INTRODUCTION

With this paper, I hope to provide an approach to systems biology research in dairy cattle, focusing on the transition period and early lactation. The observations, inferences, and opinions are based on 40 yr of research in dairy cattle and other animals the author has been able to conduct. The work ranged from basic molecular biology to practical animal feeding management but always with the approach to purposefully and directly design experiments to ask, “How is this all connected?”

The primary goal of this work is to stimulate an increase in coordinated, systems-based research and analysis in the animal agriculture community worldwide.

Although there are several researchers using a quantitative approach in dairy cattle research, in many countries the effort is limited and could be increased. Objectives of such a research approach should include critical pre-study reasoning as to what should be studied, why it should be studied, why the system indeed works the way it does, and how the work, when completed, will contribute to a deeper understanding of the system. Although more systems work is being funded, we need a more thorough application of biomathematical reasoning to provide more efficient, faster, and quantifiable improvement in understanding dairy cattle biology and application on farm. Hopefully, we can seize opportunities ahead to solve what in effect is actually a quite simple problem: how to feed everyone in the world a highly nutritious diet that includes animal products. Our call

TRIENNIAL LACTATION SYMPOSIUM:
Systems biology of regulatory mechanisms of nutrient metabolism in lactation\(^1,2\)

J. P. McNamara\(^3\)

Department of Animal Sciences, Washington State University, Pullman 99164-6310

ABSTRACT: A major role of the dairy cow is to convert low-quality plant materials into high-quality protein and other nutrients for humans. We must select and manage cows with the goal of having animals of the greatest efficiency matched to their environment. We have increased efficiency tremendously over the years, yet the variation in productive and reproductive efficiency among animals is still large. In part, this is because of a lack of full integration of genetic, nutritional, and reproductive biology into management decisions. However, integration across these disciplines is increasing as the biological research findings show specific control points at which genetics, nutrition, and reproduction interact. An ordered systems biology approach that focuses on why and how cells regulate energy and N use and on how and why organs interact through endocrine and neurocrine mechanisms will speed improvements in efficiency. More sophisticated dairy managers will demand better information to improve the efficiency of their animals. Using genetic improvement and animal management to improve milk productive and reproductive efficiency requires a deeper understanding of metabolic processes throughout the life cycle. Using existing metabolic models, we can design experiments specifically to integrate data from global transcriptional profiling into models that describe nutrient use in farm animals. A systems modeling approach can help focus our research to make faster and larger advances in efficiency and determine how this knowledge can be applied on the farms.

Key words: dairy cattle, lactation, metabolic control, modeling, nutrition, reproduction, systems biology


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\(^3\)Corresponding author: mcnamara@wsu.edu
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and challenge as animal scientists is no less than this: How do we ensure a safe and sustainable high-quality protein food supply worldwide for future generations?

MODELING IN DAIRY CATTLE RESEARCH

Given the approach and background presented above, let us define the critical “Why do we do research?” From Baldwin (1995, p. 1): “There is general agreement among most informed authors that products of animal agriculture will continue to contribute to the world food supply. However, the key challenge of ascertaining how much animals should contribute has not been resolved.” Furthermore, “Our inability to undertake quantitative evaluations of impacts of competing human nutrition strategies on human food availability is due in large part to the fact that current plant and animal production models are normally restricted to single species and have not been interfaced” Baldwin (1995, p. 1–2). Finally, “This is a long-term goal that will require the availability of advanced dynamic, mechanistic models of ruminant digestion and metabolism…” Baldwin (1995, p. 2).

Because of the work of Dr. Baldwin and others, there exists a solid and validated framework for systems models of the cow and other livestock that provide a basis for integrating data on genomics, transcriptomics, proteomics, and metabolomics into our animal models (Baldwin et al., 1987a,b; Baldwin, 1995; McNamara, 2005; Hanigan et al., 2009; Gregorini et al., 2013a,b). The model is titled “Molly” and the full history can be found in the previous references. Updates, challenges, and improvements have continued to demonstrate the worth of this approach to direct research in dairy cattle biology (McNamara and Baldwin, 2000; Hanigan et al., 2009). The objective of this model was simple: “To develop a dynamic, mechanistic model of digestion and metabolism in lactating dairy cows suitable for evaluation of hypotheses regarding underlying energetic relationships and patterns of nutrient use” (Baldwin et al., 1987a, p. 107).

In 1968, Baldwin published an article titled “Estimation of theoretical calorific relationships as a teaching technique: A review.” In it, he described the aggregate biochemical pathways that are the components of the NE system of feeding cattle (Lofgren and Garrett, 1968). This connection between the mechanisms of nutrient flux and practical cattle feeding established a solid research approach, using biomathematical models of nutrient use in farm animals, which does in fact continue today in many countries. This writer just would like there to be more people taking this approach, and in my opinion, if students and researchers read these papers, we will be better able to speed our understanding of agricultural animal systems today.

The Molly model describes aggregated pathway biochemistry in a simple fashion based on sound biological and chemical evidence. There was not an attempt to model every reaction but to model at the level of biological control most pertinent to the animal system. It is not empirical at the animals system, because the objective is to describe the animal system at the pertinent level of chemical interconversions of nutrients in the cells. For a thorough discussion of the purposes and practices of metabolic models, see Baldwin (1995). To expand the systems approach, we can integrate “omics” data, which were not available at that time, to help identify the mechanisms involved in control of productive functions and ruling out those not.

SYSTEMS BIOLOGY IN DAIRY CATTLE RESEARCH

Systems biology means different things to different people. But at a minimum, it is at least the recognition that each piece of the system (e.g., gene, enzyme, pathway, cell, organ) has a specific function related to the outcome of the entire system, not just the subsystem in which the molecule acts. For example, synthesis of ATP from the tricarboxylic acid cycle and electron transport chain in the liver provides immediate support to anabolic reactions in a liver cell, but the protein synthesized is exported into the blood to serve other needs of the system. When the work in metabolic control of pathway flux evolved into study of gene transcription and protein translation, and when sophisticated techniques were developed to study those processes and in some circles, systems biology was taken to be relevant only to genomic, transcriptomic, or proteomic work. However, the genome, transcriptome, and proteome exist only to serve the needs of the entire system (the “Why?” question: growth or reproduction, for example). The fields of genomics and transcriptomics have provided a wealth of knowledge but in large part, this information has not been fully integrated into our biological models and decision making systems. Measuring transcripts of mRNA or defining QTL and SNP tell us about a part of the system, but knowledge at those levels needs to be integrated into control of metabolic, endocrine, and cell signaling pathways and then into animal-level nutritional, genetic, and reproductive management.

In animal agriculture, we have had a specific objective to improve efficiency and productivity and, therefore, have focused on the system, usually at the empirical level of input and output, such as BW, lean gain, milk, and egg production. In addition, the animal sciences has also conducted excellent basic biology for several decades, and these 2 approaches, combined, have done much to improve animal efficiency,
welfare, and productivity. However, my primary message here is that we, as members of a scientific field, have not always done as well as we might to integrate these 2 approaches, but we have the ability to improve. It is difficult to do a focused study on specific cellular systems and also collect the relevant blood, organ, and animal level data. Many facilities and scientists do not have the equipment or training to make cellular or molecular measurements in the context of a production trial. Nevertheless, we have made great strides; we understand a significant amount about molecule, pathway, cell, organ, and animal level functions and, therefore, are poised to use a full systems approach to come to a richer understanding of the links between genetic and environmental control. Many animal scientists do seek out and use relevant basic knowledge from other disciplines to apply to animal production, and we need to continue to expand that effort.

The cow is an example of a complex system of many parts focused on the dominant physiological state of lactation, with a multifactorial goal of feeding the present generation and initiating the next one. However, and usually for good reason, the primary approach has been reductionistic, focusing on specific disciplines (e.g., nutrition, genetics, reproduction). This provided a significant amount of understanding and improvement in the field, but until recently (i.e., within the last 15 yr or so), most of the scientists in these disciplines did not interact and test more integrated hypotheses. For example, high milk production and fertility were not thought to be compatible for inclusion together in a study or the thinking was that reproductive traits have low heritability compared with milk production, so we will focus on milk production. Newer, more integrated approaches now clearly have shown the interconnectness of genetics, nutrition, and reproduction in dairy cattle and other species (Butler, 2003; Chagas et al., 2007; Vazquez et al., 2010; Lean et al., 2011). The applications of research results on genetics, nutrition, and reproduction have resulted in some a number of dairy farms with greater than 40 kg milk production and pregnancy rates greater than 20%, but we can do better. A full systems approach could accelerate improvements in production, efficiency, and sustainability.

In the rest of this paper, I will provide just one example of a long-term systems biology research program that we have been able to conduct, which combined studies at organ, cell, and molecular levels within a defied whole animal system, as is the lactating dairy cow. The purpose of this research programs has been to obtain and integrate data across several levels of biological organization into order to define and eventually control the underlying patterns and control of efficiency in dairy cattle.

**SYSTEMS BIOLOGY IN EARLY LACTATING DAIRY COWS**

The study in animal sciences, including lactation biology, has always been a story of combinations of basic and applied science. As knowledge developed on nutrient requirements and animal efficiencies based on respiration calorimetry, emerging fields were asking questions about the underlying biochemistry, physiology, and endocrinology that controlled the animal level functions. By the 1960s, respiration calorimetry and comparative slaughter techniques had been used to great effect to lay a solid energetic and chemical balance base of knowledge at the animal level. It was realized that further understanding would require research in tissue and pathway biochemistry, in vitro studies, and endocrinological studies and, eventually, a better understanding of genetic control at the cell, hormone, and pathway level. Clearly, advances in laboratory technology in enzymology, use of radioisotopes, and, eventually, molecular techniques in DNA and RNA chemistry allowed the more mechanistic and basic approach to flourish. This rich history unfolded from the 1950s through the present day in many research laboratories. The lead in systems biology of lactation was the group at the University of California (Davis) under R. L. “Lee” Baldwin (1995). For example, in 1968, Baldwin calculated the energy efficiency of milk synthesis at any given composition from the biochemical pathway stoichiometry, almost simultaneously with empirical calculations from respiration calorimetry and energy and N balance studies. Not surprisingly, there was agreement that, assuming the proper amounts and balance of precursors were available, the efficiency of milk synthesis in the mammary gland was about 83% (Baldwin, 1968). That was a constant percentage, variable with variation in milk composition, regardless of the amount of milk produced. It was, therefore, clear that any increases in efficiency must come from either 1) increasing total milk energy secretion at similar maintenance costs (i.e., dilution of maintenance costs) or 2) improving the efficiency of metabolic functions in organs such as the digestive system, liver, muscle, and adipose tissue. It was also known that the efficiency of storing body fat from carbohydrate was approximately 40% and from fat approximately 90%. The efficiency of muscle growth was only about 25 to 35%, depending on the stage of maturity and the balance of AA provided. This was, of course, due to the normal and required cost of muscle protein turnover (as noted years later by Cornish-Bowden [2005]). Therefore, if improvements in efficiency were to be made, we needed to understand the underlying functions of the organs of the body.
We used a systems modeling approach to ask the question, “What patterns of metabolic flux exist in dairy cattle of varying genetic merit and intakes?” Also, “Related to that flux, which genes are changing transcription in the adipose tissue?” This was in direct, if delayed, response to a challenge laid out years earlier by Baldwin (1980, p. 1420): “when considerable biological variation exists, opportunities for improvement are embedded within the variation...” and “…observed efficiencies considerably below theoretical are also observed. This raises 2 important questions. First, could we learn to identify animals that are capable of attaining maximum efficiencies and based on genetic selection improve the average efficiency of animal production? Second, if we knew exactly what types of unfortunate metabolic decisions that the less efficient animals were making, could we manipulate the metabolism of those animals such that their efficiencies would approach those of the best?” In retrospect, after 30 yr have passed, it is clear that many scientists have since then done exactly that but many have not taken on the full task of integrating the gene level processes with metabolism and then integrating all the metabolic functions with the physiological and animal levels.

Many researchers all over the world began to delve more specifically into endocrine, genetic, and physiological aspects of pathway biochemistry. This example from Washington State University is just one. Taking to heart the questions of Dr. Baldwin, we embarked on a long-term set of studies to determine genetic and nutritional control of adipose tissue in pregnant and lactating dairy cattle. The sole central goal of this research program, now spanning 30 yr, was to challenge, improve, and extend the scope and utility of the mechanistic dynamic metabolic model of Baldwin and colleagues (Baldwin et al., 1987a,b,c; Baldwin, 1995). The study of adipose tissue was well justified considering the teleological importance of adipose tissue to mammalian development, seasonal adaptability, and lactation. The contribution of adipose tissue to energy metabolism was significant indeed, but the knowledge of the endocrine role of adipose tissue was in its infancy (McNamara and Hillers, 1986a,b). Over several years we were able to ascertain, in brief, that anabolic pathways in adipose tissue were primarily a function of energy intake and body size whereas lipolytic pathways were primarily related to and controlled by level of milk production including genetic merit, stage of lactation, and parity (as first reported in full in McNamara and Hillers [1989] and summarized in McNamara [1994, 2012]). As usual, those discoveries led to new questions. What were the mechanisms involved? Which endocrine systems were involved and to what extent? How was basic genetic “genomic” variation contributing and which enzymes or pathways were controlled by transcriptional or post-translational mechanisms or both? It is worth noting that all of these discoveries came before the advent of reverse transcriptase-PCR and more rapid and inexpensive high-throughput methods. For example, Per Belfrage (University of Lund) spent 20 yr purifying enough hormone-sensitive lipase (HSL) to get the AA sequence so they could figure out the gene sequence (Holm et al., 1988; Langin et al., 1993), and that was a path that this author did not want to follow.

Nevertheless, our work on the mechanisms continued and, in the last 12 yr, we have been able to collect a significant amount of information on gene transcription and the relation to pathway activity in adipose tissue of the cow (Sumner and McNamara, 2007; Sumner-Thomson et al., 2011; Rocco et al., 2013; Khan et al., 2013). In summary, data were collected from several studies done at Washington State University with first- to fourth-parity cows, from 28 d prepartum to 120 d in milk (DIM), and included total feed intake, nutrient composition of intake, milk and component output, body fat and protein, and transcript levels for several key metabolic control proteins and enzymes expressed in adipose tissue as variables measured (McNamara and Hillers, 1989; Harrison et al., 1995; Phillips et al., 2003; McNamara and Valdez, 2005; Sumner and McNamara, 2007; Sumner-Thomson et al., 2011; Rocco et al., 2013; Khan et al., 2013). These cows were all on similar or the same diets, from the same herd, and spread over several years.

The Molly model in a recently published version (Hanigan et al., 2009) was used to simulate the metabolism of each cow (n = 126 cows from 3 studies cited above) from 0 to 120 DIM. Input variables included daily feed intake and chemical composition, initial BW, fat, and protein content. Outputs included milk components and pathway fluxes for lipid and glucose in mammary, body and visceral energy and protein, and changes in body fat and protein. Simulations were then continued until d 305 to predict potential overall efficiency.

The observed data on body fat, body protein, and visceral protein all varied (P < 0.05) widely among animals in their daily flux, with genetic merit (i.e., predicted transmitting ability for milk [PTAM]) and total NE absorbed being the greatest contributors to variance. Means for all cows were 112 Mcal/d (range = 89 to 139) for intake energy, 32.3 Mcal/d (range = 19.9 to 41.9) for maintenance, −0.51 Mcal/d (range = −1.74 to −0.015) for change in body energy, and 0.843 Mcal/d (range = 0.826 to 0.862) for NE efficiency [milk energy/(energy absorbed – maintenance energy)]. The model outputs were compared with observed outputs by regression analyses. The model predicted response to dietary energy, dietary fiber, and dietary protein content within 1
Systems biology of lactating dairy cattle

We must pinpoint the critical control mechanisms that vary metabolic rates in the liver, gut tissues, muscle, and fat and ask the questions “Can these efficiencies be changed?” and more importantly “Can they be changed without altering the basic system to the detriment of the animal?” The answer is, of course, yes, because we can identify those animals that are the most efficient utilizers of nutrients and identify their control points.

**EFFECT OF VARIATION IN ONE ELEMENT OF ONE SUBSYSTEM (ADIPOSE TISSUE) ON THE EFICIENCY OF THE ENTIRE SYSTEM**

We asked the question “Is transcriptional control of proteins in the adipose tissue a major contributor to the patterns of efficiency, in the cow, also, to what extent; and to what extent is posttranslational control significant?” In 2007, we reported for the first time the level of transcripts for HSL, perilipin, and the β-1, β-2, and β-3 adrenergic receptors in adipose tissue of lactating dairy cows. All of these transcripts increased in amount during lactation, with a peak at approximately 90 DIM, which is when milk production was greatest. This indicated a role for increased transcription in control of overall lipolytic activity, but the pattern was more subtle. The increase in message did not peak until lactation also did, indicating that this is not an “early response” to the negative energy balance and increased milk production of early lactation. Rather, this seems to be a secondary response over time. This does not mean it is not important; just because it was not “the first physiological response” does not mean it is not quantitatively important. In any system, all control is relevant.

When we asked the question of the proportion of control of metabolic activity by means of using multiple regressions, we began to learn more about the system relating transcriptional control with lipolysis. When we regressed the expression of the β-2 adrenergic receptor on BW, BCS, and empty body fat, this accounted for about 10% of the variation. When we focused the regression comparing β-2 adrenergic receptor transcript on the maximally stimulated rate of lipolysis, again only about 10% of the variation could be defined (Sumner and McNamara, 2007), indicating that about 10% of the control of lipolysis during lactation can be attributed to an increase in message for this receptor. This is reasonable given all the other levels of control on lipolysis and that, in fact, amount of adrenergic receptor is controlled in a loop of increased stimulation, reduced receptor activity, and attenuation of response (i.e., a “governor,” if you will, to avoid rapid mobilization).

The other systems analysis we did was to ask how the message for HSL related to lipolytic rate in adipose tissue. Even though HSL catalyzes this reaction, we found only about 12 to 17% of the variation in stimulated lipolysis explained by an increase in HSL mRNA, and there was no relationship between HSL message and basal (i.e., nonstimulated) lipolysis (Sumner and McNamara, 2007). Therefore, the inference is that the majority of the control of HSL activity is posttranslational or physiological through activation of the sympathetic nervous system and increased protein phosphor-

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**Table 1. Energetic efficiencies of dairy cattle in early lactation as simulated in Molly from observed data**

<table>
<thead>
<tr>
<th>Quintile of efficiency</th>
<th>Milk energy, % of GEI</th>
<th>Milk energy, % of ABSE</th>
<th>Milk energy, % of ABSE + BE</th>
<th>Mammary efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top 20%</td>
<td>26</td>
<td>43</td>
<td>44</td>
<td>84</td>
</tr>
<tr>
<td>Average</td>
<td>23</td>
<td>38</td>
<td>38</td>
<td>84</td>
</tr>
<tr>
<td>Lower 20%</td>
<td>21</td>
<td>34</td>
<td>34</td>
<td>85</td>
</tr>
<tr>
<td>SD</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

2 GEI = GE intake
3 ABSE = absorbed energy (sum of VFA, glucose, AA, and fat).
4 BE = body energy retained.
5 Milk energy production divided by mammary energy uptake. This is the thermodynamic maximal value.
ylation. This finding is consistent with our previous measurements of lipolysis in the cow and with what we demonstrated years earlier in the rat model (i.e., sympathetic nervous system activity is changed in adipose depots during lactation; McNamara and Murray, 2001).

Now we know that basal lipolysis is, in fact, catalyzed by a different enzyme, adipose tissue triacylglycerol lipase (ATGL; Pinent et al., 2008). Recent work with dairy cattle has confirmed involvement of several proteins in control of lipolysis in adipose tissue (Elkins and Spurlock, 2009; Koltes and Spurlock, 2011). They also confirmed specific relationships between expression of gene transcripts and animal level production (Koltes and Spurlock, 2011). This continues to demonstrate the need for a systematic approach to define the quantitative contribution of all control in the system. It also demonstrates the importance of lipolysis to survival, as the amount of control on this very simple reaction is a redundant system, such that if one method of control is not working (genetically or in a disease state), another form of control will likely still be functional. An example is that a knockout of the β-2 adrenoreceptor in mice leads to obesity because of decreased lipolysis by phosphorylated HSL, yet the adipose tissue can still mobilize triacylglycerols through the constitutive expression of ATGL (Chruscinski et al., 1999).

From this same study, we then conducted an analysis of the gene transcriptome in bovine adipose tissue during the transition from pregnancy to lactation (Sumner-Thomson et al., 2011). We obtained adipose tissue by biopsy at 30 d prepartum and 14 d postpartum and extracted the RNA. This was hybridized to the Affymetrix Genechip Bovine Genome Array (Affymetrix, Santa Clara, CA). Animals averaged 29.8 kg/d of milk for the first 60 DIM (SEM = 1.3, range 18.6 to 44.8). They lost 42.6 kg of BW (SEM = 8.4, range 9.1 to –113.6) and 0.38 BCS units (SEM = 0.10, range 0 to –1.0) from 0 to 14 DIM. This is a normal range for dairy cattle housed and fed alike and gives a glimpse of the yet unknown effects of genetic variance in a similar population. Anabolic pathway genes decreased from 30 d prepartum to 14 DIM (P < 0.05), including (mean percentage change in signal strength units with mean signal strength set to 125) sterol response element binding protein (–25.1%; SEM = 6.2), glucose transport 1 (–57.3%; SEM = 14.1), thyroid hormone receptor spot 14 (–30.8%; SEM = 7.4), lipoprotein lipase (–48.4%; SEM = 7.7), and acetyl-CoA carboxylase (–60.6%; SEM = 13.0). The regression coefficients of transcript change on milk production were 0.18 for
acetyl-CoA carboxylase and 0.26 for ATP citrate lyase ($P < 0.05$). Lipolytic control elements mRNA transcripts increased, with much variation among animals, including Ca channel subunit (338%; SEM = 203), β-2 adrenergic receptor (52.0%; SEM = 8.8), and HSL mRNA (23.0%; SEM = 17.9). The regression coefficients of transcript change on milk production were 0.30 and 0.25 for β-2 adrenergic receptor and HSL mRNA, respectively. These latter regressions explain somewhat more of the variation than the ones for HSL and β-2 adrenergic receptor in lipolysis, which is intriguing. These results led us to conduct further, more in-depth studies to integrate transcriptional control into the metabolic model.

We have since conducted additional studies on transcriptional control of metabolism and efficiency in the cow. We identified first- and second-parity animals based on their sire genetic merit and/or previous production and then fed them to requirements or to 90% of requirements for energy by pair feeding. We then sampled adipose tissue at intervals during late pregnancy and early lactation to measure lipogenesis, lipolysis, and transcript amounts through use of the Affymetrix Bovine Gene array (Affymetrix) as well as reverse transcriptase-PCR for some of the genes. Hopefully, we then can see more specifically how the mRNA transcripts, rates of metabolism, body composition, energy intake, and milk energy secretion are fully related to each other in the system. To date, we do not have the full regressions completed, but some interesting patterns certainly are emerging (Summer-Thomson et al., 2011).

A total of 48 cows were grouped by their sire PTAM—high genetic (HG; PTAM = 870 kg) or low genetic (LG; PTAM = 378 kg)—and half of each group was fed either to energy requirements (NORM) or to 90% of energy requirements (LOW). Other components were fed to requirements. Feed intake from 21 to 1 d prepartum was 13.6 kg DM/d for NORM cows and 12.7 kg DM/d for LOW cows (SEM = 1.5). From 1 to 56 DIM, it was 21.2 and 17.4 kg/d (SEM = 1.4). Milk production was 36.1 and 33.3 kg/d for HG and LG cows, respectively, from 27 to 56 DIM ($P < 0.05$). Adipose tissue biopsies at –21, –7, 7, 28, and 56 d relative to parturition (±2 d around the mean) were used to measure lipolysis, lipogenesis, and gene expression. Rates of lipogenesis were less during lactation and less in low energy fed cows whereas lipolysis rates were greater for both conditions ($P < 0.05$). The mRNA abundance of the β-2 adrenergic receptor, HSL, and the collapse periligin was several fold greater ($P < 0.05$) in animals on restricted energy. The mRNA for caveolin-1 and caveolin-2 decreased 20 to 40% ($P < 0.05$) in lactation consistent with the increase in lipolysis and HSL message. The gene expression array showed coordinated decreases in genes regulation lipogenesis (thyroid hormone receptor spot 14 [–26%], acetyl-CoA carboxylase [–76%], lipoprotein lipase [–57%], and ATP citrate lyase [–22%] as examples) and no change or moderate increases in those controlling lipolysis (Table 2).

We also conducted regression analyses of gene expression on milk production. For the genes listed, the regression coefficients for gene expression versus milk production in the first month of lactation are in parentheses: glucose transporter 1 ($r^2 = 0.34$), IGFBP3 ($r^2 = 0.67$), thyroid hormone receptor spot 14 ($r^2 = 0.38$), lipoprotein lipase ($r^2 = 0.18$), and leptin ($r^2 = 0.31$). All of these genes controlling anabolic reactions were negatively related with milk production. These regression coefficients give us some mathematical insight into how much control might be exerted on the anabolic pathways by gene expression. There was only a small relationship between milk production and expression of mRNA for lipolytic control genes, again indicating that most control on lipolysis is posttranslational through the sympathetic nervous and adrenal physiological systems.

Finally, we took these studies one step closer to the total system. If, in fact, there is specific adaptation in enzyme activity and pathway flux in adipose tissue during lactation, under varied control by transcriptional and posttranslational modification, what could be the potential total contribution to animal-level variance in efficiency if single enzymes in the adipose tissue were genetically altered? This is in keeping with the spirit and letter of systems biology, to determine the potential or actual contribution of changes in subsystems to the overall behavior of the system.

The Molly model was used as the framework for the simulation analysis that asked this question: If the substrate sensitivity ($K_s$) and maximal velocity ($V_{max}$)
of the key control points of lipogenesis (acyl-CoA carboxylase and fatty acid synthetase) and of lipolysis (primarily HSL for stimulated lipolysis) were changed (for example, by genetic variation in enzyme amount or activity), what, if any, would be the effect on the overall energy efficiency of the animal? Previous knowledge on the energy cost of triacylglycerol recycling (lipogenesis, esterification, lipolysis, and reesterification) estimated this at only 1 to 3% of the total cost of energy storage; however, that is 1 to 3% every day for 305 d. What is the cumulative effect on body and milk composition? In addition, the energy cost of protein turnover is quite large at 5 ATP equivalents per peptide bond. The energy cost of ion pumping (directly related to rate of energy metabolism) can account for 50% of maintenance costs. Visceral protein turnover alone can be 8 to 12% per day. What would happen if the rate of protein turnover varied significantly from animal to animal?

Input and output data from cows in our studies detailed above were used as the baseline for the study; that is, we used the feed intake, feed composition, starting BW and body composition, and milk component output to set the mean of the cows simulated. We then altered the parameter values in Molly for the Vmax of lipogenesis from one-half of default to 2 times default, which would be justified by the range of our measures in adipose tissue metabolism over several studies. Note that the Ks and Vmax are controlling values in the total of pathway flux, which is also controlled by substrate availability and hormones, such that a 1:1 change from Vmax and Ks to flux would not be expected.

The results were intriguing indeed. Changing the Vmax for lipogenesis altered the total accumulation of body fat by about 0.4 kg/d (Fig. 4) and the total ME for maintenance by about 0.5 Mcal/d, or just under 3% (Fig. 5). After 305 d, this resulted in a change in ending body fat of about 40 kg (more body fat with more lipogenesis). In addition, the milk fat percentage was changed 0.03% (greater milkfat with lower lipogenesis), a number that is small but not insignificant. Again when accumulated over time, that accounts for about 35 kg of milk fat. Markedly, these changes occurred with no change in milk yield (38 kg/d), in keeping with the greater priority for these functions in the system.

For changes in visceral energy cost of ion pumping and protein turnover, the effect was even greater, as expected. We also did simulations that altered the cost of basal metabolism (ion pumping and protein turnover) by one-half default, default, and 2 times default (the parameter KNaV, which represents the cost of energy metabolism in the viscera [liver and organs including mammary gland but not muscle or adipose]) from 0.15 to 0.3 (default) to 0.6 changed the ME cost of maintenance by almost 4 Mcal/d or 18% and also changed the accumulation of body fat by 0.4 kg/d. There was the same change in milk fat percent and a range of 40 kg of body fat at the end of lactation.

### Table 2. Genes coding for metabolic control in adipose tissue of lactating dairy cattle

<table>
<thead>
<tr>
<th>Gene coding for anabolism</th>
<th>GenBank accession no.</th>
<th>–7</th>
<th>7</th>
<th>28</th>
<th>d 28/d –7</th>
<th>P-value</th>
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<tbody>
<tr>
<td>LPL</td>
<td>BG688620</td>
<td>4,045</td>
<td>2,229</td>
<td>1,552</td>
<td>–57%</td>
<td>0.00</td>
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<tr>
<td>FABP5</td>
<td>NM_174315.2</td>
<td>4,378</td>
<td>4,265</td>
<td>3,075</td>
<td>–22%</td>
<td>0.88</td>
</tr>
<tr>
<td>GLUT4</td>
<td>NM_174604.1</td>
<td>49</td>
<td>37</td>
<td>36</td>
<td>–26%</td>
<td>0.53</td>
</tr>
<tr>
<td>THHHP</td>
<td>CK849521</td>
<td>2,679</td>
<td>1,148</td>
<td>625</td>
<td>–71%</td>
<td>0.01</td>
</tr>
<tr>
<td>ACRB5</td>
<td>CB433477</td>
<td>471</td>
<td>401</td>
<td>351</td>
<td>–22%</td>
<td>0.00</td>
</tr>
<tr>
<td>ACACB1</td>
<td>NM_174224.2</td>
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<th>Gene coding for catabolism</th>
<th>GenBank accession no.</th>
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<th>7</th>
<th>28</th>
<th>d 28/d –7</th>
<th>P-value</th>
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<tbody>
<tr>
<td>LIPE, mRNA</td>
<td>CK769629</td>
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<td>ACRB2</td>
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<td>CAV1</td>
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<td>CAV 2</td>
<td>CB170971</td>
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<td>77</td>
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<td>–52%</td>
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1Samples biopsied at times around calving as indicated. Results are signals from the Bovine Affymetrix Gene Array (Affymetrix, Santa Clara, CA), normalized to an average signal strength of 125.

2Biopsies of subcutaneous adipose tissue were taken 7 d prepartum and 7 and 28 d in milk.

3Gene name for sequence (from HUGO Gene Nomenclature Committee [http://www.genenames.org]).

4Signal strength at 28 d in milk as a percentage of 7 d prepartum.
Again, this was with no change in milk production and with no change in intake resulting in reduced body fat with increased maintenance costs. In this model exercise, the intake was held constant more for technical reasons than biological, but it is more likely that there would be a significant increase in intake in cows in the field with increasing fat or protein turnover, increasing the cost of production. Alternatively, more efficient cows (less fat or protein turnover and ion pumping) would require less feed.

Most people who work with cows and with multiple dairies routinely will have observed and understand that such variation in the field (that is variation among cows within a pen, among pens within a farm, and among farms) is real; many cows at the same level of milk production will nevertheless vary significantly in feed intake and body condition over time. The direct relationship between single pathways, enzymes, and genes in one tissue to the overall energetics of the animal is a significant factor in overall efficiency, and it may be that such data, as collected here and used directly in a systems model analysis, can lead to more improved selection techniques for improved efficiency.

Although this is a good example of a systems approach, it is still just one aspect of the cow. The work did not include a detailed analysis of the specific genotypes and phenotypes. Such work will require a larger research team, greater resources, and more and better techniques brought to bear in more integrated and deeper studies, a path on which we must continue on to make true progress in fully understanding the system of the dairy cow.

**Summary and Conclusions**

A major barrier to improvement of models and, more importantly, our understanding of the dairy cow remains the lack of an accurate description of the phenotype of the animal being modeled, expressed as, for example, gene transcription control, enzyme activity, hormone and receptor kinetics, and intracellular signaling. Significant changes in the cost of production can arise from just 1 or 2 small changes in gene transcription. Attitudes toward systems research are changing as more sophisticated data sets and techniques become available. In addition, the new funding paradigm at the USDA National Institute of Food and Agriculture (Washington, DC) will help scientists to integrate understanding of genetics, nutrition, and physiology on lactation and reproduction.

The dairy cow and the dairy industry are systems from the cell level to international markets and food needs. We need a reinvigorated, multi-investigator, multidisciplinary, integrated approach to solve the present and future problems of productive efficiency, including milk production and reproduction; this research effort will require construction and testing of mechanistic biomathematical models. Finally, we need to train students, scientists, and professionals in the importance of using integrative biology and biomathematical models to help improve the overall efficiency of the dairy industry.
LITERATURE CITED


