolism and obesity. Commonalities and differences in regulation of lipid metabolism between humans, rodents and food animals are emphasized throughout this review.

Keywords: Regulation of lipid deposition, humans, food animals, animal models for obesity

Experimental Biology and Medicine **2016; 241: 1272–1280. DOI: 10.1177/1535370216654996**

Introduction

Clinically oriented scientists and animal scientists often focus on a common biology. Unfortunately, differences in the underlying rationales for conducting the respective research have traditionally created an arbitrary barrier preventing cross-pollination of intellectual efforts between human and veterinary medicine and various disciplines within the animal sciences. However, the One Health concept, borne out of an acknowledgement that human, animal (both domestic and wild) and environmental health is inextricably linked, suggests that much can be learned by a greater exchange of ideas across the relevant literature of these fields.[1,2] For instance, the shift from traditional production systems toward intensive animal agriculture has facilitated impressive increases in production efficiency, improvements in animal health, enhanced food safety, and a decrease in the carbon footprint of animal production, all driven by basic research aimed at better understanding the genetic, nutritional and endocrine regulation of mammalian physiology.[3,4] Concomitant with this transformation, there has been a growing interest in the utility of developing novel large animal models for biomedical research. Such models, aimed at addressing key bottlenecks in our understanding of health and disease, could speed advancements in human health.[5,6]

In this paper, we describe the background for and research conducted in the authors' laboratories primarily on lipid metabolism in cattle and pigs. While the specific aim of our research programs is to support the efficient production of highly desirable, lean-low fat muscle foods, our work with these species also has applications for human obesity and metabolic disease. In order to demonstrate the potential for translational large animal research, these efforts will be compared to work on lipid metabolism done in rodents and humans where applicable.

Synergism between food animal research and biomedicine

Traditionally, large animal research has been motivated by a desire to improve the output and sustainability of the food

supply chain. The National Research Council Committee on Twenty-First Century Systems Agriculture (NRCC) has defined agricultural sustainability in terms of four goals that can be summarized as (1) meeting the human need for food and biofuels, (2) enhancing environmental quality, (3) sustaining the economic viability of agriculture and (4) improving the quality of life for those involved in farming and their surrounding communities.[7] This has meant that animal scientists have focused upon developing strategies to more efficiently produce animal products that satisfy consumer demand. During the last 50 years, recommendations by physicians to limit consumption of animal fat have negatively impacted the demand for fatty animal products. In response, livestock have been genetically selected to exhibit greater feed efficiency and leanness, and much research has been directed at better understanding the regulation of adipose tissue development in food animals.[8,9] Resulting market trends have thus been toward producing rapidly growing and increasingly leaner food animals.

Based upon the NRCC definition of sustainability, food animal industries in the U.S., such as the modern beef industry, are resounding success stories. For instance, approximately 85%of U.S. grazing land is unsuitable for crop production. Utilizing this land for grazing livestock could more than double the landmass that is available to produce food for human consumption. Between the years of 1977 and 2007, technical advances in genetics, production, and processing reduced the number of animals required to produce 1 billion kg of beef by 30% and the amount of feed required by 19%thus reducing the land, water and carbon footprints associated with these endeavors.[3] However, there remains a great need for further basic research aimed at better understanding the growth and reproductive physiology of large animal models as these advances come at a time when the world population is projected to exceed 9 billion souls by the year 2050 with demand for agricultural products growing 1.5% annually.[10] Additionally, mounting evidence indicates climate change will occur in coming decades with significant depressions in food production expected due to seasonality if warm season temperatures rise as climate models predict.[11] In response to these anticipated crises, the United Nation's Food and Agriculture Organization projects that 70% of the world's additional food needs will have to be satisfied by improving existing production methods and by developing new technologies all aimed at increasing production efficiency through decreasing inputs into the food supply chain while increasing outputs.[10]

Importantly, the need to better understand the regulation of energy balance, adipose tissue development, and immune function in large animal models mirrors a similar need for humans. Economic globalization has inextricably linked human populations more closely than ever before making the spread of infectious disease an ever growing threat.[12] Furthermore, a worldwide obesity epidemic represents a critical threat to public health by spurring a sharp rise in obesity-associated mortality rates through increased risk to a growing number of individuals for developing diabetes, heart disease, stroke, arthritis, and certain cancers.[13–17] The alarming increase in the incidence of obesity and type 2 diabetes (NIDDM) among juveniles since 1980 suggests that the societal impact of obesity-related issues will only intensify.[18,19] If US trends based upon historical data continue, the prevalence of obesity in US adults will increase from 32% currently, to approximately 50% by 2030, with estimated associated healthcare costs increasing $48–66 billion per year in the US due to expected increases in obesity-induced diabetes, heart disease, stroke, and cancers.[20] By 2050, 60% of men and 50% of women could be clinically obese worldwide.[21] Interestingly, companion animals face some of the same obesity-related health problems as humans do. Mirroring human medicine, the financial burden of obesity is also affecting small animal veterinary medicine through greater incidences of insulin resistance and type 2 diabetes, high blood pressure, heart and respiratory disease, osteoarthritis, cranial cruciate ligament injury, kidney disease, many forms of cancer in pets resulting in decreased life expectancy of up to 2.5 years.[22,23]

Never before has the sustainability of human and animal health, food production and safety and environmental stewardship necessitated a multidisciplinary view of the systems affected with solutions having a balanced impact on humans, animals and the environment such that when enacted potential solutions to threats do not benefit one element to a significant detriment of the others.[2] Better understanding factors regulating energy balance, satiety and adipose tissue development would address the need to manipulate body composition in humans and livestock. Such advances would simultaneously improve production efficiency, allow animal products that are both healthier and more enjoyable to consume, and improve the health of humans through insights that might be applied to human obesity. Likewise, novel animal models of hyperphagic obesity and its complications are needed in order to more quickly develop effective strategies for the intervention or long-term prevention of obesity in susceptible individuals. Such models would also allow advances in food production systems as well.

Lowering of excess lipid deposition in farm animals

Fat deposition patterns and fatty acid content has been an important area of food animal research dating as far back as the 1940s.[24] Early work centered primarily on genetic selection, nutritional strategies, and management practices that would encourage fat deposition and intramuscular fat or ''marbling'' (IMF) as animals approached harvest weight given that animal fat was historically considered a valued commodity. However, by the 1970s, a recognition grew that fat content of the then traditional muscle foods was excessive. This was coupled with the emergence of important efforts to modify human nutrition in order to lower saturated fatty acid intake, promote more polyunsaturated fatty acid intake and develop healthier eating practices in light of research suggesting a link between dietary fat and cardiovascular disease.[25]

Given these rapidly changing market trends, two approaches emerged, one long term and one much shorter term in application, to significantly lower the fat content in muscle foods. The longer term approach involved

continued genetic selection for leaner food animals and such strategies have largely been very effective in altering the fat content of animal products.[26] However, given the lack of selectable markers that allows specific traits to be manipulated independently, often such genetic selection was performed blindly by incorporating multi-allelic phenotypic criteria such as growth rate or gross indices of adiposity into selection programs without knowledge of the underlying physiological drivers. This has meant that closely linked genes that may not promote an advantageous phenotype have also been enhanced. While genetic selection over time has resulted in dramatically leaner livestock, it needs to be recognized that food items that do not have favorable organoleptic properties are a difficult item to sell in the marketplace. Since lipids enhance flavor and the perception of tenderness, extremely lean muscle foods are less desirable. Unfortunately, important organoleptic traits such as flavor, juiciness, tenderness, color, and water-holding capacity are often adversely affected in heavier muscled, leaner carcasses which has negatively impacted consumer demand.[27–29] Thus, while genetic selection programs have effectively altered the body composition of livestock, there still exists a great need to better understand the molecular regulation of muscle and adipose tissue development so that adiposity can be finely regulated in ways that maximize production efficiency without adversely impacting the sensory aspects of muscle foods.

The shorter term approach to manipulating adiposity involved efforts to pharmacologically control fat synthesis and lipolysis in growing animals. It is these efforts aimed at developing metabolic modifying agents that have revealed the most insight regarding the regulation of body composition and adiposity. Two such agents, beta-adrenergic agonists (epinephrine analogs) and somatotropin (recombinant growth hormone), emerged as potential pharmacological tools to specifically inhibit de novo fatty acid synthesis and enhance lipolysis ultimately inducing nutrient partitioning away from adipose tissue in support of greater muscle hypertrophy.[30] We conducted a series of studies on the response to these agents in lean and obese pigs focusing particularly on the mechanism of ractopamine® (RAC, Eli Lilly; Indianapolis, IN, USA) action on protein and lipid synthesis.[31–43] These studies clearly indicated that RAC administration acutely stimulated lipolysis through the cyclic-AMP-PKA pathway while chronically acting at the level of gene transcription to downregulate lipogenic genes and upregulate lipolytic genes. However, rapidly induced receptor feedback mechanisms ultimately blunt this effect limiting the long-term effectiveness of these pharmacologic agents in pigs. Eventually, a limited number of beta-adrenergic agonists were approved by the FDA for agricultural use, but somatotropin never reached market development for application to pork or beef production within the United States.

In general, work on weight regulation therapy through pharmacological means has not been extremely successful in humans. In part this is due to lack of specific targeting by some drugs or a lack of long-term effectiveness.[44–46] This is critical in humans as fat gains are achieved over a long period. A pharmacological approach in humans should be seen as a proactive-preventative for long-term expanding fat deposition. Unfortunately, such medications may need to be taken for a whole lifetime. To date, no such agents have been discovered or evaluated for long-term efficacy and safety.

Experiments aimed at elucidating the regulation of lipid deposition in livestock were first conducted using biopsies and post-harvest adipose tissue dissections. These approaches facilitated classical metabolic studies of lipid synthesis and degradation rates, enzyme studies, and metabolite effects.[8,9,47] Experimental inquiries have gradually evolved toward mechanistic studies into cell signaling pathways and the regulation of key adipose-specific genes as new technologies emerged, and species-specific reagents have become more readily available for food animals of interest. When a porcine-specific chip platform became available, we conducted a preliminary microarray study using adipose tissue harvested from large White composite castrated males fed RAC for up to four weeks.[48] Generally, genes involved in de novo fatty acid synthesis including FAS, ACC, malate dehydrogenase, SCD, GLUT4, SREBP-1c were down-regulated in response to RAC administration. Expression of genes encoding enzymes involved in energy metabolism (glycolysis, citric acid cycle and electron transport chain) was also generally down-regulated in the adipose tissue of RAC-treated animals. However, since these enzymes are also under allosteric and covalent regulation, the transcriptional regulation of oxidative metabolism enzymes in adipose may not be as critical in this tissue as in liver and skeletal muscle. Regulation of lipogenic genes by RAC was further investigated using genetic lines of pigs (Duroc crossbred (fatter) and Pietrain crossbreds (leaner) pigs) displaying differing potentials to fatten. Consistently, RAC lowered FAS, ACC, SCD, and SREBP-1c expression in pigs.[49] Interestingly, lean pigs had a lower expression of lipogenic genes and a higher expression of fatty acid oxidative genes (PPARα), while fat pigs had much higher expression of lipogenic genes but a much lower expression of oxidative genes.[26] Our attempts to study the gene expression in bovine muscle and adipose tissues in response to a beta-adrenergic agonist (Optaflexx®, Elanco Animal Health, Indianapolis, IN, USA) have generally provided little insight comparable to our pig results.[50]

Swine faithfully mimic several important aspects of human physiology

The pig is well positioned as a biomedical model that can be used to overcome the limitations associated with using rodent models for the study of metabolic syndrome and obesity.[51] Pigs are phylogenetically more closely related to humans. Humans and pigs are both omnivores, and like in humans, anatomically discreet depots of brown fat are largely absent in the pig. Additionally, the vasculature, the proportion of skeletal muscle and adipose tissue to total body mass, and circulating levels of glucose are all very similar in pigs and humans.[51,52] Pigs also have nutritional requirements similar to that of humans and have a tendency toward sedentary behavior.[53,54] Pigs and humans have

similar cardiovascular systems, pancreas morphology, and pharmacokinetics.[55] Given this, there is a well-developed literature concerning the use of swine models to study atherosclerosis, cardiovascular disease, and diabetes.[56–59] Furthermore, pigs and humans exhibit similar mature body weights.[53] A pig's larger body size relative to alternative animal models makes it possible to obtain greater volumes of blood and tissue, making pigs an easier experimental model to utilize.[54] Because of their anatomical, physiological, and metabolic similarities to humans, it is reasonable to use the human criteria for swine in modeling diabetes.[55]

Much of the classic work on lipid metabolism in swine was conducted using lean and obese pigs.[47] Interestingly, this classic literature has recently been rediscovered and has taken on renewed importance given current efforts to develop translational swine models of obesity and metabolic syndrome. The pig has a long history of serving as a biomedical model with reports of such use dating back in the literature as early as the 1930s. The pig began being heavily utilized for cardiovascular research beginning in the 1950s, and this application continues through the present day.

The first reports of the pig for use specifically as a model of obesity began appearing during the early 1970s. These efforts centered upon the Ossabaw pig, a novel, small-framed, lard type, feral hog which exhibits a unique lipid metabolism due to seasonal selection pressures characteristic of Ossabaw Island. This breed, phylogenetically similar to the Iberian pig, has existed as a closed genetic population on Ossabaw Island since they were introduced during the initial exploration of the Southern Gulf coast of the U.S. by the Spanish. The dramatic seasonal variation in nutrient availability on Ossabaw Island has cultivated an extreme thrifty genotype in Ossabaw pigs allowing them to rapidly accumulate excessive adiposity during periods of caloric excess. Interestingly, feral Ossabaw pigs exhibit island dwarfism and rarely exceed 25 kg at mature body weight. However, when reared under conditions of balanced nutrition, females can reach weights ranging between 70 and 110 kg, while males are capable of attaining a somewhat larger size (100–150 kg). When in a positive energy balance, especially in response to high fat diets, these pigs develop morbid obesity, mild insulin resistance, and profound hypercholesterolemia making them an attractive biomedical model for obesity and cardiovascular disease. Their failure to develop a more extreme metabolic dysfunction despite their excessive adiposity may be related to their very efficient lipid biochemistry which is consistent with an effective metabolic regulation rather than overt dysfunction. For instance, Ossabaw pigs fail to exhibit adipose tissue inflammation, a key link between obesity and metabolic dysfunction in humans, despite the development of an obese phenotype, while these pigs can rapidly mobilize lipid stores and significantly reduce carcass adiposity when in a negative energy balance.[60] Furthermore, Ossabaw pigs display a late onset of puberty and generally have small litters while bearing piglets of low birth weights. This may also confound their ability to model the progressive nature of obesity-induced metabolic disease in humans though their reproductive phenotype also serves as a rationale for using the Ossabaw pig to study links between obesity and reproductive function.

Over the years, several experimental paradigms have been applied to this breed to model obesity. In work conducted from the early 1970s through the turn of the century, Ossabaw pigs were largely maintained on high-energy grain diets with genetically lean breeds often serving as a control group, or in the case of USDA experiment stations, fat Ossabaw pigs were compared to ones that had undergone selection for leanness. Generally, this literature revealed that Ossabaw and contemporary pigs had similar plasma concentrations of glucose and free fatty acids, but insulin levels were slightly higher in Ossabaw pigs suggesting these pigs develop mild insulin sensitivity.[61] Moreover, insulin binding was lower in liver microsomes from Ossabaw versus lean York pigs, consistent with Ossabaw pigs being moderately insensitive to insulin. Insulin binding also decreased as Ossabaw pigs approached market weight (grew fatter). Meanwhile, Ossabaw pigs had greater plasma TG, cholesterol, and HDL than contemporary pigs.[62,63] However, these older studies comparing Ossabaw (obese) to Yorkshire (lean) controls represent a confounding design because of the potential for significant breed differences and the fact that there was a large difference in body weight between groups when age was held constant and these differences in live weight were not solely due to differences in adiposity.

A newfound interest in developing the Ossabaw breed as a biomedical model for obesity-linked cardiovascular disease emerged at the turn of century.[59] The Ossabaw literature has since been largely characterized by studies in which attempts were made to create control and obese pigs through dietary manipulation. In these efforts, pubertal pigs are either fed a high-fat diet for periods spanning at least 12 weeks to create obese pigs or they are maintained on a normal diet to create a control, ''leaner'' group. This serves to eliminate the potentially confounding effect of different genetic backgrounds between experimental cohorts and eliminates the need to maintain multiple breeding herds. When fed a high-fat diet, this breed has been known to develop indices of metabolic syndrome. This literature has firmly established the Ossabaw pig as a porcine model of obesity that develops a prediabetic state, where glucose levels are relatively normal, and the pigs display mild hyperinsulinemia and dyslipidemia.[59]

There currently are no established porcine models of hyperphagic, juvenile obesity which give rise to frank metabolic disease absent the feeding of high fat diets.[54,55] To address the need to model, the progressive nature of obesity-induced disease more faithfully, we undertook efforts to assess the potential for a novel, obese swine breed that expresses an extreme obese phenotype, the Mangalica pig, to serve as an animal model for human obesity and its metabolic complications. Lean and obese groups were created by either allowing ad libitum access to feed or by limiting access to 40% of voluntary feed intake. The extreme adiposity exhibited by obese Mangalica pigs associated with increased innate immune function and higher tissue expression of proinflammatory cytokines, hyperglycemia,

hyperinsulineamia, insulin resistance, and dyslipidemia indicating the spontaneous development of metabolic syndrome and a diabetic state.[64] These observations indicate that the Mangalica pig indeed serves as a novel biomedical model for human obesity and its metabolic complications. The use of obese Mangalica pigs in this regard should allow the pursuit of new avenues of research concerning the progressive nature of obesity-induced disease.

Cholesterol trafficking responses in mature pigs to sudden changes in dietary macronutrient composition

As our population grows more obese, all manners of pharmacological and dietary practices are being studied to effect weight loss in obese individuals. Among these is the adoption of a ketogenic diet. However, very little is understood at the metabolic level concerning how an abrupt shift from a traditional carbohydrate-rich diet to a high calorie, fat one affects gene expression in the liver and adipose tissue. To address this, we exposed growing pigs to a sudden switch from a low to a high fat diet and then examined the impact on the molecular regulation of lipid trafficking in these pigs at the transcriptomic level.[48] In this study, pigs were fed either a balanced, low fat diet (LFD, 4.3% fat) consisting of ground corn and soybean meal or a tallow/corn oil supplemented diet (HFD, 40% fat) for 14 days.[65] Expression of LCAT, ACAT, ApoB, HL (hepatic lipase) and beta actin mRNA was then subsequently determined in the liver, subcutaneous adipose, loin skeletal muscle and gut samples harvested from these pigs. Mirroring gene expression profiles exhibited by humans, LCAT mRNA was present in all four tissues, while ACAT was expressed only by the liver, ApoB expression was detected in the liver, adipose and gut while HL mRNA was only detectable in liver samples. Differential gene expression in response to a sudden dietary macronutrient shift was not observed in the porcine tissues except that liver ACAT expression was down-regulated in response to the high fat diet. Interestingly, this finding differs with observations in rodents pointing to a need to better characterize transcriptomic and proteomic responses to conditions of sudden changes in diet composition in the pig as efforts to develop porcine models to study the impact of nutritional manipulation in humans progress.

Gene expression studies in bovine intramuscular and subcutaneous fat

The anatomical location of lipid deposition has important implications across species. For instance, the distribution of body fat impacts health and disease risk in humans while it is a primary factor influencing production efficiency and carcass merit in food-producing animals. Visceral fat is more closely linked to the development of metabolic and cardiovascular disease in humans due to depot differences in the metabolic activity, endocrine function and venous drainage of visceral versus subcutaneous fat.[66,67] Furthermore, in contrast to food animals, IMF is rarely described in humans. The outcomes for research in lipid metabolism in biomedicine and animal agriculture differ although the basic biological processes are the same. Human clinical research interests lie for example in how gender affects distribution of lipid between visceral vs. subcutaneous depots, impact of accumulation of intramyocellular fat on muscle metabolism and insulin sensitivity, all aspects of obesity and associated maladies. Enhanced understanding of all the topics above will support an overall goal to limit all adipose tissue accretion and achieve recovery from lipid deposition-associated maladies in humans.[68,69] In livestock, on the other hand, the ideal distribution of adipose tissue is largely determined by (1) consumer demand for meat products that exhibit certain organoleptic properties, and (2) the need to continually improve production efficiency. Intramuscular fat, i.e. the fat deposited between myofibers is generally valued by consumers due to the perception that the presence of such fat confers greater flavor and tenderness to the meat cut.[70–72] Meanwhile subcutaneous or ''trim'' fat is undesirable and wasteful as it is generally removed and discarded during the processing of the carcass.

Unfortunately, due to a poorly characterized developmental program underlying the temporal pattern of adipose tissue accumulation in livestock, IMF accretion occurs predominantly at a time when significant quantities of unwanted visceral and subcutaneous fat stores have already accumulated on the carcass.[73] Thus, modern harvest weights represent a balance between achieving desired marbling and tolerating diminishing feed efficiency.[70,71] Given this, cattle are currently finished on high energy, grain-based diets but increasing competition for grain inputs threatens the viability of this strategy.[74,75]

Rodent models have revealed transcriptional networks that coordinate adipose tissue hyperplasia and hypertrophy.[76] In this well-characterized paradigm, the sequential expression of nuclear transcription factors, *C/EBPβ, PPARγ*, and *C/EBPα* results in transactivation of adipocyte-specific genes such as the insulin receptor (*IR*), glucose transporter 4 (*GLUT4*), *SCD1*, *LPL*, and *FAS*, leading to terminal differentiation of preadipocytes, an ability to respond to homeorhetic hormones such as insulin, and the induction of metabolic pathways related to lipid metabolism.[77] Presently, *PPARγ* is considered the master regulator of adipocyte differentiation, whereas C/EBPα is thought to potentiate differentiation by upregulating genes that confer insulin sensitivity on the adipocyte.[77–79] On the other hand, the transcription factor cascade necessary to trigger the adipogenic gene program appears to be blocked by *PREF1* in part by blocking transcription of *C/EBPβ*, whereby *COUP-TF* appears to block this cascade primarily through antagonizing *PPARγ*.[80–84] Additionally, morphogenic proteins such as Wnts, SHH, and BMPs appear to prevent the recruitment of preadipocytes from mesenchymal stem cells.[85–87] Thus, both factors that decide the fate of mesenchymal stem cells and factors acting directly to alter the preadipocyte transcriptome are important regulators of adipogenesis. Unfortunately, knowledge of factors controlling these processes and how production practices influence such factors is limited in cattle, especially concerning mechanisms that control the timing and extent

of depot development.[88] Consequently, it has proven difficult to devise strategies that enhance marbling and simultaneously limit adipose tissue accretion in undesirable depots that negatively impact feed efficiency and yield grade in livestock.

Our labs have conducted serial sampling protocols in order to describe the molecular regulation of lipid metabolism in response to nutritional transitions that food animals experience during the production cycle. The overarching goal is to better understand the mechanisms underlying the temporally regulated development of each fat depot. In one such study, the expression of candidate regulatory genes was examined in subcutaneous adipose tissue of growing heifers in response to age and finishing strategy whereby one group was transitioned onto a lower energy, forage-based diet while the other transitioned to a high energy, grain-based diet.[89] As expected, carcass adiposity increased with age and was significantly greater in animals transitioned onto grain-based rations versus forage-finished animals. These changes in adiposity correlated with increases in mRNA for transcription factors *PPARγ*, *BMP2*, and *SMAD1* in subcutaneous adipose tissue concomitant with decreases in mRNA for *SHH*, *COUP-TF1*, *COUP-TF2*, and *PREF-1* in this fat depot. Expression levels for metabolic genes (*LPL*, *SCD1*, *FAS*) were largely unaffected by age, but were significantly increased by grain finishing relative to animals that remained on forage. The patterns of expression of morphoregulatory and transcription factor genes measured in subcutaneous adipose tissue as cattle fattened illustrates the presence of stimulatory and inhibitory regulatory gene networks that appear coordinately regulated in opposing fashions.[89] These data suggest that there is considerable similarity in the regulation of adipose tissue development, at least at the molecular level, between humans, rodents, and cattle.

In another study, steers were reared on dormant pasture or high quality grass pasture. Biopsies were serially collected from developing subcutaneous adipose and skeletal muscle (containing intramuscular adipose tissue).[50,90] Monitoring adipose tissue-related genes (IMF) in total skeletal muscle tissue samples were previously established as a valid practice.[91] The mRNA for *PPARγ*, *PGC-1α* (reflecting mitochondrial function), and *CPT-1b* increased in intramuscular adipose tissue with age while *CEBP/α*, *UCP-2*, and *FABP-4* mRNA expression was not changed.[90] Furthermore, *PSM11*, one of the non-ATPase subunits of the 26S proteasome complex and a sentinel for protein turnover, did not differ between treatments. Unexpectedly, *Pref-1* mRNA was increased in IMF with age, an observation that is inconsistent with the increased overall adiposity of steers with age. The mRNA expressions for *GPAT*, *ZFP423*, *FABP4*, and *UCP2* in subcutaneous adipose tissue were largely unaffected by age or forage quality in these animals. Finally, it was noted that animal performance related directly to feed intake. As the rye grass pasture productivity regressed, gains slowed considerably. From the limited gene panel conducted, no consistent correlations were observed in mRNA expression as related to actual animal average daily gain over time, but expression of *FABP4* and *CEBP/α* mRNA correlated to total gain in all steers. These data suggest that like in humans, there are significant differences in gene expression, and presumably function across adipose tissue depots.

Potential link between energy balance, feed efficiency and adipose tissue

Given that adipose tissue development appears influenced by nutritional management in cattle, the residual feed intake model has been used as a method to create extreme cohorts consisting of very efficient and very inefficient animals that can be used as a basis for studying the underlying mechanisms linking energy balance and growth in animals. Residual feed intake (RFI) is a heritable feed efficiency measure that allows cattle to be ranked based on individual variation in feed intake that is independent of growth rate and other production traits, essentially being calculated by subtracting actual feed intake of the animal from a theoretical expected value given the growth rate and body weight exhibited by the animal.[92] Since the hypothalamus functions to integrate metabolic, neural, and endocrine signals to coordinate feeding behavior, energy balance, and developmental trajectory in animals, we undertook studies to examine potential associations between the expression of hypothalamic genes and RFI status in growing steers.[93–96]

In one recent study, two divergent cohorts consisting of inefficient and efficient individuals were created by assessing RFI in growing steers.[94] When examining mRNA expression of candidate satiety regulating genes in the hypothalamic collected from these animals, mRNA for *NPY, RLN3, and MC4R* was significantly lower in the arcuate nucleus of efficient animals relative to their inefficient counterparts. In contrast, *POMC* mRNA expression was elevated in the arcuate nucleus of efficient steers. These profiles suggest that efficient animals have a neuropeptide profile in the feeding center of their brain consistent with satiety which could explain the lower feed intake characteristically exhibited by efficient animals. Meanwhile *leptin* mRNA expression was significantly higher in the adipose tissue of efficient steers consistent with lower levels of *NPY* and higher expression of *POMC* in their hypothalami. This difference in leptin mRNA expression occurred despite no differences in adiposity between the efficient and inefficient cohorts. To further assess the role of hypothalamic genes in regulating feed efficiency, microarray studies have also been conducted on hypothalamic tissue RNA samples harvested from efficient and inefficient RFI steers.[94,96] Of the 24,000+ probes included on the Affymetrix Bovine Genome Array, 891 were found to be significantly different between efficient and inefficient RFI animals. Ingenuity Pathway Analysis software revealed that the pathways most heavily represented in the differentially expressed genes were consistent with the known functions of the central nervous system, specifically; increased cellular movement, cell-to-cell communication and cellular development were highly significant ($P=1.34\times10^{-24}$, 9.54×10^{-20}, 3.14×10^{-17}, respectively). In terms of canonical pathways, dendritic cell maturation and interleukin signaling were identified as activated in inefficient versus efficient steers. Likewise, mRNA for the proinflammatory cytokine genes,

tumor necrosis factor alpha (*TNFα)* and interleukin-6 (*IL-6)* was higher in the arcuate nucleus of inefficient versus efficient steers as measured by real-time PCR. Thus, increased inflammatory tone appears to contribute to decreased production efficiency in otherwise healthy, growing cattle. Importantly, these studies indicate a role for adipose tissue development in regulating feed efficiency in growing animals potentially due to differences in the endocrine function of adipocytes independent of the traditional consideration of the energetic costs of adipose tissue accretion. Given the implication that increased inflammatory tone in the hypothalamus contributes to inefficiency and the realization that adipose tissue expresses a functional innate immunity pathway, these data point to another potential mechanism whereby adipose tissue might alter satiety, muscle development and feed efficiency through the secretion of proinflammatory cytokines as well.

Conclusion

In conclusion, lipid metabolism research in humans and farm animals has different final goals. For humans, the research is critical to better understand the development, cure and/or prevention of lipid dysmetabolism, cardiovascular diseases and obesity. In farm animals, the research goal is to produce lean and well-flavored muscle foods in the most sustainable fashion. Results to date from studies of adipose and liver lipid metabolism have shown many fundamental similarities in biochemical processes and molecular regulation of lipid metabolism across species. The utility of farm animals (especially the pig) from numerous perspectives may suggest that pigs, along with rodents, may be an appropriate animal model for human lipid metabolism and diseases studies.

Authors' contributions: Both authors shared equally in the work and preparation of the manuscript.

ACKNOWLEDGEMENTS

This work was supported by the Alabama Agricultural Experiment Station.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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