Functional Role of PPARs in Ruminants: Potential Targets for Fine-Tuning Metabolism during Growth and Lactation

Massimo Bionaz,1 Shuowen Chen,2 Muhammad J. Khan,2 and Juan J. Loor2

1Animal and Rangeland Sciences, Oregon State University, Corvallis, OR 97330, USA
2Animal and Nutritional Sciences, University of Illinois, Urbana, IL 61801, USA

Received 24 November 2012; Revised 1 March 2013; Accepted 1 March 2013

Characterization and biological roles of the peroxisome proliferator-activated receptor (PPAR) isotypes are well known in monogastrics, but not in ruminants. However, a wealth of information has accumulated in little more than a decade on ruminant PPARs including isotype tissue distribution, response to synthetic and natural agonists, gene targets, and factors affecting their expression. Functional characterization demonstrated that, as in monogastrics, the PPAR isotypes control expression of genes involved in lipid metabolism, anti-inflammatory response, development, and growth. Contrary to mouse, however, the PPARγ gene network appears to control milk fat synthesis in lactating ruminants. As in monogastrics, PPAR isotypes in ruminants are activated by long-chain fatty acids, therefore, making them ideal candidates for fine-tuning metabolism in this species via nutrients. In this regard, using information accumulated in ruminants and monogastrics, we propose a model of PPAR isotype-driven biological functions encompassing key tissues during the peripartal period in dairy cattle.

1. Introduction

In humans, mouse, and rat, nuclear receptors (NR), including PPARs, form a transcription factor family of 47–49 members [1]. Activity of NR allows for long-term (hours to days) control of metabolism because they can affect mRNA expression of target genes, including metabolic enzymes [2]. Thus, NR represent an important regulatory system in cells, tissues, and organs playing a central role in metabolic coordination of the entire organism.

Peroxisome proliferator-activated receptors (PPARs) were originally identified in Xenopus frogs [3] as novel members of the NR that induced the proliferation of peroxisomes in cells, a process that was accompanied by activation of the promoter of the acyl-CoA oxidase gene (ACOX1) encoding the key enzyme of peroxisomal long-chain fatty acid (LCFA) β-oxidation. The PPARα was the first member or isotype of the PPARs to be discovered in mammals during the search of a molecular target for liver peroxisome proliferators [4]. Those compounds include hypolipidemic drugs, that is, fibrates (e.g., clofibrate, fenofibrate, or Wy-14643), whose main effect is to lower blood triacylglycerol (TAG) and regulate cholesterol concentrations [5].

Initial characterization of PPARα (gene symbol PPARA in human and ruminants) in the adult mouse revealed that it was highly expressed in liver, kidney, and heart [4]. Shortly after PPARα was discovered, the isotypes PPARγ (gene symbol PPARG) and PPARβ/δ (gene symbol PPARD) were cloned [3, 6]. In monogastrics, PPARA is highly abundant in liver, intestine, heart, and kidney; PPARG is abundant in adipose and immune cells, while PPARD is ubiquitously expressed [7, 8]. In the mouse, both PPARγ isoforms γ1 and γ2 act in white and brown adipose tissue to promote adipocyte differentiation and lipid storage. While PPARγ2 is mainly expressed in adipocytes, PPARγ1 is expressed at modest levels also in other cells/tissues [9]. Expression of PPARβ/δ in murine resembled closely that of PPARα and was the sole isoform expressed in brain [6]. More recent studies in rats have established that PPARβ/δ is expressed ubiquitously throughout the body but is substantially more abundant in skeletal muscle than PPARα or PPARγ [7].
The PPARs form and function as heterodimers with retinoid-X-receptor (RXR). Once the ligand binds (e.g., LCFA, fibrates, thiazolidinedione (TZD)) to the ligand-binding domain (LBD), it produces a covalent modification of the PPAR structure [10] activating the NR. The activated PPAR/RXR binds to a specific DNA sequence (PPAR response element, PPRE) in the promoter region of specific target genes inducing or repressing their expression. The PPRE is a direct repeat of a hexanucleotide (AGGTCA) separated by a single nucleotide (i.e., DR-1). The DR-1 varies for each of the PPAR isotypes, thus differing greater or lower strength to the PPAR/RXR complex for binding to PPRE and the strength of activation [11]. All PPAR isotypes are activated by ligand concentrations in the μM range or below, at least in nonruminants [12–14].

2. Role of PPAR in Monogastrics

The PPAR isotypes play multiple roles in mammals. There are a vast number of excellent reviews discussing those aspects in detail (e.g., [2, 5, 15–19]). Among others, the PPAR isotypes play important roles in regulating lipid and glucose metabolism, controlling inflammatory response, regulating tissue repair and differentiation, and cancer progression. Although with contrasting roles, PPAR isotypes affect blood vessel formation [20]. The PPARγ is pivotal in controlling the switch between adipogenesis and osteogenesis [17, 21] and insulin sensitivity [22], and it has an important neuroprotective role [23]. Similarly, it is well established that PPARα plays a crucial role in hepatic fatty acid catabolism in mitochondria, peroxisome, and microsomes [18]. The PPARβ/δ controls fatty acid catabolism in skeletal muscle and heart [2]. The PPAR isotypes are known to play important roles in all the reproductive tissues studied to date (reviewed in [24]). Due to the important functions played by the PPAR isotypes, PPARα and PPARγ have long been considered promising drug targets for human metabolic disorders as they regulate lipid and/or glucose homeostasis by controlling uptake, synthesis, storage, and clearance [25].

3. PPAR Isotype Expression in Ruminant Tissues

Judging from the published literature, the interest on PPAR isotypes in ruminants, particularly their role in lipid metabolism, has been modest compared to the vast literature in nonruminants, including human. Therefore, information about protein and gene expression abundance in ruminants is relatively scant. In order to help close this gap of knowledge we have performed Real-Time RT-PCR (qPCR) analysis to provide an evaluation of the relative distribution of PPAR isotypes in bovine tissues/cells. This PPAR isoform has been the most-studied in ruminants. Our results from qPCR analysis (Figure 1(a)) indicated that PPARγ expression is very high in all adipose tissues, followed by rumen, Madin-Darby Bovine Kidney cell line (MDBK), and placenta with moderate-to-low mRNA expression in small intestine, beef cattle longissimus muscle, hoof corium, lung, and mammary gland. In contrast, the lowest expression of PPARγ was detected in liver, kidney, dairy calf semitendinosus muscle, bovine mammary alveolar cell line (MAC-T), and blood polymorphonuclear leukocytes (PMN) (Figure 1(a)). In an early study bovine PPARγ mRNA expression (via northern blot) was characterized in several tissues [29]. Similar to our data (Figure 1(a)), a greater expression of PPARγ was detected in adipose tissue followed by spleen, lung, and ovary. Although lower, expression was also detected in mammary gland and small intestine. Expression was absent in pancreas and almost undetectable in liver. In other tissues the expression was very low or nondetectable. The PPARγ is highly expressed in adipose tissue of mice [6], human [9], and chicken [30], all of which agree with the relative high expression in bovine adipose tissues (Figure 1(a)). Similar to mouse [6], human [9], pig [31], chicken [30], and beef bulls [32], the expression of PPARγ in bovine liver, or other tissues such as kidney and intestine, was very low (Figure 1(a)).

We and others have previously detected expression of PPARγ in bovine mammary tissue and the MAC-T cell line using qPCR [26, 33, 34]. In a recent study in our laboratory comparing gene expression between mammary gland and MAC-T cells, the former had greater expression of PPARγ both during pregnancy and lactation [35]. The relatively high expression of PPARγ in MDBK cells detected (Figure 1) confirmed previous observations [36]. Expression of PPARγ was detected also in goat mammary, although at a significant lower level compared to bovine [37].

The PPARγ is expressed at all stages during bovine embryo development (both in the inner mass and in the trophoblast of sheep) and in the placentas of bovine (caruncles) and sheep (cotyledons) [38] and in the placenta (cotyledons and caruncles) of bovine [39] and sheep [40], with an evident expression in the trophoblasts [41]. Lutein cells [42] and uterus [43] express PPARγ, but not bovine endometrial cells [44], while endometrial cells of pregnant ewes express this NR [41]. The expression of PPARγ in ovary was confirmed in sheep [45] and the same study reported expression in pituitary gland but not hypothalamus. In previous studies it has been shown that this PPAR isoform is expressed in bovine aortic endothelial cells [46], beef cattle skeletal muscle (including intramuscular fat) [47], ovine intramuscular fat [48], bovine perimysial preadipocytes [49], and bovine retinal pericytes [50]. In several beef cattle breeds, PPARγ had a similar degree of expression in perirenal and omental adipose depots, followed by intramuscular fat and, in a minor quantity, in the longissimus muscle [47, 51].

The expression of various PPARγ isoforms in buffalo was recently evaluated [52] and found to be expressed in all tissues tested: ovary (follicles and corpus luteum), mammary gland, adipose tissue, liver, spleen, and lung. The isoforms PPARγ1a and 1b were highly expressed in ovarian tissue followed by
Figure 1: (a) Relative transcript abundance of each PPAR isotype in several bovine tissues and cells. We measured gene expression of PPAR isotypes in 14 different tissues including tissues from adult dairy cattle: adipose tissue (subcutaneous, mesenteric, and omental), small intestine (jejunum), liver, hoof corium, lung, kidney, mammary gland, blood polymorphonuclear leukocytes (PMN), and placenta; from dairy calves: rumen papillae and semitendinosus muscle (D-muscle); skeletal muscle of beef cattle (Longissimus lomboarum); and two cell lines: Madin-Darby Bovine Kidney (MDBK) and bovine mammary alveolar cells (MAC-T). The total RNA was extracted and qPCR performed as previously described [26]. The qPCR data were normalized by the geometrical mean of 5 internal control genes (PPP1R11, RPS15A, ACTB1, MRPL39, and UXT). For the difference of each PPAR isotype abundance between tissues, the qPCR data were transformed using a 6-point standard curve prior statistical analysis using PROC GLM of SAS (version 9.3) with tissue as main effect. Dissimilar letters denote significant differences (P < 0.05). (b) Tissue-specific relative mRNA abundance between PPAR isotypes. The % relative abundance of the three PPAR isotypes in each tissue was calculated using the delta Ct method as previously described [27]. The final data for PPARG and PPARD were obtained as % relative to PPARA. N.B.: the y-axis values in (a) are least square means of the Ct values transformed using the standard curve and then log2-transformed. The values in (b) are calculated without use of a standard curve. Therefore, the values in (a) are radically different compared to the values in (b) and the two cannot be compared.

3.2. PPARα. This isotype has been less studied compared with PPARγ. The bovine PPARα gene is located in chromosome 5 in cattle [53]. The qPCR analysis of the relative mRNA abundance of PPARA highlighted, as in mice [6], human [54], and pig [31], that PPARA is very abundant in kidney (Figure 1(a)). Contrary to this general feature, even though the PPARA in liver of chicken is expressed at lower level than kidney, its expression in liver is similar to other tissues [30]. In contrast to what is observed in human [54], our data revealed that the relative abundance of PPARA was not statistically different between jejunum and adipose tissues of bovine (Figure 1(a)). In general the data in Figure 1(a) reveals a more widespread expression of this PPAR isotype among the tissues and cells evaluated compared to PPARG. The highest expression was observed in kidney and liver followed by adipose tissues, small intestine, and dairy cattle semitendinosus muscle. Beef cattle longissimus muscle and
mammary cells [69, 70], rumen [64], and uterus [43]. The source of energy. Previous studies have observed expression in blood neutrophils, placenta, and rumen tissue, that expression observed in liver, followed by semitendinosus muscle, and, then, intermuscular adipose tissue [32]. In ewes, its expression was detected in superficial endometrium and trophoblast during early pregnancy [41]. Lastly, expression of PPARα was demonstrated in sheep heart [67].

3.3. PPARβ/δ. As for nonruminants, the PPARβ/δ is the least-studied PPAR isotype also in ruminants, with few published information available. The results of our qPCR analysis indicate relatively similar PPARα mRNA expression in all the 14 tissues and cells assessed (Figure 1(a)); however, the greatest expression was observed in kidney and placenta, followed by adipose tissues, rumen, and MDBK cells with the lowest expression observed in hoof corium, liver, and skeletal muscle (Figure 1(a)). The relative distribution of PPARα expression among cattle tissues/cells, even though similar to that in mouse [6], is rather curious particularly considering its low expression in skeletal muscle and the marked expression in blood neutrophils, placenta, and rumen tissue, that is, tissues that probably do not rely on LCFA oxidation as source of energy. Previous studies have observed expression of PPARα in bovine liver [56], aortic endothelial cells [68], mammary cells [69, 70], rumen [64], and uterus [43]. The PPARα was also shown to be expressed in longissimus muscle of beef steers [47] and in both superficial endometrium and trophoblast of early pregnant ewes [41].

3.4. Relative Abundance between PPAR Isotypes in Cattle Tissues. To date, there is almost a complete lack of data available in the literature of a direct comparison of PPAR isotypes expression in ruminant tissues. Among the few available studies, it was observed that liver of dairy cows expresses a similar amount of PPARα and PPARδ but does not express PPARγ [44]. In a recent study where the expression of the three PPAR isotypes was evaluated in liver and muscle of beef bulls, the greatest expression was observed for PPARα, followed by PPARγ, with the lowest expression for PPARδ in liver, while, the largest expression in muscle was observed for PPARγ [71]. This relative distribution among tissues is somewhat comparable to our data (Figure 1(b)). More numerous are the studies comparing mRNA abundance between PPAR isotypes in bovine cell culture. Those have revealed that bovine endometrial cells express PPARα and PPARδ at a similar level, but not PPARγ [44]. In addition, bovine aortic endothelial cells express both PPARα and PPARγ [46] and mammary cells express both PPARγ and PPARδ [69].

When the relative mRNA abundance between the three PPAR isotypes was evaluated in several tissues from bovine (Figure 1(b)), we observed that the three adipose tissues along with rumen, MDBK cells, and placenta have a marked abundance of PPARδ and PPARγ compared with PPARα, whereas MAC-T cells and PMN have marked abundance of PPARγ but very low abundance of the other two PPAR isotypes. Despite the relatively low abundance, at least in vitro, PPARγ appears to be functional in bovine neutrophils [72] and MAC-T cells [26]. Paradoxically, given its well-established function in monogastrics, with few exceptions (i.e., MDBK and beef cattle longissimus muscle), PPARδ is more abundant than PPARγ, even in the three adipose depots (Figure 1(b)). The PPARα instead was the most abundant PPAR isotype in small intestine, liver, kidney, skeletal muscle, hoof corium, lung, and mammary gland (Figure 1(b)).

Overall, the data in Figure 1 depict a distribution of PPAR isotypes that, similar to other species, seems to underscore the putative biological role of each PPAR isotype. For instance, the expression of PPARα is more abundant in tissues where LCFA oxidation is generally higher (e.g., liver and kidney) and PPARγ is more abundant in lipogenic tissues (e.g., the three adipose tissues).

4. Sequence Homology, 3D Structure, and Activation of PPARα among Bovine, Mouse, and Human

We recently carried out an in silico analysis to compare the amino acid sequence homology of PPARα between bovine, mouse, and human [28]. The analysis revealed more than 90% conservation of this PPAR isotope between the three species, with bovine having greater overall homology to human (94.9%) than mouse (91.2%). When the four domains of the PPARα protein were compared, we observed lower conservation in the N-terminal A/B domain containing the ligand-independent activation function (AF-1), which was 86% conserved between bovine and human and 81% between bovine and mouse [17], and the largest conservation (i.e., 100%) in the DNA-binding domain. The latter suggests that the capacity of the domain for the recognition of the PPRE is highly conserved between species. This has been confirmed by the high responsiveness of rat PPRE when transfected in bovine endothelial cells [73].

The LBD is also highly conserved with greater homology of bovine with human (98%) than with mouse (92%). The lower conservation of the LBD and AF-1, which is common between species, could indicate a difference in interspecies sensitivity of PPARα activation [17] and a greater similarity between bovine and human than bovine and mouse. Surprisingly, when the transcription response of 30 putative PPARα target genes to the potent and specific PPARα agonist Wy-14643 were compared between mouse liver, human liver, and MDBK, we observed a greater number of genes with a common response between bovine and mouse (73%) than bovine and human (60%) [28]. Despite the limitation of comparing liver with kidney cells, those data indicate
a good degree of conservation of PPARα response between species. There are no published studies comparing PPARγ or PPARβ/δ response between ruminant and nonruminant species considering the same (or similar) tissue/cells. An attempt to compare the activation of PPARγ in mammary gland between dairy cattle and mouse is reported (see Section 9.2.1).

In order to further investigate the potential differences in PPARα between mouse and bovine we performed an *in silico* 3-dimensional (3D) structure analysis of the publicly available PPARα protein sequence [28]. The alignment analysis identified an overall high degree of conservation of PPARα amino acid sequence between the two species; however, when the overlap of the 3D structure of the PPARα of the two species was performed, we observed important differences in spatial structure of the LBD. In particular, the residues Leu462 and Tyr466 of the LBD in bovine result in a completely different spatial position compared with mouse (Figure 2). When the electrostatic potential of the surface was visualized, it was apparent that the bovine PPARα has an overall more neutral charge, particularly in the ligand pocket, compared with the highly negatively charged mouse PPARα. This allowed inferring that longer and more saturated LCFA (i.e., more neutrally charged and with a more straight configuration) might be more easily accommodated (Figure 2), hence, likely be better inducers in bovine.

It has been demonstrated, however, that the activation of PPAR isotypes is highly dependent on the A/B domain rather than the LBD [74]. This last observation could explain the interspecies differences observed, considering also that the A/B domain is the least-conserved between species and also between PPAR isotypes (see below). However, this does not fully explain the results from the comparison in PPARα response between bovine, mouse, and human [28] because the conservation of the A/B domain is lower between mouse and bovine than between human and bovine, despite the greater similarity in response between bovine and mouse compared to bovine and human [28].

5. Structural Similarity between PPAR Isotypes in Bovine

Approximately 80% of the 34 amino acid residues in the binding cavity of the three PPAR isotypes (α, β/δ, and γ) are conserved in humans and rodents [75]. The main features dictating the ligand specificity across the PPAR isotypes appear to be the topology of the ligand binding cavity; for example, the PPARβ/δ cavity is much narrower than PPARα and PPARγ and, thus, cannot accommodate bulky polar heads found in thiazolidinedione (TZD) [75, 76]. In contrast, TZD is a potent ligand of PPARγ. Once inside the cavity, the side chains of the ligand (e.g., hydrogen, carboxyl groups) interact with the amino acid residues to achieve a stable configuration.

In bovine, the three PPAR isotype proteins have low conservation overall, with PPARα being more similar to PPARβ/δ (59%) than PPARγ (52%) [28]. The three proteins have a large degree of conservation in the DNA binding domain (>80%), but a low degree of conservation in the A/B domain (<21%) [28]. The PPARα has a greater degree of conservation in the LBD with PPARβ/δ (71%) than PPARγ (64%) [28]. This last observation suggests that among the three isotypes, the expected response to agonists should be more similar between PPARα and PPARβ/δ as it is the case in nonruminants [2]. This would imply that activation of PPARα and PPARβ/δ could result in similar outcomes, for example, fatty acid catabolism.

The 3D depiction of the bovine PPAR isotypes surface reveals a difference in the ligand pocket (Figure 2) [28]. The PPARα appears to have a larger pocket compared with the other two PPAR isotypes. In addition, analysis of the electrostatic potential of the surface indicates a greater negative charge in PPARγ than PPARα and PPARβ/δ, with the latter being mostly positively charged. Those observations suggest a greater capacity of PPARα for binding neutral charged and/or more structurally rigid compounds. Clearly, this inference is only speculative.

6. Ruminant PPAR Response to Synthetic and Natural Agonists

The effect of PPAR agonists in nonruminants has been tested in different models using *in vitro* systems with specific assays such as the Coactivator-Dependent Receptor Ligand Assay (CARLA) [18] or the transfection of PPRE with firefly luciferase (e.g., [96]). An additional assay available today is the direct measurement of activation of PPAR isotypes after nuclear isolation by the presence of PPRE immobilized onto the bottom of cell culture wells; however, such assays have not been developed for ruminants [61]. The use of these techniques with greater sensitivity, precision, and reliance in ruminants has been scant [61]. Most of the studies performed in ruminants are based on measurements of changes in expression of genes or proteins after treatment with PPAR isotype-specific agonists.

6.1. Ruminant PPAR Response to Synthetic Agonists. Several synthetic PPAR agonists are available today for nonruminants [18]. Among the most commonly used are Wy-14643 and fenofibrate as PPARα agonists and TZD and rosiglitazone as PPARγ agonists. Very few synthetic agonists of PPARβ/δ are known (e.g., GW501516). Besides agonists, a few antagonists have been developed, for example, the PPARγ specific antagonists GW9662 [97] and BADGE [98], the PPARα antagonists T0070907 [99] and GW6471 [100], and the PPARβ/δ antagonists GSK0660 [101] and GSK3787 [102]. The use of the specific agonists in combination with antagonists could be a valid, though indirect, approach to uncover both the existence of an active PPAR isoform in cells or tissues and PPAR target genes.

Supplementary Table 1 (see Supplementary Material available online at http://dx.doi.org/10.1155/2013/684159) contains a summary of studies performed to date using specific PPAR agonists in ruminants. From the data, it is evident that most of the studies dealt with bovine with few ones in sheep and goat. A large amount of the bovine studies were performed with bovine endothelial cells. Those cells have been widely used as a model to study endothelial physiology and
Figure 2: Inter-species and inter-isotypes three-dimensional PPAR protein structure comparisons. (a) Three-dimensional surface structure of bovine (residue 202–470; UniProtKB/TrEMBL Q5EA13) and mouse (residue 202–468; UniProtKB/TrEMBL P23204) PPARα ligand binding domain (LBD). The upper and lower panels include two views of the 3D structure of the PPARα protein in bovine and mouse species. The 3D structure is in full alignment between species. From the comparison, the difference in the ligand pocket of the PPARα between the two species is evident, with a larger and more pronounced pocket in bovine compared with mouse. In addition, the bovine PPARα appears to be more neutrally charged compared with the same protein in mouse. (b) Three-dimensional surface structure comparisons between PPARα (residue 202–470; Q5EA13), PPARγ (residue 234–505; O18971), and PPARβ/δ (residue 171–441; A4IFL4) LBD of bovine. Shown is the ligand pocket domain (green arrow) in two diverse views for each of the PPAR isotypes. The comparison highlights the larger and more neutrally charged ligand pocket in PPARα compared with the more negatively charged PPARγ ligand pocket and positively charged and small PPARβ/δ ligand pocket. The images were modified from [28]. Legend: red = negative charge; white = neutral charge; blue = positive charge. The 3D analyses were performed using Swiss-Pdb Viewer software (freely available at http://spdbv.vital-it.ch/).
pathology, particularly for the inflammatory status related to arteriosclerosis, that is, with a clear biomedical purpose and not to understand ruminant biology. Overall those studies established important roles of PPAR in endothelial cells [46, 92, 103, 104]. In particular the activation of PPARγ and PPARα appears to have a protective role for endothelium (Supplementary Table 1).

The first study performed using a PPAR agonist with a clear aim to understand the biology of ruminants was performed in 1998 by a German group [42] where it was observed that PPARγ controls progesterone synthesis in luteal cells isolated from dairy cows. Subsequent studies in granulosa cells of sheep confirmed the role of PPARγ in controlling progesterone synthesis [45].

In 1998, a Japanese group demonstrated that activation of PPARγ is central for adipogenic differentiation of vascular stromal cells from bovine adipose tissue [105] and intramuscular fibroblast-like cells [106]. In 2001, another Japanese group demonstrated that in vivo injection of the PPARα agonist 2,4-TZD partially reversed the insulin resistance induced by TNFα in dairy steers. The phenomenon was explained by the activation of PPARγ in adipose tissue [107]. A year later a group of researchers from a pharmaceutical company fed the PPARα agonist Wy-14643 to lactating goats [108]. The authors reported an overall increase in hepatic β-oxidation and aromatase activity by Wy-14643 and decreased cholesterol in blood (with numerical decrease of TAG as well). No effects were observed on liver size, milk composition, or content of hepatic cytochrome P450. The low magnitude of changes and the unexpected lack of effect of the treatment on P450 led the authors to conclude that the goat is a weak responder to PPARα agonists.

The two studies in vivo mentioned above were critical for animal bioscientists interested in PPAR because they demonstrated that PPARα in liver and PPARγ in adipose tissue of ruminants are active and likely play similar roles as in monogastrics: regulation of β-oxidation for PPARα and regulation of adipogenesis and insulin sensitivity for PPARγ. Since then, few additional in vivo studies using PPAR agonists with agricultural aims have been performed (Supplementary Table 1). Recently, we tested the effects of oral administration for 14 days of the PPARα agonist clofibrate on liver of weaned dairy calves [78] (see also Supplementary Table 1). The treatment had several expected effects such as the increase in expression of several PPARα target genes (see Section 7 for details about PPAR targets in ruminants), but the magnitude of response was lower than usually observed in rodents; thus, we concluded, as for the work performed on goats, that the bovine hepatic PPARα is a weaker responder compared to rodents.

The above observations from in vivo studies of a weak response in ruminants might be explained by the inherent differences in digestive physiology. Contrary to monogastrics, in ruminants, the digestion of any feed is markedly affected by the process of fermentation in the rumen via microorganisms. None of the above studies have assessed the effect of the rumen on PPAR agonists. In this regard, it could have been interesting to measure the blood concentration of the agonists. Interestingly, the human PPAR isotypes also appear to have a lower response compared with rodents [19]. It can also be the case that Wy-14643, a recognized potent PPARα agonist in rodents, is not as potent in ruminants. In accord with this, we have observed in bovine cells a greater increase in expression of PPARα target genes by saturated LCFA compared to Wy-14643 [28]. Those responses indicate a species-specific response to PPAR induction and a different effect of agonists between species.

The results obtained during the in vivo study of the Japanese group mentioned above led to a series of in vivo experiments in pregnant and lactating dairy cows [82, 84, 109, 110]. The purpose of those studies was to evaluate the effects of PPARγ activation on preventing metabolic problems typical of the peripartal period. The specific PPARγ agonist 2,4-TZD was used (via injection) for that purpose (Supplementary Table 1). The treatment with 4 mg/kg BW daily of 2,4-TZD during the last two or three weeks prepartum until parturition decreased substantially the NEFA post-partum. Such effect was ascribed to enhanced insulin sensitivity and PPARγ expression in adipose. In addition, the treatment improved the overall metabolic health postpartum, as reflected in greater feed intake, lower hepatic lipid accumulation, and greater glycogen content in the liver. Overall, the data also suggested an improved fertility (i.e., lower open days) in cows treated with 2,4-TZD.

This series of in vivo experiments reported above (see also Supplementary Table 1) was the first demonstration that PPAR isoforms can play a pivotal role in the physiology and metabolism of dairy cattle. It also underscores the concrete possibility of fine-tuning the PPAR isotype activity through appropriate treatments in order to improve overall performance and health of dairy cattle.

An elegant in vivo study performed recently in pregnant sheep involved the injection of rosiglitazone into the fetuses for >10 days beginning at ca. 25 days before term [81]. The experiment demonstrated that activation of PPARγ had a similar effect on fetuses as overnutrition of the pregnant mother, which is known to induce obesity in later life in offsprings. For instance, rosiglitazone treatment increased expression of lipoprotein lipase and adiponectin in adipose tissue and PPARα and PPARγ coactivator 1 alpha (PPARGC1A) in liver of fetuses (Supplementary Table 1).

Several in vitro studies using synthetic agonists have demonstrated that activation of PPAR isotypes (except gamma) affects fertility by increasing the expression and/or production of prostaglandins, for example, prostaglandin (PG) F2α, and PGE2 in bovine endometrial cells [44, 77]. Other in vitro studies were carried out in order to test the response to PPAR isotypes in two bovine cell lines (MDBK and MAC-T) with the purpose of determining PPARα and PPARγ target genes [26, 28, 36, 61]. Besides target genes, those studies also uncovered several biological functions of PPAR isotypes in ruminants. For instance, the activation of PPARγ in MAC-T cells with rosiglitazone provided a demonstration that PPARγ controls expression of several genes known to be involved in milk fat synthesis [26] while activation of PPARα controls lipid metabolism at the cellular and organismal level.
(i.e., by controlling expression of several signaling molecules) [28].

All the above studies clearly demonstrated an active role of PPAR isotypes in ruminants. The studies also established that PPAR isotypes can be manipulated by using synthetic agonists; however, from a practical stand-point the suggestion of using synthetic agonists is not feasible, namely, because of the high costs that would be incurred. Clearly that could be circumvented if natural ligands are identified.

6.2. Ruminant PPAR Response to Natural Agonists

6.2.1. LCFA. The great interest in PPARs in the area of nutrition stems from the ability to bind and be activated (or inhibited) by LCFA or chemically related derivatives [18, 111, 112].

Monogastrics. In monogastrics all PPAR isotypes are sensitive to fatty acids, particularly LCFA. Although the potency varies with each PPAR isotype, the most potent PPAR endogenous ligands in nonruminants are linoleic acid, linolenic acid, arachidonic acid, and also derivatives of arachidonic acid such as leukotriene B4 (LTB4) or PG [12]. In general it is safe to conclude that PPAR isotypes in most monogastric species studied to date have a greater sensitivity towards unsaturated than saturated [17, 18]. However, in nonruminants both saturated and unsaturated LCFA enhance PPAR transactivation in vitro (e.g., [12, 113, 114]).

In vivo data have been more variable and in some instances high dietary fat activated PPAR target genes regardless of whether the dietary lipid was mostly polyunsaturated (PUFA), monounsaturated, or saturated (e.g., [115]). At the cellular level studies with endogenous ligands such as free LCFA or LCFA-CoA (i.e., activated 16:0, 18:2n-6, 18:3n-3, and 20:4n-6) have demonstrated (at least for PPARα) that both forms of the FA exhibit high affinity (i.e., low nanomolar dissociation values) for the ligand-binding domain of PPAR [114]. This point is important because intranuclear concentrations of free LCFA and LCFA-CoA range between 120–500 nM and 8 nM, respectively [116].

From a mechanistic standpoint it is important to point out that FA binding proteins (FABP, particularly FABP1 and FABP4) are important in channeling intracellular nonactivated (i.e., without addition of the CoA group) LCFA not only to the various organelles but also to the nucleus where the LCFA can activate PPAR. The essential role of FABP in transporting LCFA into the nucleus for the activation of PPAR isotypes was first reported in rodent liver where the amount of FABP protein significantly correlated with transactivation of PPAR in response to LCFA (linoleic acid, linolenic acid, and arachidonic acid) as well as other chemical ligands [117].

Ruminants. To our knowledge there are only two published studies where PPRE luciferase was used to test activation of PPAR isotypes in bovine cells [62, 68]. In one study, however, only activation of PPARβ/δ was assessed and no LCFA were tested. In another study the activation of PPARα by free LCFA or oleic acid was demonstrated in bovine aortic endothelial cells [62]. So far the effect of LCFA on ruminant PPAR activity has been evaluated primarily in an indirect way through measuring changes in expression of target genes after addition of specific LCFA. This model has limitations, one being the capacity of LCFA to bind and activate additional transcription factors (TF). Besides PPARs, also Hepatic Nuclear Factor 4 (HNF4α), Liver X Receptor (LXR), and RXR can bind LCFA, as shown in human, mouse, and rat [118]; however, in those species the LXRβ and the RXRs appear to be weakly activated by natural LCFA while PPARα, PPARβ/δ, and PPARγ are strongly activated [119]. The greater sensitivity of PPAR compared with other TF provides some support for the use of target gene expression as a proxy for evaluating activation of PPARs by LCFA. Another limitation of the indirect approach is the inability to distinguish the activation between PPAR isotypes. Using the above indirect approach it was demonstrated that ruminant PPAR are activated by several physiologically relevant LCFA (Table 1).

The LCFA experiments in ruminants were mainly performed with MAC-T and MDBK cells and focused on PPARα and PPARγ [26, 28, 36, 61]. In both cell types the LCFA clearly induced expression of genes previously shown using specific agonists (Wy-14643 and rosiglitazone) to be PPARα and PPARγ target genes (see Table 2 and Section 7 for details). The potency of saturated was greater than unsaturated LCFA. In particular, in MDBK cells we observed weaker induction of target genes as the degree of unsaturation increased [28]. Above all it was observed that palmitate and stearate induced a very strong activation of transcription of PPARα and PPARγ target genes [26, 28]. Those data were suggestive of an evolutionary adaptation of the PPAR in ruminants to respond to saturated LCFA, which are the most abundant LCFA in the circulation of ruminants [120, 121] compared to monogastrics [122, 123] due to extensive ruminal hydrogenation of unsaturated LCFA. However, our studies suggested that the LCFA activated gene expression not only through PPAR isotypes but also other TF, probably the ones mentioned above, or even other unknown TF [28]. This point, as well as the role of coactivators and their relative abundance [76], deserves further investigation in order to select with greater confidence the most suitable mixture of LCFA for modulating metabolism in ruminants.

Because intracellular LCFA pools are a mixture of saturated and unsaturated LCFA, it is interesting that PPARγ (and maybe other PPAR isotypes) is capable of binding two LCFA simultaneously, at the least in monogastrics [124]. This suggests that there could exist a mechanism whereby the composition of LCFA in the cytosol dictates the “strength” of the response, that is, the ability to bind two LCFA simultaneously could allow PPARγ to give a graded response to the varying composition of the intracellular LCFA pool [124].

6.2.2. Glucose. Besides LCFA, it has been also reported that glucose binds and activates PPARα in mouse connecting glucose with lipid metabolism [125]. This has not been confirmed in ruminants; however, it has been shown that ruminant PPARβ/δ binds and is activated by glucose [68].
Specifically, it was demonstrated in bovine endothelial cells that when PPARβ/δ is activated by glucose, it downregulates glucose transport in order to prevent hyperglycemia.

6.2.3. Other Natural Agonists/Antagonists. As with nonruminants, PPARγ in bovine vascular endothelial and mammary cells is activated by PGJ2 [46, 69]. The PPARγ is inhibited and its expression decreased by the oxidative stress intermediate H₂O₂ in bovine endothelial cells [94, 126]. Nitric oxide appears to be an inhibitor because it decreased the expression of the PPARGCA, a known PPARγ target gene [94]. This compound decreased the expression of PPARGCA during the first 12 h after treatment but increased the expression of the same gene in the longer term (> 24 h) [127]. The increase in expression of PPARGCA was demonstrated to be crucial for the mechanism of protection from oxidative stress [127]. In bovine articular chondrocytes, the presence of oxidized LDL increased expression of vascular endothelial growth factor (VEGF) through PPARγ [128].

The activation of PPARα by Wy-14643 resulted in a general increase in lipid metabolism-related genes including several involved in lipid synthesis, such as lipin 1 (LPIN1) and sterol regulatory element binding transcription factor 1 (SREBF1) [28]. Interestingly, expression of both genes was not induced in a previous study using the same model [61]. The only difference between the two studies was the addition of insulin in the latter [28]. In support of a potentially important role of insulin for PPAR activation, in a recent study with MDBK, we observed a faster response in expression of PPARα target genes after addition of insulin [61]. Therefore, insulin in bovine seems essential for PPAR activation but may be more crucial for some genes (e.g., LPIN1 and SREBF1 versus carnitine palmitoyltransferase 1A (CPTIA)) [28, 61].

The increased expression of SREBF1 with Wy-14643 in the MDBK study might also be due to the activation of PPARγ because we observed that activation of PPARγ with rosiglitazone increased expression of SREBF1 in MAC-T cells [26]. The activation of PPARγ in MAC-T cells appeared to be robust [26]; however, the use of 10 μM TZD for 12 h in MDBK cells did not affect expression of any gene tested using microarray technology, suggesting that activity of PPARγ in MDBK is extremely low or inexistent (Bionaz et al. unpublished data). This observation is intriguing considering that overall expression of PPARγ in MDBK is relatively high compared with other tissues/cells (Figure I(a)), and higher than PPARα (Figure I(b)). Furthermore, the response to PPARα agonists is consistently high in those cells [28]. Therefore, it cannot be excluded that the increase in expression of SREBF1 after addition of Wy-14643 was due exclusively to PPARα activation.

7. PPAR Isotype Target Genes in Ruminants

In several of our studies, the overall response of PPARα and PPARγ in bovine cells was strong and consistent [26, 28, 61, 129]. Those studies allowed uncovering several bovine-specific PPARα target genes (Table 2), and several were already established as PPARα targets in other species. Among bovine-specific PPARα target genes, the osteopontin (SPP1) gene had a large increase in expression after Wy-14643 treatment in bovine kidney cells [28] contrary to what has been observed in human and mouse [130, 131]. Between bovine, human, and mouse, only 67% of the putative PPARα target genes tested responded in a similar fashion, suggesting a species-specific response of PPAR [28].

## Table 1: Activation of PPAR isotypes in ruminants by main long-chain fatty acids or glucose.

| LCFA/glucose | Effect on PPAR isotype | Method$ | References |
|--------------|-----------------------|---------|------------|
| 16:0         | +++                   | n/a     | Indirect [26, 28, 61] |
| 18:0         | +++                   | n/a     | Indirect [26, 28] |
| c9:18:1      | ++                    | n/a     | Indirect [26, 28, 62] |
| t10:18:1     | n/a*                  | n/a     | Indirect [26] |
| 20:0         | ++                    | n/a     | Indirect [26, 28] |
| 20:4n-6      | ++                   | n/a     | Indirect [68, 77] |
| 20:5n-3      | ++                   | n/a     | Indirect [26, 28] |
| 22:6n-3      | +                    | n/a     | Indirect [26, 28] |
| Glucose      | No                    | n/a     | Luciferase [68] |

$ +/+ : strong agonist; ++: agonist; +: weak agonist; ±: mixture between agonist and antagonist.

$The 12-HETE, a metabolite of the 20:4n-6 is the actual agonist.

$Indirect: the effect on PPAR isotype target genes was uncovered by the use of specific PPAR synthetic agonists; luciferase: the use of the PPRE-luciferase construct to test activation of PPAR by agonists.

$A mixture (ca. 50% each) of the t10,c12- and c9,t11-conjugated 18:2

$Not available.
| Gene     | HUGO gene name                        | Tissue/cells | PPAR           | Reference |
|----------|---------------------------------------|--------------|----------------|-----------|
| **Fatty acid import and activation** |                        |              |                |           |
| ACSL1    | Acyl-CoA synthetase long-chain family member 1 | MDBK, Liver | PPARα          | [28, 61]  |
| ACSL3    | Acyl-CoA synthetase long-chain family member 3 | MDBK         | PPARα          | [28]      |
| CD36     | Thrombospondin receptor                | BAEC, MAC-T, Muscle | PPARγ          | [79, 26]  |
| FABP4    | Fatty acid binding protein 4           | MDBK, MAC-T, sP.adipose, bS.adipose | PPARγ          | [28, 80]  |
| **Fatty acid synthesis** |                        |              |                |           |
| ACACA    | Acetyl-CoA carboxylase alpha            | MAC-T, MAC-T | PPARγ          | [26, 82]  |
| FASN     | Fatty acid synthase                    | bS. adipose, bS. adipose | PPARγ          | [82]      |
| INSIG1   | Insulin induced gene 1                 | MAC-T | PPARγ          | [26]      |
| SCD      | Stearoyl-CoA desaturase (delta-9-desaturase) | MDBK         | PPARα          | [28]      |
| SREBF1   | Sterol regulatory element binding factor 1 | MAC-T, MAC-T, MDBK | PPARγ          | [26, 28]  |
| **Fatty acid oxidation** |                        |              |                |           |
| ACADVL   | Acyl-CoA dehydrogenase, very long chain | MDBK, Liver | PPARα          | [61]      |
| ACOX1    | Acyl-coenzyme A oxidase 1              | MDBK         | PPARα          | [61]      |
| CPT1A    | Carnitine palmitoyltransferase 1A (liver) | MDBK         | PPARα          | [28, 36, 61] |
| CPT2     | Carnitine palmitoyltransferase 2       | PAEC         | PPARγ          | [85]      |
| CRAT     | Carnitine O-acetyltransferase          | PAEC         | PPARγ          | [85]      |
| CYP4A11  | Cytochrome P450, family 4, subfam. A, polypeptide 11 | Liver | PPARα          | [78]      |
| **Triacylglycerol synthesis** |                        |              |                |           |
| AGPAT6   | 1-Acylglycerol-3-phosphate O-acyltransferase 6 | MAC-T | PPARγ          | [26]      |
| DGAT1    | Diacylglycerol O-acyltransferase 1      | MAC-T | PPARγ          | [26]      |
| LPIN1    | Lipin 1                                | MAC-T | PPARγ          | [26]      |
| LPIN3    | Lipin 3                                | MDBK         | PPARα          | [28, 78]  |
| **Cholesterol synthesis** |                        |              |                |           |
| HMGCR    | 3-Hydroxy-3-methylglutaryl-CoA reductase | MDBK | PPARα          | [28]      |
| Gene       | HUGO gene name                        | Tissue/cells | PPAR<sup>2</sup> | Reference |
|------------|---------------------------------------|--------------|-----------------|-----------|
| SREBF2     | Sterol regulatory element binding transcription factor 2 | MAC-T        | PPARγ          | [26]      |
| ANGPTL4    | Angiopoietin-like 4                   | Liver        | PPARα          | [56, 86]** |
| FGF21      | Fibroblast growth factor 21           | Liver        | PPARα          | [86]<sup>1</sup>++ |
| EDN1       | Endothelin 1                          | BAEC         | PPARα          |           |
| LEP        | Leptin                                | bS. adipose  | PPARγ          | [84]      |
| NOS3       | Nitric oxide synthase 3 (endothelial cell) | BAEC        | PPARα          | [88]      |
| PTGS2      | Prostaglandin-endoperoxide synthase 2 | pBESC, MAC-T | PPARγ, PPARα   |           |
| SPP1       | Osteopontin                           | MDBK         | PPARα          | [28]      |
| VEGF       | Vascular endothelial growth factor     | BAEC         | PPARγ          | [89]      |

### Signaling molecules

| Gene       | HUGO gene name                        | Tissue/cells | PPAR<sup>2</sup> | Reference |
|------------|---------------------------------------|--------------|-----------------|-----------|
| CDKN2A     | Cyclin-dependent kinase inhibitor 2A   | BAEC         | PPARγ          | [90]      |
| GAPDH<sup>6</sup> | Glyceraldehyde-3-phosphate-dehydrogenase | s. ASC       | PPARγ, PPARβ/δ |           |
| OLR1       | Oxidized low density lipoprotein receptor 1 | BAEC        | PPARα          | [92]      |
| PC         | Pyruvate carboxylase                   | Hepatoma<sup>*</sup> | PPARα          | [93]      |
| SLC2A1     | Solute carrier family 2, member 1     | BAEC         | PPARβ/δ         | [68]      |
| TERF2      | Telomeric repeat binding factor 2      | BAEC         | PPARγ          | [90]      |

### Other functions

| Gene       | HUGO gene name                        | Tissue/cells | PPAR<sup>2</sup> | Reference |
|------------|---------------------------------------|--------------|-----------------|-----------|

| PPAR activation-related functions |
|-----------------------------------|

<sup>1</sup>Acronyms: BAEC: Bovine Aortic Endothelial Cells; BEND: Bovine Endometrial Cells; bEPC: bovine renal Epithelial cells; BRCP: Bovine Retinal Capillary Pericytes; bS. Adipose: bovine subcutaneous adipose; pBESC: primary (16-day cycle) bovine endometrial stromal cells; MDBK: Madin-Darby Kidney Cell Line; PAEC: ovine pulmonary arterial endothelial cells; sP.adipose: sheep perirenal adipose; s. ASC: sheep adipose stem cells; s. Muscle: sheep muscle.

<sup>2</sup>The PPAR activated by the treatment with a different effect on expression of the target gene (↓ induction; ↑ inhibition; ⇔ no change).

<sup>*</sup>Rat hepatoma was transfected with bovine PC promoter region.

<sup>**</sup>The increase in expression was with P < 0.10 but P > 0.05.

<sup>++</sup>The activity and not the mRNA expression of GAPDH was measured.

<sup>1</sup>Inferred based on the high correlation of expression between PPARG and FABP4.

<sup>1</sup>Inferred based on hepatic mRNA expression in studies with peripartal cows and undernutrition ketosis [56, 86, 95] (see main body of the paper for details).
the same tissue/cell. This is not surprising considering that several conditions can change the activity of PPAR isotypes, for example, the addition of insulin mentioned above. However, another important factor that might explain the different response between cell types or experiments is the abundance and activity of coregulators [132].

Some unexpected findings can be seen from data reported in Table 2. For instance, the well-established PPARγ target in nonruminants FABP4 [133] does not appear to be affected by activation of PPARγ in ruminants, at least in MAC-T cells [26] but was induced by activation of PPARα in MDBK cells [28]. In a study performed in intramuscular fat of growing beef steers, it was observed a very high correlation between the expression of FABP4 and PPARG suggesting a dependence of FABP4 expression from PPARγ [80]. Contrary to such observation, in a recent study in pregnant overfed versus normal fed energy dairy cows, no change in expression of FABP4 was observed but a greater expression of PPARG in subcutaneous adipose [134]. As for others, this unexpected finding in ruminant cells needs to be further confirmed; however, it underscores the limitation of using nonruminant data in the context of bovine.

Another cause of discrepancy might be due to methodological differences between studies, such as the methods used to perform qPCR. Most of the target genes reported in Table 2 were uncovered using qPCR. This technique relies on the identification and use of proper internal control genes [135], which is seldom conducted. As a result, some of the data generated by qPCR may lack accuracy prompting for a more routine application of all quality controls. In order to overcome several of the critical limitations often found in work reporting qPCR data, the minimum information for Publication of quantitative Real-Time PCR experiments (MIQE) [136] was created. Adherence to those guidelines will help standardize protocols, thus, enhancing data reliability. The use of such guidelines should be required by a greater number of scientific journals.

8. Effect of NEFA, Energy in the Diet and Fetal Reprogramming, on PPAR Isotypes

8.1. NEFA. The provision of LCFA to mammalian cells is from NEFA originating from adipose tissue lipolysis or from lipolysis of chylomicron or very low density lipoproteins (VLDL). The activation of bovine PPARα by NEFA was demonstrated recently in bovine aortic endothelial cells, where it was observed that PPARα activity was increased by release of free FA from VLDL via the action of lipoprotein lipase (LPL) [62]. In the same experiment it was demonstrated that ~10 μM of released NEFA in the media activated PPARα by ca. 80% compared to 10 μM of the specific PPARα agonist Wy-14643. A similar concentration of oleic acid alone activated bovine PPARα up to ca. 60% compared to Wy-14643. The activation of PPARα was due to free FA uptake by the cells as demonstrated by the strong linear relationship between activation of PPARα and uptake of LCFA [62]. In addition, the activation of PPARα was proportionally inhibited by amount of albumin in the medium [62]. The results from the same study also indicated that the free FA released by the LPL, and not the circulating plasma FA (i.e., albumin-bounded NEFA), are the ones able to activate PPARα. The authors explained this by proposing that the high concentration of LCFA needed for PPARα activation can be achieved only by local release by lipase of LCFA from lipoproteins. Those results need to be further confirmed because of their important implications in the fine-tune activation of PPARs by dietary approaches.

The activation of PPAR by FA entering the cells via the unsaturable process is supported by the fact that endogenous activation of PPARα in vivo seems to occur mainly with high levels of LCFA that occur under fasting conditions in nonruminants [137]. In addition, we have shown in bovine cells that the expression of PPARα target genes is faster and more pronounced if cells are treated with free palmitate instead of palmitate bound to albumin [61].

The above-mentioned findings are relevant to dairy cattle soon after parturition when the hypoinsulinemia due to negative energy balance (NEB) reduces insulin sensitivity, and uncoupling of the growth hormone-insulin-like growth factor-1 axis results in substantial increase in NEFA, a mixture of LCFA whose composition can be partly altered through dietary approaches.

Evidence of increased activation and/or expression of PPARs due to the surge in NEFA has been reported in cattle. In particular, it has been observed that during the transition from pregnancy to lactation, characterized by a large surge of plasma NEFA, there is upregulation in expression of several PPAR target genes (e.g., CPT1A, ACOXI, see Table 2) in liver of dairy cattle, with a concomitant increase in expression of PPARα [57, 138, 139]; however, not all the studies found this to be a consistent response [140].

8.2. Nutrient Restriction. Nutrient restriction in dairy cows, causing a concomitant increase in blood NEFA, enhanced expression of PPARα and PPARδ in liver [56] and protein expression of PPARγ in the hypothalamus [141]. Similarly, a 60-day period of body weight loss in beef cows was associated with greater expression of all three PPAR isotypes in biceps femoris muscle and several PPAR target genes, compared with cows that maintained body weight [142]. Overall, the data indicated that the NEB, with a consequent increase in NEFA, appears to induce expression and activation of all PPAR isotypes, but particularly of PPARα and PPARδ.

8.3. High Dietary Energy. High dietary energy during pregnancy in dairy cows was associated with lower expression of liver PPARα early post-partum [143]. High dietary energy in weaned Angus steers, but not Angus × Simmental steers, was associated with lower expression of PPARδ in Longissimus lumborum muscle [47].

8.4. Dietary Energy and Fetal Reprogramming. In ovine, nutrient restriction in ewes during early pregnancy (between 28 to 80 days gestation) increased expression of PPARα in the adipose tissue of the near-term fetus [144]. However, this was true only if the ewes were fed to requirements after this period of pregnancy; the adipose tissue of fetuses from ewes fed ad libitum from 80 days of pregnancy to term had lower PPARα
expression [144]. The above data clearly indicate that level of energy in the diet of the mother has a strong effect on the fetal transcriptome, that is, fetal reprogramming.

The fetal reprogramming of PPAR due to dietary energy level also has been observed when animals were overfed energy during pregnancy, such that fetuses of those dams had greater expression of PPAR and other lipogenic genes [145]. In contrast, either control or a high-energy diet in the periconception period or during pregnancy did not affect expression of PPAR in perirenal, omental, or subcutaneous adipose tissue of 4-month-old lambs [146]. Interestingly, intrafetal administration of a PPAR agonist, rosiglitazone, increased expression of LPL, a putative PPAR target gene, in perirenal adipose tissue of sheep fetuses [81]. No effect was observed for PPAR itself. In contrast, in the same study rosiglitazone increased expression of PPARA in liver.

9. Biological Effects of PPAR Activation in Ruminants

Most of the biological roles of PPAR uncovered in monogastrics can likely be extrapolated to ruminants; however, before those roles can be considered established also in ruminants, experiments need to be performed. Due to the modest amount of research performed to date, the biological significance of PPAR isotypes in ruminants is not well established. In this section we provide an overview of the existence of conserved roles between monogastrics and ruminants, the biological roles of PPAR uncovered in monogastrics [2], and the biological roles of PPARA in controlling lipid metabolism of PPAR in liver.

9.1. Control of Adipogenesis and Lipid Metabolism

9.1.1. PPARy. As for nonruminants [21], PPARy plays a pivotal role in adipogenesis in ovine and bovine [91,147], and in dairy cows its expression is high in adipose tissue (Figure 1) and appears to control lipogenesis by acutely responding to energy level in the diet [82, 134, 148, 149]. The importance of PPARy in adipogenesis has been highlighted also by the identification of this as one of the candidate genes related to bovine marbling [150]. Besides lipogenesis, PPARy might also play a role in LCFA oxidation as recently observed in lamb pulmonary arterial endothelial cells [85]. In that study it was demonstrated that PPARy controls the expression of carnitine palmitoyltransferase 2 (CPT2) and carnitine O-acetyltransferase (CRAT), both genes involved in the entry of LCFA into the mitochondria, while it controls the translation of CPT1A but not its expression [85].

9.1.2. PPARα. The activation of goat PPARα in vivo increased fatty acid oxidation in liver [108]. The oral administration of Wy-14643 increased palmitate oxidation in liver of dairy calves with a concomitant increase in expression of several genes known to be PPARα targets (see Table 2) involved in FA oxidation in nonruminants [78]. Therefore, it is apparent that the activation of PPARα in ruminants controls catabolism of fatty acids. Other pieces of evidence supporting that conclusion include the fact that FA catabolism in mitochondria and peroxisome increases during the transition from pregnancy into lactation [151]. This appears to be consequence of the large surge of NEFA and the concomitant increase in expression of few key genes rather than an increase of overall pathway flux [152]. However, the expression of PPARA in liver of dairy cattle increases from pregnancy to early post-partum [57, 138]. In the same time, several PPARα target genes involved in lipid metabolism have a similar increase in expression as PPARA in liver during the transition from pregnancy to lactation; those include ACOX1 and acyl-coenzyme A dehydrogenase, medium chain (ACADM) [57, 138]. Finally, the use of Wy-14643 in MDBK cells increased expression of several genes involved in lipid catabolism [28, 61] (Supplementary Table 1). One of those key genes is the well-known PPARα target CPTIA [57, 138].

9.1.3. PPARβ/δ. Compared with PPARα and PPARy, the role on lipid metabolism of PPARβ/δ activation in ruminants is less clear. The PPARβ/δ was shown to have a role in adipogenesis in sheep because its activation increased activity of GAPDH [91]. An involvement of PPARβ/δ in adipogenesis also was reported by several experiments performed in monogastrics [2]. However, a contrasting role of PPARy and PPARβ/δ was observed in primary bovine mammary cells, where several PPARy ligands reduced the expression of PPARβ/δ [69]. PPARα unarguably has a primary role in controlling fatty acid oxidation in rodents; however, PPARβ/δ also controls fatty acid oxidation in skeletal muscle, heart, and brown and white adipose tissue [2]. Several data indirectly suggest a similar role in ruminants. It was observed that during nutrient restriction [56] and during body weight loss in muscle of beef cows [142], both situations that enhance LCFA oxidation, there was a concomitant increase in expression of PPARA and PPARD.

In summary, the pivotal role of PPARα in controlling adipogenesis and lipogenesis in adipose tissue, which was clearly established in nonruminants, can also be considered established in ruminants. The control of fatty acid oxidation by PPARα in ruminants appears supported by the data published to date. The few data available also suggest a role for PPARβ/δ in lipid catabolism in ruminants.

9.2. Control of Milk Fat Synthesis by PPARy in Dairy Cattle.

Milk fat synthesis in dairy cows appears to be controlled at least in part by PPARy. This was originally suggested by the increase in expression of PPARG in mammary gland of dairy cows between pregnancy and lactation [33]. In the same study, a large increase in expression of a network of genes potentially involved in milk fat synthesis and for the most part putative PPARy target genes was observed. Based, on those data we then tested, and demonstrated, the hypothesis that PPARy controls expression of key genes involved in milk fat synthesis, including SREBF1 [26].

A pivotal role of milk fat synthesis regulation by SREBP1 has been originally proposed based on the consistent reduction of SREBF1 expression by t10,c12-CLA, a minor unsaturated FA produced during ruminal biohydrogenation of
In an *in vivo* experiment the activation of PPARγ pre-partum by TZD affected adipose tissue post-partum but, apparently in contrast to the above data, decreased milk fat production [109]. This result is not completely surprising considering that the TZD treatment was provided pre-partum when there is a large abundance of PPARγ in adipose tissue and a low abundance in mammary gland [33], whereas, when PPARγ is expected to increase in mammary gland due to the onset of lactation [33], the TZD was no longer supplemented and the amount of NEFA, which could have played a role in activating PPARγ, was decreased in cows treated with TZD [109]. In addition, the adipose tissue competes with mammary gland for lipogenic substrates, especially if the insulin sensitivity is high, as demonstrated by the reduced milk fat by injection of insulin in cows [156]. From this point of view it would be interesting to test the effect of TZD injection post-partum on milk fat synthesis in dairy cows.

Besides PPARγ and SREBP1, data from another laboratory suggested that LXR also plays a role in controlling *de novo* FA synthesis [157]. It is recognized that in order to demonstrate the central role of PPARγ, SREBP1, LXR, or their combination in controlling milk fat synthesis in dairy cows, there is need for more fundamental studies, for instance, via gene-specific knock-outs. Recently, two studies from the same laboratories [158, 159] used siRNA specific for *SREBF1* in order to define the role on controlling milk fat synthesis of this transcription factor. From the studies it was shown that basal transcription of genes involved in *de novo* FA synthesis in bovine mammary epithelium is partly under control of SREBP1. Some of the same genes were induced when LXR was activated using a specific agonist. Studies using siRNA specific for *PPARG* in bovine mammary cells are lacking. In the context of milk fat synthesis regulation, we deem more relevant the unbiased discovery of the role of LCFA in affecting the transcriptome by binding specific TF than demonstrating a more crucial role of one or another TF.

9.2.1. Is PPARγ Crucial for Milk Fat Synthesis Also in Mouse? Contrary to dairy cows [33], in mouse the mammary *PPARG* expression decreased between pregnancy to lactation [160], also after accounting for the large disappearance of adipose tissue [161]. In porcine mammary gland, the *PPARG* was not affected by lactation [162]. The expression of *PPARG* in mouse and pig mammary gland suggests that PPARγ likely does not control milk fat synthesis in monogastrics. In order to further study the role of PPARγ on milk fat synthesis in monogastrics, we have performed an *in vitro* experiment in mouse mammary epithelial cells (HC11; Figure 3). The experiment also was performed with the purpose of comparing the data previously generated with bovine mammary cells [26]. For this reason, the experiment was performed in HC11 with the same experimental design as the one previously performed in MAC-T cells [26]. Most of the treatments in HC11 were the same as in MAC-T cells with the exception of the PPARγ inhibitor GW9662.

As observed in MAC-T cells, the saturated LCFA palmitate increased expression of several lipogenic genes in HC11 but, differently than in MAC-T cells [26], the effect appeared to be PPARγ-independent due to the extremely low
expression and activity of PPARγ (Figure 3(a)). Those findings are intriguing because, together with the greater abundance of PPARA compared with PPARγ in MAC-T cells (Figure 1(b)), suggests that the observed increase in mammary lipogenic genes due to palmitate are via PPARα or other TF rather than PPARγ in immortalized mammary cells from cattle and mouse.

Contrary to what was observed in MAC-T cells [26] and in vivo in mouse mammary gland [163], the cis10,trans12-CLA failed to inhibit the expression of lipogenic genes in HC11 (Figure 3(a)). This observation is surprising considering that the Srebp1 expression is relatively high and with similar level in HC11 compared with MAC-T cells (Figure 3(b)). Only EPA decreased expression of few lipogenic genes in HC11; among

**Figure 3:** Effect of PPARγ activation on genes coding for proteins involved in milk fat synthesis in mouse mammary epithelial cells HC11. The experiment was performed with the purpose to test the effects of 50 μM of the PPARγ activator rosiglitazone, the PPARγ inhibitor GW9662, or 100 μM of several long-chain fatty acids (trans-10,cis 12-conjugated linoleic acid (CLA), eicosapentaenoic acid (EPA), or palmitate (16:0)) for 12 hours in HC11 cells and compare the data with results using the same experimental design (except the GW9662 treatment) in MAC-T cells [26]. All the procedures with few modifications were as previously described [26]. The RNA was extracted and qPCR performed for several genes known to be involved in milk fat synthesis and significantly upregulated by rosiglitazone in MAC-T cells and the same 3 internal control genes used [26]. In (a), the effect of treatments on HC11 cell is reported. For that experiment, the qPCR data were calculated as fold change relative to control and log2 transformed prior statistical analysis using Proc GLM of SAS with treatment as main effect and replicate as random. Dissimilar letters denote significant differences between treatments (P < 0.05). In (b), a comparison in mRNA abundance between measured genes in the control group of HC11 and MAC-T cells is presented. The relative mRNA abundance was calculated as previously described [26] but as fold difference relative to the geometric mean of the median Ct values of the 3 internal control genes instead as % relative abundance. The same analysis was performed for the MAC-T cells using data previously published [26]. The PPARγ was detectable only for few samples in HC11 cells and LPL was barely detectable in both HC11 and MAC-T cells.
those the SCD was downregulated by EPA also in MAC-T cells [26]. The relative abundance of genes measured in HC11 compared to MAC-T cells (Figure 3(b)) revealed that lipogenic gene expression is overall greater in HC11 than MAC-T, with exception of SCD that is more abundant in MAC-T cells. The PPAR had low expression in both cell lines but was virtually absent in HC11, while clearly detectable in MAC-T cells. This observation likely accounted for the fact that the PPAR agonist rosiglitazone and the inhibitor GW9662 had little effect on the expression of most genes in HC11 (Figure 3(a)). On the contrary, rosiglitazone increased the expression of all those genes in MAC-T cells [26].

The virtual absence of Ppar expression in HC11 (Figure 3(b)) together with the lack of decrease in expression of milk fat-related genes by CLA despite the large expression of SREBF1 seems to indicate a role of PPARγ, and more likely PPARγ-SREBP1 crosstalk, in translating the lipogenic inhibition, and particularly milk fat depression effect, of CLA (and likely EPA) usually observed in vivo. However, the data also point to a more complex nutrigenomics response to LCFA, likely involving additional TF besides SREBP1 and PPARγ.

Overall, the comparison between the mouse and the bovine mammary epithelial cell lines, with all the limitations of in vitro experiments, highlights a crucial difference between rodents and bovine in the genomic control of milk fat synthesis. The data clearly uncovered no roles for PPARγ in controlling milk fat synthesis in mouse. Those observations suggest caution when inferring physiological responses using data from a different species.

9.3. Control of Inflammatory Response. The activation of PPARγ, PPARα, and PPARβ/δ has anti-inflammatory effects in nonruminants [19, 164] and some data are available in ruminants suggesting a similar effect. The first demonstration that PPARγ might play an anti-inflammatory role in ruminants was carried out by a Japanese group by injecting for 9 days human recombinant TNFα plus TZD in dairy steers. They observed that the TZD treatment partially reversed the insulin resistance caused by TNFα [107]. The TZD effect was probably due to enhanced insulin signaling through PPARγ activation by also counteracting the effect of TNFα [165]. The anti-inflammatory effect of PPARγ in ruminants is elicited not only by counteracting the effect of TNFα, but also by reducing the production of this cytokine. This was demonstrated recently when treatment of bovine peripheral blood mononuclear cells with 100 µM of t10,c12-CLA or 10 µM of rosiglitazone attenuated the production of TNFα in vitro, with a stronger effect observed in cells treated with rosiglitazone [166].

In bovine primary mammary epithelial cells (bMEC), the activation of PPARγ by several agonists caused downregulation of several proinflammatory cytokines and increased expression of the chemokine CCL2 and TNFα [69]. In contrast, PGJ2 enhanced markedly the expression of both interleukin 8 (IL8) and chemokine (C-X-C motif) ligand 6 (CXCL6) and had no effect on other cytokines [69]. The same study also demonstrated that the generation of proinflammatory mediators in bMEC treated with lipopolysaccharide (LPS) can be modulated by synthetic PPARγ agonists. These findings support a role of PPARγ in mastitis resistance in dairy cows.

Some additional evidences support an anti-inflammatory role of PPAR in ruminants. The activation of PPARα has shown to limit leukocyte adhesion to the bovine endothelium [167]. The expression of PPARγ is reduced by intramammary infection with Escherichia coli [168] and PPAR signaling was evidently inhibited by intramammary infection with Streptococcus uberis [169]. The PPARγ and PPARα were also markedly downregulated in PMN soon after an inflammatory challenge; however, the expression of PPARδ increased markedly and was substantially more abundant than the other isotypes (Moyes et al. unpublished data). In contrast, the expression of PPARα and PPARγ in liver was not affected after intramammary treatment with Escherichia coli that induced a strong hepatic acute-phase reaction [170]; however, the most-impacted biological effect of the treatment was the reduction of lipid metabolism in the liver, particularly steroid synthesis and PPAR signaling [171]. The involvement of PPARβ/δ in the process of inflammation was recently underscored when an intramammary infusion of LPS led to marked upregulation of PPARδ and several proinflammatory genes in liver of dairy cows (e.g., TNF, NFκB) [172].

The potential role of PPAR isotypes on inflammation can also be inferred by the fact that the expression of the PPARα agonist ANGPTL4 (Table 2) increases markedly in response to inflammation not only in mouse liver [173] but also in bovine liver [172], and it has been proposed to serve as a positive acute phase protein (+APP) [173]. In that context, it is interesting that the expression of ANGPTL4 in adipose tissue increases markedly after parturition [134, 174], when the animals experience inflammatory-like conditions [175, 176]. Whether the upregulation of ANGPTL4 in adipose tissue after parturition denotes a response of the tissue to an inflammatory state remains to be determined; however, there is evidence of activation of immune-related pathways in adipose tissue soon after parturition [177].

9.4. Control of Intertissue Metabolic Adaptations during Changes in Nutritional Status and Physiological State. In monogastries, the PPARα targets angiopoietin-like 4 (ANGPTL4) [178] and fibroblast growth factor 21 (FGF21) [179, 180] have been identified as extra-hepatic signals (hepatokines) that play an important role in the coordination of tissue adaptations to fasting, undernutrition, and the transition into lactation in bovine [56, 95, 174]. Although direct proof of bovine PPARα activation as the trigger for the marked upregulation of liver FGF21 after parturition [86, 95] is not available, the fact that the upregulation of FGF21 was observed in animals with greater NEFA [86] is suggestive of FGF21 as a PPARα target in bovine. The link between PPARα activation and ANGPTL4 was previously discussed with data from cows suffering from undernutrition-driven ketosis [56] and was partly confirmed in vitro [28]. However, it was recently observed that hepatic ANGPTL4 and PPARδ (not PPARα) expression was upregulated during acute inflammation suggesting that in bovine this PPAR isotype also may regulate expression of the hepatokine [172].
molecular work would need to be carried out to clarify the validity of the observed relationship in terms of a functional link.

9.5. Other Roles. The use of PPARγ agonists decreases protein synthesis, but as demonstrated in bovine aortic endothelial cells, the mechanism appears to be independent of PPARγ [181]. As with nonruminants, the activation of PPARγ improves insulin sensitivity in dairy cows [82]. The activation of PPARα in the liver might also increase gluconeogenesis. This was inferred by the impaired gluconeogenesis in PPARα-null mice [182]; however, none of the main enzymes involved in gluconeogenesis are known to be PPARα targets in nonruminants [182]. One of the three known promoter regions of bovine pyruvate carboxylase (PC), a key enzyme in gluconeogenesis, was activated by Wy-14643 when transfected as a construct with firefly luciferase into rat hepatoma cells, indicating a potential control of expression of this enzyme by PPARα in ruminants [93]. However, the PC expression was not induced in MDBK cells treated with Wy-14643 or single LCFA [28]. The expression of PC was instead induced by cocktails of LCFA and particularly the concentration mimicking NEFA composition in dairy cows around parturition [183]. Therefore, an increase in gluconeogenesis via the activation of PPARα in ruminants still needs to be fully proven.

It has been demonstrated that the high-glucose-induced downregulation of the glucose transport system in bovine endothelial cells is mediated by PPARβ/δ [68]. It was shown that activation of PPARβ/δ inhibits the expression of the solute carrier family 2 member 1 (or facilitated glucose transporter GLUT1) coupled with an increase in expression of calreticulin, a protein that increases degradation of GLUT1 mRNA. The condition tested in the study (i.e., high glucose) has probably little implication for ruminants, considering the low level of circulating glucose compared with nonruminants (<4 mM in dairy cows [176] versus ca. 5 mM in human and >6 mM in mouse [184]). However, the control of glucose transport by PPARβ/δ could have implications in milk synthesis, considering that GLUT1 is one of the most important glucose transporters and its expression increases drastically during lactation in mammary tissue of dairy cows [185]. Thus, this PPAR isoform could play a pivotal role in provision of glucose for lactose synthesis. Interestingly, in mammary gland during lactation, PPARD is significantly downregulated [186] concomitant with an increase in expression of several glucose transporters, including GLUT1 [70, 185]. If the suggested link is real, this offers the opportunity of using PPARβ/δ antagonists in order to improve milk production.

More recently, it was demonstrated that PPARD transcript in rumen epithelium of neonatal dairy calves is substantially more abundant than PPARA (see also Figure I(b)), and its expression increased markedly from the milk-fed stage to the roughage-fed (i.e., high-structural fiber) stage at ~10 weeks of age [64]. The increase correlated with greater mass of the rumen, which suggested a potential link between PPARβ/δ and mechanisms driving ruminal epithelial cell development and proliferation [64].

10. What Controls Abundance of PPAR in Tissues?

The sensitivity of various tissues to PPAR isotype-specific agonists is closely related with the abundance of the specific isotype and other essential factors such as the abundance of coactivators or corepressors, LCFA, and hormones [76, 187–189]. As for nonruminants, the abundance of various isotypes in tissues appears to be directly related with the specific function they perform; for example, PPARγ abundance is relatively high in lipogenic tissues while PPARα is relatively high in tissues with elevated FA catabolic capacity (see Figure I(a)). Besides tissue-specific distribution, other factors can control the abundance of PPAR isotypes in tissues.

Among factors controlling PPAR isotypes expression in ruminants (Supplementary Table 2), it is evident that several lipid molecules, some nutritionally relevant such as LCFA and retinoids, and propionate (likely indirectly via glucose and insulin) can affect expression of PPAR isotypes, with a different sensitivity based on tissue type. The expression of ruminant PPAR isotypes is also affected by physiological status, level of energy in the diet, mechanical cues (e.g., laminar flow, mechanical load), oxygen and peroxide levels, hormones, and other growth factors (Supplementary Table 2). In addition, data from several groups also suggest that the activation of PPARγ increases expression of its own gene and, in the case of sheep, also the expression of PPARA (Supplementary Table 2). Interestingly, in bovine mammary epithelial cells several PPARγ agonists decreased the expression of PPARD, with one case (ciglitazone) in which PPARG also was downregulated [69].

Overall the data presented in Supplementary Table 2 suggest that it is possible to increase or decrease the abundance, hence the sensitivity, of PPAR isotypes in ruminant tissues. Among the factors affecting the PPAR isotype expression, the more interesting from a nutrigenomics point of view are the LCFA and the level of dietary energy because they can be easily manipulated.

11. PPAR Isoype Activation during the Peripartal Period in Dairy Cattle: A Hypothesis

11.1. The Peripartal Condition. The transition from pregnancy into lactation (also called simply “transition period”) is one of the most stressful stages of the life of dairy cattle [190]. Physiologically, the transition period is a complex phenomenon intertwining various metabolic activities (e.g., lipid, glucose, protein) and functions (e.g., inflammatory response) of several organs and tissues (e.g., adipose tissue, mammary, liver, uterus, and immune system) [152, 190]. A key feature of the transition period from a metabolic and health standpoint is the increase in plasma of NEFA and ketone bodies (KB), both of which can be toxic above certain thresholds, and by a general decrease in both insulin sensitivity (except for the mammary gland) and blood insulin concentration [191]. The transition period is also characterized by inflammatory-like conditions as consequence of
the release of proinflammatory cytokines, which along with NEFA affects directly liver functionality leading to poor performance [192].

The metabolic load placed on the liver of periparturient cows is exacerbated by this inflammatory-like conditions and also by the decrease in feed intake and the ensuing NEB, which often occurs as early as 10 days prior to parturition (reviewed in [193]). All of the above increase the risk of dairy cattle for developing metabolic disorders such as fatty liver [194] and ketosis [195, 196], but more importantly these disorders are tightly connected with other typical peripartal diseases [197]. Therefore, a smooth transition period is an important target in order to optimize performance and overall welfare of dairy cows. Interestingly, most of the above-described conditions (e.g., high NEFA, insulin insensitivity, fatty liver, inflammatory-like conditions) with the exception of the NEB are common to the metabolic syndrome that afflicts human [198].

11.2. PPAR Isotype Activation to Help Transition Dairy Cattle. It has been proposed previously that the PPAR isotypes are ideal targets for the prevention and cure of the metabolic syndrome in humans [199]. The use of PPARγ agonists is a clinical approach currently in practice to treat insulin resistance, one of the main problems related with the metabolic syndrome [22, 200]. Similarly, it was proposed earlier that PPAR isotypes play a pivotal role in the physiological adaptation of dairy cattle to the transition period [36, 201]. It was proposed that fine-tuning the activity of PPARα and PPARγ, in particular, by nutritional approaches at specific time/s during the transition period might be a way to prevent and/or help the cows overcome metabolic disorders. Among nutritional approaches in order to affect PPARs, the saturated LCFA appear to be the most promising based on in vitro data (see above and [28]). The effects of saturated LCFA on PPARs activation and the consequent improvement of lipid metabolism appear to be supported by recent in vivo data [202]. In that study it was observed that the adaptations in lipid metabolism in dairy cows fed high-saturated fat compared with a low-fat control diet or a high-linseed diet (high in unsaturated LCFA) for up to 5 weeks pre-partum was better.

In Figure 4 a qualitative hypothetical model describing the potential role of PPAR isotypes in transition dairy cows is depicted. That model rests on the well-established fact that the liver, adipose, rumen, skeletal muscle, immune system, and mammary gland play a crucial role in the adaptations leading to the onset of lactation. Other organs such as uterus, kidney, and pancreas are also crucial in this context but less is known about their molecular adaptations to lactation. In particular, data partly reviewed above strongly support a pivotal role of PPAR isotypes in the regulation of fertility and pregnancy; however, the overall effect of PPAR isotypes activation on fertility is not fully clear. In addition, the PPAR isotypes likely play a more important role before pregnancy compared with early lactation, when the cows are not yet cycling. Once the role of PPAR isotypes is better defined for the reproductive organs, it can become an important component of the overall model proposed.

Dairy cattle during the transition from pregnancy into lactation experience a multitiered set of adaptations aimed at allowing the mammary gland to begin and maintain lactogenesis. From a physiological perspective, the inherently low capacity of animals to consume enough dietary energy and the detrimental inflammatory-like conditions due to release of proinflammatory cytokines lead to the marked release of LCFA into the bloodstream from the adipose tissue. Those LCFA are mostly metabolized by the liver. A greater level of dietary energy in the form of nonstructural carbohydrate provided to the animal early postpartum can partly alleviate the negative shortfall in energy status; such approach would enhance production of short-chain fatty acids (SCFA), of which propionate metabolism via gluconeogenesis could serve as a trigger for greater insulin secretion [193]. The latter has been shown to promote rumen epithelial cell proliferation and might work in concert with PPARs to coordinate metabolism and development of these cells [64]. During the peripartal period, proinflammatory cytokines are released and induce the liver to produce +APP [176], taking away hepatic resources for normal liver functions (e.g., glucose synthesis, lipid metabolism, and ureagenesis) [175, 176, 197]. This condition effectively exacerbates the tissue’s capacity to coordinate appropriately metabolism of lipid and to provide the required glucose to mammary gland for milk synthesis. The marked NEFA concentration is only partly oxidized by liver with the rest accumulating as TAG. The TAG are then packed into VLDL for release into the bloodstream, but at a lower rate relative to monogastics [203]. An excessive accumulation of TAG can have detrimental effects on liver function [194].

We propose that the increased abundance pre-partum of PPAR isotypes and the timely and isotype-specific activation pre- or post-partum might be beneficial in preparing and allowing the animal to face the above-described conditions favoring a smooth transition into lactation. In particular the following:

(i) the greater abundance and activation of PPARγ pre-partum in adipose tissue can prevent the large NEFA surge due partly to an increase in insulin sensitivity, leading to reduced lipid overload on the liver with a consequent reduction of fatty liver, ketone body production, and any potential satiety effects (as consequence of high FA oxidation) [204]. The activation of PPARγ postpartum in mammary gland can allow to increase or to maintain the amount of milk fat in the early stages of lactation when NEFA provide endogenous LCFA for the mammary gland. Several pieces of evidence support such expected effects in adipose tissue [82, 109] and mammary tissue [26, 33, 186];

(ii) the greater abundance and activation of PPARα in liver and skeletal muscle in postpartum relative to prepartum can increase oxidation of NEFA leading to a lower accumulation of lipid in the liver. The greater oxidation capacity of liver (i.e., increase ketone body synthesis per unit of NEFA oxidized) would help prevent any substantial alteration in the production of
ketone bodies due to the systemic decrease of NEFA as a consequence of PPARγ activation. The activation of PPARα in liver might also increase gluconeogenesis rate, an essential process in ruminants particularly for milk synthesis. Another expected response would be increased VLDL synthesis and secretion by preventing the negative effect of the acute-phase reaction as a consequence of inflammatory-like conditions on apolipoproteins and other molecules involved in VLDL synthesis and TAG export. This suggestion is based on several pieces of evidence such as the observed negative association between apolipoprotein B100 or other VLDL components with inflammatory-like conditions in ruminants [170, 175, 176, 194, 205, 206];

(iii) the activation of PPARα, PPARγ, and particularly PPARβ/δ in immune (e.g., neutrophils, macrophages) and endothelial cells might contribute to a reduction of the NEFA surge induced by proinflammatory cytokines [207] and also increase insulin sensitivity and prevent the negative effect of acute phase reaction on liver functionality [166, 176].
The hypothetical model for fine-tuning PPAR isotypes for prevention of metabolic disorders in transition dairy cows we propose (Figure 4) is indirectly supported by several in vivo and in vitro studies, but a number of major details remain to be understood. One of the most important pertains to the effects of LCFA on PPAR isotype activation and, particularly, on how they could be used to target activation of a particular PPAR isotype at a particular stage of the transition period. More detailed and mechanistic studies with LCFA, for example, effective dose/s of individual LCFA or mixtures, are essential in order for these nutrients to have practical application as proposed in our model (Figure 4).

12. Conclusions and Perspectives

The understanding of physiological roles of PPAR isotypes in ruminants has advanced incrementally over the last decade. There is enough direct and indirect evidence compiled to conclude that these NR are biologically relevant in this species. The data suggest that the harmonized activity of PPAR isotypes across tissues is one facet of the multilayered set of control points that evolved to coordinate metabolism and physiological responses to endogenous and exogenous ligands. The transition from pregnancy to lactation provides the clearest example of the need for control points to ensure the nourishment of the neonate offspring, while ensuring the fitness of the dam. At a fundamental level, the functional activity of PPARs during this physiological state provides an elegant example of the multilayered concept because it links biological molecules with cellular responses that encompass several tissues. The model proposed based on the most-current knowledge is quite complex and its full evaluation obviously requires an integrative systems approach, that is, several tissues at various levels (e.g., cells and the underlying molecular networks) need to be studied simultaneously considering their dynamic adaptation.

If the model/hypotheses proposed hold, it would become the first “true” nutrigenomics application in dairy cattle biosciences. Benefits would go beyond simply establishing the physiological role of PPAR isotypes in ruminants. Improving the transition from pregnancy to lactation means to provide benefits for farmers, dairy cattle, and the society as a whole. We envisage farmers modulating the LCFA in the diets of dairy cattle to fine-tune metabolism through PPAR isotypes. Today, the continuous development of high-throughput technologies and bioinformatics tools permits the study of complex phenomena as is the case of the transition from pregnancy to lactation in dairy cattle [152]. This is an exciting era for expanding scientific knowledge and, apparently, the proper one for nutrigenomics.

Abbreviations

+APP: Positive acute phase proteins
ACOX1: Acyl-coenzyme A oxidase 1, palmitoyl
AF-1: Activation Function 1 or ligand-independent activation function
ANGPTL4: Angiopoietin-like 4
bMEC: Bovine primary mammary epithelial cells
CARLA: Coactivator-dependent receptor ligand assay
CCL2: Chemokine (C-C motif) ligand 2
CLA: Conjugated linoleic acid
CPT1A: Carnitine palmitoyltransferase 1A (liver)
CPT2: Carnitine palmitoyltransferase 2
CRAT: Carnitine O-acetyltransferase
CXCL6: Chemokine (C-X-C motif) ligand 6
EPA: Eicosapentaenoic acid
FA: Fatty acid(s)
FABP: Fatty acid binding protein
FGF21: Fibroblast growth factor 21
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
GLUT1: Glucose transporter 1
IL-8: Interleukin 8
INSIG1: Insulin induced genes 1
INSIG2: Insulin induced genes 2
KB: Ketone bodies
LBD: Ligand binding domain
LCFA: Long-chain fatty acid(s)
LCFA-CoA: LCFA-coenzyme A (i.e., activated LCFA)
LDL: Low density lipoprotein(s)
LPIN1: LiPIN 1 (coding for a phosphatidate phosphatase)
LPL: Lipoprotein lipase
LPS: Lipopolysaccharide
LXR: Liver X receptor
MAC-T: Bovine mammary alveolar cells transfected with simian virus-40 (SV-40) large T-antigen
MDBK: Madin-Darby bovine kidney cells
MIQE: Minimum Information for Publication of Quantitative Real-Time PCR Experiments
NEB: Negative energy balance
NEFA: Nonesterified fatty acid(s)
NFKB1: Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NR: Nuclear receptor(s)
PC: Pyruvate carboxylase
PG: Prostaglandin(s)
PGJ2: 15-Deoxy-delta-12,14-prostaglandin J2
PMN: Polymorphonuclear cells
PPAR: Peroxisome proliferator-activated receptor
PPRE: PPAR response element
PUFA: Polyunsaturated fatty acid(s)
qPCR: Quantitative Real-Time reverse transcription polymerase chain reaction
RXR: Retinol X receptor
SCAP: SREBP cleavage-activating protein
SCD: Stearoyl-CoA desaturase
SCFA: Short-chain fatty acid(s)
siRNA: Short interference RNA
SREBF1: Sterol regulatory element-binding transcription factor 1
TAG: Triacylglycerol(s)
TF: Transcription factor(s)
TNF: Tumor necrosis factor(s)
TZD: Thiazolidinedione
VEGF: Vascular endothelial growth factor
VLDL: Very low density lipoprotein(s)
Wy-14643: Pirinixic acid.

Acknowledgments

The mouse mammary epithelial cell line HC11 and protocols for cultivation and differentiation (i.e., induction into lactation [208, 209]) were gently provided by Dr. Daniel G. Peterson (Animal Science Department, California Polytechnic State University). The comments from Dr. Afshin Hosseini were also helpful in completing the final version of the paper. The authors thank the three anonymous reviewers for their suggestions that greatly helped to improve the paper.

References

[1] Z. Zhang, P. E. Burch, A. J. Cooney et al., “Genomic analysis of the nuclear receptor family: new insights into structure, regulation, and evolution from the rat genome,” Genome Research, vol. 14, no. 4, pp. 580–590, 2004.

[2] B. Desvergne, L. Michalik, and W. Wahli, “Transcriptional regulation of metabolism,” Physiological Reviews, vol. 86, no. 2, pp. 465–514, 2006.

[3] C. Dreyer, G. Krey, H. Keller, F. Givel, H. Helftenbein, and W. Wahli, “Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors,” Cell, vol. 68, no. 5, pp. 879–887, 1992.

[4] I. Issemann and S. Green, “Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators,” Nature, vol. 347, no. 6294, pp. 645–650, 1990.

[5] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, “From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions,” Progress in Lipid Research, vol. 45, no. 2, pp. 120–159, 2006.

[6] S. A. Klawer, B. M. Forman, B. Blumberg et al., “Differential expression and activation of a family of murine peroxisome proliferator-activated receptors,” Proceedings of the National Academy of Sciences of the United States of America, vol. 91, no. 15, pp. 7355–7359, 1994.

[7] O. Braissant, F. Foufelle, C. Scotto, M. Dauçà, and W. Wahli, “Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat,” Endocrinology, vol. 137, no. 1, pp. 354–366, 1996.

[8] P. S. Jones, R. Savory, P. Barratt et al., “Chromosomal localisation, inducibility, tissue-specific expression and strain differences in three murine peroxisome proliferator-activated-receptor genes,” European Journal of Biochemistry, vol. 233, no. 1, pp. 219–226, 1995.

[9] A. Vidal-Puig, M. Jimenez-Liñan, B. B. Lowell et al., “Regulation of PPAR γ gene expression by nutrition and obesity in rodents,” The Journal of Clinical Investigation, vol. 97, no. 11, pp. 2553–2561, 1996.

[10] T. Waku, T. Shiraki, T. Oyama et al., “Structural insight into PPARγ activation through covalent modification with endogenous fatty acids,” Journal of Molecular Biology, vol. 385, no. 1, pp. 188–199, 2009.

[11] M. Heinäniemi, J. O. Uski, T. Degenhardt, and C. Carlberg, “Meta-analysis of primary target genes of peroxisome proliferator-activated receptors,” Genome Biology, vol. 8, no. 7, article R147, 2007.

[12] B. M. Forman, J. Chen, and R. M. Evans, “The peroxisome proliferator-activated receptors: ligands and activators,” Annals of the New York Academy of Sciences, vol. 804, pp. 266–275, 1996.

[13] G. Krey, O. Braissant, F. L’Horset et al., “Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay,” Molecular Endocrinology, vol. 11, no. 6, pp. 779–791, 1997.

[14] H. E. Xu, M. H. Lambert, V. G. Montana et al., “Molecular recognition of fatty acids by peroxisome proliferator-activated receptors,” Molecular Cell, vol. 3, no. 3, pp. 397–403, 1999.

[15] A. Yessoufou and W. Wahli, “Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels,” Swiss Medical Weekly, vol. 140, article w13071, 2010.

[16] L. Michalik and W. Wahli, “Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease,” Biochimica et Biophysica Acta, vol. 1771, no. 8, pp. 991–998, 2007.

[17] P. Escher and W. Wahli, “Peroxisome proliferator-activated receptors: insight into multiple cellular functions,” Mutation Research, vol. 448, no. 2, pp. 121–138, 2000.

[18] B. Desvergne and W. Wahli, “Peroxisome proliferator-activated receptors: nuclear control of metabolism,” Endocrine Reviews, vol. 20, no. 5, pp. 649–688, 1999.

[19] T. Varga, Z. Czimmerer, and L. Nagy, “PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation,” Biochimica et Biophysica Acta, vol. 1812, no. 8, pp. 1007–1022, 2011.

[20] D. Bishop-Bailey, “PPARs and angiogenesis,” Biochemical Society Transactions, vol. 39, pp. 1601–1605, 2011.

[21] I. Takada, A. P. Kouzmenko, and S. Kato, “Wnt and PPAR-gamma signaling in osteoblastogenesis and adipogenesis,” Nature Reviews. Rheumatology, vol. 5, no. 8, pp. 442–447, 2009.

[22] J. M. Olefsky and A. R. Saltiel, “PPARγ and the treatment of insulin resistance,” Trends in Endocrinology and Metabolism, vol. 11, no. 9, pp. 362–368, 2000.

[23] W. Gillespie, N. Tyagi, and S. C. Tyagi, “Role of PPAR-γ in the regulation of adipocyte differentiation,” Biochemical Society Transactions, vol. 48, no. 2, pp. 73–81, 2011.

[24] P. Froment, F. Gizzard, D. Defever, B. Staels, J. Dupont, and P. Monget, “Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition,” Journal of Endocrinology, vol. 189, no. 2, pp. 199–209, 2006.

[25] J. Sonoda, I. Pei, and R. M. Evans, “Nuclear receptors: decoding metabolic disease,” FEBS Letters, vol. 582, no. 1, pp. 2–9, 2008.

[26] A. K. G. Kadegowda, M. Bionaz, L. S. Piperowa, R. A. Erdman, and J. J. Loor, “Peroxisome proliferator-activated receptor-γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents,” Journal of Dairy Science, vol. 92, no. 9, pp. 4276–4289, 2009.

[27] M. Bionaz and J. J. Loor, “ACSL1, APOA1, FABP3, LPIN1, and SLC27A6 are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation,” The Journal of Nutrition, vol. 138, no. 6, pp. 1094–1024, 2008.

[28] M. Bionaz, B. J. Thering, and J. J. Loor, “Fine metabolic regulation in ruminants via nutrient-gene interactions: saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-α activation,” The British Journal of Nutrition, vol. 107, pp. 179–191, 2012.
M. Yiallourides, S. P. Sebert, V. Wilson et al., "The differential expression of porcine peroxisome proliferator-activated receptors alpha and gamma gene in various chicken tissues," *Domestic Animal Endocrinology*, vol. 28, no. 1, pp. 105–110, 2005.

H. Sundvold, E. Grindflek, and S. Lien, "Tissue distribution of porcine peroxisome proliferator-activated receptor α: detection of an alternatively spliced mRNA," *Gene*, vol. 273, no. 1, pp. 105–113, 2001.

M. Cherfaoui, D. Durand, M. Bonnet et al., "Expression of enzymes and transcription factors involved in n-3 long chain PUFA biosynthesis in limousin bull tissues," *Lipids*, vol. 47, pp. 391–401, 2012.

M. Bionaz and J. J. Loor, "Gene networks driving bovine milk fat synthesis during the lactation cycle," *BMC Genomics*, vol. 9, article 366, 2008.

O. Mani, M. T. Sorensen, K. Sejrsen, R. M. Bruckmaier, and C. Albrecht, "Differential expression and localization of lipid transporters in the bovine mammary gland during the pregnancy-lactation cycle," *Journal of Dairy Science*, vol. 92, no. 8, pp. 3744–3756, 2009.

R. Sharma, M. Bionaz, A. K. G. Kadegowda et al., "Transcriptomics comparison of MacT cells and mammary tissue during pregnancy and lactation," *Journal of Dairy Science*, vol. 92, article M145, 2009.

M. Bionaz, C. R. Baumrucker, E. Shirk, J. P. Vanden Heuvel, E. Block, and G. A. Varga, "Short communication: characterization of Madin-Darby bovine kidney cell line for peroxisome proliferator-activated receptors: temporal response and sensitivity to fatty acids," *Journal of Dairy Science*, vol. 91, no. 7, pp. 2808–2813, 2008.

L. Bernard, M. B. Torbati, B. Graulet, C. Leroux, and Y. Chilliard, "Long-chain fatty acids differentially alter lipogenesis in bovine and caprine mammary slices," *Journal of Dairy Research*, vol. 80, no. 1, pp. 89–95, 2013.

M. Mohan, J. R. Malayer, R. D. Geisert, and G. L. Morgan, "Expression patterns of retinoid X receptors, retinaldehyde dehydrogenase, and peroxisome proliferator activated receptor gamma in bovine preattachment embryos," *Biology of Reproduction*, vol. 66, no. 3, pp. 692–700, 2002.

J. R. Miles, C. E. Farin, K. F. Rodriguez, J. E. Alexander, and P. W. Farin, "Angiogenesis and morphometry of bovine placenta in late gestation from embryos produced in vivo or in vitro," *Biology of Reproduction*, vol. 71, no. 6, pp. 1919–1926, 2004.

M. Yiallourides, S. P. Sebert, V. Wilson et al., "The differential effects of the timing of maternal nutrient restriction in the ovine placenta on glucocorticoid sensitivity, uncoupling protein 2, peroxisome proliferator-activated receptor-γ and cell proliferation," *Reproduction*, vol. 138, no. 3, pp. 601–608, 2009.

L. Cammas, P. Reinaud, N. Bordas, O. Dubois, G. Germain, and G. Chapigny, "Developmental regulation of prostacyclin synthase and prostacyclin receptors in the ovine uterus and conceptus during the peri-implantation period," *Reproduction*, vol. 131, no. 5, pp. 917–927, 2006.

B. Lührke, T. Viergutz, S. K. Shahi et al., "Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor y in lutein cells," *Journal of Endocrinology*, vol. 159, no. 3, pp. 429–439, 1998.

G. S. Coyne, D. A. Kenny, S. Childs, J. M. Sreenan, and S. M. Waters, "Dietary n-3 polyunsaturated fatty acids alter the expression of genes involved in prostaglandin biosynthesis in the bovine uterus," *Theriogenology*, vol. 70, no. 5, pp. 772–782, 2008.

L. A. MacLaren, A. Guzeloglu, F. Michel, and W. W. Thatcher, "Peroxisome proliferator-activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2 prostaglandin production," *Domestic Animal Endocrinology*, vol. 30, no. 3, pp. 155–169, 2006.

P. Froment, S. Fabre, J. Dupont et al., "Expression and functional role of porcine peroxisome proliferator-activated receptor-γ in ovarian folliculogenesis in the sheep," *Biology of Reproduction*, vol. 69, no. 5, pp. 1665–1674, 2003.

Y. Chiba, T. Ogita, K. Ando, and T. Fujita, "PPARγ ligands inhibit TNF-α-induced LOX-1 expression in cultured endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 286, no. 3, pp. 541–546, 2001.

D. E. Graugnard, P. Piantoni, M. Bionaz, L. L. Berger, D. B. Faulkner, and J. J. Loor, "Adipogenic and energy metabolism gene networks in Longissimus lumborum during rapid post-weaning growth in Angus and Angus × Simmental cattle fed high-starch or low-starch diets," *BMC Genomics*, vol. 10, article 142, 2009.

Z. G. Huang, L. Xiong, Z. S. Liu et al., "The developmental changes and effect on IMF content of H-FABP and PPARγ mRNA expression in sheep muscle," *Yi Chuan Xue Bao*, vol. 33, no. 6, pp. 507–514, 2006.

M. Taniguchi, L. L. Guan, B. Zhang, M. V. Dodson, E. Okine, and S. S. Moore, "Adipogenesis of bovine perimyascular preadipocytes," *Biochemical and Biophysical Research Communications*, vol. 366, no. 1, pp. 54–59, 2008.

J. Kim, Y. S. Oh, and S. H. Shinn, "Troglitazone reverses the inhibition of nitric oxide production by high glucose in cultured bovine retinal pericytes," *Experimental Eye Research*, vol. 81, no. 1, pp. 65–70, 2005.

P. W. Huff, M. Q. Ren, F. I. Lozeman, R. J. Weselake, and J. Wegner, "Expression of peroxisome proliferator-activated receptor (PPARγ) mRNA in adipose and muscle tissue of Holstein and Charolais cattle," *Canadian Journal of Animal Science*, vol. 84, no. 1, pp. 49–55, 2004.

I. Sharma, R. Monga, N. Singh, T. K. Datta, and D. Singh, "Ovary-specific novel peroxisome proliferator activated receptors-gamma transcripts in buffaloes," *Gene*, vol. 504, pp. 245–252, 2012.

H. Sundvold, I. Olsaker, L. Gomez-Raya, and S. Lien, "The gene encoding the peroxisome proliferator-activated receptor (PPARA) maps to chromosome 5 in cattle," *Animal Genetics*, vol. 28, no. 5, p. 374, 1997.

S. Kersten, M. Rakshandehroo, B. Knoch, and M. Müller, "Peroxisome proliferator-activated receptor alpha target genes," *PPAR Research*, vol. 2010, Article ID 612089, 2010.

M. Bionaz, J. K. Drackley, S. L. Rodriguez-Zas et al., "Uncovering adaptive hepatic gene networks due to prepartum plane of dietary energy and physiological state in periparturient Holstein cows," *Journal of Dairy Science*, vol. 90, pp. 678–687, 2007.

J. J. Loor, R. E. Everts, M. Bionaz et al., "Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows," *Physiological Genomics*, vol. 32, no. 1, pp. 105–116, 2007.
[57] J. J. Loor, H. M. Dann, R. E. Everts et al., “Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function,” *Physiological Genomics*, vol. 23, no. 2, pp. 217–226, 2005.

[58] J. J. Loor, H. M. Dann, N. A. Janovick-Guretzky et al., “Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling,” *Physiological Genomics*, vol. 27, no. 1, pp. 29–41, 2006.

[59] M. Bionaz, F. Samadi, M. J. D’Occhio, and J. J. Loor, “Altered liver gene expression and reproductive function in postpartum suckled beef cows on different planes of nutrition,” *Journal of Dairy Science*, vol. 90, pp. 649–649, 2007.

[60] K. T. Selberg, C. R. Staples, N. D. Luchini, and L. Badinga, “Dietary trans octadecenoic acids upregulate the liver gene encoding Peroxisome Proliferator-Activated Receptor-α in transition dairy cows,” *Journal of Dairy Research*, vol. 72, no. 1, pp. 107–114, 2005.

[61] B. J. Thering, M. Bionaz, and J. J. Loor, “Long-chain fatty acid effects on peroxisome proliferator-activated receptor-α-regulated genes in Madin-Darby bovine kidney cells: optimization of culture conditions using palmitate,” *Journal of Dairy Science*, vol. 92, no. 5, pp. 2027–2037, 2009.

[62] M. A. Ruby, B. Goldenson, G. Orasanu, T. P. Johnston, J. Plutzky, and R. M. Krauss, “VLDL hydrolysis by LPL activates PPAR-α through generation of unbound fatty acids,” *Journal of Lipid Research*, vol. 51, no. 8, pp. 2275–2281, 2010.

[63] S. M. Waters, J. P. Kelly, P. O’Boyle, A. P. Moloney, and D. A. Kenny, “Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Delta9-desaturase in muscle of beef cattle,” *Journal of Animal Science*, vol. 87, no. 1, pp. 244–252, 2009.

[64] A. Naeem, J. K. Drackley, J. Stamey, and J. J. Loor, “Role of metabolic and cellular proliferation genes in ruminal development in response to enhanced plane of nutrition in neonatal Holstein calves,” *Journal of Dairy Science*, vol. 95, pp. 1807–1820, 2012.

[65] S. A. Balaguier, R. A. Pershing, C. Rodriguez-Sallaberry, W. W. Thatcher, and L. Badinga, “Effects of bovine somatotropin on uterine genes related to the prostaglandin cascade in lactating dairy cows,” *Journal of Dairy Science*, vol. 88, no. 2, pp. 543–552, 2005.

[66] D. E. Graugnard, *Immunne Function, Gene Expression, Blood Indices and Performance in Transition Dairy Cows Affected by Diet and Inflammation*, University of Illinois, Urbana, Ill, USA, 2011.

[67] N. E. Buroker, X. H. Ning, and M. Portman, “Cardiac PPARα protein expression is constant as alternate nuclear receptors and PGC-1 coordinately increase during the postnatal metabolic transition,” *PPAR Research*, vol. 2008, Article ID 279531, 2008.

[68] Y. Riahi, Y. Sin-Malia, G. Cohen et al., “The natural protective mechanism against hyperglycemia in vascular endothelial cells: roles of the lipid peroxidation product 4-hydroxyxodecadienal and peroxisome proliferator-activated receptor-α,” *Diabetes*, vol. 59, no. 4, pp. 808–818, 2010.

[69] Y. S. Lutzow, C. Gray, and R. Tellam, “15-deoxy-A12,14-prostaglandin J2 induces chemokine expression, oxidative stress and microfilament reorganization in bovine mammary epithelial cells,” *Journal of Dairy Research*, vol. 75, no. 1, pp. 55–63, 2008.

[70] M. Bionaz, K. Periasamy, S. L. Rodriguez-Zas, W. L. Hurley, and J. J. Loor, “A novel dynamic impact approach (DIA) for functional analysis of time-course omics studies: validation using the bovine mammary transcriptome,” *PloS One*, vol. 7, article e32455, 2012.

[71] M. Arevalo-Turrubiarte, L. Gonzalez-Davalos, A. Yabuta et al., “Effect of 2,4-thiazolidinedione on limousin cattle growth and on muscle and adipose tissue metabolism,” *PPAR Research*, vol. 2012, Article ID 891841, 8 pages, 2012.

[72] X. S. Reveleo and M. R. Waldron, “Effects of in vitro insulin and 2,4-thiazolidinedione on the function of neutrophils harvested from blood of cows in different physiological states,” *Journal of Dairy Science*, vol. 93, no. 9, pp. 3990–4005, 2010.

[73] N. Marx, T. Bourcier, G. K. Sukhova, P. Libby, and J. Plutzky, “PPARγ activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARγ as a potential mediator in vascular disease,” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 3, pp. 546–551, 1999.

[74] C. Tudor, J. N. Feige, H. Pingali et al., “Association with coregulators is the major determinant governing peroxisome proliferator-activated receptor mobility in living cells,” *Journal of Biological Chemistry*, vol. 282, no. 7, pp. 4417–4426, 2007.

[75] V. Zoete, A. Grosdidier, and O. Michielin, “Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators,” *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 915–925, 2007.

[76] A. Bugge and S. Mandrup, “Molecular mechanisms and genome-wide aspects of PPAR subtype specific transactivation,” *PPAR Research*, vol. 2010, Article ID 169506, 2010.

[77] E. L. R. Sheldrick, K. Derecka, E. Marshall et al., “Peroxisome proliferator-activated receptors and the control of levels of prostaglandin-endoperoxide synthase 2 by arachidonic acid in the bovine uterus,” *Biochemical Journal*, vol. 406, no. 1, pp. 175–183, 2007.

[78] N. B. Litherland, M. Bionaz, R. L. Wallace, J. J. Loor, and J. K. Drackley, “Effects of the peroxisome proliferator-activated receptor-α agonists clofibrate and fish oil on hepatic fatty acid metabolism in weaned dairy calves,” *Journal of Dairy Science*, vol. 93, no. 6, pp. 2404–2418, 2010.

[79] Y. Liu, Y. Zhu, F. Rannou et al., “Laminar flow activates peroxisome proliferator-activated receptor-γ in vascular endothelial cells,” *Circulation*, vol. 110, no. 9, pp. 1128–1133, 2004.

[80] E. Albrecht, T. Gotoh, F. Ebara et al., “Cellular conditions for intramuscular fat deposition in Japanese Black and Holstein steers,” *Meat Science*, vol. 89, no. 1, pp. 13–20, 2011.

[81] B. S. Muhlhauser, J. L. Morrison, and I. C. McMillen, “Rosiglitazone increases the expression of peroxisome proliferator-activated receptor-γ target genes in adipose tissue, liver, and skeletal muscle in the sheep fetus in late gestation,” *Endocrinology*, vol. 150, no. 9, pp. 4287–4294, 2009.

[82] K. M. Schoenberg and T. R. Overton, “Effects of plane of nutrition and 2,4-thiazolidinedione on insulin responses and adipose tissue gene expression in dairy cattle during late gestation,” *Journal of Dairy Science*, vol. 94, no. 12, pp. 6021–6035, 2011.

[83] G. Invernizzi, A. K. G. Kadegowda, M. Bionaz et al., “Palmitate affects larger gene networks in MACT cells compared with trans-10,cis-12-CLA or PPAR-gamma activation via Rosiglitazone,” *Journal of Dairy Science*, vol. 92, article 321, 2009.

[84] K. M. Schoenberg, K. L. Perfield, J. K. Farney et al., “Effects of prepartum 2,4-thiazolidinedione on insulin sensitivity, plasma concentrations of tumor necrosis factor-alpha and leptin, and adipose tissue gene expression,” *Journal of Dairy Science*, vol. 94, no. 11, pp. 5523–5532, 2011.
[85] S. Sharma, X. Sun, R. Rafikov et al., “PPAR-gamma regulates carnitine homeostasis and mitochondrial function in a lamb model of increased pulmonary blood flow,” *PLoS One*, vol. 7, article e41555, 2012.

[86] M. J. Khan, D. E. Graugnard, and J. J. Loor, “Endocannabinoid and PPARα signaling gene network expression in liver of peripartal cows fed two levels of dietary energy prepartum,” *Journal of Dairy Science*, vol. 93, article 1124, 2020.

[87] P. Delerive, F. Martin-Nizard, G. Chinietti et al., “Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway,” *Circulation Research*, vol. 85, no. 5, pp. 394–402, 1999.

[88] Y. Wang, Y. Wang, Q. Yang et al., “Effects of bezafibrate on the expression of endothelial nitric oxide synthase gene and its mechanisms in cultured bovine endothelial cells,” *Atherosclerosis*, vol. 187, no. 2, pp. 265–273, 2006.

[89] D. H. Cho, Y. J. Choi, S. A. Jo, and I. Jo, “Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone: evidence for involvement of peroxisome proliferator-activated receptor (PPAR) y-dependent and PPARγ-independent signaling pathways,” *Journal of Biological Chemistry*, vol. 279, no. 4, pp. 2499–2506, 2004.

[90] C. Werner, C. Gensch, J. Pöss, J. Haendeler, M. Böhml, and U. Laufs, “Troglitazone activates aortic telomerase and prevents stress-induced endothelial apoptosis,” *Atherosclerosis*, vol. 216, no. 1, pp. 23–34, 2011.

[91] B. Soret, H. J. Lee, E. Finley, S. C. Lee, and R. G. Vernon, “Regulation of differentiation of shear subcutaneous and abdominal preadipocytes in culture,” *Journal of Endocrinology*, vol. 161, no. 3, pp. 517–524, 1999.

[92] K. Hayashida, N. Kume, M. Minami, H. Kataoka, M. Morimoto, and T. Kita, “Peroxisome proliferator-activated receptor α ligands increase lectin-like oxidized low density lipoprotein receptor-1 expression in vascular endothelial cells,” *Annals of the New York Academy of Sciences*, vol. 947, pp. 370–372, 2001.

[93] H. M. White, S. L. Kosler, and S. S. Donkin, “Differential regulation of bovine pyruvate carboxylase promoters by fatty acids and peroxisome proliferator-activated receptor-α agonist,” *Journal of Dairy Science*, vol. 94, no. 7, pp. 3428–3436, 2011.

[94] M. Sommer and G. Wolf, “Rosiglitazone increases PPARγ in renal tubular epithelial cells and protects against damage by hydrogen peroxide,” *American Journal of Nephrology*, vol. 27, no. 4, pp. 425–434, 2007.

[95] K. M. Schoenberg, S. L. Giesy, K. J. Harvatine et al., “Plasma FGF21 is elevated by the intense lipid mobilization of lactation,” *Endocrinology*, vol. 152, pp. 4652–4661, 2011.

[96] J. P. Vanden Heuvel, “Peroxisome proliferator-activated receptors: a critical link among fatty acids, gene expression and carcinogenesis,” *The Journal of Nutrition*, vol. 129, no. 2, pp. 575S–580S, 1999.

[97] H. M. Wright, C. B. Clish, T. Mikami et al., “A synthetic antagonist for the peroxisome proliferator-activated receptor γ inhibits adipocyte differentiation,” *Journal of Biological Chemistry*, vol. 275, no. 3, pp. 1873–1877, 2000.

[98] T. Dworzanski, K. Celinski, A. Korolczuk et al., “Influence of the peroxisome proliferator-activated receptor gamma (PPARγ) agonist, rosiglitazone and antagonist, bifenophenol-a-diglycidyl ether (BADGE) on the course of inflammation in the experimental model of colitis in rats,” *Journal of Physiology and Pharmacology*, vol. 61, no. 6, pp. 683–693, 2010.

[99] B. Rakic, S. M. Sagan, M. Noestheden et al., “Peroxisome proliferator-activated receptor α antagonism inhibits hepatitis C virus replication,” *Chemistry and Biology*, vol. 13, no. 1, pp. 23–30, 2006.

[100] H. E. Xu, T. B. Stanley, V. G. Montana et al., “Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARα,” *Nature*, vol. 415, no. 6873, pp. 813–817, 2002.

[101] B. G. Shearer, D. J. Steger, J. M. Way et al., “Identification and characterization of a selective peroxisome proliferator-activated receptor β/δ (NRIC2) antagonist,” *Molecular Endocrinology*, vol. 22, no. 2, pp. 523–529, 2008.

[102] B. G. Shearer, R. W. Wiethe, A. Ashe et al., “Identification and characterization of 4-chloro-N-(2-[5-trifluoromethyl]-2-pyridyl) sulfonyl ethylbenzamide (GSK3787), a selective and irreversible peroxisome proliferator-activated receptor δ (PPARδ) antagonist,” *Journal of Medicinal Chemistry*, vol. 53, no. 4, pp. 1857–1861, 2010.

[103] S. T. de Dios, K. M. Hannan, R. J. Dilley, M. A. Hill, and P. J. Little, “Troglitazone, but not rosiglitazone, inhibits Na/H exchange activity and proliferation of macrovascular endothelial cells,” *Journal of Diabetes and Its Complications*, vol. 15, no. 3, pp. 120–127, 2001.

[104] Y. Fukunaga, H. Itoh, K. Doi et al., “Thiazolidinediones, peroxisome proliferator-activated receptor γ agonists, regulate endothelial cell growth and secretion of vasoactive peptides,” *Atherosclerosis*, vol. 158, no. 1, pp. 113–119, 2001.

[105] M. Ohyama, K. Matsuda, S. Torii et al., “The interaction between vitamin a and thiazolidinedione on bovine adipocyte differentiation in primary culture,” *Journal of Animal Science*, vol. 76, no. 1, pp. 61–65, 1998.

[106] S. I. Torii, T. Kawada, K. Matsuda, T. Matsui, T. Ishihara, and H. Yano, “Thiazolidinedione induces the adipose differentiation of fibroblast-like cells resident within bovine skeletal muscle,” *Cell Biology International*, vol. 22, no. 6, pp. 421–427, 1998.

[107] S. Kushibiki, K. Hodate, H. Shingu et al., “Insulin resistance induced in dairy steers by tumor necrosis factor alpha is partially reversed by 2,4-thiazolidinedione,” *Domestic Animal Endocrinology*, vol. 21, no. 1, pp. 25–37, 2001.

[108] G. D. Cappon, R. C. M. Liu, S. R. Frame, and M. E. Hurtt, “Effects of the rat hepatic peroxisome proliferator, Wyeth 14,643, on the lactating goat,” *Drug and Chemical Toxicology*, vol. 25, no. 3, pp. 255–266, 2002.

[109] K. L. Smith, W. R. Butler, and T. R. Overton, “Effects of prepartum 2,4-thiazolidinedione on metabolism and performance in transition dairy cows,” *Journal of Dairy Science*, vol. 92, no. 8, pp. 3623–3633, 2009.

[110] K. L. Smith, S. E. Stebulis, M. R. Waldron, and T. R. Overton, “Prepartum 2,4-thiazolidinedione alters metabolic dynamics and dry matter intake of dairy cows,” *Journal of Dairy Science*, vol. 90, no. 8, pp. 3660–3670, 2007.

[111] C. Bocos, M. Gottlicher, K. Gearing et al., “Fatty acid activation of peroxisome proliferator-activated receptor (PPAR),” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 53, no. 1–6, pp. 467–473, 1995.

[112] E. Duplus and C. Forest, “Is there a single mechanism for fatty acid regulation of gene transcription?” *Biochemical Pharmacology*, vol. 64, no. 5–6, pp. 893–901, 2002.

[113] M. Gottlicher, E. Widmark, Q. Li, and J. A. Gustafsson, “Fatty acids activate a chimera of the clobifiram acid-activated receptor and the glucocorticoid receptor,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 10, pp. 4653–4657, 1992.
[114] H. A. Hostetler, A. D. Petrescu, A. B. Kier, and F. Schroeder, “Peroxisome proliferator-activated receptor α interacts with high affinity and is conformationally responsive to endogenous ligands,” Journal of Biological Chemistry, vol. 280, no. 19, pp. 18667–18682, 2005.

[115] S. Bonilla, A. Redonnet, C. Noël-Suberville, V. Pallet, H. Garcin, and P. Higuere, “High-fat diets affect the expression of nuclear retinoid acid receptor in rat liver,” The British Journal of Nutrition, vol. 83, no. 6, pp. 665–671, 2000.

[116] H. Huang, O. Starodub, A. McIntosh, A. B. Kier, and F. Schroeder, “Liver fatty acid-binding protein targets fatty acids to the nucleus. Real time confocal and multiphoton fluorescence imaging in living cells,” Journal of Biological Chemistry, vol. 277, no. 32, pp. 29139–29151, 2002.

[117] C. Wolfrum, C. M. Borrmann, T. Börchers, and F. Spener, “Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α and γ-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus,” Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 5, pp. 2323–2328, 2001.

[118] S. A. Khan and J. P. Vanden Heuvel, “Reviews: current topics role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review),” Journal of Nutritional Biochemistry, vol. 14, no. 10, pp. 554–567, 2003.

[119] J. P. Vanden Heuvel, J. T. Thompson, S. R. S. Frame, and P. J. Gillies, “Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-α, -β, and -γ, liver X receptor-β, and retinoid X receptor-α,” Toxicological Sciences, vol. 92, no. 2, pp. 476–489, 2006.

[120] M. Zachut, A. Arieli, H. Lehrer, L. Livshitz, S. Yakoby, and U. Moallem, “Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat,” Journal of Dairy Science, vol. 93, no. 12, pp. 5877–5889, 2010.

[121] M. M. Or-Rashid, R. Fisher, N. Karrow, O. AlZahal, and B. W. McBride, “Plasma fatty acid profile of gestating ewes supplemented with docosahexaenoic acid,” Canadian Journal of Animal Science, vol. 89, pp. 138–138, 2009.

[122] S. Peltier, L. Portois, W. J. Malaisse, and Y. A. Carpenter, “Fatty acid profile of plasma and liver lipids in mice depleted in long-chain polyunsaturated (n-3) fatty acids,” International Journal of Molecular Medicine, vol. 22, no. 4, pp. 559–563, 2008.

[123] J. Ma, A. R. Folsom, J. H. Eckfeldt et al., “Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters,” The American Journal of Clinical Nutrition, vol. 62, no. 3, pp. 572–578, 1995.

[124] T. Itoh, L. Fairall, K. Amin et al., “Structural basis for the activation of PPARγ by oxidized fatty acids,” Nature Structure and Molecular Biology, vol. 15, no. 9, pp. 924–931, 2008.

[125] H. A. Hostetler, H. Huang, A. B. Kier, and F. Schroeder, “Glucose directly links to lipid metabolism through high affinity interaction with peroxisome proliferator-activated receptor α,” The Journal of Biological Chemistry, vol. 283, no. 4, pp. 2246–2254, 2008.

[126] C. Blanquicett, B. Y. Kang, J. D. Ritzenthaler, D. P. Jones, and C. M. Hart, “Oxidative stress modulates PPARγ in vascular endothelial cells,” Free Radical Biology and Medicine, vol. 48, no. 12, pp. 1618–1625, 2010.

[127] S. Borniquel, I. Vallee, S. Cadenas, S. Lamas, and M. Monsalve, “Nitric oxide regulates mitochondrial oxidative stress protection via the transcriptional coactivator PGC-1α,” The FASEB Journal, vol. 20, no. 11, pp. 1889–1891, 2006.

[128] S. Kanata, M. Akagi, S. Nishimura et al., “Oxidized LDL binding to LOX-1 upregulates VEGF expression in cultured bovine chondrocytes through activation of PPAR-γ,” Biochemical and Biophysical Research Communications, vol. 348, no. 3, pp. 1003–1010, 2006.

[129] M. Bionaz, C. R. Baumrucker, E. Shirk et al., “Characterization of Madin-Darby bovine kidney cell line for peroxisome proliferator-activated receptors: temporal response and sensitivity to fatty acids,” Journal of Dairy Science, vol. 92, no. 9, pp. 4715–4715, 2009, vol. 91, p. 2808, 2008.

[130] J. H. Lee, A. Banerjee, Y. Ueno, and S. K. Ramaiah, “Potential relationship between hepatobiliary osteopontin and peroxisome proliferator-activated receptor α expression following ethanol-associated hepatic injury in vivo and in vitro,” Toxicological Sciences, vol. 106, no. 1, pp. 290–299, 2008.

[131] Y. Oyama, N. Akuzawa, R. Nagai, and M. Kurabayashi, “PPARγ ligand inhibits osteopontin gene expression through interference with binding of nuclear factors to A/T-rich sequence in THP-1 cells,” Circulation Research, vol. 90, no. 3, pp. 348–355, 2002.

[132] M. Kishimoto, R. Fujiki, S. Takezawa et al., “Nuclear receptor mediated gene regulation through chromatin remodeling and histone modifications,” Endocrine Journal, vol. 53, no. 2, pp. 157–172, 2006.

[133] G. M. Thompson, D. Trainor, C. Biswas, C. LaCerte, J. P. Berger, and L. J. Kelly, “A high-capacity assay for PPARγ ligand regulation of endogenous aP2 expression in 3T3-L1 cells,” Analytical Biochemistry, vol. 330, no. 1, pp. 21–28, 2004.

[134] P. Ji, J. S. Osorio, J. K. Drackley, and J. J. Loor, “Overfeeding a moderate energy diet prepartum does not impair bovine subcutaneous adipose tissue insulin signal transduction and induces marked changes in peripartal gene network expression,” Journal of Dairy Science, vol. 95, pp. 4333–4351, 2012.

[135] A. K. G. Kadegowda, M. Bionaz, B. Thering, L. S. Piperova, R. A. Erdman, and J. J. Loor, “Identification of internal control genes for quantitative polymerase chain reaction in mammary tissue of lactating cows receiving lipid supplements,” Journal of Dairy Science, vol. 92, no. 5, pp. 2007–2019, 2009.

[136] S. A. Bustin, V. Benes, J. A. Garson et al., “The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments,” Clinical Chemistry, vol. 55, no. 4, pp. 611–622, 2009.

[137] S. Kersten, J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli, “Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting,” The Journal of Clinical Investigation, vol. 103, no. 11, pp. 1489–1498, 1999.

[138] G. Schlegel, J. Keller, F. Hirche et al., “Expression of genes involved in hepatic carnitine synthesis and uptake in dairy cows in the transition period and at different stages of lactation,” BMC Veterinary Research, vol. 8, article 28, 2012.

[139] H. A. van Dorland, S. Richter, I. Morel, M. G. Doerr, N. Castro, and R. M. Bruckmaier, “Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows,” Journal of Dairy Science, vol. 92, no. 5, pp. 1924–1940, 2009.

[140] M. Carriquiry, W. J. Weber, S. C. Fahrenkrug, and B. A. Crooker, “Hepatic gene expression in multiparous Holstein cows treated with bovine somatotropin and fed n-3 fatty acids in early
lactation,” *Journal of Dairy Science*, vol. 92, no. 10, pp. 4889–4900, 2009.

[141] B. Kuhla, S. Gors, and C. C. Metges, “Hypothalamic orexin A expression and the involvement of AMPK and PPAR-gamma signalling in energy restricted dairy cows,” *Archiv für Tierzucht-Archives of Animal Breeding*, vol. 54, pp. 567–579, 2011.

[142] K. M. Brennan, J. J. Michal, J. J. Ramsey, and K. A. Johnson, “Body weight loss in beef cows: I. The effect of increased β-oxidation on messenger ribonucleic acid levels of uncoupling proteins two and three and peroxisome proliferator-activated receptor in skeletal muscle,” *Journal of Animal Science*, vol. 87, no. 9, pp. 2860–2866, 2009.

[143] N. A. Janovick-Guretzky, H. M. Dann, J. J. Loor, and J. K. Drackley, “Prepartum plane of dietary energy alters hepatic expression of inflammatory and fatty acid oxidation genes in dairy cows,” *The FASEB Journal*, vol. 21, pp. A374–A374, 2007.

[144] J. Bispham, D. S. Gardner, M. G. Gnanalingham, T. Stephenson, M. E. Symonds, and H. Budge, “Maternal nutritional programming of fetal adipose tissue development: differential effects on mRNA abundance for uncoupling proteins, peroxisome proliferator activated and prolatin receptors,” *Endocrinology*, vol. 146, no. 9, pp. 3943–3949, 2005.

[145] B. S. Muhlhausler, J. A. Duffield, and I. C. McMillen, “Increased maternal nutrition stimulates peroxisome proliferator activated receptor-γ, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth,” *Endocrinology*, vol. 148, no. 2, pp. 878–885, 2007.

[146] L. Rattanatray, S. M. MacLaughlin, D. O. Kleemann, S. K. Walker, B. S. Muhlhausler, and I. C. McMillen, “Impact of maternal periconceptional overnutrition on fat mass and expression of adipogenic and lipogenic genes in visceral and subcutaneous fat depots in the postnatal lamb,” *Endocrinology*, vol. 151, no. 11, pp. 5195–5205, 2010.

[147] P. García-Rojas, A. Antaramian, L. González-Dávalos et al., “Induction of peroxisomal proliferator-activated receptor γ and peroxisomal proliferator-activated receptor γ coactivator 1 by unsaturated fatty acids, retinoic acid, and carotenoids in preadipocytes obtained from bovine white adipose tissue,” *Journal of Animal Science*, vol. 88, no. 5, pp. 1801–1808, 2010.

[148] J. J. Loor, M. Bionaz, and G. Invernizzi, “Systems biology and animal nutrition: insights from the dairy cow during growth and the lactation cycle,” in *Systems Biology*, vol. 1, pp. 15–31, 2008.

[149] D. E. Bauman, K. J. Harvatine, and A. L. Locke, “Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis,” *Annual Review of Nutrition*, vol. 31, pp. 299–319, 2011.

[150] E. Monaco, A. Lima, M. Bionaz et al., “Morphological and transcriptomic comparison of adipose and bone marrow derived porcine stem cells,” *Journal of Tissue Engineering and Regenerative Medicine*, vol. 2, pp. 20–33, 2009.

[151] P. Sertzig, M. Seifert, W. Tilgen, and J. Reichrath, “Peroxisome proliferator-activated receptors (PPARs) and the human skin: importance of PPARs in skin physiology and dermatologic diseases,” *American Journal of Clinical Dermatology*, vol. 9, no. 1, pp. 15–31, 2008.

[152] B. A. Corl, S. T. Butler, W. R. Butler, and D. E. Bauman, “Short communication: regulation of milk fat yield and fatty acid composition by insulin,” *Journal of Dairy Science*, vol. 89, no. 11, pp. 4172–4175, 2006.

[153] J. W. McFadden and B. A. Corl, “Activation of liver X receptor (LXR) enhances de novo fatty acid synthesis in bovine mammary epithelial cells,” *Journal of Dairy Science*, vol. 93, no. 10, pp. 4651–4658, 2010.

[154] C. Oppi-Williams, J. K. Suagee, and B. A. Corl, “Regulation of lipid synthesis by liver X receptor alpha and sterol regulatory element-binding protein 1 in mammary epithelial cells,” *Journal of Dairy Science*, vol. 96, no. 1, pp. 112–121, 2013.

[155] L. Ma and B. A. Corl, “Transcriptional regulation of lipid synthesis in bovine mammary epithelial cells by sterol regulatory element binding protein-1,” *Journal of Dairy Science*, vol. 95, pp. 3743–3755, 2012.

[156] M. C. Rudolphi, J. L. McManaman, T. Phang et al., “Metabolic regulation in the lactating mammalian gland: a lipid synthesizing machine,” *Physiological Genomics*, vol. 28, no. 3, pp. 323–336, 2007.

[157] M. Bionaz and J. J. Loor, “Comparative Mammomics of milk fat synthesis in Mus musculus vs. Bos Taurus,” *Journal of Dairy Science*, vol. 91, pp. 566–567, 2008.

[158] D. P. Shu, B. L. Chen, J. Hong et al., “Global transcriptional profiling in porcine mammary glands from late pregnancy to peak lactation,” *Omics*, vol. 16, pp. 123–137, 2012.

[159] X. Lin, J. J. Loor, and J. H. Herbein, “Trans10,cis12-18:2 is a more potent inhibitor of de novo fatty acid synthesis and desaturation than cis9,trans11-18:2 in the mammary gland of lactating mice,” *The Journal of Nutrition*, vol. 134, no. 6, pp. 1362–1368, 2004.

[160] D. Bishop-Bailey and J. Bystrom, “Emerging roles of peroxisome proliferator-activated receptor-β/δ in inflammation,” *Pharmacology and Therapeutics*, vol. 124, no. 2, pp. 141–150, 2009.

[161] H. Hauner, “The mode of action of thiazolidinediones,” *Diabetes/Metabolism Research and Reviews*, vol. 18, no. 2, pp. S10–S15, 2002.

[162] M. C. Perdomo, J. E. Santos, and L. Badinga, “Trans-10, cis-12 conjugated linoleic acid and the PPAR-gamma agonist rosiglitazone attenuate lipopolysaccharide-induced TNF-alpha production by bovine immune cells,” *Domestic Animal Endocrinology*, vol. 41, no. 3, pp. 118–125, 2011.

[163] W. Ahmed, G. Orasanu, V. Nehra et al., “High-density lipoprotein hydrolysis by endothelial lipase activates PPARα: a candidate mechanism for high-density lipoprotein-mediated repression of leukocyte adhesion,” *Circulation Research*, vol. 98, no. 4, pp. 490–498, 2006.

[164] S. Mitterhuemer, W. Petzl, S. Krebs et al., “Escherichia coli infection induces distinct local and systemic transcriptome responses in the mammary gland,” *BMC Genomics*, vol. 11, no. 1, article 138, 2010.
[167] K. M. Moyes, J. K. Drackley, D. E. Morin et al., “Gene network and pathway analysis of bovine mammary tissue challenged with Streptococcus uberis reveals induction of cell proliferation and inhibition of PPAR signaling as potential mechanism for the negative relationships between immune response and lipid metabolism,” BMC Genomics, vol. 10, article 542, 2009.

[170] L. Jiang, P. Sørensen, C. Røntved, L. Vels, and K. L. Ingvartsen, “Gene expression profiling of liver from dairy cows treated intra-mammary with lipopolysaccharide,” BMC Genomics, vol. 9, article 443, 2008.

[171] J. J. Loom, K. M. Moyes, and M. Bonaz, “Functional adaptations of the transcriptome to mastitis-causing pathogens: the mammary gland and beyond,” Journal of Mammary Gland Biology and Neoplasia, vol. 16, no. 4, pp. 305–322, 2011.

[172] D. E. Graugnard, K. M. Moyes, E. Trevisi et al., “Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepelant energy intake and postpartal intramammary inflammatory challenge,” Journal of Dairy Science, vol. 96, pp. 918–935, 2013.

[173] B. Lu, A. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold, “The acute phase response stimulates the expression of angiopoietin like protein 4,” Biochemical and Biophysical Research Communications, vol. 391, no. 4, pp. 1737–1741, 2010.

[174] D. A. Koltes and D. M. Spurlock, “Adipose tissue angiopoietin-like protein 4 messenger RNA changes with altered energy balance in lactating Holstein cows,” Domestic Animal Endocrinology, vol. 43, no. 4, pp. 307–316, 2012.

[175] G. Bertonio, E. Trevisi, X. Han, and M. Bonaz, “Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows,” Journal of Dairy Science, vol. 91, no. 9, pp. 3300–3310, 2008.

[176] M. Bonaz, E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertonio, “Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows,” Journal of Dairy Science, vol. 90, no. 4, pp. 1740–1750, 2007.

[177] K. Shahzad, J. Sumner-Thomson, J. P. McNamara, and J. J. Loom, “Analysis of bovine adipose transcriptomics data during the transition from pregnancy to early lactation using two bioinformatics approaches,” Journal of Dairy Science, vol. 94, article M258, 2011.

[178] S. Kersten, “Regulation of lipid metabolism via angiopoietin-like proteins,” Biochemical Society Transactions, vol. 33, no. 5, pp. 1059–1062, 2005.

[179] A. Kharitononenkov, T. L. Shiyanova, A. Koester et al., “FGF-21 as a novel metabolic regulator,” The Journal of Clinical Investigation, vol. 115, no. 6, pp. 1627–1635, 2005.

[180] E. Hondares, M. Rosell, F. J. Gonzalez, M. Giralt, R. Iglesias, and E. Villarroya, “Hepatic FGF21 expression is induced at birth via PPARA in response to milk intake and contributes to thermogenic activation of neonatal brown fat,” Birth via PPAR and FGF21, Cell Metabolism, vol. 19, no. 3, pp. 206–212, 2010.

[181] D. H. Cho, Y. J. Choi, S. A. Jo et al., “Troglitazone acutely inhibits protein synthesis in endothelial cells via a novel mechanism involving protein phosphatase 2A-dependent p70 S6 kinase inhibition,” American Journal of Physiology—Cell Physiology, vol. 291, no. 2, pp. C317–C326, 2006.

[182] S. Mandard, M. Müller, and S. Kersten, “Peroxisome proliferator-activated receptor α target genes,” Cellular and Molecular Life Sciences, vol. 61, no. 4, pp. 393–416, 2004.

[183] H. M. White, S. L. Koser, and S. S. Donkin, “Gluconeogenic enzymes are differentially regulated by fatty acid cocktails in Madin–Darby bovine kidney cells,” Journal of Dairy Science, vol. 95, no. 3, pp. 1249–1256, 2012.

[184] S. Andrikopoulos, A. R. Blair, N. Deluca, B. C. Fam, and J. Proietto, “Evaluating the glucose tolerance test in mice,” American Journal of Physiology—Endocrinology and Metabolism, vol. 295, no. 6, pp. E1323–E1332, 2008.

[185] M. Bonaz and J. J. Looor, “Gene networks driving bovine mammary protein synthesis during the lactation cycle,” Bioinformatics and Biology Insights, vol. 5, pp. 83–98, 2011.

[186] M. Bonaz, K. Periasamy, S. L. Rodriguez-Zas et al., “Old and new stories: revelations from functional analysis of the bovine mammary transcriptome during the lactation cycle,” PLoS One, vol. 7, article e33268, 2012.

[187] J. Berger and D. E. Moller, “The mechanisms of action of PPARs,” Annual Review of Medicine, vol. 53, pp. 409–435, 2002.

[188] N. Viswakarma, Y. Jia, L. Bai et al., “Coactivators in PPAR-regulated gene expression,” PPAR Research, vol. 2010, Article ID 250126, 21 pages, 2010.

[189] A. J. Lengi and B. A. Corl, “Factors influencing the differentiation of bovine preadipocytes in vitro,” Journal of Animal Science, vol. 88, no. 6, pp. 1999–2008, 2010.

[190] J. K. Drackley, “ADSA foundation scholar award: biology of dairy cows during the transition period: the final frontier?” Journal of Dairy Science, vol. 82, no. 11, pp. 2259–2273, 1999.

[191] P. Holtenius and K. Holtenius, “A model to estimate insulin sensitivity in dairy cows,” Acta Veterinaria Scandinavica, vol. 49, no. 1, article 29, 2007.

[192] E. Trevisi, M. Amadori, I. Archetti, N. Lacetera, and G. Bertonio, “Inflammatory response and acute phase proteins in the transition period of high-yielding dairy cows,” in Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases, F. Veas, Ed., InTech, Rijeka, Croatia, 2011.

[193] M. S. Allen, B. J. Bradford, and K. J. Harvatine, “The cow as a model to study food intake regulation,” Annual Review of Nutrition, vol. 25, pp. 523–547, 2005.

[194] G. Bobe, J. W. Young, and D. C. Beitz, “Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows,” Journal of Dairy Science, vol. 87, no. 10, pp. 3105–3124, 2004.

[195] P. Holtenius and K. Holtenius, “New aspects of ketone bodies in energy metabolism of dairy cows: a review,” Zentralblatt für Veterinärmedizin. Reihe A, vol. 43, no. 10, pp. 579–587, 1996.

[196] R. B. Walsh, J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Dufield, “The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows,” Journal of Dairy Science, vol. 90, no. 6, pp. 2788–2796, 2007.

[197] J. K. Drackley, H. M. Dann, G. N. Douglas et al., “Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders,” Italian Journal of Animal Science, vol. 4, no. 4, pp. 323–344, 2005.

[198] L. M. Sordillo, G. A. Contreras, and S. L. Aitken, “Metabolic factors affecting the inflammatory response of periparturient dairy cows,” Animal Health Research Reviews / Conference of Research Workers in Animal Diseases, vol. 10, no. 1, pp. 53–63, 2009.

[199] M. C. E. Bragt and H. E. Popejus, “Peroxisome proliferator-activated receptors and the metabolic syndrome,” Physiology and Behavior, vol. 94, no. 2, pp. 187–197, 2008.

[200] L. Guo and R. Tabrizchi, “Peroxisome proliferator-activated receptor γ as a drug target in the pathogenesis of insulin resistance,” BMC Genomics, vol. 10, article 256, 2009.
resistance,” *Pharmacology and Therapeutics*, vol. 111, no. 1, pp. 145–173, 2006.

[201] M. Bionaz, *Studi sui Rapporti fra Funzionalità Epatica e Fenomeni Infiammatori al Parto: Conseguenze sulle Performance Produttive e Riproduttive*, Università cattolica del Sacro Cuore, Piacenza, Italy, 2004.

[202] J. B. Andersen, C. Ridder, and T. Larsen, “Priming the cow for mobilization in the periparturient period: effects of supplementing the dry cow with saturated fat or linseed,” *Journal of Dairy Science*, vol. 91, no. 3, pp. 1029–1043, 2008.

[203] D. Gruffat, D. Durand, B. Graulet, and D. Bauchart, “Regulation of VLDL synthesis and secretion in the liver,” *Reproduction Nutrition Development*, vol. 36, no. 4, pp. 375–389, 1996.

[204] M. S. Allen, B. J. Bradford, and M. Oba, “Board-invited review: the hepatic oxidation theory of the control of feed intake and its application to ruminants,” *Journal of Animal Science*, vol. 87, no. 10, pp. 3317–3334, 2009.

[205] U. Bernabucci, B. Ronchi, L. Basiricò et al., “Abundance of mRNA of apolipoprotein B100, apolipoprotein E, and microsomal triglyceride transfer protein in liver from periparturient dairy cows,” *Journal of Dairy Science*, vol. 87, no. 9, pp. 2881–2888, 2004.

[206] N. Kato, “Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows,” *Journal of Veterinary Medical Science*, vol. 64, no. 4, pp. 293–307, 2002.

[207] G. A. Contreras and L. M. Sordillo, “Lipid mobilization and inflammatory responses during the transition period of dairy cows,” *Comparative Immunology, Microbiology and Infectious Diseases*, vol. 34, no. 3, pp. 281–289, 2011.

[208] R. K. Ball, R. R. Friis, C. A. Schoenenberger, W. Doppler, and B. Groner, “Prolactin regulation of beta-casein gene expression and of a cytosolic 120-kd protein in a cloned mouse mammary epithelial cell line,” *The EMBO Journal*, vol. 7, no. 7, pp. 2089–2095, 1988.

[209] C. W. Hsieh, C. Huang, I. Bederman et al., “Function of phosphoenolpyruvate carboxykinase in mammary gland epithelial cells,” *Journal of Lipid Research*, vol. 52, no. 7, pp. 1352–1362, 2011.