TRIENNIAL LACTATION SYMPOSIUM: Nutrigenomics in dairy cows: Nutrients, transcription factors, and techniques[1,2]

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ABSTRACT: Nutrigenomics in dairy cows is a relatively new area of research. It is defined as the study of the genomewide influences of nutrition altering the expression of genes. Dietary compounds affect gene expression directly or indirectly via interactions with transcription factors. Among those, the most relevant for nutrigenomics are ligand-dependent nuclear receptors, especially peroxisome proliferator-activated receptors (PPAR) and liver X receptor. Among other transcription factors, a prominent nutrigenomic role is played by the sterol regulatory element-binding protein 1 (SREBP1). Data from studies on dairy cows using gene expression and gene reporters among the main molecular methods used to study nutrigenomics in dairy cows are indicative of a network of multiple transcription factors at play in controlling the nutrigenomic responses. Fatty acids, AA, and level of feed and energy intake have the strongest nutrigenomic potential. The effect of *t*10,*c*12 CLA on depressing milk fat synthesis via inhibition of SREBP1 was among the first and likely the best-known nutrigenomic example in dairy cows. Although long-chain fatty acids (LCFA) are clearly the most potent, a nutrigenomic role for short-chain fatty acids is emerging. Available data indicate that saturated compared with unsaturated LCFA have a more potent nutrigenomic effect in vitro, likely through PPAR. In vivo, the effect of saturated LCFA is more modest, with contrasting effects among tissues. Nutrigenomic effects of AA are emerging, particularly for the regulation of milk protein synthesis–associated genes. The level of energy in the diet has a strong and broad nutrigenomic effect and appears to "prime" tissue metabolism, particularly liver. We are at the frontier of the nutrigenomics era in ruminants and initial data strongly indicate that this scientific branch (and spinoffs such as nutriepigenomics) can play a critical role in future strategies to better feed dairy cattle.

Key words: dairy cow, long-chain fatty acids, nutrigenomics, transcription factor

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INTRODUCTION

Nutrigenomics is defined as the study of "the genome-wide influences of nutrition" (Muller and Kersten, 2003, p. 315) and how this "affects the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup" (Kaput and Rodriguez, 2004, p. 166). This definition underscores the basic fact that dietary nutrients alter gene expression either directly or indirectly (Raqib and Cravioto, 2009), hence affecting protein expression, metabolic and/or signaling status of cells, and, as a consequence, tissues, organs, and the entire organism.

The concept that food components affect biological functions by interacting with the molecular milieu of cells has revolutionized the field of nutrition (Mutch et al., 2005). In this context, nutrients no longer just provide energy and "building blocks" for cells but constitute signals detected by cellular sensors that provoke a change in the biology of the cells; therefore, the nutritional molecules are bioac-

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tive (Muller and Kersten, 2003). However, other components of the diet also can have nutrigenomic effects without being specifically bioactive; one example is restriction of dietary energy (Abete et al., 2012). The fact that nutritional compounds can interact with the genome, particularly through transcription regulators, opens the possibility for fine-tuning the biology of the organism through refined manipulation of the diet.

Nutrigenomics is a recent field of research in companion animals and livestock species (Dawson, 2006; Fekete and Brown, 2007; de Godoy and Swanson, 2013). In dairy cows, the field can be considered in its infancy but, as argued by Coffey (2007), holds great potential to improve health and productivity.

NUTRIGENOMICS AND TRANSCRIPTION REGULATORS

The word nutrigenomics is composed of "nutri" (= nutrient) and "genomics." The term genomics, strictly speaking, is related to the structure, function, and sequencing of genomes (de Godoy and Swanson, 2013). However, the word nutrigenomics does not imply the effect of nutrients on the sequence of DNA; rather, it encompasses the nutrient–gene interactions through the intermediate action of transcriptional regulatory factors (**TF**) in the short to medium term and epigenetics factors in the medium to long term. Bioactive nutrigenomic molecules are able to directly or indirectly activate or repress TF. Therefore, the name nutrient–gene interaction is understood to be a TF-mediated interaction with the genome rather than a direct binding of nutrients to the genome. The word nutrigenomics also can be spelled out as "nutri-gene-omics." The word "omics" denotes large-scale data or information to understand life obtained through high-throughput techniques (Yadav, 2007). Therefore, nutrigenomics can be defined as the whole or complete effect of nutrients on expression of genes. Due to the dual definition of nutrigenomics, nutrigenomic experiments are both the ones performed measuring few genes and the ones using large-scale analysis.

General Aspects

The short-to-medium-term regulation of expression of genes is determined by *cis*-regulatory elements that include the promoter of the genes located just upstream of the transcription starting site and *cis*-regulatory modules, including enhancers and silencers, that are present from few kilobases to megabases upstream of the transcription starting site (Shlyueva et al., 2014). When the DNA is in the euchromatin structure, the gene and its upstream regions become accessible to TF, which are proteins that specifically bind short DNA sequences (i.e., 6 to 12 nucleotides) called response elements located in the enhancer regions of the genes (Shlyueva et al., 2014). Upon binding the DNA, the transcription of genes is induced by a cascade of events encompassing binding or recruiting other TF, coactivators, chromatin remodeling proteins, and the RNA polymerase components.

In humans, there are approximately 2,000 estimated different TF (Vaquerizas et al., 2009) that often work in a combinatorial way (i.e., multiple TF are bound to the same enhancer regions upstream of the target gene; Villar et al., 2014); however, just around 100 have been experimentally verified for their DNA-binding and regulatory functions (Vaquerizas et al., 2009; Yip et al., 2012). Most up-to-date information about TF can be found on specialized websites, such as AnimalTFDB (Zhang et al., 2015). The AnimalTFDB website had collected information for almost 1,300 TF and almost 400 transcription cofactors for *Bos taurus*. The TF do not work in isolation but a regulatory network of transcription factors is essential to coordinate the response to external stimuli and translate this into changes in gene expression (Cheatle Jarvela and Hinman, 2015).

Main Transcriptional Factors with Nutrigenomic Potential in Dairy Cows

In the early 21st century, Muller and Kersten (2003) summarized the nutrients capable of directly affecting TF in monogastrics. The short list of TF included ligand-dependent nuclear receptors (**LdNR**), comprising peroxisome proliferator-activated receptors (**PPAR**), liver X receptors (**LXR**), and hepatic nuclear factor 4 (**HNF4**), which are able to bind and be activated by macronutrients, including fatty acids for all (Khan and Vanden Heuvel, 2003) and metabolites of cholesterol (oxysterols) for LXR (Zhao and Dahlman-Wright, 2010). The vitamin-specific LdNR are among the micronutrient responders. These include retinoid X receptors (**RXR**) and retinoic acid receptors (**RAR**) activated by retinoic acids (metabolites of the vitamin A; Minucci et al., 1997), vitamin D receptor (**VDR**), and pregnane X receptor activated by vitamin E (Landes et al., 2003).

There also are several non-LdNR TF that control the expression of genes when activation by nutrients; however, such activation is not direct but mediated by other factors. These include sterol regulatory element-binding proteins sterol regulatory element-binding protein (**SREBP1**; gene name *SREBF1*), which are affected by long-chain fatty acids (**LCFA**; Georgiadi and Kersten, 2012) and glucose (Uttarwar et al., 2012); Spot14 or thyroid hormone responsive protein (THRSP), which is affected by polyunsaturated fatty acids (**PUFA**; Cunningham et al., 1998); carbohydrate responsive element binding protein (**ChREBP**; gene name *MLXIPL*),

Table 1. Ligand-dependent nuclear receptors (LdNR) with potential nutrigenomic role

Subfamily	Common name	Protein symbol	Gene name	Gene symbol	RXR[1]	Endogenous agonist	Main function
NR1B1	Retinoic acid receptor α	RARα	*Retinoid X receptor, alpha*	*RARA*	Yes	Retinoic acid	Development, differentiation, apoptosis, and CLOCK[2] genes
NR1B2	Retinoic acid receptor β	RARβ	*Retinoid X receptor, beta*	*RARB*	Yes	Retinoic acid	Embryonic morphogenesis, cell growth, and differentiation
NR1B3	Retinoic acid receptor γ	RARγ	*Retinoid X receptor, gamma*	*RARG*	Yes	Retinoic acid	Limb bud development, skeletal growth, and matrix homeostasis
NR1C1	Peroxisome proliferator-activated receptor α	PPARα	*Peroxisome proliferator-activated receptor, alpha*	*PPARA*	Yes	Fatty acids	Fatty acid metabolism, inflammation, and tissue regeneration
NR1C2	Peroxisome proliferator-activated receptor β/δ	PPARβ/δ	*Peroxisome proliferator-activated receptor, delta*	*PPARD*	Yes	Fatty acids	Fatty acid metabolism, tissue regeneration, and epidermal proliferation
NR1C3	Peroxisome proliferator-activated receptor γ	PPARγ	*Peroxisome proliferator-activated receptor, gamma*	*PPARG*	Yes	Fatty acids	Adipogenesis, insulin sensitivity, and lipogenesis
NR1H2	Liver X receptor α	LXRα	*Nuclear receptor subfamily 1, group H, member 3,*	*NR1H3*	Yes	Oxysterols/ fatty acids (?)	Cholesterol homeostasis, macrophage functions, and inflammation
NR1H3	Liver X receptor β	LXRβ	*Nuclear receptor subfamily 1, group H, member 2*	*NR1H2*	Yes	Oxysterols/ fatty acids (?)	
NR1I1	Vitamin D receptor	VDR	*Vitamin D (1,25- dihydroxyvitamin D3) receptor*	*VDR*	Yes	Vitamin D	Mineral metabolism and immune response
NR1I2	Pregnane X receptor	PXR	*Nuclear receptor subfamily 1 group I member 2*	*NR1I2*	Yes	Vitamin E	Detoxification
NR2A1	Hepatocyte nuclear factor 4 α	HNF4α	*Hepatocyte nuclear factor 4* α	*HNF4A*	No	Fatty acids (?)	Development of the liver, kidney, and intestines
NR2B1	Retinoid X receptor α	RXRα	*Retinoid X receptor* α	*RXRA*	Yes	9-*cis*-retinoic acid	Forming heterodimers with other LdNR and differentiation of leukocytes
NR2B2	Retinoid X receptor β	RXRβ	*Retinoid X receptor* β	*RXRB*	Yes	9-*cis*-retinoic acid	Embryonic morphogenesis and cell growth and differentiation

[1]Heterodimer formation with retinoid X receptors (RXR).

[2]CLOCK = circadian locomotor output cycles kaput.

which is activated indirectly by glucose 6-phosphate and xylulose-5-phosphate (Li et al., 2006; Oosterveer and Schoonjans, 2014); and CCAAT/enhancer-binding protein, activating transcription factor 4, and nuclear factor kappa-light-chain-enhancer of activated B cells, which respond to AA deprivation (Kilberg et al., 2012). Georgiadi and Kersten (2012) recently provided a review of TF involved in sensing fatty acids also including, besides the ones reported above, Toll-like receptor 4 and nuclear factor erythroid 2-related factor 2. Few of the above TF have been studied in dairy cattle as potential targets for nutrigenomic approaches. In the following sections, we provide an overview of the relatively few investigated LdNR and non-LdNR TF and their nutrigenomic roles in dairy cows.

NUTRIGENOMIC ROLES IN DAIRY COWS OF LIGAND-DEPENDENT NUCLEAR RECEPTORS

The LdNR are part of the nuclear receptor superfamily. There are 48 known LdNR in humans, but only 27 have known endogenous ligands that are in general lipophilic compounds, including hormones, fatty acids, and xenobiotics. The LdNR with nutrigenomic importance are those with the ability to bind and be activated by molecules with a direct or indirect dietary origin, such as fatty acids and vitamins (Burris et al., 2013; Table 1). Alternatively, the LdNR also can be activated independently from the agonist or agonists through phosphorylation, as it has been clearly demonstrated for PPAR (Burns and Vanden Heuvel, 2007). Once activated, LdNR bind the DNA as a dimer, either homo- or heterodimer (Burris et al., 2013).

The cellular localization of the LdNR is almost exclusively nuclear with some observed to be present in the cytosol (Hager et al., 2000). In the absence of agonist, however, most LdNR are sequestered by a complex formed by the inactive LdNR associated with heat shock protein 90, heat shock protein 70, and other proteins (Khan and Vanden Heuvel, 2003). Upon activation by agonist, the LdNR separates from the complex. Several LdNR contain a nuclear localization signal that allows for their transport into the nucleus via the nuclear shuttling protein importin α (Lange et al., 2007).

The type II nuclear hormone receptors, which include PPAR, RXR, VDR, and RAR (Table 1), are nor-

mally located in the nucleus but also can be present in the cytoplasm (Patel et al., 2005). Specific agonists and, more potently, RXR activation induce a nuclear localization of the PPAR (Akiyama et al., 2002). More recently, it was demonstrated that different agonists can differentially affect the import and the export of PPAR from the nucleus (Umemoto and Fujiki, 2012). Therefore, besides the presence of agonist and abundance of the LdNR and its coactivators, the activity of the LdNR also is determined by the shuttling of the LdNR between cytoplasm and nucleus.

Peroxisome Proliferator-Activated Receptors

Biological Roles of Peroxisome Proliferator-Activated Receptors in Dairy Cows. The PPAR are LdNR that work as heterodimers with RXR (Table 1) and have a prominent role in controlling expression of genes involved in lipid metabolism and inflammation. An extensive review on PPAR and their potential nutrigenomic role in dairy cows was recently published (Bionaz et al., 2013). The review discussed the role of PPARα in controlling lipid metabolism and inflammation in liver, the potential role of PPARβ/δ in controlling glucose uptake in mammary tissue, and the potential role of PPARγ in controlling milk fat synthesis and mastitis (for the latter, also see Mandard and Patsouris [2013]). Additional evidence of an important role of PPARγ in controlling milk fat synthesis was recently provided by overexpression of the transcription factor specific protein 1 (Sp1) in goat mammary epithelial cells that increased expression of several milk fat synthesis–related genes including *PPARG* (but not *SREBF1* or *NR1H3*; Zhu et al., 2014). Lack of a role for PPARγ in milk fat synthesis was indicated by data from a study with lactating mice where the PPARγ agonist, rosiglitazone, failed to reverse the milk fat depression induced by *trans*-10,*cis*-12 CLA (*t*10,*c*12 CLA; Vyas et al., 2014). This was not surprising because a lack of a role of PPARγ in murine mammary epithelial cells was previously demonstrated (Bionaz et al., 2013).

Preliminary evidence that PPARγ activation affects the response to mastitis was provided by an in vivo experiment in lactating dairy goats used as model for dairy cows (Richards et al., 2014). Goats were injected with 2,4-thiazolidinedione (**2,4-TZD**), a synthetic PPARγ agonist, for 1 wk before an intramammary infusion of *Streptococcus uberis* to induce subclinical mastitis. The goats injected with 2,4-TZD had an overall reduction of somatic cells in milk. Further analyses revealed a positive effect of 2,4-TZD on liver and neutrophils response to inflammation. Surprisingly, no overall effects of 2,4-TZD on expression of putative PPARγ target genes in adipose or mammary tissue were detected (da Rosa et al., 2015). A lack of 2,4-TZD effect on expression of putative PPARγ target genes also was reported in dairy cow adipose tissue (Schoenberg and Overton, 2011). To test if 2,4-TZD is a PPARγ agonist, we have used a luciferase gene reporter assay in bovine mammary epithelial (**MacT**) and Madin-Darby bovine kidney (**MDBK**) cells (Fig. 1). The data clearly indicated that 2,4-TZD does not activate PPAR. However, the activation of PPAR is tightly related to the simultaneous activation of RXR by its specific agonist, the vitamin A derived 9-*cis*-retinoic acid (Wang et al., 2010). When cells were treated with 2,4-TZD in the presence of 9-*cis*-retinoic acid in the media, we observed a tremendous activation of PPAR by 2,4-TZD (Fig. 1). Therefore, the use of 2,4-TZD alone does not seem to be an effective treatment to activate PPARγ, and in vivo supplementation with vitamin A might be necessary.

The potential role of PPARβ/δ in controlling glucose uptake in mammary tissue, as previously proposed (Bionaz et al., 2013), was only partly substantiated by an experiment performed in bovine mammary alveolar (epithelial) cells in our laboratory (Lohakare et al., 2015). It also was recently demonstrated that PPARβ/δ controls the expression of one of the carnitine transporters in MDBK cells (Zhou et al., 2014); therefore, it can affect lipid peroxidation in the mitochondria.

Peroxisome Proliferator-Activated Receptors and Long-Chain Fatty Acids. The PPAR are of extreme interest in nutrigenomics due to their capacity for binding to and be activated by LCFA in both monogastrics and ruminants (Bionaz et al., 2013; Nakamura et al., 2014). The studies performed using LCFA in dairy cows uncovered a species-specific sensitivity of PPAR receptors to LCFA. In monogastrics, the PPAR are more sensitive to PUFA compared with saturated LCFA (Desvergne and Wahli, 1999; Xu et al., 1999; Khan and Vanden Heuvel, 2003). In particular, linoleic acid and CLA are potent PPAR activators (Vanden Heuvel, 1999; Vanden Heuvel et al., 2006), with some contrasting data reported for PPARγ. For instance, PPARγ has a low to null response to these LCFA in humans, mice, and rats (Vanden Heuvel et al., 2006) and *t*10,*c*12 CLA antagonized the activity of PPARγ in human preadipocytes (Brown et al., 2003). Others have reported CLA binding to and activating PPARγ in human cells (Belury et al., 2002) and in vivo in mice (Bassaganya-Riera et al., 2004).

For both PPARα and PPARγ, the studies performed in dairy cows using expression of target genes clearly uncovered a "preference" for saturated LCFA, particularly palmitate and stearate (Bionaz et al., 2013). In general, unsaturated LCFA only weakly transactivate PPAR in bovine cells, and the effect diminishes as the degree of unsaturation increases (Kadegowda et al., 2009; Bionaz et al., 2012b). However, greater expression of *CPT1A*, a

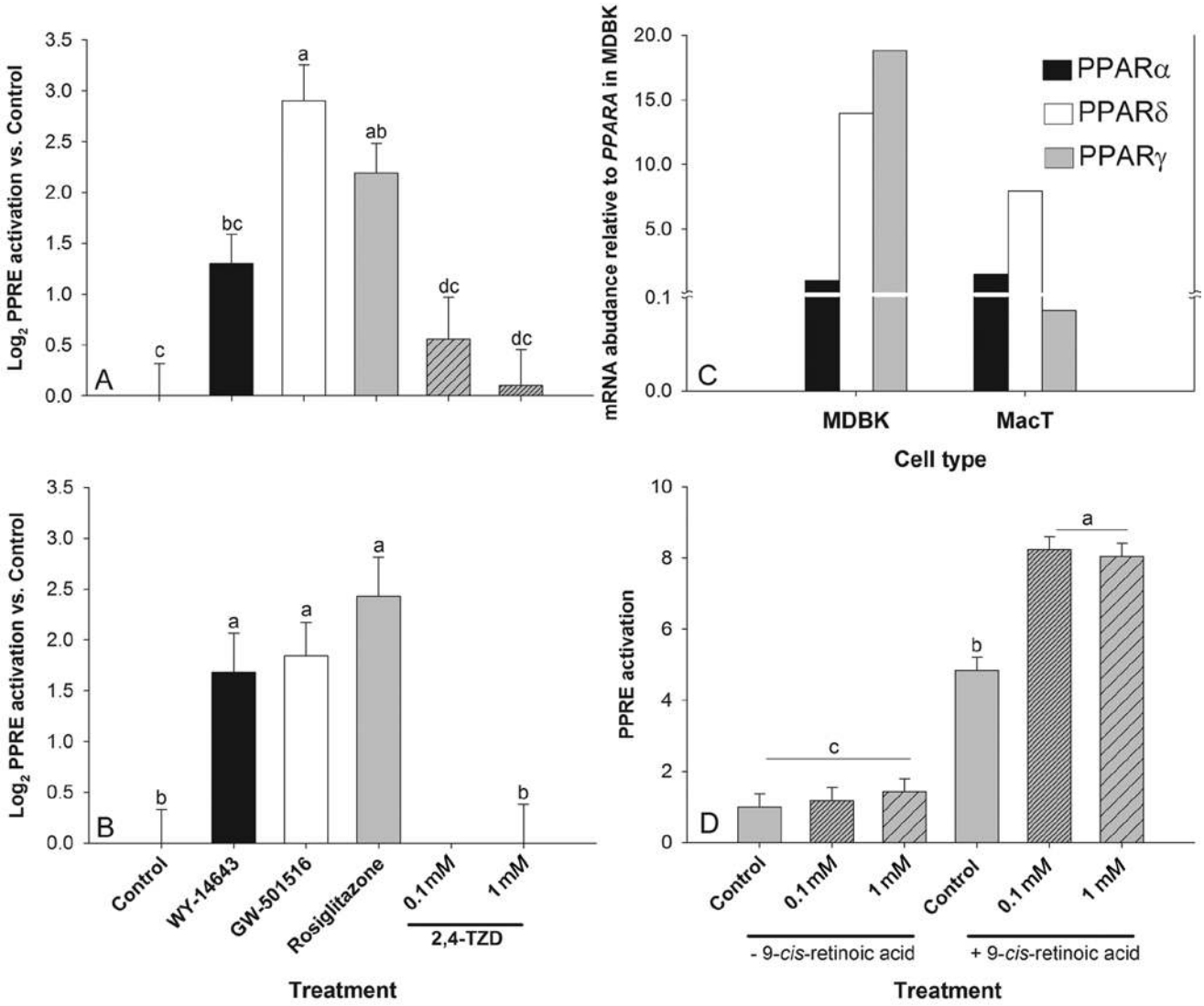

Figure 1. Isotype-specific activation for peroxisome proliferator-activated receptors (PPAR) in bovine cells. Madin-Darby bovine kidney (MDBK; panel A) and bovine mammary alveolar (epithelial; MacT; panel B) cells were transfected with 3x PPAR response element (PPRE) luciferase (generously provided by Dr. Siva Kolluri, Oregon State University, Corvallis, OR) using Lipofectamine 2000 (ThermoFisher Scientific, Waltham, MA). Twelve hours after transfection, the cells were treated with 100 μ*M* of the PPARγ agonist rosiglitazone, 100 μ*M* of the PPARα agonist Wy-14643, 100 n*M* of the PPARβ/δ agonist GW-501516, and 100 μ*M* or 1 m*M* of 2,4-thiazolidinedione (2,4-TZD). The 1 m*M* dose of 2,4-TZD was based on the estimated blood concentration of the same compound injected daily into the goats in a previous experiment (Richards et al., 2014). Both cell types respond to the PPAR-isotype-specific agonists, with MacT cells having a similar PPAR activation among isotype-specific agonists, whereas MDBK cells had a greater response when treated with a PPARβ/δ agonist. The magnitude of PPAR activation in response to PPARγ- and PPARα-specific agonists was similar between the 2 cell types despite PPARγ having a large difference in mRNA abundance (panel C; original data were published in Bionaz et al. [2013]). This indicates a greater sensitivity of PPARγ in MacT cells that is independent of the abundance of the PPAR isotype. In panel D, the addition of 9-*cis*-retinoic acid is essential for in vitro activation of PPAR by 2,4-TZD. The MacT cells were cotransfected with 3x PPRE luciferase and Renilla (pRL-SV40P; Addgene, Cambridge, MA) using Lipofectamine 3000 (ThermoFisher Scientific). Twenty-four hours after transfection, cells were cultured in OptiMEM media (ThermoFisher Scientific) supplemented either with or without 10 μ*M* 9-*cis*-retinoic acid (retinoid X receptor agonist) and cells were treated in 4 replicates with 30 μL ethanol (control) or 0.1 or 1 m*M* 2,4-TZD for 24 h. For all experiments (panels A, B, and D), luciferase activity was measured with a luminometer (for data in panel D also Renilla activity was measured and used to normalized luciferase data). The luminometer data were normalized to the mean of the control group, checked for normal distribution, and $\log_2$ transformed (only for data in panels A and B) before statistical analysis using ANOVA with treatment as the main effect and replicate as the random effect. $^{a–d}$Within each cell type, treatment means bearing different letters differed ($P < 0.05$). Reported are the least squares means ± SEM relative to control (CTR).

PPARα target gene, was observed in vitro with increased concentration of unsaturated LCFA (Bionaz et al., 2008, 2012b). This might indicate a difference of PPAR isotypes in the response to LCFA, as previously observed in monogastrics (Vanden Heuvel et al., 2006).

Recently, the use of a luciferase gene-reporter assay demonstrated that *t*10,*c*12 CLA is a weak activator of PPAR in MacT cells (Ma et al., 2014). In our laboratory, we tested the activation of PPAR by palmitate, *t*10,*c*12 CLA, and the PPARγ agonist rosiglitazone in MacT cells and nonpurified primary goat mammary cells transiently transfected with 3x PPAR response element (PPRE) luciferase (Fig. 2). The strong activation of PPARγ by rosiglitazone in MacT cells confirmed previous obser-

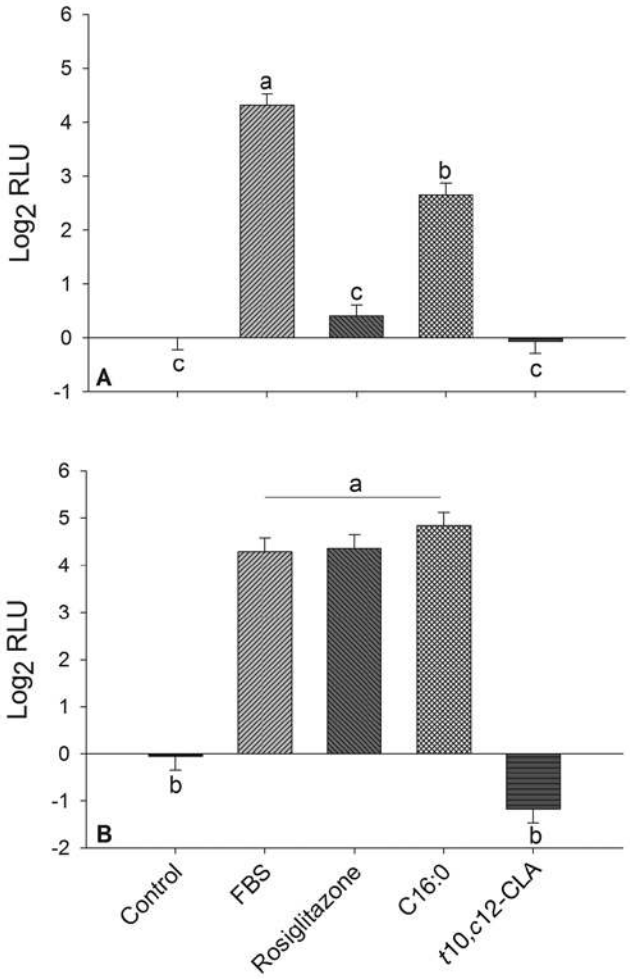

Figure 2. In goat mammary epithelial cells (panel A), the peroxisome proliferator-activated receptor (PPAR) γ agonist rosiglitazone had only a numerical effect on PPAR activation whereas it strongly activated PPAR in bovine mammary epithelial (MacT) cells (panel B). The use of 10% fetal bovine serum (FBS) had a similar magnitude of activation of PPAR in goat and bovine mammary epithelial cells. In goat mammary epithelial cells (panel A), the 10% FBS had the strongest effect followed by palmitate, whereas in MacT, palmitate, FBS, and rosiglitazone had a similar effect. Lastly, palmitate but not *trans*-10,*cis*-12 CLA activates PPAR in ruminant cells. Overall, the data are indicative of a difference in response to PPAR agonist between the 2 cells types that differ by species but also by being the goat cells primary cultures whereas MacT are a cell line. Because we used a general PPAR response element (i.e., it is recognized by all 3 PPAR isotypes), we were unable to differentiate the PPAR activation between isotypes; therefore, the activation by FBS and palmitate could occur through any of the PPAR isotypes and it is likely not PPARγ specific. Nonpurified primary goat mammary cells (obtained from a Saanen dry goat) and bovine mammary alveolar (MacT) cells ($n = 4$ per treatment) were cultivated in high-glucose Dulbecco's modified Eagle medium (DMEM) media in a 96-well plate. The DMEM media was replaced with an OptiMEM media (ThermoFisher Scientific, Waltham, MA) and cells transfected with 3x PPRE-luciferase plasmid. After 24 h, transfected cells were treated with 100 μ*M* rosiglitazone (PPARγ synthetic agonist), C16:0, *trans*-10,*cis*-12 CLA, or 10% FBS in OptiMEM media. In the control group, 30 μL ethanol was added. Cells were harvested 24 h later for luciferase activity measurement via a luminometer (RLU = relative light unit). Data were normalized to the mean of the control group and $\log_2$ transformed before statistically analysis using GLM of SAS (SAS Inst. Inc., Cary, NC) with treatment as the main effect and replicate as the random effect. $^{a–c}$Within each cell type, treatment means bearing different letters differed ($P < 0.05$).

vations derived from expression of putative target genes (Kadegowda et al., 2009). In contrast, goat mammary cells had a weak (only numerical) activation of PPARγ by rosiglitazone, confirming, to some extent, the low activation recently detected in goat primary mammary cells (Shi et al., 2013; Zhao et al., 2014). In both cell types, we detected a strong activation of PPAR by palmitate but none with *t*10,*c*12 CLA (Fig. 2). As previously revealed by gene expression analysis (Bionaz et al., 2013), we also observed that palmitate has a similar or stronger activation of PPAR compared with the specific PPARγ agonist rosiglitazone. The activation of specific PPAR isotypes via palmitate is not possible to decipher with the method used. However, we previously observed that saturated LCFA increases expression of both PPARα and PPARγ target genes in bovine and goat cells, often with a stronger effect than synthetic agonists (Kadegowda et al., 2009; Bionaz et al., 2012b; Zhao et al., 2014). The greater PPAR sensitivity to natural than to synthetic agonists has not been observed in monogastrics (Vanden Heuvel et al., 2006). The marked response of PPAR to saturated LCFA in ruminants makes evolutionary sense considering that saturated LCFA are the primary LCFA absorbed from the small intestine due to the extensive rumen biohydrogenation of dietary unsaturated LCFA.

Liver X Receptor and Control of Milk Fat Synthesis

In nonruminants, the LXR are LdNR with a prominent role in controlling cholesterol synthesis (Desvergne et al., 2006). There are 2 LXR isoforms: LXRα (gene symbol *NR1H3*) and LXRβ (gene symbol *NRH2*). The LXR are activated mainly by oxysterols and other derivatives of cholesterol metabolism and, to a lesser extent, by fatty acids (Table 1). In monogastrics (Desvergne et al., 2006) and dairy cows (Harvatine et al., 2014), *NR1H3* is highly expressed in the liver and *NR1H2* is ubiquitously expressed. Bovine mammary tissue has a relatively low expression of both LXR isoforms (Harvatine et al., 2014). As demonstrated by several independent studies, despite the low abundance, the LXRα controls the expression of *SREBF1* in bovine and goat mammary epithelial cells (McFadden and Corl, 2010; Oppi-Williams et al., 2012; Wang et al., 2012; Harvatine et al., 2014). Besides controlling expression of *SREBF1*, the LXRα also can control the expression of lipogenic genes in a SREBP1-independent manner and can increase lipogenesis in mammary cells (Oppi-Williams et al., 2012; Harvatine et al., 2014).

In monogastrics, the LXR has a relatively low capacity of being activated by LCFA (Vanden Heuvel et al., 2006) and its inhibition by PUFA is controversial (Clement et al., 2002; Ducheix et al., 2011). In bovine mammary cells, LXRα does not respond to *t*10,*c*12

CLA (Weerasinghe et al., 2012; Harvatine et al., 2014; Ma et al., 2014); therefore, in bovine LXR, isotypes do not participate in the transcription inhibition induced by *t*10,*c*12 CLA (Harvatine et al., 2014). Besides a potential role in controlling milk fat synthesis in dairy cows, the nutrigenomic importance of LXR during lactation also arises from its multiple established functions in nonruminants, including cholesterol metabolism, glucose uptake and lipogenesis in adipose tissue, lipogenesis and very low density lipoprotein formation in liver, and decreasing expression of inflammatory genes in macrophages (Calkin and Tontonoz, 2012). Indirect evidence for a role of LXR in the inflammatory response in dairy cows was provided by several studies in rumen epithelium (Li et al., 2011; Steele et al., 2011) and mammary tissue (Moyes et al., 2009).

Overall, the nutrigenomic potential of LXR is modest but promising. All of the work in dairy cows performed so far focused on its role on milk fat depression; therefore, only a nutrigenomic role by unsaturated LCFA was evaluated. It would be of interest to evaluate if LXR are activated by saturated LCFA in dairy cows.

Other Ligand-Dependent Nuclear Receptors with Potential Nutrigenomic Roles in Dairy Cows

Vitamin D Receptor. The VDR is a LdNR (Table 1) activated specifically by vitamin D. Horst et al. (1994) published a comprehensive review on the role of calcium and vitamin D in dairy cows. They discussed the regulation of VDR by several factors, including vitamin D level and retinoic acid. The fact that VDR forms a heterodimer with RXR, the target for the vitamin A metabolite 9-*cis*-retinoic acid (Table 1), might allow for nutrigenomic interplay between vitamin D and vitamin A. Due to its role in calcium metabolism, VDR is crucial in dairy cows and especially early postpartum when the incidence of milk fever is greatest. Besides calcium metabolism, VDR also can play important roles in overall animal health including a long-term "nutriepigenomics" role (Haussler et al., 2008; Tapp et al., 2013; Saccone et al., 2015). In monogastrics, VDR has a very high specificity for binding vitamin D but also can be activated by other natural compounds including ω6 and ω3 PUFA (Haussler et al., 2008). Therefore, a potential exists for improving milk fever in dairy cows through nutrigenomic intervention using LCFA.

The Pregnane X Receptor. This is a LdNR involved in controlling the expression of detoxification-related genes, particularly cytochrome P450 genes (Ihunnah et al., 2011). Besides being activated by xenobiotics, it also is activated by the metabolites of vitamin E (Table 1), by vitamin K2, and several herbal extracts (Chang, 2009; Ihunnah et al., 2011). The study of pregnane X receptor (**PXR**) in dairy cows could be important due to its role in gluconeogenesis, triglycerides synthesis, and bone mineral homeostasis in monogastrics (Ihunnah et al., 2011).

The Hepatic Nuclear Factor 4 Alpha. This LdNR is highly expressed in liver and is involved in transactivation of GH receptor, as also demonstrated in dairy cows (Jiang and Lucy, 2001). Its expression increases in bovine liver early postpartum (Loor et al., 2005) and on treatment with GH (Eleswarapu and Jiang, 2005). In monogastrics, the acyl-CoA thioester of myristic acid (C14:0) and palmitic acid (C16:0) are agonists whereas ω3 and ω6 PUFA and stearic acid (C18:0) are antagonists of HNF4α (Hertz et al., 1998). To our knowledge, there are no published studies where the activation of HNF4α by LCFA was tested in bovine cells.

NUTRIGENOMIC ROLES OF OTHER TRANSCRIPTION REGULATORS

Sterol Regulatory Element-Binding Transcription Factor/Protein 1 (SREBP1)

Sterol Regulatory Element-Binding Transcription Factor/Protein 1 (SREBP1) and Milk Fat Synthesis. The study of the reasons causing milk fat depression has revealed a pivotal role of SREBP1 in milk fat synthesis (Bauman et al., 2011). Loor and Herbein (1998) and Baumgard et al. (2001) demonstrated an inverse relationship between milk fat and content of *t*10,*c*12 CLA in dairy cows. In MacT cells, Peterson et al. (2004) observed an inverse relationship between *t*10,*c*12 CLA and de novo fatty acid synthesis due to a decrease in expression of fatty acid synthase (*FASN*), acetyl-CoA carboxylase α (*ACACA*), and stearoyl-coenzyme A desaturase (*SCD*). As reviewed by Bauman et al. (2011), this was the first nutrigenomic effect of LCFA described in dairy cows. In a subsequent study, Harvatine and Bauman (2006) reported that *t*10,*c*12 CLA and a milk fat–depressing diet (i.e., high energy and low forage plus high oil) significantly reduced the bovine mammary tissue expression of *SREBF1* and, as consequence, *FASN*, *LPL*, and *INSIG1*. A similar decrease in expression of *FASN* and *LPL* in bovine mammary tissue during milk fat depression induced by feeding fish oil was initially reported by Ahnadi et al. (2002). The authors also were the first to propose a potential role of SREBP1 in coordinating the transcriptional responses to milk fat depression. Using a gene-reporter assay, the inhibition of SREBP1 activity by *t*10,*c*12 CLA was recently confirmed (Ma et al., 2014). A definitive proof of a crucial role of SREBP1 in milk fat synthesis was re-

cently provided using gene reporter assays (Lengi and Corl, 2010; Ma, 2012; Ma and Corl, 2012).

In monogastrics, it was previously reported that *cis*-9,*trans*-11 CLA (*c*9,*t*11 CLA) and unsaturated LCFA in general decreased the activity of SREBP1 by inhibiting its cleavage in the Golgi and, thus, its maturation, nuclear transport, and transcriptional activity (Hannah et al., 2001; Roche et al., 2002). Similarly, Peterson et al. (2004) demonstrated that this was the main mechanism for the inhibition of SREBP1 by *t*10,*c*12 CLA. However, the *t*10,*c*12 CLA does not bind SREBP1 directly, but a potential mechanism for the inhibition of SREBP1 maturation by CLA was provided by a recent study by Lee et al. (2010). In that study, it was demonstrated that oleic acid inhibits the activity of the Fas-associated factor family member 2, Ubxd8. The Ubxd8 protein is present in the endoplasmic reticulum membrane and facilitates the degradation of INSIG1 protein; therefore, inhibition of Ubxd8 activity increases the INSIG1 and reduces the activity of SREBP1. It remains to be determined if *t*10,*c*12 CLA depresses SREBP1 maturation and activity via inhibition of Ubxd8. Oleic acid does not induce milk fat depression in cows and does not seem to have any nutrigenomic effect in bovine cells (Bionaz et al., 2008, 2012b; Kadegowda et al., 2009).

Sterol Regulatory Element-Binding Transcription Factor/Protein 1 (SREBP1) and Milk Protein Synthesis. The SREBP1 also can play a role in the regulation of protein synthesis by cross talking with mammalian target of rapamycin (**mTOR**) through RAC-α serine/threonine-protein kinase (AKT1; Porstmann et al., 2008, 2009). This effect can be relevant in dairy cows when considering milk protein synthesis; however, it remains an unexplored possibility.

Despite the important roles of SREBP1 in milk synthesis, the inability of this TF to bind directly and be activated by dietary or endogenous compounds limits practical nutrigenomic interventions with the exception of using the milk fat–depressing phenomenon to help in improving negative energy balance in early postpartum cows (Odens et al., 2007).

Other Transcription Factors with Nutrigenomic Potential in Dairy Cows

Few other TF with nutrigenomic potential have been considered in dairy cows. The expression of both Spot14 and ChREBP is decreased in bovine mammary tissue and epithelial cells due to milk fat depression and *t*10,*c*12 CLA treatment (Harvatine and Bauman, 2006; Bauman et al., 2011; Harvatine et al., 2014). Overexpression of Spot14 in bovine mammary cells increases triacylglycerol synthesis and expression of *FASN*, *SREBF1*, and *PPARG* (Cui et al., 2015). No binding capacity for dietary compounds is known for Spot14. The role of ChREBP in bovine mammary tissue is unclear due to its low expression (Harvatine et al., 2014). It remains to be determined if the effect observed in bovine mammary is due to a direct response to dietary compounds or a consequence of upstream TF, such as SREBP1. A glucose sensing role for ChREBP in nonruminants is known; however, little or no information exists on the possibility of using such TF for nutrigenomic purposes.

Transcriptional Factor Networks and Nutrigenomics

Besides the fact that several TF are potentially involved in sensing nutrients, especially fatty acids, the existence of a complex interactive TF network at play in the response to nutrients also is evident. We have demonstrated that PPARγ partly controls the expression of *SREBF1* (Kadegowda et al., 2009) but SREBP1 can affect the activity of PPARγ by increasing the production of natural agonists (i.e., LCFA), as observed during differentiation in 3T3-L1 (Kim et al., 1998). As observed in bovine and goat mammary cells, the manipulation of various TF by overexpression or molecular inhibition has confirmed such strong interdependence between TF (Shi et al., 2013; Li et al., 2014; Zhu et al., 2014; Cui et al., 2015).

We previously suggested that an interaction between SREBP1 and PPARγ is at play in the regulation of milk fat synthesis (Bionaz and Loor, 2008). Based on the data previously discussed, it is reasonable to expect that more than a network between 2 TF is responsible for the regulation of milk fat synthesis. Therefore, all of the TF presented previously and, in particular, LXR need to be considered. Based on data from our experiments, we previously suggested that additional (at the present unknown) TF, especially LdNR, are part of the network responsible for the translation of the nutrigenomic effects by LCFA (Bionaz et al., 2012b).

The interactive network of TF highlights the complexity that needs to be accounted for in nutrigenomic studies and interventions. The complexity is even more evident when we consider that TF interact not only at the intracellular level but also at the systemic level where activation of a TF in one tissue can induce the activation or repression of a TF in another tissue (i.e., tissue cross talk) by inducing expression of secreted signaling molecules. One example of this is the hepatokine fibroblast growth factor 21 (**FGF21**), a signaling molecule secreted by the liver with a role in controlling adipose metabolism (Itoh, 2014). The role for FGF21 in dairy cows is an active field of study (Schoenberg et al., 2011; Akbar et al., 2015). For this reason, a systems biology approach must be undertaken in implementing nutrigenomics in dairy cows with greater accuracy and efficacy.

MAIN METHODS TO STUDY NUTRIGENOMIC ROLES OF TRANSCRIPTION FACTORS

Gene Expression

The activation or inhibition of TF results in greater or lesser transcription of its target genes. For this reason, the measurement of expression of known target genes can be an indirect method to test if a compound is an agonist or antagonist of a particular TF. Such an approach has been used in dairy cows to investigate PPARα and PPARγ (Bionaz et al., 2013), SREBP1 (Harvatine and Bauman, 2006; Hussein et al., 2013), and the LXRα (McFadden and Corl, 2010; Harvatine et al., 2014).

The use of gene expression has the advantage of not disrupting the normal biology of the cells and can be applied both in vitro and in vivo. It also provides a way to infer the biological effects of the activation of a TF, especially if whole transcriptomics analysis is performed. However, this approach has some limitations. Among those is the inability to distinguish if the observed change in expression is a direct effect of the activation or inhibition of the specific TF or the effect is indirect through a secondary TF. In addition, the specific agonist can activate other TF (i.e., off-targets), as observed for PPAR isotype-specific synthetic agonists (Johnson et al., 1997). Another limitation is related to the timing of sample collection. The TF have a transient effect on transcription of specific genes, and the expression measured is highly dependent on other factors, such as the half-life of the mRNA. Therefore, the use of gene expression, which requires harvesting the cells or tissues, does not allow assess in real time to the activation of TF. Nonetheless, the use of gene expression is a legitimate method to indirectly study the activation of TF in nutrigenomic studies.

Gene Reporter

The ability to monitor activation of a TF is possible through production of a chimera plasmid by fusion of the appropriate DNA coding for a promoter including the response element of the gene of interest with the DNA sequence coding for the reporter gene. After insertion (e.g., transfection) of the chimera into cells, the response of TF is assessed through the direct or indirect measurement of the expression of the gene reporter. Gene-reporter technology can be performed through transient or permanent transfection methods. Among the methods for transient transfection, the most used are cation lipids (i.e., liposomes and lipoplexes; Junquera and Aicart, 2014). Permanent transfection is mainly achieved by using viruses (Zhang and Godbey, 2006). The initial use of gene reporter technology in bovine cells dates back 30 yr (Camper et al., 1985; Adan et al., 1991) but has been very limited since. Due to the possibility of studying with great precision the activation of TF, the use of such technology is gaining momentum in nutrigenomic studies.

Luciferase. The luciferase was originally extracted from fireflies but it is present in several other organisms. The term luciferase and luciferin are generically used for the enzyme and the substrate, respectively, which, on reaction, generate bioluminescence. The most widely used: bioluminescent proteins for gene reporter assays are firefly luciferase and Renilla. However, luciferase is arguably the most used for quantitative analysis of gene expression in mammalian cells and, often, the Renilla is used as an internal control for data normalization. This combination is due to the almost ideal characteristics of these reporters: 1) mammalian cells do not contain luciferase or Renilla, 2) the 2 compounds remain inert within cells, and 3) the current generic assays for luciferase and Renilla are rapid, simple to use, and have high sensitivity (as low as 1×10^5 luciferase molecules; Alam and Cook, 1990).

Among the limitations of these assays is the requirement for lysing the cells to assess luciferase, which limits the possibility of monitoring the dynamism of TF activation over time. Therefore, the researcher has to choose a priori a time point for cell harvesting. To overcome this limitation, cells can be harvested at several time points; however, this only partly resolves the issue and can considerably increase the cost. A new approach that does not require harvesting the cells is the use of extracellular secreted luciferases, such as the one derived from *Gaussia princeps*, which catalyzes the oxidation of the substrate, coelenterazine, leading to the emission of light (Tannous et al., 2005).

Luciferase is by far the most-used gene-reporter technology in nutrigenomic studies in dairy cows (Lengi and Corl, 2010; White et al., 2011; Harvatine et al., 2014; Ma et al., 2014) but has also been used to study bovine cell signaling (Zhou and Jiang, 2006; Wang et al., 2009), validation of promoter regions of genes (Hazelton et al., 2008), milk protein gene expression (Zhou et al., 2008), and single nucleotide polymorphisms (Keating et al., 2007; Wang et al., 2013b).

Fluorescent Proteins. The initial steps toward the use of fluorescent proteins in molecular biology were made when Prasher et al. (1992) sequenced and cloned the green fluorescent protein (**GFP**) from the *Aequorea victoria* jellyfish. Since then, the application of fluorescent proteins for tracking single molecules and live cells in vitro or in the whole organism has been vastly exploited and has impacted the fields of biochemistry, biotechnology, and cell biology. The great advantage of fluorescent proteins over luciferase is their ability to form internal chromophores without requiring accessory cofactors, enzymes, or substrates other than molecular oxygen. This

advantage allows researchers to collect "true" real-time data on a specific cellular activity without harvesting the cells. Today, there are several new fluorescent proteins besides the GFP that can be used for gene reporter assays providing a variety of different emission color such as cyan, yellow, and red (Gurskaya et al., 2001; Carter et al., 2004). Therefore, this approach is ideally suited for the investigation of interactions among TF during nutrigenomic responses. One of the major drawbacks of using live-cell imaging systems is the high initial investment in equipment (e.g., automated fluorescent microscope). In general, the use of fluorescent proteins in dairy cow–related studies is scarce. The GFP has been used as a gene reporter for selection and proof of concept in bovine embryo research (Roh et al., 2000; Eghbalsaied et al., 2013; Furusawa et al., 2013), cell survival in mammary epithelial cells (Sobolewska et al., 2009) and to determine the level of transfection to study key process in lipid metabolism in hepatocytes from newborn calves (Fu et al., 2012). To the authors' knowledge, no nutrigenomic studies in dairy cows have been published using fluorescent proteins to investigate activation of TF.

COMPOUNDS WITH NUTRIGENOMIC POTENTIAL IN DAIRY COWS

Short-Chain Fatty Acids

Among the main short-chain fatty acids (**SCFA**) produced by rumen fermentation, butyrate is the one with the greatest amount of nutrigenomic data in dairy cows. Butyrate affects the expression of a large number of genes related to cell cycle arrest, immune response, and signaling in MDBK cells (Li and Li, 2006; Li et al., 2008; Wu et al., 2012b). It plays epigenetic roles by affecting histone deacetylase activity and by changing the amount of alternative splicing in MDBK cells (Wu et al., 2012a). Expression of cyclin D1, involved in cell proliferation, was induced by butyrate supplementation in dairy goat ruminal papillae cultures (Malhi et al., 2013). Butyrate also affected the expression of genes related to glycolysis and lipogenesis in ruminal papillae of dairy cows (Laarman et al., 2013).

Surprisingly, data from a recent study indicated that SCFA, particularly propionate, decreased the expression of *GH* and prolactin (*PRL*) in dairy cow anterior pituitary cells (Wang et al., 2013a). Those data seem in contrast to the known positive effects of energy in the diet, generally associated with propionate production, with an increase of protein synthesis in bovine mammary, partly induced by GH (Bionaz et al., 2012a). More recently, MacT cells cultured with 5 m*M* acetate, 5 m*M* β-hydroxybutyrate, or a mixture of the 2 (5 m*M* each) led to a significant increase in expression of *SCD1* and *ACACA* (Jacobs et al., 2013). The observation that expression of *PPARG* was not detected in MacT cells and that treatment with either C16:0 or C18:0 did not affect the expression of any PPAR-related gene excluded the possibility of an effect via PPAR of the 2 saturated fats from acetate produced through de novo synthesis.

The above observed nutrigenomic effect of acetate is probably transduced via plasma membrane receptors. It is known that SCFA bind specific plasma membrane proteins called free fatty acid receptors (**FFAR**). The FFAR are members of the G protein-coupled receptor (**GPR**) with a pivotal role, among others, in mediating the effect of fatty acids on insulin secretion and adipocyte differentiation and metabolism. There are 4 receptors with such a role (Covington et al., 2006; Holliday et al., 2011; Georgiadi and Kersten, 2012): GPR41 (*FFAR3*) and GPR43 (*FFAR2*) that bind SCFA and GPR40 (*FFAR1*) and GPR120 (*FFAR4*) that bind medium and LCFA.

Investigations on FFAR in ruminants are relatively scarce. Yonezawa et al. (2009) have determined the expression of *FFAR2* (GPR43) and *FFAR3* (GPR41) in bovine mammary tissue during lactation and in mammary epithelial cells. The data from that study are indicative of those receptors mediating the increase in intracellular Ca^{2+}, reducing cAMP, and increasing phosphorylation of mitogen-activated protein kinases (**MAPK**). Hosseini et al. (2012) observed an increase of *FFAR3* and *FFAR2* during bovine adipogenesis in vitro but expression was not affected by insulin, propionate, or β-hydroxybutyrate. However, an increase in *FFAR3* expression due to propionate was observed in goat white adipose tissue (Mielenz et al., 2008) and from pregnancy to lactation in bovine adipose tissue (Lemor et al., 2009) despite the well-established decreased rate of adipogenesis during early lactation (McNamara, 1991). An important role for GPR43 in microbiota–host communication affecting insulin sensitivity and obesity was discovered in mice (Kimura et al., 2013); considering the high concentration of SCFA in the circulation, this finding can be of extreme relevance for ruminants.

The GPR40 has been less investigated in ruminants. Hidalgo et al. (2011) reported an increase in intracellular Ca2+, MAPK phosphorylation, production of superoxide, and granule released in bovine neutrophils due to oleic acid via GPR40. Yonezawa et al. (2008) detected mRNA of *FFAR1* in bovine mammary tissue and primary bovine mammary epithelial cells. The authors proposed that induction of proliferation of mammary cells by oleate was likely mediated by GPR40. To our knowledge, no data are available in dairy cows or ruminants for the GPR120.

In Vitro Nutrigenomic Effects of Saturated versus Unsaturated Long-Chain Fatty Acids

Relatively few in vitro studies in dairy cows have been performed to test the nutrigenomic role of LCFA. Several of these studies dealt with the role of SREBP1 and its response to a limited number of unsaturated fatty acids, especially *t*10,*c*12 CLA. Most of these studies were previously discussed in the section on SREBP1. In vitro nutrigenomic studies considering a greater number of fatty acids, including saturated, were previously reviewed by Bionaz et al. (2013). A summary of the findings from 2 studies we performed where the nutrigenomic role of a large number of LCFA was tested (Kadegowda et al., 2009; Bionaz et al., 2012b) is presented in Fig. 3. Based on those data, all saturated LCFA, but especially C16:0, have a more pronounced nutrigenomic effect compared with unsaturated LCFA.

The greater nutrigenomic effect of saturated LCFA coupled with their potent PPAR agonistic activity observed in vitro (Fig. 2) allowed us to propose that the use of saturated LCFA can be an effective and economic way to deliver nutrigenomic interventions (Bionaz et al., 2013). However, it should be kept in mind that the use of in vitro approaches to study nutrigenomics can have limitations when translated to an in vivo condition. Considering that cells in vitro are removed from their original niche and lose the surrounding cross talk (both physical and chemical) with other cells, the in vitro conditions do not mimic the in vivo situation. In fact, cells in culture are in a relatively stagnant condition compared with the dynamic fluctuation of hormones and metabolites they experience in vivo. The limitations of extrapolating from in vitro to in vivo conditions is accentuated when immortalized cells are used. Cultured cells tend to change their identity and likely the response to external stimuli. This is supported, for example, by the fact that MacT cells have a transcriptome profile widely different than mammary tissue (Hosseini et al., 2013). In particular, the study allowed the conclusion that MacT cells can be considered a relatively adequate model to study regulation of protein synthesis but are likely less responsive compared with mammary tissue to the regulation of milk fat synthesis. The latter conclusion was supported, in part, by the significantly less expression of PPARγ and some of its putative target genes (e.g., lipoprotein lipase) in MacT cells compared with mammary tissue (Hosseini et al., 2013). Therefore, in vitro experiments are useful in the nutrigenomic framework to test, with high accuracy, dose–responses of a specific TF agonist. In doing so, preliminary information to carry out in vivo experiments can be generated. Subsequent in vivo experiments must be performed before practical nutrigenomic applications can be proposed.

In Vivo Nutrigenomic Effects of Saturated versus Unsaturated Long-Chain Fatty Acids

Supplementing fat to increase the energy or caloric density in the diet of lactating dairy cows has a long history (Palmquist and Jenkins, 1980). The use of saturated fat is preferred over the use of oil because the latter tends, among other things, to induce milk fat depression but also because in ruminants, saturated fats are more digestible than unsaturated fat (Palmquist and Jenkins, 1980). Besides the provision of dietary energy, LCFA elicit several biological effects on dairy cows. Dietary LCFA, particularly PUFA, generally have a positive effect on several components of the reproductive organs and enhance fertility (Santos et al., 2008; Gulliver et al., 2012; Leroy et al., 2014). A recent review of data from several studies where C16:0 and/or C18:0 were supplemented to dairy cows concluded that, in general, there is a benefit of supplementing dairy cows with saturated LCFA (Loften et al., 2014). In particular, the authors observed in most of the studies, an increase of milk fat percentage and milk yield while improving the transition from pregnancy to lactation in dairy cows. The increase in milk fat likely is due to a greater availability of LCFA for the mammary tissue, as supported by the increase in percentage of the supplemented LCFA in the milk fat. Very recently, feeding midlactation dairy cows with a fat supplement containing 98% C18:0 increased milk fat production (Piantoni et al., 2015a) with an increase in yield of most fatty acids, including SCFA. This indicates that the increase in milk fat was also due to greater de novo fatty acid synthesis. The increase in milk fat after supplementation of saturated LCFA is, however, not always observed (Moallem et al., 2007; Duske et al., 2009; Piantoni et al., 2015b). The increase in milk fat synthesis and improvement of overall metabolism on saturated LCFA supplementation reported in the literature are likely due to the nutrigenomic effects of those saturated LCFA (Bionaz et al., 2013). Evidence of in vivo nutrigenomic effects of LCFA are accumulating, although they vary across tissues and with seemingly contrasting results.

Mammary Tissue. In an in vivo experiment, supplementation with saturated lipid (40% C16:0 and 40% C18:0) for 21 d in midlactation dairy cows did not increase milk fat synthesis compared with a control whereas a mixture of fish and soy oil induced milk fat depression (Thering et al., 2009b; Invernizzi et al., 2010b). The expression of lipogenic genes in mammary tissue was greater with saturated lipid compared with the mixture of fish and soy oil (Invernizzi et al., 2010a). However, the study did not have gene expression data for the control and a conclusion cannot be made about the transcriptomic effect of saturated fat in mammary tissue. It is likely that the observed nutrigenomic dif-

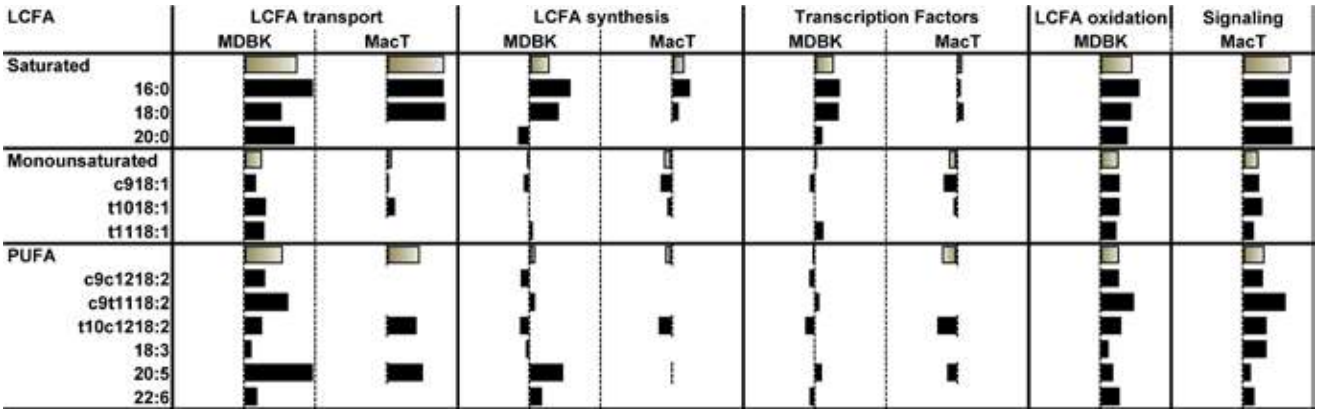

Figure 3. In vitro nutrigenomics effects of long-chain fatty acids (LCFA) in bovine cells. Data were compiled from 2 studies where Madin-Darby bovine kidney (MDBK; Bionaz et al., 2012b) and bovine mammary alveolar (epithelial; MacT; Kadegowda et al., 2009) cells were used to study the nutrigenomics effects of several LCFA. The MacT cells were treated with 100 μ*M* of LCFA 4:1 with albumin and harvested after 12 h treatment whereas the MDBK cells were treated with 150 μ*M* LCFA not bound to albumin for 6 h. Expression of 16 and 30 genes for MacT and MDBK cells, respectively, were measured using quantitative RT-PCR. The bars denote the geometrical mean of expression relative to a control of all measured genes involved in each of 5 separated functions: LCFA transport, LCFA synthesis, transcriptional regulation, LCFA oxidation, and signaling (see the original publications for the specific genes). Right horizontal bars denote increase and left horizontal bar denote decrease in expression relative to the control (vertical dotted line). Gray bars denote overall effect and black bars denote effect for each LCFA. The LCFA were clustered in SFA, MUFA, and PUFA.

ferences were mostly driven by the well-known milk fat–depressing role of the mixture of oil (Bauman et al., 2011). However, this is not always observed (Kramer et al., 2013). Overall, a strong negative nutrigenomic effect of some unsaturated LCFA is clear but a positive nutrigenomic effect of saturated LCFA in mammary tissue remains unclear but possible (Piantoni et al., 2015a).

Adipose Tissue. A lack of nutrigenomic effects by saturated LCFA but a greater expression of lipogenic genes with a mixture of oil compared with a control was observed in the adipose tissue of midlactation dairy cows (Thering et al., 2009b). In contrast, in a more recent study, Schmitt et al. (2011) observed that expression of several lipogenic genes, related transcription factors, and nuclear receptor coactivators was greater at 11 d postpartum in adipose tissue in cows supplemented for 30 d with a commercial lipid high in saturated LCFA compared with a control or unsaturated fat.

Liver. In the same experiment performed by Schmitt et al. (2011), hepatic expression also was assessed (Akbar et al., 2013b). The results uncovered a stronger nutrigenomic effect of saturated compared with unsaturated fat supplementation prepartum (i.e., 1 wk after starting the fat supplementation), but a larger nutrigenomic effect was observed for fish oil supplementation postpartum (i.e., >4 wk of supplementation). Interestingly, the data indicated that the supplementation of fat, either saturated or unsaturated, induced an overall decrease in expression of genes postpartum, including genes such as *ACOX1*, *CPT1A*, or *FGF21* that would be expected to be upregulated if PPARα was one of the target TF (Bionaz et al., 2013). These data do not seem to support a positive role of saturated fat supplementation postpartum but they do indicate that the supplementation of saturated fat might be beneficial in liver prepartum. A lack of nutrigenomic effects of ω3 PUFA in liver of transition dairy cows was observed by Carriquiry et al. (2009). The lack of nutrigenomic effect of fish oil via PPARα also was observed in liver tissue of dairy calves (Litherland et al., 2010).

Differentially Nutrigenomic Response to LCFA by Tissues. A recent study detected a contrasting nutrigenomic effect of the type of fat supplementation on liver and adipose tissue of lactating dairy cows (Hiller et al., 2013). Similar to the postpartum period in the work of Akbar et al. (2013b), compared with saturated fat, the supplementation with unsaturated fat in the work of Hiller et al. (2013) had a general decrease in expression of genes related to fatty acid metabolism (mostly involved in triglyceride synthesis) in liver, but almost the opposite was observed in adipose tissue. In another study (Duske et al., 2009), supplementation of dairy cows for 12 wk prepartum with fat containing mostly C16:0 and C18:1 compared with an isoenergetic nonfat supplement decreased milk yield and had no effect on the mRNA expression of leptin and its receptor in adipose tissue and *ACACA* expression in liver and adipose tissue.

The above data support a tissue-specific nutrigenomic response to LCFA, with saturated LCFA generally having some positive nutrigenomic effects on liver and unsaturated LCFA having positive nutrigenomic effects on adipose (although with some positive nutrigenomic effects also by saturated LCFA) and negative nutrigenomic effects on mammary tissue. This tissue-specific nutrigenomic response to LCFA is of great importance considering potential practical applications and, hence, needs to be better investigated.

The number of in vivo studies where the nutrigenomic effects of supplementing fat in dairy cows was evaluated is relatively small, and more studies need to be performed before drawing solid conclusions; however, the few published studies seem to indicate a weak nutrigenomic effect of saturated LCFA despite the very strong nutrigenomic effects observed in vitro. One of the major issues related to the in vivo supplementation of fat is the lack of precise criteria to determine the proper dose and time. Dose of agonist is an important factor in the activation of TF, especially if considering the fine-tuning of TF activity (Bionaz et al., 2013).

Fundamental open questions related to the use of LCFA for nutrigenomic applications exist. Which are the TF activated or inhibited by LCFA? Which are the LCFA with nutrigenomic activity? What dose of each LCFA maximizes the activity (or the repression) of specific TF? Are single LCFA or combinations more effective? When is the best physiological stage to supplement cows with specific LCFA? What is the nutrigenomic effect of LCFA in each tissue and what is the nutrigenomic effect at the systemic level? From this point of view, the opposite nutrigenomic responses observed in liver and adipose tissue reported above are of extreme interest. What about the other tissues?

Considerations in Using an In Vitro Approach to Study Nutrigenomic Effect of LCFA in Dairy Cows

The large nutrigenomic effects of LCFA in vitro coupled with relatively weak effects in vivo still need to be completely understood, but one of the main reasons may be that the in vivo concentration of LCFA (particularly saturated) available to the cells already is close to the maximum binding capacity of the LdNR. This is partly supported by the fact that in vitro nutrigenomic studies using LCFA are performed without the use of fetal bovine serum (**FBS**) in the media; however, adding only 10% FBS to culture media activates PPAR with a similar or stronger magnitude compared with 100 μ*M* C16:0 or the synthetic PPARγ agonist rosiglitazone (Fig. 2). There have been very few attempts to determine the fatty acid composition of FBS. For instance, Lagarde et al. (1984) analyzed the fatty acid profile of media containing 20% FBS and observed an estimated 67.5 and 32.7 μ*M* of total (with 40.2 and 13.4 μ*M* in the FFA fraction) C16:0 and C18:0, respectively. From this point of view, it is interesting that a dose of 100 μ*M* of C16:0 coupled 4:1 with albumin was necessary to induce expression of *CPT1A* in MDBK cells (Bionaz et al., 2008). Based on this last observation, the presence of C16:0 and C18:0 in FBS is likely not sufficient to explain the large activation of PPAR observed in our study (Fig. 1).

To estimate the amount of each LCFA available in vivo at a cellular level for mammary cells, we used data from 3 independent studies (Enjalbert et al., 1998; Loor et al., 2002; Loor and Herbein, 2003 [Table 2]). In the calculation, we assumed that only the LCFA from NEFA and triacylglycerol are free to be taken up by the tissues. Based on data from Enjalbert et al. (1998), the mammary gland extracts between 30 (in control cows) and 56% (in fat-supplemented cows) of LCFA from plasma, with differences among the various LCFA. Using these criteria, we estimated that the LCFA availability for mammary cells from cows not supplemented with fat is substantially less (approximate range from 60 to 130 μ*M* total LCFA) compared with fat-supplemented cows (approximate range from 145 to 650 μ*M*; Table 2). The data indicate less LCFA availability in Jersey cows compared with Holstein cows.

It is noteworthy that in all the estimated data from the 3 studies considered in Table 2, the concentration of C16:0 and C18:0 ranges from 13 to 54 μ*M* in unsupplemented cows and reaches a maximum of >300 μ*M* for C18:0 in Holstein cows supplemented with soybean oil and approximately 200 μ*M* for C16:0 in cows duodenally infused with 500 g/d of C16:0-enriched fat mixture (Table 2). Therefore, considering the saturated LCFA, the 100 μ*M* concentration of LCFA used in several in vitro nutrigenomic studies (Bionaz et al., 2008, 2012b; Kadegowda et al., 2009; Thering et al., 2009a) is in a similar range but slightly greater compared with what is available for cells in vivo in unsupplemented cows and lower than those in fat-supplemented cows. Considering the data in Table 2, for the unsaturated LCFA, especially PUFA, doses in the range of 0.1 to 10 μ*M* seem more physiologically relevant. The 195 μ*M* concentration of available C16:0 estimated from the 500 g/d of C16:0 duodenal infusion in the study of Enjalbert et al. (1998; Table 2) appears excessively high and may be toxic (Cacicedo et al., 2005; Thering et al., 2009a).

An important practical consequence can be drawn from these observations. If the concentration of LCFA is already sufficient or close to maximize the activation of LdNR, then it may be worth to concentrate the nutrigenomic effort in increasing the abundance of LdNR or their coactivators. Several LCFA, other nutritional compounds, and management strategies can affect the expression of TF, such as appears to be possible with PPAR (Bionaz et al., 2013). The idea of increasing the activity of PPAR in vivo also is supported by a recent study where use of saturated fat supplementation in transition cows increased the expression of several PPAR coactivators in adipose tissue (Schmitt et al., 2011). It remains to be determined if this affects the nutrigenomic activity of PPAR.

Table 2. Estimated micromolar concentration of long-chain fatty acids available to mammary cells after extraction from NEFA and triglycerides in blood. Data are from 3 independent studies carried out on Holstein and Jersey dairy cows at early and mid lactation without or with supplementation of several sources of fat (either orally or abomasal infusion). The percentage extraction of each fatty acid used in the calculation was from Enjalbert et al. (1998).

	Jersey 60–90 DIM[1]				Holstein 45–60 DIM[2]			Holstein 100 DIM[3]			
Fatty acid	CTR	CAN	MIX	SOY	CTR	CAN	SOY	CTR	C16:0	C18:0	C18:1
C14:0	1.8	5.0	4.0	4.9	na[4]	na	na	3.3	10.1	8.4	11.1
cis-9 14:1	0.2	0.3	0.2	0.6	na	na	na	na	na	na	na
C16:0	12.8	41.5	37.3	48.8	20.6	125.2	146.7	22.9	195.3	62.4	47.0
cis-9 16:1	0.1	0.7	0.3	0.6	na	na	na	na	na	na	na
C18:0	27.5	61.5	57.6	63.3	54.0	284.4	318.5	52.6	99.9	128.6	58.7
cis-9 18:1	2.8	9.8	10.8	17.9	na	na	na	23.0	45.7	44.1	122.1
trans-11 18:1	11.1	30.5	22.3	22.0	5.0	16.5	28.3	6.0	8.1	11.6	5.1
cis-9,*cis*-12 18:2	2.0	6.0	5.7	8.6	5.4	10.2	18.6	3.7	9.8	2.9	11.9
cis-9,*trans*-11 18:2	0.0	0.7	0.8	1.9	na	na	na	na	na	na	na
18:3	0.6	1.0	0.7	1.2	1.3	3.3	3.9	na	na	na	na
20:3	0.2	0.3	0.1	0.7	na	na	na	na	na	na	na
Other	2.2	5.2	4.5	5.9	22.8	79.6	70.6	21.2	2.0	26.7	6.0
Total	61.2	162.6	144.4	176.4	116.2	582.6	649.8	132.8	370.9	284.6	262.0

[1]Twenty-four lactating Jersey cows between 60 and 90 d were supplemented for 4 wk with 35 g/d of canola oil (CAN), soybean oil (SOY), or 50% of each. Original data are from Loor et al. (2002).

[2]Four lactating Holstein cows were used in a 4 × 4 Latin square and supplemented for 7 d with 30g/d of CAN or SOY plus a control group (not supplemented [CTR]). The groups supplemented with addition of CLA are not shown. Original data are from Loor and Herbein (2003).

[3]Four lactating Holstein cows with a duodenal cannula were used in a 4 × 4 Latin square and supplemented through abomasal infusion of 500 g of 3 fat mixtures containing 98.6% palmitate (C16:0), 92.3% stearate (C18:0), or 79.9% oleic acid (C18:1). Original data are from Enjalbert et al. (1998).

[4]na = not detected data or not available.

Amino Acids

Bionaz et al. (2012a) provided a comprehensive review of the literature up to 2012 regarding the effects of AA on milk protein synthesis. The conclusion from the review was that the activity of the main pathway regulating protein synthesis, with mTOR as central hub, is basically inhibited in bovine mammary tissue and induced by a cooperation between insulin, IGF-1, GH, AA (e.g., leucine), and glucose leading to greater translation of mammary proteins. In addition, the available data indicated that the posttranscriptional changes elicited by AA, insulin, and glucose appear to fine-tune the protein synthesis but a large effect on milk protein synthesis is coordinated by alterations in mRNA expression of genes related to glucose transport and AA uptake.

At the time of the review by Bionaz et al. (2012a), the inhibitory effect of adenosine monophosphate-activated protein kinase (**AMPK**) on the mTOR pathway was hypothetical and based on nonruminant literature. Recent work using MacT cells provided confirmation that activation of AMPK via 2-deoxyglucose (inhibits glucose metabolism) led to a quick and marked decrease in global rates of protein synthesis (Burgos et al., 2013). In a subsequent study, Appuhamy et al. (2014) evaluated various essential AA alone or in combination with glucose and acetate on mTOR and AMPK phosphorylation in MacT cells. The study confirmed the positive role of energy on milk protein synthesis detected by Burgos et al. (2013) and confirmed the positive effects via phosphorylation of essential AA on activation of the mTOR pathway with a concomitant increase in casein synthesis. Therefore, at the present time, data indicate a physiological link between mammary availability of essential AA and glucose as drivers of phosphorylation status and function of mTOR and AMPK. Further in vivo studies need to verify these data.

Recent molecular studies have been more focused on examining the nutrigenomic role of individual AA on milk protein synthesis in vitro with primary bovine mammary cells. Some of the focus has been on Lys and Met, thought to be the most limiting AA for milk synthesis (Bionaz et al., 2012a). Nan et al. (2014) provided evidence that peak synthesis of casein at a Lys to Met ratio of 3:1 was driven partly by an increase in mTOR phosphorylation but also upregulation of mRNA expression of *MTOR* itself, casein and lactalbumin genes, and the transcription regulator E74-like factor 5 (*ELF5*). Another study provided evidence that Arg, a conditionally essential AA, also is capable of increasing the expression of casein genes along with *MTOR*, *RPS6KB1*, and *STAT5* and decreasing the expression of the translation inhibitor 4EBP1 when supplemented at a level equivalent to 2x the concentration found in casein (Wang et al., 2014).

NUTRIGENOMIC ROLE OF LEVEL OF FEED INTAKE

Liver

Velez and Donkin (2005) observed that temporary feed restriction increases the gene expression of pyruvate carboxylase in liver of dairy cows. A strong temporal nutrigenomic effect by prepartum feed intake (restricted and high feed intake) in the liver of dairy cows was reported by Loor et al. (2006) and Bionaz and Loor (2012). In a more recent systems biology analysis of the same data by Shahzad et al. (2014), a strong "priming" effect of feed intake level on liver of dairy cows was uncovered. In particular, it was observed that cows experiencing prepartum feed restriction had a liver primed to better face the postpartum metabolic challenges. Extensive transcriptomic effects on liver also were observed in early postpartum dairy cows when subjected to restricted grazing (60% equated to optimal level of forage) compared with adequately fed animals (Grala et al., 2013). The functional analysis uncovered a general decrease in the liver metabolism likely to spare energy for the other tissues. In addition, a strong inhibition of cholesterol synthesis but an activation of PPAR signaling was observed. Similar overall nutrigenomic effects, but of a lesser magnitude, were detected in the liver of feed-restricted midlactation dairy cows (Akbar et al., 2013a). Sigl et al. (2013) reported that short-term feed restriction was enough to induce change in expression of lipid-related genes in liver of early postpartum dairy cows.

Adipose Tissue

In data partly published (Janovick et al., 2009; Loor et al., 2011; Bionaz and Loor, 2012), the magnitude of the transcriptomics effect of prepartum dietary feed intake level on the subcutaneous adipose tissue was similar to the liver; however, there did not seem to be a priming effect on the adipose tissue as was the case for the liver. Furthermore, the nutrigenomic effect with the high intake (i.e., high dietary energy level) prepartum was more acute in adipose tissue compared with the liver and, compared with a control group, substantially induced the gene networks involved in triglyceride accumulation. The greater synthesis of triglyceride in cows receiving high prepartum feed intake was also supported by the greater BCS (Janovick and Drackley, 2010).

Mammary Tissue and Immune Cells

Boutinaud et al. (2008) detected a decrease in gene expression of 2 glucose transporters (*SLC2A1* and, with a tendency, *SLC5A1*) in mammary epithelial cells isolated from milk of feed-restricted dairy cows. More recently, Sigl et al. (2014) reported that a temporary feed restriction during early and mid lactation in dairy cows induced a large effect on expression of genes involved in milk protein synthesis. In 2 studies performed in sheep and goats, it was observed that feed restriction decreased the expression of several milk fat–related genes in mammary tissue (Holsinger, 1997; Schaafsma, 2008). At 2 wk postpartum, neutrophils from cows with high prepartum feed intake had less phagocytosis and greater expression of several genes involved in inflammatory response and metabolism (Moyes et al., 2014).

Overall, the data discussed above support a nutrigenomic role of level of feed or dietary energy intake in dairy cows; however, the mechanism for such effect is complex and remains unclear. Together with energy content, other dietary components also are increased or decreased through changes in total feed intake. Therefore, the nutrigenomic effect of level of feed intake is complex and likely involves a multitude of TF. To account for this and the interaction among tissues, a systems biology approach should be used. The extensive transcriptomics effects as a consequence of changes in level of feed intake, as observed in liver and adipose tissue, do not allow for a practical approach to fine-tune metabolism, as is the case for the use of specific nutritional compounds; however, the priming effect observed in the liver of feed-restricted cows is of extreme interest and could be used to improve the overall "capacity" of tissues, especially the liver, to face metabolic and inflammatory challenges.

NUTRIEPIGENOMICS IN DAIRY COWS

Nutriepigenomics encompasses the study of how bioactive food compounds can affect the health and performance of individuals through epigenetic modifications. Although this field of study is relatively new, in humans, it already has shed light on our understanding of the implications of adequate maternal and early life nutrition on epigenetic alterations and how these translate into phenotypic modifications (Burdge and Lillycrop, 2010).

"Over," "outside of," or "around" are meanings of the Greek-derived term "epi" and, as such, epigenetics can be defined as "on-top-of genetics," meaning inherited characteristics, phenotypes, and chemical entities that are superimposed on the DNA and do not follow basic Mendelian laws. The epigenetic changes can be passed on from cells to cells as they divide and from one generation to the next. The catalog of the presence or absence of epigenetic changes or marks is called "the epigenome" and the makeup of the epigenome may differ even between individuals with identical genetic code based on distinct nutritional exposures (Levesque et al., 2014). In

fact, epigenetic changes via nutriepigenomics can occur continually throughout life. The body of knowledge on the role of specific nutrients on main epigenetic factors that can potentially influence gene expression, such as DNA methylation, histone modifications, and noncoding RNA, is still limited in dairy cows.

Deoxyribonucleic Acid Methylation

Methylation of DNA is the most extensively studied epigenetic mechanism (Jaenisch and Bird, 2003). Among the 4 nucleotides that DNA comprises, cytosine is the target of methylation, especially if associated with guanine. The cytosine–guanine dinucleotide pair in the DNA sequence is known as CpG, and DNA regions with high frequency of this dinucleotide are often referred to as "CpG islands." A high degree of methylation (e.g., hypermethylation) of CpG islands across the promoter region of a gene reduces its levels of expression, whereas a low level of methylation (e.g., hypomethylation) at CpG islands is indicative of active gene expression.

There is a significant amount of data in monogastrics but also in sheep indicating that dietary methyl donors cannot affect only the epigenome of the dam but also of its offspring (Parle-McDermott and Ozaki, 2011), that is, the process of fetal programming. In monogastrics, methyl donor–deficient diets during pregnancy can cause intrauterine growth retardation with a concomitant predisposition to insulin resistance and fatty liver (Gueant et al., 2014). In sheep, the effects of methyl donors can have long-term health implications (Sinclair et al., 2007). Similar to prenatal dietary methyl donors, the prepartal plane of nutrition of dams elicits epigenetic effects on their offspring. This has been demonstrated in beef, sheep, and, to a lesser extent, dairy cows (Bispham et al., 2005; Micke et al., 2010; Penagaricano et al., 2013, 2014; Wang et al., 2015).

In dairy cows, DNA methylation can play an important role in the physiological adaptation to environmental stimuli, such as change in milking frequency, physiological state (Nguyen et al., 2014), mastitis (Vanselow et al., 2006), and milk protein synthesis (Singh et al., 2012). Although epigenetic alterations were not directly measured, supplementation of rumen-protected Met (**RPM**) from parturition to approximately 70 d in milk elicited marked changes in the transcriptome of whole preimplantation embryos (Penagaricano et al., 2013). The authors discussed the potential involvement of epigenetics in the induction of transcriptomic differences, but further studies will have to be conducted to not only study RPM effects on epigenome but also study the functional link between embryo transcriptome changes due to RPM end future performance (e.g., milk production, mammary development, and fertility).

In dairy cows, the effect of nutrition on fetal programming has not yet received enough attention; however, data reviewed recently by Bach (2012) clearly support a role of nutritional strategies during the prepartal period in fetal programming. This is further supported by the observed impact of prepartal maternal energy intake of cows on immunometabolic profile, immune cell function, and antioxidant capacity of calves (Gao et al., 2012; Osorio et al., 2013). Furthermore, data from neonatal calves in a recent study provide evidence that a relatively short-term period of supplementation of RPM (during approximately the last 4 wk of gestation) leads to alteration in hepatic expression of metabolically important genes (e.g., *PCK1*, *CPT1A*, *ACOX1*) and also in neutrophil genes associated with innate immune function (e.g., *SELL*, *TLR2*; Jacometo et al., 2015a,b).

MicroRNA

The microRNA (**miRNA**; average 22 nucleotides) play a major role in controlling the availability of mRNA for translation through posttranscription regulation (Lim et al., 2005). Studies dealing with "miRNAomics" in dairy cows have been partly reviewed by Loor et al. (2013). A more recent study uncovered not only specific hepatic miRNA (e.g., miRNA-122 and miRNA-192) that increase in abundance during postpartum negative energy balance in dairy cows but also downregulation of other miRNA, such as miRNA-143 that putatively targets genes involved in lipid metabolism (Fatima et al., 2014).

In monogastrics, evidence is accumulating about a role of micronutrients (i.e., vitamins and minerals) in modulating miRNA with important implications for overall health (Beckett et al., 2014). To our knowledge, no similar data in this context are available in ruminants. In addition, work to identify the miRNA expressed in bovine mammary cells (Li et al., 2012; Bu et al., 2015) will certainly lead to future studies to elucidate the link between nutrition and miRNA.

SUMMARY AND CONCLUSIONS

The nutrigenomic era in dairy cows has just begun. There are several transcription factors with great potential for nutrigenomic interventions to fine-tune the metabolism of dairy cows to improve performance, health, and milk quality. Among these, PPAR stand out as the most promising. However, a network of TF is more likely involved in the functional outcomes of the nutrigenomic effects of dietary compounds. The fatty acids, particularly LCFA, are the most potent nutrigenomic compounds in the diet. In dairy cows, the use of gene expression technologies confirmed by studies using gene

reporter techniques has uncovered a strong nutrigenomic role of saturated LCFA, likely acting as PPAR agonists. Nutrigenomic effects of those LCFA are striking in vitro but appear more modest in vivo. In contrast to effects of saturated LCFA, the nutrigenomic role of unsaturated LCFA is not as great in vitro, with the exception of a negative effect on expression of lipid-related genes by several PUFA, but the in vivo nutrigenomic effect is not nil. The relatively modest nutrigenomic effects of LCFA observed in vivo may be consequence of lack of knowledge about LdNR or other TF, nutrigenomic activity of LCFA, and best time and dose to maximize the bioactive role of LCFA. Other components of the diet have nutrigenomic roles, including the level of nutrient intake, which can be used to prime the liver (and other tissues?) to better face metabolic challenges, and AA, of which initial studies revealed an interesting nutrigenomic role in controlling milk protein synthesis. The field of nutriepigenomics is quite new in dairy cows but holds great promise. Lastly, proper use of available methods and development of new ones is an essential part of nutrigenomic discovery. At present, the combination of gene expression and gene reporter technologies is likely the most potent available means to investigate nutrient–gene interactions.

Despite the modest nutrigenomic effects observed in vivo, overall results from nutrigenomic experiments thus far produced appear promising; however, partly due to the complexity of the systems at study, practical nutrigenomic applications are not yet at hand. We anticipate that practical nutrigenomic dietary interventions will likely not become available in the near future. To get to practical applications, more fundamental research needs to be performed.

Nutrigenomics data produced in dairy cows clearly underscores the fact that the current system to build diets for high-producing dairy cows is blind to the nutrigenomic effects of dietary compounds that, by affecting the metabolism of the animal, likely modify its dietary requirements. Therefore, we predict that as more data from nutrigenomic studies in dairy cows become available, besides using nutrigenomic properties of compounds in the feedstuff to improve metabolism, we also will witness the inclusion of these effects in the ration formulation systems.

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