TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Adipokines affect mammary growth and function in farm animals^{1,2}

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ABSTRACT: The essential role of mammary fat pads in mammary growth and morphogenesis was the first indication that biologically active molecules, secreted from adipocytes or other stromal cells, could regulate endocrine cues for growth and function of the mammary gland. The presence of leptin and adiponectin receptors in mammary tissues suggested that locally produced or circulating adipokines could affect mammary growth and function. Herein, we present the current knowledge on the role of adipokines in mammary cell proliferation and differentiation and in lactogenesis and galactopoiesis in farm animals. We also address the role of milk adipokines in the neonate.

Accumulating evidence suggests that adipokines could act as metabolic sensors, regulating mammary growth and function in periods of metabolic adaptations such as late pregnancy and early lactation. Indeed, different experiments reported that adiponectin and leptin expression varies according to physiological stages and nutritional status of the animal. The current review also demonstrates that adipokines, such as leptin and adiponectin, are important regulators of the action of lactogenic hormones in the mammary gland. Findings also suggest important roles for adipokines in growth and intestinal maturation of the neonate.

Key words: adipokines, adiponectin, farm animals, leptin, mammary development, milk

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INTRODUCTION

The adipose tissue is now considered an endocrine organ secreting numerous biologically active molecules, collectively termed adipokines. Several of these adipokines regulate energy expenditure and food intake and are essential for the maintenance of

metabolic homeostasis (Stern et al., 2016). These functions are of particular importance in livestock species that are under negative energy balance during late pregnancy and early lactation due to the important nutrient drain toward the mammary gland. The synthesis and secretion of over 350 adipokines by mammary adipose tissue (Celis et al., 2005) and the presence of adipokine receptors in mammary tissue and cells suggest that some of these adipokines may play key roles in mammary development and function. This hypothesis is strengthened by earlier studies demonstrating the essential role of mammary fat pads (MFP) in mammary growth and morphogenesis (Hovey et al., 1999). This review summarizes current knowledge on the role of leptin and adiponectin, the most widely studied adipokines, in mammary growth and function in farm animals. The effects of other adipokines potentially playing

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important roles in mammary development will also be succinctly covered.

LEPTIN

Leptin is a 16-kDa polypeptide that is mainly produced and secreted by white adipose tissue (WAT; Zhang et al., 1994), and its plasma concentrations are associated with the amount of body fat in humans and rodents (Maffei et al., 1995). Leptin transcripts and proteins are also found in several peripheral tissues such as the skeletal muscle, stomach, pituitary, placenta, ovary, and mammary gland (Wylie, 2011). The Lep^{ob}/Lep^{ob} mice, which synthesize a truncated leptin protein, show reduced energy expenditure and hyperphagia, leading to severe obesity (Zhang et al., 1994). In addition, these mice have impaired reproductive and endocrine functions and minimal mammary development (Gonzalez et al., 2000; Hu et al., 2002). Leptin plays key roles in several physiological processes including energy expenditure and feed intake, and in conditions of metabolic disturbance, leptin also affects reproduction and immune responses (Roh et al., 2016). It is also involved in glucose homeostasis and lipid metabolism independently of its central energy metabolism functions (Bates et al., 2005; Huang et al., 2006).

Local Synthesis of Leptin in Mammary Tissue and Cells

Leptin (*LEP*) transcript and protein were detected in mammary tissue of gilts, dairy cows, sheep, and goats (Table 1). Adipocytes, alveolar epithelial, and myoepithelial mammary cells all express leptin (Table 1) as do the bovine mammary epithelial (MAC-T) cells, where leptin expression is upregulated in response to insulin (10 ng/mL) and insulin-like growth factor I (IGF-I; 0.1 to 10 ng/mL). However, this effect disappears with greater concentrations of insulin or IGF-I (100 ng/mL; Smith and Sheffield, 2002). Abundance of *LEP* mRNA was also downregulated when high concentrations of lactogenic hormones (10 µg/mL dexamethasone + 10 µg/mL insulin + 10 µg/mL prolactin [PRL]) were added to bovine primary mammary epithelial cells (PMEC; Yonekura et al., 2006).

Transcripts of *LEP* were detected in mammary tissues of farm animals at different physiological stages including prepuberty, pregnancy, lactation, and the dry-off period (Table 1). In prepubertal dairy heifers, *LEP* gene expression is approximately 100-fold greater in the mammary stromal tissue than in the parenchyma, whereas it is absent in PMEC (Thorn et al., 2006). This suggests that *LEP* transcripts observed in the parenchymal tissue may originate from embedded

adipocytes. In the same study, heifers that were fed a high plane (12.7 MJ ME and 228 g CP per kilogram DM to reach 1,202 g ADG) of nutrition showed greater (3-fold) *LEP* mRNA abundance in stromal and parenchymal tissues and greater circulating leptin concentrations compared with heifers on a normal plane (8.4 MJ ME and 116 g CP per kilogram DM to reach 715 g ADG) of nutrition (Thorn et al., 2006). The high plane heifers also had less mammary parenchymal weight and more stromal weight than normal plane heifers, suggesting that mammary development of prepubertal heifers can be affected by their nutritional status, which modulates locally produced or circulating adipokines such as leptin (Thorn et al., 2006). Physiological state also modulates leptin expression in mammary tissue of dairy sheep, with greater *LEP* gene expression being found in early (d 15) and mid pregnancy (d 80) followed by low expression at d 106 and 112 of pregnancy (Bonnet et al., 2002b). The observed decrease in mammary leptin transcripts between d 80 and 112 may reflect the important reduction in adipocyte numbers previously reported in mammary glands of ewes during that same period (Smith et al., 1989). During lactation, *LEP* gene expression remains low, with minimal variations between early and peak lactation in dairy sheep and goat mammary tissue (Bonnet et al., 2002a; Rasmussen et al., 2008). Interestingly, Bonnet et al. (2002b) reported that leptin cellular localization in ewe mammary glands differs according to the physiological stage, being detected in adipocytes in early pregnancy, in alveolar epithelial cells before parturition, and in myoepithelial cells on the first day of lactation. A similar observation was made in dairy goat mammary tissue (Li et al., 2010). These findings suggest that leptin may act as a paracrine/autocrine factor in the ruminant mammary gland. Moreover, the presence of *LEP* mRNA within alveolar epithelial cells during lactation clearly demonstrates a local production of leptin by mammary cells and possible secretion in milk (Sayed-Ahmed et al., 2004).

In gilts, *LEP* transcripts were detected in mammary parenchyma (Farmer et al., 2016a,b). We have also observed *LEP* transcripts in extraparenchymal tissue, with approximately 2 times lower expression levels than the WAT *LEP* gene (M.-F. Palin et al., personal communication). Backfat thickness of gilts had no effect on *LEP* mRNA abundance in parenchymal tissue at the end of gestation (Farmer et al., 2016b), whereas greater *LEP* expression levels were found in the extraparenchymal tissue of high-backfat (21 to 26 mm) gilts compared with the low-backfat (12 to 15 mm) gilts (M.-F. Palin et al., personal communication). Gene expression of *LEP* in mammary extraparenchymal tissue

Table 1. Leptin and leptin receptor transcripts and proteins in mammary tissues and cells of farm animals

Species	Parenchyma	Extraparenchyma/ fat pad	Mammary cells	Physiological stage
Leptin				
Gilt	Transcript (+) ¹	Transcript (+)	NR ²	End of gestation (Farmer et al., 2016a,b)
Cow	Transcript (+)	NR	MAC-T ³ (+)	Unknown (Smith and Sheffield, 2002)
	Transcript (+)	Transcript (+)	Adipocyte and alveolar epithelial (+)	Lactating, nonpregnant, and dry off (Sayed-Ahmed et al., 2004)
Sheep	Transcript (+)	Transcript (+)	NR	Lactation (Feuermann et al., 2006)
	Transcript (+)	Transcript (+)	PMEC ⁴ (-) ¹	Prepubertal (Thorn et al., 2006)
	NR	Transcript (+)	NR	Pregnancy and lactation (Laud et al., 1999)
	Transcript (+)	NR	Adipocyte, epithelial, and myoepithelial (+)	Pregnancy and lactation (Bonnet et al., 2002a)
Goat	Transcript (+)	NR	NR	Periparturient (Rasmussen et al., 2008)
	Protein (+)	Protein (+)	Adipocyte, ductal, and alveolar epithelial (+)	Prepubertal, pregnancy, and lactation (Li et al., 2010)
Leptin receptor				
Gilt	Transcript <i>Ob-Rb</i> (±) ¹	Transcript <i>Ob-Rb</i> (+)	NR	End of gestation (Farmer et al., 2016a,b)
Cow	Transcript, <i>Ob-Ra</i> (-), and <i>Ob-Rb</i> (±)	Transcript, <i>Ob-Ra</i> (-), and <i>Ob-Rb</i> (+)	<i>Ob-Rb</i> : MAC-T (±) and PMEC (+) <i>Ob-Ra</i> : MAC-T (-) and PMEC (-)	Prepubertal (Silva et al., 2002)
	Transcript, <i>Ob-Ra</i> (+), and <i>Ob-Rb</i> (+)	Transcript, <i>Ob-Ra</i> (+), and <i>Ob-Rb</i> (+)	Adipocyte and alveolar epithelial: <i>Ob-Ra</i> (+) and <i>Ob-Rb</i> (+)	Lactating, nonpregnant, and dry off (Sayed-Ahmed et al., 2004)
	Transcript, <i>Ob-Ra</i> (+), <i>Ob-Rb</i> (±), and <i>Ob-R_{total}</i> (+)	Transcript, <i>Ob-Ra</i> (+), <i>Ob-Rb</i> (±), and <i>Ob-R_{total}</i> (+)	MAC-T and PMEC: <i>Ob-Ra</i> (-), <i>Ob-Rb</i> (-), and <i>Ob-R_{total}</i> (±)	Prepubertal (Thorn et al., 2006)
	Transcript, <i>Ob-R_{short}</i> (+), and <i>Ob-R_{long}</i> (+)	NR	Alveolar epithelial: <i>Ob-R_{total}</i> (+)	Pregnancy and lactation (Laud et al., 1999)
Sheep	Transcript <i>Ob-R_{total}</i> (+)	NR	NR	Periparturient (Rasmussen et al., 2008)
	Protein <i>Ob-Rb</i> (+)	Protein <i>Ob-Rb</i> (+)	Adipocyte, ductal, and alveolar epithelial (+)	Prepubertal, pregnancy, and lactation (Li et al., 2010)

¹(+) = detected; (-) = absent; (±) = negligible expression.

²NR = not reported.

³MAC-T is a bovine mammary epithelial cell line.

⁴PMEC = primary mammary epithelial cells.

may therefore be regulated by the body condition of the gilts.

Regulation of Leptin Receptor Expression in Mammary Tissue and Cells

The effect of leptin in peripheral tissues is mediated through the leptin receptor (Ob-R), a member of the class I cytokine receptor family. A total of 6 alternatively spliced Ob-R isoforms have been identified in rodents and humans and can be grouped into short (Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf), long (Ob-Rb), and secreted (Ob-Re) isoforms. Long and short Ob-R isoforms were also identified in farm animals (Kawachi et al., 2007; Pérez-Montarelo et al., 2013). These receptors share identical extracellular and transmembrane domains but present different intracellular portions (Wylie, 2011). The *db/db* mice, which express only the short leptin receptor isoforms, present a severe obesity

phenotype and an impaired mammary gland development caused by minimal ductal development (Hu et al., 2002). It was demonstrated that Ob-Rb can activate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) intracellular signaling pathways and that both Ob-Rb and Ob-Ra mediate leptin internalization (Wylie, 2011). The Ob-Ra isoform is found in several peripheral tissues where its intracellular signaling activity remains to be demonstrated (Wylie, 2011).

Transcripts of the short (*Ob-Ra*), long (*Ob-Rb*) and *Ob-R_{total}* isoforms of leptin receptors were detected in the mammary tissue of gilts, dairy cows, and sheep and Ob-Rb proteins in dairy goats (Table 1). The *Ob-Rb* transcript was either undetectable or at very low concentrations in parenchymal tissue of prepubertal and pubertal dairy cows (Silva et al., 2002; Thorn et

al., 2006), whereas it was found in pregnant, lactating, and drying-off cows (Sayed-Ahmed et al., 2004) and in pregnant and lactating sheep (Laud et al., 1999). Lactogenic hormones may therefore be needed for priming of *Ob-Rb* gene expression in mammary cells of ruminants but not in gilts, where *Ob-Rb* transcripts were barely detectable in parenchymal tissue at the end of gestation (Farmer et al., 2016b). As observed for the leptin transcript (see above), cellular locations of *Ob-Rb* protein differed according to the physiological state. It was detected in adipocytes and ductal epithelial mammary cells in prepubertal and early pregnant goats, in alveolar epithelial cells during late pregnancy and lactation, and in adipocytes and ductal epithelial mammary cells during the dry-off period (Li et al., 2010). Transcripts of *Ob-Ra* are expressed in mammary parenchymal and extraparenchymal tissues of dairy cows and sheep, although contradictory results were reported (Table 1). For instance, Silva et al. (2002) were unable to detect *Ob-Ra* in prepubertal cows' mammary tissue, whereas Thorn et al. (2006) reported the presence of this isoform in parenchymal and extraparenchymal tissue at the same physiological stage. These last authors also reported that *Ob-Ra* and *Ob-R_{total}* transcripts were present in parenchymal and extraparenchymal mammary tissues but at levels less than 0.8% of those in the hypothalamus. Nevertheless, most studies indicate that *Ob-Ra* predominates over *Ob-Rb* transcripts in mammary parenchymal tissue of farm animals (Table 1). Although it is currently difficult to draw a clear picture of the leptin receptor expression profile in mammary tissues of farm animals, the presence of *Ob-Ra* and *Ob-Rb* mRNA in mammary epithelial cells confirmed a local synthesis of leptin receptor transcripts (Sayed-Ahmed et al., 2004).

Effect of Leptin on Mammary Cell Proliferation and Differentiation

To determine the effect of leptin on mammary epithelial cell proliferation, MAC-T cell and PMEC models were used. Silva et al. (2002) reported that adding physiological doses of recombinant ovine leptin (**oleptin**) to MAC-T cells had no effect on cell proliferation, whereas the highest doses of **oleptin** (64 and 100 ng/mL) decreased IGF-I-stimulated cell proliferation. These findings are in contrast to those of Thorn et al. (2006), who observed no effect of leptin (100 ng/mL) on MAC-T cell or PMEC proliferation. Although valuable, studies using isolated epithelial cells do not reflect the physiological environment of the whole mammary gland, which contains different cell types such as adipocytes and immune cells and which is affected by locally produced and systemic hormones and

factors. To investigate the effect of leptin *in vivo*, Silva et al. (2008) performed intramammary infusions of recombinant **oleptin** in prepubertal Holstein heifers. As expected, intramammary infusion of IGF-I increased epithelial cell proliferation compared with saline infusions. Conversely, infusions of leptin or IGF-I + leptin decreased mammary cell proliferation compared with saline or IGF-I treatments (Silva et al., 2008). However, leptin concentrations in parenchymal tissue of mammary glands infused with **oleptin** were 25-fold greater than concentrations when either saline or IGF-I were infused, corresponding to supraphysiological doses of leptin (Silva et al., 2008). Additional studies are needed to determine whether infusion of physiological doses of **oleptin** will provide similar findings. Plasma leptin can be increased through greater nutrient intake. Indeed, plasma leptin concentrations were greater (2.26 ± 0.098 ng leptin/mL) in Holstein heifers fed a high plane of nutrition (e.g., 29% CP and 0.32 Mcal GE intake per kilogram of BW to reach 950 g/d BW gain) compared with 1.99 ± 0.050 ng leptin/mL for heifers being restricted-fed (e.g., 22% CP and 0.20 Mcal GE intake per kilogram BW to reach 650 g/d BW gain; Meyer et al., 2006). However, this long-term diet-induced increase in systemic leptin concentrations did not affect mammary epithelial cell proliferation. Physiological serum concentrations of leptin vary between 3 and 10 ng/mL in cattle (Ehrhardt et al., 2000), suggesting that the increase in plasma leptin of heifers fed the high plane of nutrition was quite moderate (Meyer et al., 2006). Interestingly, a high plane of nutrition (e.g., 12.7 MJ ME and 228 g CP per kilogram DM to reach 1,202 g of ADG) in prepubertal heifers increased weight of the MFP and *LEP* mRNA abundance in the MFP compared with restricted feeding (8.4 MJ ME and 116 g CP per kilogram DM to reach 715 g of ADG; Thorn et al., 2008).

To our knowledge, there is only 1 paper addressing the effect of leptin on bovine mammary epithelial cell differentiation. In that study, a 3-dimensional primary cell culture model of mammary epithelial cells collected from a pregnant cow was used (Yamaji et al., 2007). The model was validated with the addition of human hepatocyte growth factor (**HGF**), which allowed the formation of ductal-like organoids after 14 d of culture. Human leptin alone had no effect on cell differentiation, and treating cells with HGF + leptin significantly decreased the average size of organoids. The inhibitory effect of leptin on HGF-induced organoid formation was more pronounced when lactogenic hormones were added to the culture media (Yamaji et al., 2007). Of interest, Thorn et al. (2010) observed a complete arrest of mammary ductal growth in *s/s* mice, which present a mutation at tyrosine 1138 of the

Ob-Rb receptor, preventing the activation of STAT3 and related intracellular signaling pathways. Although this last study was performed in mice, it brings additional evidence that leptin may indeed affect mammary cell differentiation.

Effect of Leptin on Lactogenesis and Galactopoiesis

Some studies used mammary gland explant culture models, which offer the advantage of including all mammary cell types, to investigate the role of leptin in lactogenesis and galactopoiesis. Using this type of in vitro model, Feuermann et al. (2004) first reported that PRL increases *Ob-R_{total}* (25-fold) and *LEP* (2.2-fold) mRNA abundances in mammary explants from lactating cows. Conversely, PRL had no effect on *Ob-R_{total}* and *LEP* gene expression when mammary explants were from postpubertal calves (Feuermann et al., 2004), probably because of the low expression of PRL receptors at this physiological stage. Significant increases in α -casein and β -lactoglobulin mRNA abundances and in fatty acid synthesis were also observed with the addition of leptin and PRL to mammary explants of lactating cows, whereas leptin alone had no effect (Feuermann et al., 2004). These results demonstrate that leptin, in combination with PRL, may play a role during lactogenesis by increasing milk protein and fatty acid synthesis. The same research team further reported that the addition of PRL to mammary gland explants from lactating cows increases *LEP* mRNA abundance and leptin protein synthesis and secretion (Feuermann et al., 2006). Addition of PRL also increased the α -casein mRNA abundance in these explants, but leptin alone had no effect. When leptin and PRL were added to explant cultures, the effect on α -casein transcript abundance was greater than with PRL treatment alone, and this increase was abolished with the addition of a leptin antagonist (Feuermann et al., 2006). Moreover, the PRL-induced increase in α -casein mRNA abundance was much greater when mammary epithelial cells were coincubated with mammary fat explants, and this increase was abolished with the addition of a leptin antagonist. Findings using mammary fat-epithelial co-cultures show that leptin synthesis and secretion from mammary fat explants can affect milk protein synthesis in lactating dairy cows (Feuermann et al., 2006). Using a similar mammary explant culture model, Feuermann et al. (2008) demonstrated that addition of PRL and leptin increased DNA synthesis (e.g., proliferation) and decreased apoptosis, whereas leptin alone had no effect. Interestingly, the expression of mTOR protein was also increased in mammary explants treated with PRL and leptin but not with leptin or PRL alone. Because mTOR is a serine/

threonine protein kinase playing a central role in protein synthesis and cell proliferation (Saxton and Sabatini, 2017), we may suggest that the observed increase in mTOR protein strengthens the hypothesis that leptin regulates the action of PRL on mammary epithelial cell proliferation and on milk protein synthesis.

In mammary explants from lactating cows, leptin alone upregulated the *estrogen receptor α* (*ER- α*) mRNA abundance in a dose-dependent manner and addition of leptin + PRL + estrogen resulted in the highest expression of *ER- α* . Leptin antagonist was also shown to downregulate the PRL-induced expression of *ER- α* transcript (Feuermann et al., 2009). Addition of PRL alone to mammary gland primary cell cultures had a limited effect on *ER- α* expression, and in co-cultures with fat explants, PRL increased *ER- α* expression. This suggests that fat-derived factors, such as leptin, are needed to mediate PRL-induced regulation of *ER- α* expression in mammary epithelial cells.

Milk Leptin and Its Biological Significance in the Neonate

Leptin is present in colostrum and milk of farm animals (Table 3). In dairy cows, plasma and milk leptin concentrations progressively increase from the first week of pregnancy up to the dry-off period, and leptin concentrations are greater in milk than in plasma from wk 21 to 28 of pregnancy (Parola et al., 2007). These authors suggested that the high local and systemic leptin concentrations found at the end of lactation might be needed to initiate mammary involution and start a new cycle of epithelial cell differentiation. This is supported by the observed increase in apoptotic cells and the activation of signal transducer and activator of transcription 3 (STAT3) phosphorylation with the addition of recombinant leptin to bovine mammary explant cultures (Li et al., 2010). High circulating leptin concentrations may also affect feed intake at the end of gestation. A decline in milk leptin concentrations is generally observed during the first week postpartum in dairy cows (Pinotti and Rosi, 2006; Parola et al., 2007), goats (Whitley et al., 2005), sheep (Tygesen et al., 2008), and sows (Woliński et al., 2014), with the greatest leptin concentrations being present in colostrum. Leptin pooling in colostrum may be due to the presence of leaky tight junctions (Nguyen and Neville, 1998). In sheep, the greater concentrations of milk leptin at parturition were suggested to be due to leptin accumulation in the udder before parturition (McFadin et al., 2002). During lactation, greater leptin concentrations are found in colostrum and milk than in blood of farm animals (Table 3), suggesting that a certain amount of milk leptin is synthesized and secreted by the mammary gland. High leptin concentrations

Table 2. Adiponectin and adiponectin receptor transcripts in mammary tissues and cells of farm animals

Species	Parenchyma	Extraparenchyma/ fat pad	Mammary cells	Physiological stage
<i>Adiponectin (ADIPOQ)</i>				
Gilt	Transcript (+) ¹	Transcript (+)	NR ¹	End of gestation (d 110; Farmer et al., 2016a,b)
Cow	Transcript (±) ¹	Transcript (+)	PMEC ² (–) ³	2–5 mo of lactation (Feuermann et al., 2006)
	Transcript (+)	NR	PMEC (–)	Nonpregnant, lactation, and dry off (Ohtani et al., 2011)
	Transcript (±)	NR	NR	Periparturient (d –30 to +21; Giesy et al., 2012)
	Transcript (±)	NR	NR	Lactation (Saremi et al., 2014)
	Transcript (±)	NR	Alveolar epithelial cells (+)	Lactation (Lecchi et al., 2015)
<i>Adiponectin receptors (ADIPOR1 and ADIPOR2)</i>				
Gilt	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	NR	End of gestation (d 110; Farmer et al., 2016a,b)
Cow	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	NR	PMEC: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	Nonpregnant, lactation, and dry off (Ohtani et al., 2011)
	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	NR	NR	Periparturient (d –30 to +21; Giesy et al., 2012)
	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	NR	NR	Lactation (Saremi et al., 2014)
	Transcript: <i>ADIPOR1</i> (+) and <i>ADIPOR2</i> (+)	NR	NR	Lactation (Lecchi et al., 2015)

¹NR = not reported.²PMEC = primary mammary epithelial cells.³(+) = detected; (–) = absent; (±) = negligible expression.

in colostrum may favor leptin absorption in neonates before gut closure (Le Dividich et al., 2005). Such absorption was previously demonstrated by Wolinski et al. (2003), with greater leptin concentrations detected in the plasma of sow-reared piglets compared with that of piglets receiving only milk formula. Whitley et al. (2009) also showed greater serum leptin concentrations in newborn piglets that were allowed to suckle vs. being subjected to delayed suckling. The effect of ingested leptin on intestinal length and villi height in neonatal piglets (Wolinski et al., 2003) and the presence of leptin receptors in the porcine small intestine (Hansen et al., 2008) suggest a role for leptin in intestinal maturation. Because leptin is involved in energy metabolism and feed intake, the possible effect of milk leptin on newborn growth was also investigated. In goats, there was no correlation between milk leptin concentrations and newborn weight (Whitley et al., 2005). Moreover, neonates submitted to delayed suckling on the first day after birth had similar ADG and weaning weights compared with piglets allowed to suckle (Whitley et al., 2009). Woliński et al. (2014) showed negative correlations between leptin colostrum concentrations and neonate BW on d 1. On the other hand, injections of human leptin to piglets from d 3 to 8 increased their growth rate (on d 9), suggesting a possible role of circulating leptin for piglet growth (Litten et al., 2005). Further studies are clearly needed, especially for the long-term effects of milk leptin. Other roles have been suggested for leptin

in the neonate, including thermoregulation in newborn lambs (Mostyn et al., 2002) and regulation of immunity (Lacetera et al., 2010).

ADIPONECTIN

Adiponectin is a 30-kDa protein hormone mainly produced and secreted by the WAT. It is one of the most abundant adipokines in circulation, and its serum concentrations are inversely associated with body fat mass (Lara-Castro et al., 2006). Different adiponectin isoforms are detected in blood including low molecular weight (approximately 90 kDa), medium molecular weight (approximately 180 kDa), and high molecular weight (HMW; ≥250 kDa) oligomeric complexes having different biological activities (Kadowaki et al., 2006). Obesity and type 2 diabetes are associated with reduced concentrations of circulating adiponectin, particularly the HMW forms (Weyer et al., 2001; Basu et al., 2007). Adiponectin can affect several physiological processes in different tissues and organs including cell growth and survival, fatty acid oxidation, protein synthesis and degradation, inflammatory processes, and glucose uptake (see review by Brochu-Gaudreau et al. [2010]).

Table 3. Circulating and milk concentrations of leptin and adiponectin in human and farm animals at different lactation stages

Species	Blood concentration	Colostrum and milk concentration	Lactation period
Leptin, ng/mL			
Pig	Serum, 2.8 ± 0.2 to 4.0 ± 0.2	Whole milk, 36 ± 6.0 Skim milk, 18 ± 4.0	d 1 to 22 (Estienne et al., 2000; Bonnet et al., 2002a)
	Serum, 2.34 to 3.83	Whole colostrum, 41.8 to 42.8 ± 1.17 Whole milk, 33.7 to 35.0 ± 1.51	d 1 to 18 (Farmer et al., 2007)
	Plasma, 2.09 ± 0.13 to 2.19 ± 0.21	Whole colostrum, 56.2 ± 7.1 Whole milk, 24.7 ± 1.3	d 1 to 7 (Woliński et al., 2014)
Cow	Plasma, 2.17 ± 0.10 to 2.64 ± 0.10	Skim milk, 6.1 to 13.9 ± 0.48	d 1 to 30 (Pinotti and Rosi, 2006)
	Plasma, 4.3 ± 1.4	Whole milk, 3.8 ± 1.0 to 9.0 ± 0.9	wk 1 to 28 (Parola et al., 2007)
Goat	NR ¹	Whole milk, 7.8 ± 0.6 Skim milk, 5.0 ± 0.0	d 270 to 300 (Bonnet et al., 2002a)
	Plasma, 2.58 to 2.80 ± 0.11	Whole milk, 7.64 to 7.81 ± 0.83	d 133 to 163 (Rosi and Rapetti, 2004)
Sheep	Plasma, approximately 0.7 to 1.4	Whole colostrum, approximately 1.35 to 1.55 Whole milk, approximately 0.9 to 1.35	d 1 to 28 (Tygesen et al., 2008)
	NR ¹	Whole milk, 3.4 ± 1.0 to 73.2 ± 13.8 Skim milk, 0.2 to 1.5 ± 0.9	d 7 to 120 (Bonnet et al., 2002a)
Human	Serum, 2.42 to 3.27	Skim milk, 0.83 to 1.18	<2 to 4–6 mo (Savino et al., 2016)
	NR ¹	NR ¹	NR ¹
Adiponectin, µg/mL			
Cow	Plasma, 32.1 ± 1.0	Skim milk, 0.6 ± 0.03	wk 2 and 12 (Singh et al., 2014)
	Plasma, approximately 2.5 to 23	Colostrum (d 1), 56.1 to 75.9 Colostrum (d 3), 2.67 to 3.32	d 1 to 3 (Kesser et al., 2015)
Goat	Plasma, 26.2 ± 4.2 to 33.0 ± 3.0	Skim milk, 2.4 ± 0.2 to 3.9 ± 0.2	d 20 to 80 (Guzel et al., 2017)
Human	Serum, 8.37 ± 3.36	Skim milk, 21.57 ± 5.12 ng/mL	1 to 24 mo (Woo et al., 2012)

¹NR = not reported.

Local Synthesis of Adiponectin in Mammary Tissues and Cells

The interest for adiponectin in ruminants and its potential effects on mammary development and function is relatively new. Studies demonstrating mammary adiponectin expression in dairy cows and gilts are presented in Table 2. Feuermann et al. (2006) were the first to report the presence of *adiponectin* (*ADIPOQ*) transcripts in mammary explants and mammary fat explants from lactating cows. However, no *ADIPOQ* transcript could be detected in PMEC, suggesting that *ADIPOQ* transcripts observed in mammary explants may be due to embedded adipocytes. *Adiponectin* transcripts were also detected in the mammary gland of Holstein heifers (65 d old), with a 42-fold greater expression in MFP than in parenchymal tissue (Piantoni et al., 2010). This raises the possibility that adiponectin from MFP may act in a paracrine manner on parenchymal cells, which express adiponectin receptors (Table 2). Ohtani et al. (2011) reported that mammary *ADIPOQ* gene expression is modulated by the cow's lactation stage, with greater expression found in the nonpregnant and dry-off stages and lesser expression

during peak and late lactation. These results suggest that mammary adiponectin expression might be affected by lactogenic hormones. This is supported by the inhibitory effect of PRL on adiponectin release, previously demonstrated in human WAT (Brandebourg et al., 2007). However, this has never been investigated in mammary tissues of farm animals. Interestingly, a PRL-dependent STAT5 binding site was identified in the murine adiponectin promoter (White et al., 2016), suggesting that PRL may indeed regulate *ADIPOQ* transcription. However, the observed decrease of *ADIPOQ* gene expression in lactating cows (Ohtani et al., 2011) may be caused by a reduction of mammary stromal adipose cells, known to be less abundant during lactation. Weak *ADIPOQ* transcript abundance was also observed in the mammary tissue of periparturient cows, with estimated mammary *ADIPOQ* transcript levels never exceeding 0.05% of those found in WAT (Giesy et al., 2012). Similarly, Saremi et al. (2014) estimated the *ADIPOQ* mRNA abundance in WAT to be 900 times higher compared with its expression in mammary parenchyma. In a recent paper, Lecchi et al. (2015) detected *ADIPOQ* transcripts in the cow's

mammary parenchymal tissue but at a quite negligible expression level. In this study, adiponectin protein was detected in epithelial cells of the secretory alveoli, supporting the capacity of mammary epithelial cells to synthesize adiponectin, although in low abundance.

In pigs, only 2 studies have reported the presence of *ADIPOQ* transcripts in mammary tissues (Farmer et al., 2016a,b). Gene expression of *ADIPOQ* was confirmed in parenchymal tissue of gestating gilts (d 110), but its weak expression suggests that transcripts may originate from embedded adipocytes. This hypothesis is strengthened by positive correlations found between parenchymal *ADIPOQ* mRNA abundance and percent fat ($r = 0.43$) and DM ($r = 0.34$) in parenchyma and the negative correlation with parenchymal protein percent ($r = -0.38$). The *ADIPOQ* transcript was also found in extraparenchymal tissue of these gilts with 2 times greater expression level in WAT than in extraparenchymal tissue (M.-F. Palin et al., personal communication). There was no correlation between *ADIPOQ* mRNA abundance in extraparenchymal tissue of gilts and mammary composition variables, suggesting that the impact of locally produced adiponectin is quite limited on mammary composition in gestating gilts.

Regulation of Adiponectin Receptor Expression in Mammary Tissue and Cells

The pleiotropic effects of adiponectin are mediated through 2 adiponectin receptors (ADIPOR1 and ADIPOR2) that are detected in several peripheral tissues and organs (Brochu-Gaudreau et al., 2010). Adiponectin receptors expression is reduced in the WAT of obese subjects, and mice lacking both ADIPOR1 and ADIPOR2 are glucose intolerant and hyperinsulinemic (Yamauchi et al., 2007). Mice knockout studies provided key information on the opposing effects of ADIPOR1 and ADIPOR2 on energy metabolism. Indeed, ADIPOR1^{-/-} mice present a decrease in energy expenditure and become obese and glucose intolerant, whereas ADIPOR2^{-/-} mice show an increase in energy expenditure and are resistant to high-fat-diet-induced obesity (Bjursell et al., 2007).

Transcripts for *ADIPOR1* and *ADIPOR2* were detected in mammary parenchymal tissue of cows at different stages of lactation and also in bovine PMEC (Table 2), with *ADIPOR1* mRNA abundance being 2 times greater than *ADIPOR2* (Giesy et al., 2012). The presence of adiponectin receptors in mammary parenchymal cells suggests that adiponectin from the circulation or locally produced may affect mammary development and function. Ohtani et al. (2011) were the first to report the presence of *ADIPOR1* and *ADIPOR2* transcripts in bovine mammary tissue. They also

showed that *ADIPOR1* mRNA abundance in the parenchymal tissue was greater at peak lactation than during the dry-off period, whereas *ADIPOR2* was not affected by lactation stages. Interestingly, *ADIPOR2* mRNA abundance was increased by lactogenic hormones and GH in PMEC but not the *ADIPOR1* transcript (Ohtani et al., 2011). In a recent paper, Saremi et al. (2014) reported that the mRNA abundance of *ADIPOR1* and *ADIPOR2* increased with days in milk (d 1 to 105). Conversely, there was no difference in *ADIPOR1* and *ADIPOR2* gene expression in mammary biopsies collected 30 d before calving, at parturition, and on d 21 of lactation (Giesy et al., 2012). Collectively, these results suggest that *ADIPOR1* and *ADIPOR2* gene expression in mammary tissue is affected by the cow's lactation stage and that lactogenic hormones and GH can upregulate *ADIPOR2* gene expression but not that of *ADIPOR1*. In the pig, we were able to detect *ADIPOR1* and *ADIPOR2* transcripts in mammary parenchymal and extraparenchymal tissues of pregnant gilts (d 100; Farmer et al., 2016a,b; M.-F. Palin et al., personal communication). We also observed that *ADIPOR1* and *ADIPOR2* gene expression in parenchymal and extraparenchymal tissues is not affected by body condition (backfat thickness) of gilts.

Milk Adiponectin and Its Biological Significance in the Neonate

Adiponectin was detected in human breast milk, goat milk, and colostrum and milk of cows (Table 3). In dairy cows, milk adiponectin concentrations are approximately 100-fold greater than those of leptin (Table 3). Milk adiponectin concentrations in cows are also 7 to 142 times greater than in human milk, despite the observation that blood concentrations are quite similar (Table 3). These differences may be explained by the hemochorial placenta in humans, which allows the transfer of HMW maternal proteins to the fetus, whereas the epitheliochorial placenta of cows prevents HMW protein transfer. Therefore, newborn calves rely on colostrum for supply of adiponectin and during the periparturient period, bovine mammary epithelial cells have leaky tight junctions, thereby allowing the transfer of large molecules from blood into the colostrum (Nguyen and Neville, 1998). In dairy cows, the important decrease in colostrum adiponectin observed between d 1 and 3 postpartum (Table 3; Kesser et al., 2015) suggests a transfer and accumulation of adiponectin in the mammary gland due to the presence of leaky tight junctions on d 1 followed by a reduction of adiponectin concentration on d 3, likely related to closure of tight junctions. These authors also demonstrated that circulating adiponectin concentrations in

newborn calves are associated with colostrum intake. A sharp increase (3- to 4-fold) in calf's serum adiponectin was observed immediately after the first colostrum intake. The transfer of HMW adiponectin from colostrum to the calf serum was also demonstrated by western blot analyses (Kesser et al., 2015). These findings suggest that milk adiponectin resists gut proteolysis, is absorbed by the intestinal mucosa, and transferred to the circulation of newborn calves.

The putative role of milk adiponectin remains to be determined. However, the inverse correlation between somatic cell count and adiponectin concentrations in goat milk suggests a role for adiponectin in udder health (Guzel et al., 2017). The observation that adiponectin-deficient mice have reduced intestinal epithelial cell differentiation (Saxena et al., 2013) and the presence of adiponectin receptors in the intestine of human (Yoneda et al., 2008) and mouse embryos (Zhou et al., 2005) further suggest a role for adiponectin in neonatal intestinal maturation. The HMW adiponectin is considered the most active isoform based on its activation of AMP-activated protein kinase (AMPK), a known energy sensor, and on its role in improving insulin sensitivity (Brochu-Gaudreau et al., 2010). Because the primary adiponectin isoform found in cow's milk is the HMW isoform, it was suggested that adiponectin could also play a role in calf growth and energy metabolism. The influence of human milk adiponectin on infant growth was shown (Woo et al., 2012; Weyermann et al., 2007), but this was never investigated in farm animals.

OTHER ADIPOKINES

There are few studies on the role of other adipokines in mammary development and functions in live-stock species. Some adipokines known to be important regulators of energy metabolism, food intake, and insulin sensitivity were found in human milk (Çatlı et al., 2014). Among these, apelin, ghrelin, and resistin were detected in the colostrum and milk of farm animals (Rosi and Rapetti, 2004; Mesmin et al., 2011; Woliński et al., 2014; Guzel et al., 2017), but their potential role in neonatal growth and health has never been investigated. In a recent paper, Suzuki et al. (2015) reported that chemerin transcripts were not detected in MAC-T cells and weak transcript levels were observed in the bovine mammary gland, probably due to the presence of embedded adipocytes. The Chemerin receptor (*chemerin chemokine-like receptor 1* [*CMKLR1*] and *C-C motif chemokine receptor-like 2* [*CCRL2*]) transcripts were detected in mammary tissue and in MAC-T cells, suggesting a possible role for chemerin in the bovine mammary gland. This was further demonstrated

by the observation that addition of human chemerin to MAC-T cells increased the *CMKLR1* and *CCRL2* mRNA abundance, along with genes involved in fatty acid and casein synthesis, glucose uptake, and insulin sensitivity (Suzuki et al., 2015). Moreover, the addition of lactogenic hormones to MAC-T cells down-regulated the *CMKLR1* gene expression. It was also suggested that ghrelin could play a role in the regulation of mammary functions. During lactation (d 30 to 180) and dry off (d 30), ghrelin and its receptor, the growth hormone secretagogue (GHS) receptor type 1a (GHSR-1a), were detected in alveolar and ductal epithelial cells of goat mammary tissues (Zhang et al., 2013). The mRNA abundance of *ghrelin* (*GHRL*) and *GHSR-1a* in goat mammary tissue is also modulated according to lactation stage, with greatest values being observed on d 60 of lactation for both genes (Zhang et al., 2013). Most importantly, the addition of human ghrelin to mammary explant cultures of lactating goats increased the synthesis of β -casein in a dose-dependent manner, whereas the addition of a GHSR-1a antagonist abolished the observed increase. Ghrelin was also shown to increase the proliferation of PMEC (Zhang et al., 2013).

SUMMARY AND CONCLUSIONS

In farm animals, mammary growth and function depend on steroid and lactogenic hormones derived from the ovaries, the pituitary gland, and placenta. However, accumulating evidence suggests that their actions are locally mediated through biologically active molecules including growth factors and adipokines. Hence, this review clearly shows that adipokines, such as leptin and adiponectin, are important regulators of lactogenic hormone actions in the mammary gland. Although this review mainly covered the direct effects of locally produced adipokines on mammary growth and function, circulating adipokines may also act centrally with possible effects on the secretion of lactogenic hormones. Moreover, systemic adipokines may also act as energy sensors in the regulation of metabolic adaptations occurring in late pregnancy and lactation. Most of the work on the effects of adipokines on mammary growth and function was conducted on leptin and adiponectin, and studies on ghrelin, apelin, resistin and chemerin are just emerging. Therefore, additional work is needed to determine the role of these new adipokines in mammary gland development, milk synthesis, and growth and development of the neonate.

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