1. Introduction

With livestock animals as matter of research, physiology needs to consider a complex system with numerous interactions and feedback mechanisms. This system does not only comprise the biological organism of the animal itself, but also the herd with the interactions between animals, and the underlying technical and human subsystems involving the natural environment and also socio-economic factors. In view of the worldwide declining arable land and increasing population, the use of land for "food, feed or fuel" needs to be balanced. The ability of livestock, in particular ruminants, to make use of grasslands otherwise not accessible for food production, as well as the option of using human food "waste" for feed provides an opportunity to feed more people and also presents other advantages.

With this background, animals that are able to efficiently convert nutrients into milk or meat are required. Genetic selection for increased productivity can have negative side effects on animal health and welfare. For example, in dairy cows increasing milk yield is commonly related to metabolic disorders, to reduced fertility, lameness and compromised immune function and thus increased susceptibility towards infectious disease e.g. mastitis. In consequence, the turnover rates within a herd increase resulting in a shorter productive life of the animals. To reduce these negative effects different managerial, nutritional, and genetic approaches are used. Nowadays, different feed supplements are introduced aiming to improve health of farm animals and thus counteracting the short productive life.

1.1. Negative energy balance and insulin sensitivity during early lactation

During early lactation, dairy cows undergo a state of negative energy balance (**NEB**) and cows have to mobilize body resources, mainly body fat, to cover the output of energy via milk. Voluntary feed intake does not increase as fast as milk production increases in early lactation; in adaptation to this, homeorhetic changes of hormonal regulation and, in consequence, metabolism occur, leading to a repartitioning of nutrients mainly from fat reserves to the mammary gland (Bell and Bauman, 1997).

Glucose is a primary fuel for fetal life. The placenta translocates glucose from maternal blood circulation in an insulin-independent manner (Bell and Bauman, 1997). This process is facilitated by the development of insulin resistance in peripheral tissues. In

early lactation, the peripheral insulin sensitivity is further reduced, e.g. in muscle and adipose tissue (**AT**) allowing to direct glucose towards the mammary gland (Bell, 1995). Similar to placenta, glucose uptake is an insulin-independent process in the mammary gland tissue as well. The endocrine mechanisms affecting insulin sensitivity are well-documented but the role of AT derived messenger molecules, i.e. of adipokines and also of their receptors in these adaptive processes are only at the outset of being known and understood.

1.1.1. Homeorhetic adaptations around parturition

The importance of a successful transition from pregnancy to early lactation is well accepted and determines the profitability of dairy cows. Health problems, nutrient deficiency, or poor management may impede the ability of cows to reach maximal (production) efficiency (Drackley, 1999). The most critical time for dairy cows is early lactation during which the energy requirements increase up to 4 fold in a short time from pregnancy and dry period to lactation (Carriquiry et al., 2009). In compensation of the new requirements, cows start to mobilize body fat and muscle tissue. Increasing growth hormone serum concentrations directly after parturition (Gross et al., 2011; Doepel et al., 2002) force liver to increase gluconeogenesis and AT to reduce lipogenesis (Renaville et al., 2002). Growth hormone increases the utilization of nonesterified fatty acids (**NEFA**) indirectly via IGF-1 in muscle and in mammary gland by increased blood flow as reviewed by Renaville et al. (2002). Most of the energy requirement during lactation is needed for milk production i.e. in 4 days after parturition, 97% of net energy and 83% of metabolizable energy based on energy and protein intake is used for milk production (Drackley, 1999). The nutrient uptake of the mammary gland is insulin independent therefore other insulin dependent tissues start to increase fatty acid oxidation and to decrease glucose uptake and utilization because of the low plasma insulin concentrations and the decrease in insulin sensitivity in early lactation (Butler et al., 2003). Liefers et al. (2003) showed that the leptin (***LEP***) concentration is high in the dry period and declines to a nadir at parturition thus they suggest that, at least in short term, *LEP* reflects energy balance consequently it is low during early lactation. Recent studies on obesity summarized by Ahima and Osei (2008) identified many new molecules related to insulin sensitivity i.e. adiponectin (***ADIPOQ***) and its receptors (***ADIPOR1*** and ***ADIPOR2***), *LEP* and its receptors [e.g. *LEP* receptor isoform b (***LEPRB***)], interleukin-6 (***IL-6***), tumor necrosis factor-α (***TNF-α***), peroxisome proliferator-activated receptor-γ (***PPARγ***),

PPARγ2, etc. Many of these molecules are AT derived and are named adipokines. Although different aspects of these molecules are well established in monogastrics, there is less information available in ruminants.

1.1.2. Lipid metabolism during the transition period

As already mentioned in previous sections, early lactation is accompanied with NEB and reduced insulin sensitivity. In normal conditions when tissues are insulin responsive, AT deposits fatty acids in the form of triglycerides. Under reduced insulin sensitivity conditions or low insulin concentrations in serum, a situation typical for early lactation, AT starts lipolysis and thus serum NEFA concentrations increase. Extreme rates of lipolysis in AT increase the uptake of NEFA by liver and the liver triglyceride content (Figure 1). In early lactation, cows have a high total lipid and triacylglycerol content in liver whereas glycogen content decreases (Drackley et al., 2005). In severe cases hepatic lipidosis or fatty liver may happen. Otherwise, NEFA is metabolized to ketone bodies or

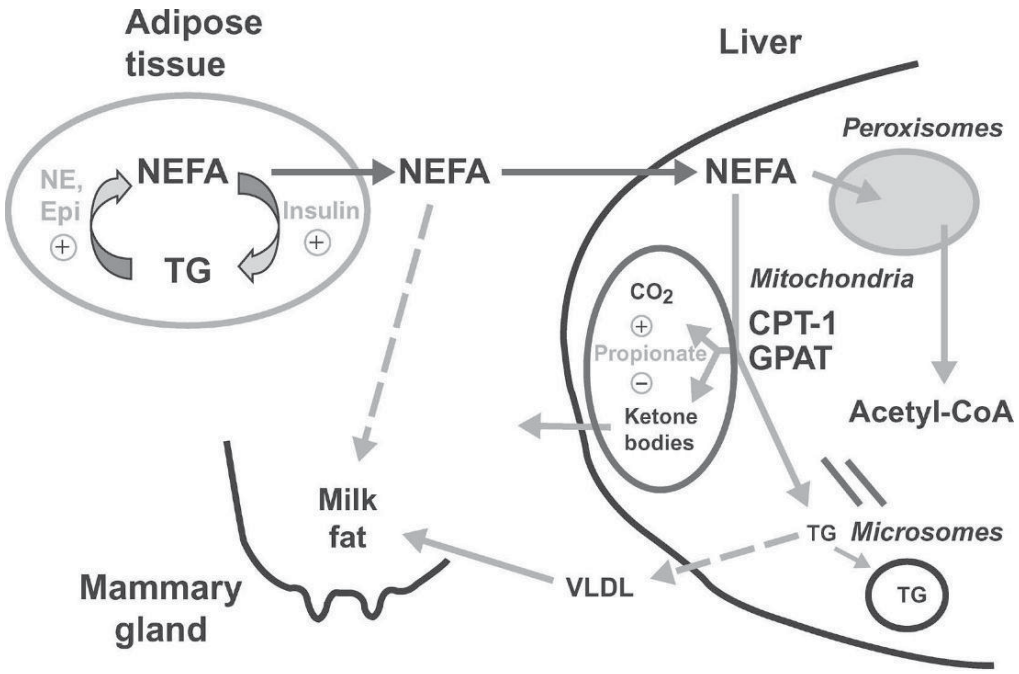


Figure 1: Schematic of metabolic relationships between AT, liver, and mammary gland during the transition period; NE = norepinephrine, Epi = epinephrine, CPT-1 = carnitine palmitoyltransferase-1, GPAT = glycerol-3-phosphate acyltransferase, TG = triglyceride, CoA = coenzyme A, VLDL = very low density lipoprotein (adapted from Drackley et al., 2006).

is released from liver in the form of very low density lipoproteins (**VLDL**). Both NEFA and VLDL are also used in milk fat production as well (Drackley, 1999; Drackley et al., 2006).

1.2. Regulators of insulin sensitivity

1.2.1. *ADIPOQ* system

ADIPOQ was identified in 2001 in bovine fetal serum as a glycoprotein. *ADIPOQ* shows 92 and 82% identity with murine and human *ADIPOQ*, respectively (Sato et al., 2001). *ADIPOQ* is secreted mainly from AT and exerts insulin sensitizing effects via adiponectin receptor 1 and 2 (***ADIPOR1/2***; Figure 2) in humans (Kadowaki and Yamauchi, 2005). The *ADIPOR1* is ubiquitously expressed, including abundant expression in skeletal muscle. The *ADIPOR2* is most abundantly expressed in liver (Kadowaki et al., 2006). *ADIPOQ* reduces the tissue triglyceride content in muscle and liver thus increasing insulin sensitivity via increasing the expression of *PPARα*. *ADIPOQ* also activates AMP activated protein kinase thereby stimulating ß-oxidation and thus decreasing the triglyceride content in liver and in muscle tissue (Kadowaki and Yamauchi, 2005).

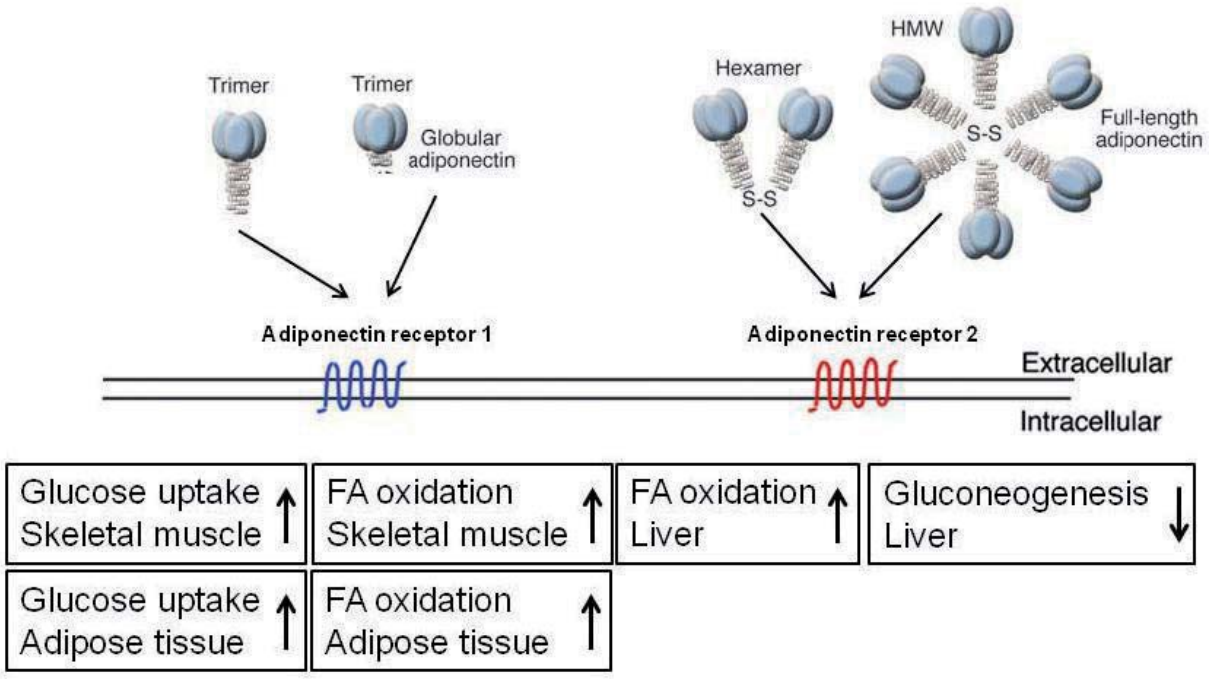


Figure 2: Adiponectin action and its receptors and their functions in different tissues (revised after Kadowaki and Yamauchi, 2005 using Wu et al., 2003 and Puigserver et al., 2001).

In dairy cattle, the mRNA abundance of *ADIPOQ* in subcutaneous (**s.c.**) AT has been reported to be constant (Lemor et al., 2009) or to decrease (Koltes and Spurlock, 2012) during the transition from pregnancy to lactation. In intestinal AT, a down-regulation of *ADIPOQ* mRNA from the dry period to peak and late lactation was demonstrated (Komatsu et al., 2007). For *ADIPOR1* and *ADIPOR2* in s.c.AT, constant (Sadri et al., 2010b) or decreasing values were reported (Lemor et al., 2009). Reduced abundance of *ADIPOQ* and its receptors in AT point to reduced insulin sensitivity during early lactation. *ADIPOQ* is negatively correlated with fat mass and adipocyte size, respectively (Cnop et al., 2003; Skurk et al., 2007).

Studying mammary tissue samples, Ohtani et al. (2011) showed a stable expression of *ADIPOR1* and *ADIPOR2* in peak [60 day postpartum (**p.p.**)] and late (240 day p.p.) lactation. Indeed, *ADIPOR2* had the same level of expression in the dry period in comparison to the lactation period whereas *ADIPOR1* was down-regulated.

1.2.2. *LEP* system

LEP was first identified in mice (Zhang et al., 1994) and subsequently in sheep (Dyer et al., 1997) and cattle (Ji et al., 1998). *LEP* is highly conserved across all species, with human *LEP* showing 84 and 83% homology with mouse and rat *LEP* (Zhang et al., 1994). The word *LEP* comes from the Greek word *Leptos* which means thin. *LEP* regulates feed intake via its effects on the central nervous system. Increasing *LEP* suppresses feed intake and increases energy expenditure (Ricci and Bevilacqua, 2012).

LEP is the first adipokine identified and is secreted mainly but not exclusively from AT and its expression is proportional to fat mass and adipocyte size (Delavaud et al., 2002). *LEP* affects energy homeostasis and improves insulin sensitivity (Houseknecht and Portocarrero, 1998). Serum *LEP* is reduced p.p. vs. antepartum (**a.p.**), but at the level of the mRNA in s.c.AT from tail head of multiparous cows no changes were observed (Lemor et al., 2009; Sadri et al., 2010b). A down-regulation of AT *LEP* mRNA was reported comparing 3 to 10 weeks dry cows with cows in peak lactation, thus AT *LEP* mRNA is higher in dry cows (Komatsu et al., 2007; Thorn et al., 2008).

LEP exerts its effects via its receptors. *LEPR* was first identified in mice (Tartaglia et al., 1995). Multiple splice variants of the *LEPR* gene encode at least 6 different isoforms of *LEPR* in rodents (Ahima and Flier, 2000; Ingvartsen and Boisclair, 2001). They share a common extracellular ligand binding domain but differ in size of the intracellular domain (Figure 3). The long form of *LEPR* - *LEPRB* - has the primary biological role (Bartha et

al., 2005). The *LEPRB* contains intracellular motifs required for activation of the ***JAK*** (janus kinase)-***STAT*** (signal transducers and activators of transcription) signal transduction pathways being the signal transduction pathways attributed to *LEP* (Bjørbæk et al., 1997; Vaisse et al., 1996). The *LEPRB* is mainly expressed in different brain regions, in particular the paraventricular, ventromedial, and arcuate nuclei of the hypothalamus (Williams et al., 1999). *LEPRB* is also expressed in peripheral tissues (Chelikani et al., 2003). In dairy cows' AT, Lemor et al. (2009) reported an increase in mRNA abundance of *LEPRB* comparing days p.p. vs. a.p. consistent with the observations of Thorn et al. (2008) reporting a 10 fold increase in *LEPR* mRNA abundance in AT and a 2 fold increase for the same receptor in liver tissue from late pregnancy to early lactation. Hypoinsulinemia associated to NEB in early lactation might be responsible for the mentioned induction of *LEPRB* in liver and AT (Lemor et al., 2009; Thorn et al., 2008).

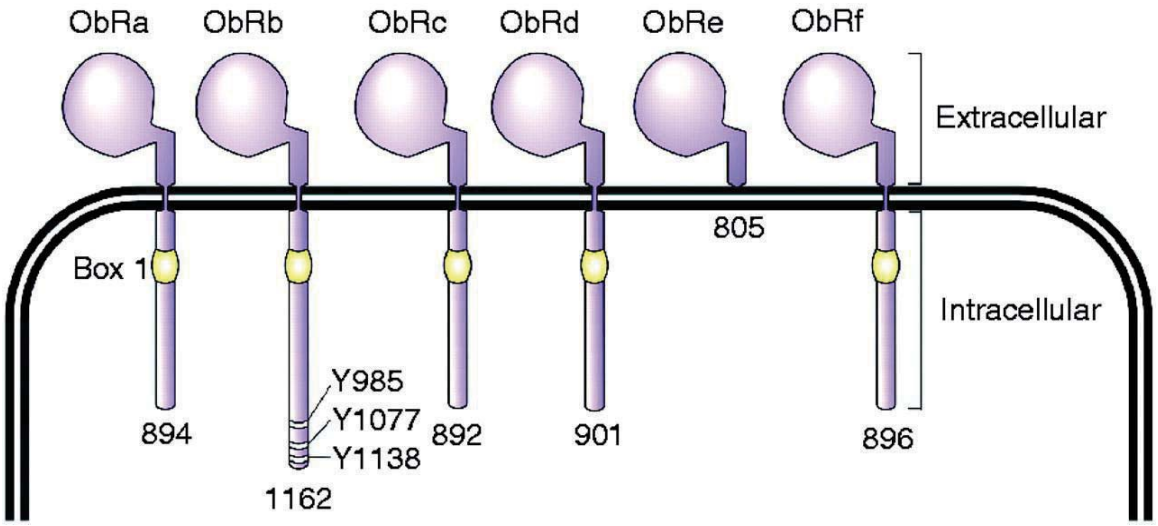


Figure 3: The leptin receptor has six isoforms obtained by alternative splicing, which are designated *ObRa*, *ObRb*, *ObRc*, *ObRd*, *ObRe*, and *ObRf* (also named *LEPRA*, *B*, *C*, *D*, *E*, and *F* respectively). The number below each *LEPR* form indicates the number of amino acids characteristic of each isoform. The box 1 motif is required for *JAK* interaction and activation. However, only the long form (*ObRb*) contains motifs for the complete activation of the JAK/STAT pathway. Three tyrosine residues, whose phosphorylation is important for leptin signaling, are indicated in *LEPRB*: Y985 interacts with the SH2-containing protein tyrosine phosphatase 2, Y1077 with STAT5, and Y1138 with STAT3 (adapted from Marroquí et al., 2012).

1.2.3. *PPARγ* and *PPARγ2*

Peroxisomes are subcellular organelles found in most plant and animal cells that perform diverse metabolic functions including β-oxidation of fatty acids, and cholesterol metabolism. The *PPAR* were identified in 1990 and belong to a nuclear hormone receptor superfamily (Tyagi et al., 2011). Nuclear hormone receptor proteins form a class of ligand activated proteins that, when bound to specific sequences of DNA, serve as on-off switches for transcription within the cell nucleus (Abbott, 2009). The *PPAR* regulate gene expression via binding to specific DNA sequences, peroxisome proliferator response elements (***PPRE***), in the promoter regions of target genes (Figure 4). Prior to DNA binding, *PPAR* forms a heterodimer with the retinoid X receptor (***RXR***), another member of the nuclear receptor super family (Abbott, 2009).

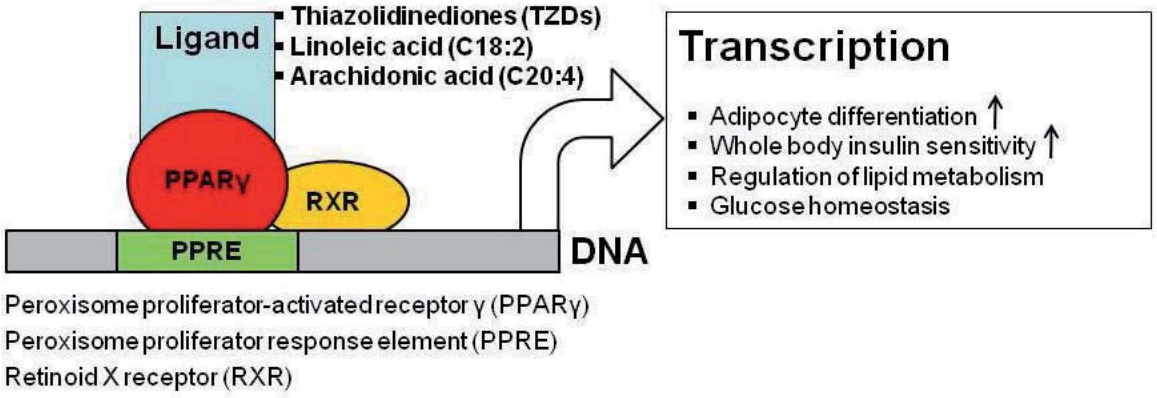


Figure 4: Signal transduction of genes regulated by peroxisome proliferator-activated receptor γ (revised after Houseknecht et al., 2002 using Rosen and MacDougald, 2006 and Herrmann et al., 2009).

The family of *PPAR* includes three members (Figure 5): *PPARα*, *PPARδ*, and *PPARγ*. They play an essential role in energy metabolism; however, they differ in the spectrum of their activity. *PPARγ* regulates energy storage in AT, whereas *PPARα* is expressed predominantly in the liver, and to a lesser extent, in muscle, in the heart, and in bone. *PPARδ*, ubiquitously expressed in the whole body, regulates energy expenditure. *PPARγ* is further subdivided in four isoforms (Evans et al., 2004):

- γ1 - expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas, and spleen.

- γ2 - expressed mainly in AT.
- γ3 - expressed in macrophages, large intestine, and AT.
- γ4 - expressed in endothelial cells.

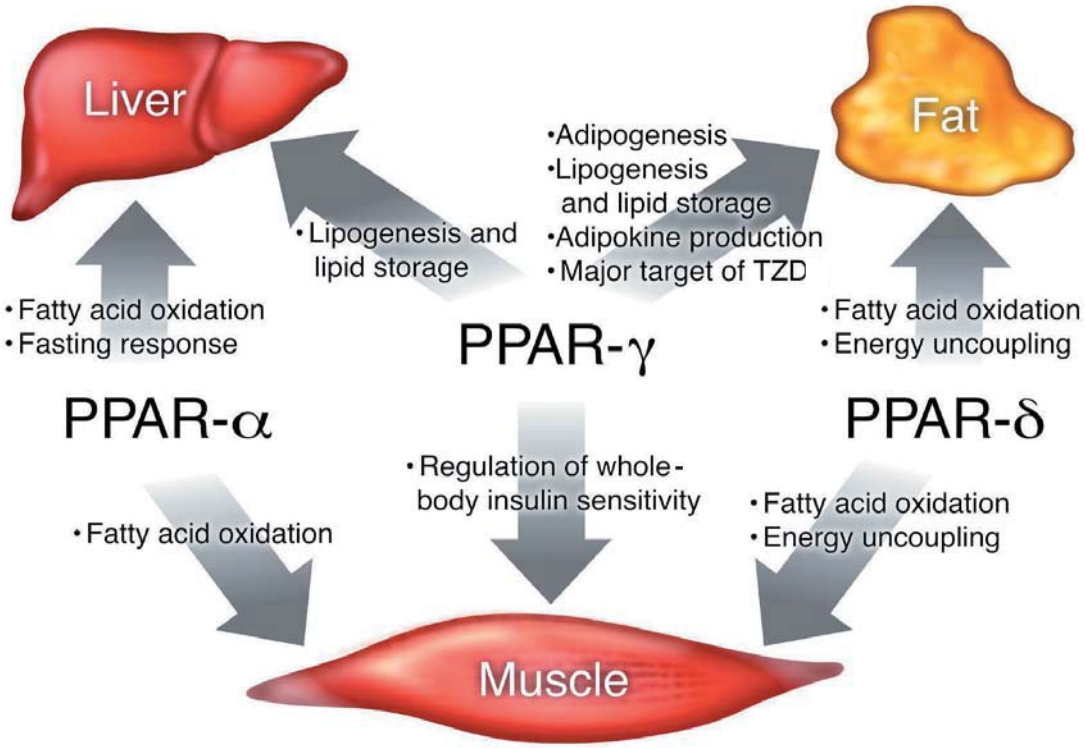


Figure 5: Metabolic integration of *PPAR* regulating energy homeostasis in liver, muscle, and adipose tissue (adapted from Evans et al., 2004). TZD: Thiazolidinediones, *PPAR*: peroxisome proliferator-activated receptor.

PPARγ2 is a nuclear receptor highly expressed in AT (Herrmann et al., 2009). It is important for adipocyte differentiation and regulates insulin sensitivity by transcriptionally activating adipocyte-specific genes involved in insulin signaling, glucose and fatty acid uptake, and lipid-storage (Herrmann et al., 2009). There are different agonists for *PPARγ*. Linoleic and arachidonic acid are two natural agonists. Thiazolidinediones (**TZD**) e.g. Ciglitazone, Pioglitazone, and Rosiglitazone are synthetic agonists (Nosjean and Boutin, 2002). *PPARγ2* agonists increase plasma *ADIPOQ* concentrations and thus improve whole body insulin sensitivity in monogastrics (Combs et al., 2004).

1.2.3.1. *PPARγ2* in AT

The peripartal plasticity of AT in response to changes in hormones and specific nutrients is well documented and is under control of regulators of transcription like *PPARγ* (Loor,

2010). *PPARγ* is a key regulator of insulin sensitivity in non-ruminants (Rosen and MacDougald, 2006). Therefore, nutritional regulation of *PPARγ* around parturition might support insulin responsiveness in bovine AT. The *PPARγ* mRNA is stably expressed after parturition up to 3 weeks p.p. in AT of dairy cows without differences between cows with high or low lipolytic activity as indicated by plasma NEFA concentrations (van Dorland et al., 2011). In addition, no difference between *PPARγ* mRNA expression in AT of cows in peak and late lactation was observed as well (Komatsu et al., 2007). *PPARγ* mRNA abundance in AT increases in cows fed with a high energy diet in comparison to low energy diet. In the same experiment, they found no effect of TZD administration to diet on AT *PPARγ* mRNA (Schoenberg and Overton, 2011). For the first time in dairy cattle, Schoenberg et al. (2011b) reported that TZD up-regulate expression of *PPARγ* mRNA in s.c. tail head fat as well as *LEP* mRNA similar to monogastrics. Therefore, *PPARγ* has an important regulatory effect in bovine AT similar to monogastrics.

1.2.3.2. *PPARγ2* in the mammary gland

In bovine mammary tissue, *PPARγ2* is probably localized in epithelial cells (Bionaz and Loor, 2008). Bionaz and Loor (2008) supported this hypothesis by the low expression of adipocyte specific genes indicating a low number of adipocytes in lactating mammary parenchyma. *PPARγ2* is up-regulated up to 3 fold at the onset and throughout 240 days in lactation in comparison to 15 d a.p. Previously, similar data was reported for *PPARγ*. Although lactating cows were compared to cows dried-off for 3 to 10 weeks a.p., *PPARγ* was stably expressed during the dry period and peak and late lactation (Komatsu et al., 2007). *PPARγ2* agonists i.e. rosiglitazone and long chain fatty acids were not directly influencing mammary *PPARγ2* mRNA expression. However, *PPARγ2* putative target genes (mostly genes related to lipid synthesis) were up-regulated by treatment with *PPARγ2* agonists (Kadegowda et al., 2009).

1.2.3.3. *PPARγ2* in liver

Liver has a major role in energy metabolism during the transition period. Studies in monogastrics associated liver *PPARγ* with triglyceride homeostasis (Gavrilova et al., 2003) as reviewed by Rogue et al. (2010). Deletion of *PPARγ* in liver causes impairment in insulin sensitivity (Gavrilova et al., 2003; Matsusue et al., 2003). A decrease in the triglyceride content of the liver, a reduction in mRNA abundance of genes dealing with

lipid metabolism, and an increase in triglycerides and free fatty acids content occurs in plasma of mice with *PPARγ* gene deficiency. In addition, *PPARγ2* but not *PPARγ1* in murine liver was up-regulated in response to a high butter fat diet (Yamazaki et al., 2011) Thus, high fat diets will induce fatty liver in a *PPARγ2* dependent manner.

In dairy cows, feeding a mixture of 50% each of the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 isomer of conjugated linoleic acid (**CLA**) to dairy cows during late pregnancy and early lactation had no effect on *PPARγ* expression in liver tissue biopsies (Sigl et al., 2010). This observation regarding to changes in *PPARγ* mRNA a.p. and p.p. based on the real-time PCR quantification cycle values revealed no changes in this time period. In an experiment where cows were grouped based on plasma *beta-hydroxybutyrate* (**BHB**) concentrations (van Dorland et al., 2009), no difference in liver *PPARγ* mRNA was found by BHB grouping or by days around parturition (day 70 a.p. to 98 p.p.). In another experiment, hepatic gene expression profiling between cows in mild NEB and severe NEB revealed no shift in *PPARγ* mRNA abundance (McCarthy et al., 2010).

1.2.3.4. *PPARγ2* in muscle and pancreas

In a murine muscle cell line, *PPARγ2* follows the same regulatory pathways as in adipocytes, i.e. *PPARγ2* is reduced in inflammation (*TNF-α* treatment) and the omega-3 polyunsaturated fatty acid eicosapentaenoic acid attenuates this effect (Magee et al., 2012). Indeed, muscle *PPARγ* mRNA abundance increased more than 10 fold in growing steers from 10 to 22 months of age when the size of intramuscular adipocytes was increasing independently of breed (Albrecht et al., 2011). A very low abundance of *PPARγ2* in muscle tissue in comparison to AT was demonstrated as well (Huff et al., 2004).

The *PPARγ* is expressed in pancreatic ß-cells and it could be related to lipotoxic effects of fatty acids in these cells (Kawai et al., 2002). Overexpression of *PPARγ* suppresses insulin secretory capacity of pancreatic islets (Ito et al., 2004). Moreover, *PPARγ* inhibits glucagon gene transcription in islets of pancreatic tissues (Krätzner et al., 2008). Nevertheless, it remains controversial whether *PPARγ* contributes to the function of ß-cells (Welters et al., 2004).

1.2.3.5. Conflicting information available about *PPARγ* isoforms

Two isoforms are defined for *PPARγ* gene in cattle i.e. *PPARγ1* (NIH gene back accession number: Y12419) and *PPARγ2* (accession number: Y12420) (Sundvold et al.,

1997). The two isoforms show 90% homology. At the mRNA level, the isoforms can be differentiated only if at least one of the primer pairs is located at the first 259 bp of *PPARγ2* or at the first 155 bp of *PPARγ1* (Figure 6).

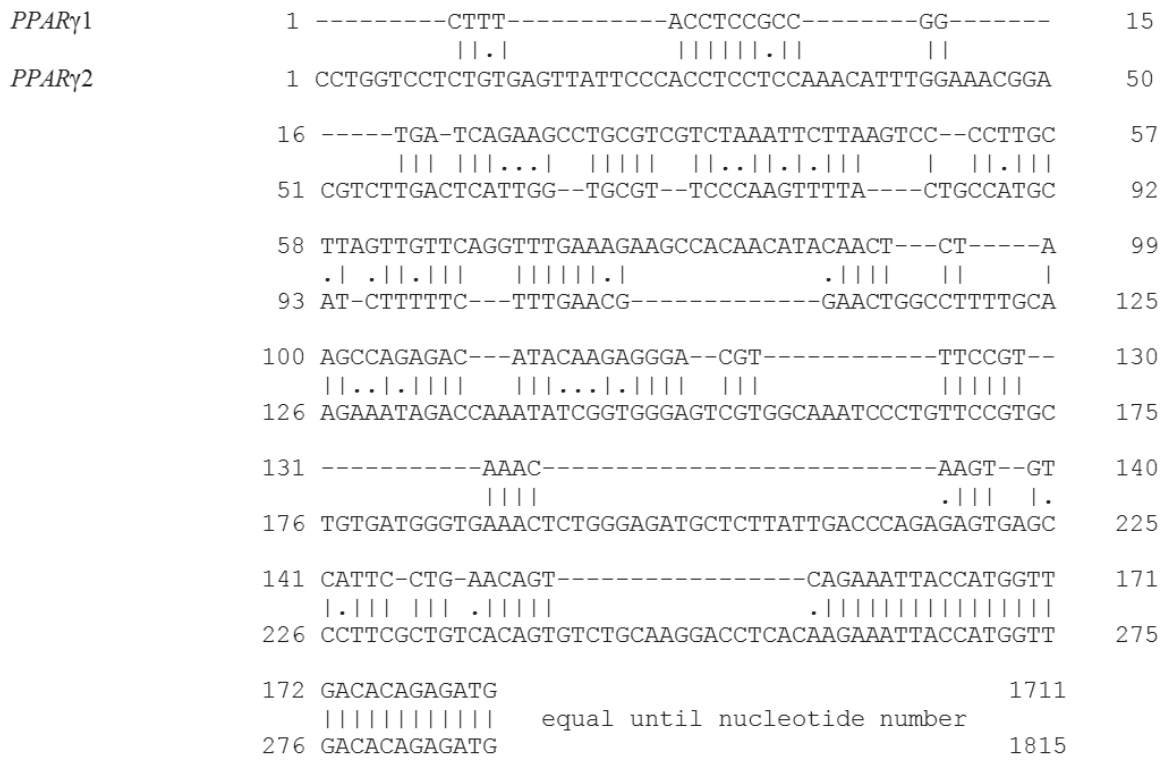


Figure 6. Alignment of bovine peroxisome proliferator-activated receptor γ 1 (*PPARγ1*, NIH gene bank accession number: Y12419) and *PPARγ2* (accession number: Y12420) sequences. Horizontal lines indicate missing base pairs. Vertical lines define homologue sequences. Dots are used for differences between the two sequences. Digits define the number of nucleotides.

Otherwise the primer will not be specific to the isoform. There is another sequence submitted in NCBI database under the name of *PPARγ* (accession number: NM_181024) while is actually 100% identical to *PPARγ2* (accession number: Y12420) and in many cases it is misleading; for example, Kadegowda et al. (2009), Bionaz and Loor, (2008), Schmitt et al. (2011), and Ji et al. (2012) reported a *PPARγ* primer which is actually specific to the *PPARγ2* isoform based on alignment analysis. My survey of the published data (Harvatine et al., 2009; Huff et al., 2004; Komatsu et al., 2007; MacLaren et al., 2006; Mani et al., 2009; McCarthy et al., 2010; Schoenberg and Overton, 2011;

Schoenberg et al., 2011a, b; Sigl et al., 2010; Soliman et al., 2007; van Dorland et al., 2009; van Dorland et al., 2011) revealed that actually many of the published data regarding to *PPARγ* or *PPARγ2* are presenting both isoforms.

1.2.4. *TNF-α* and *IL-6*

TNF-α is a proinflammatory cytokine produced by different cells, mainly by stimulated monocytes. *TNF-α* is involved in inflammation, cell apoptosis, and insulin sensitivity (Antuna-Puente et al., 2008; Galic et al., 2010; Gnacińska et al., 2009; Kershaw and Flier, 2004). High concentrations of *TNF-α* lead to reduced insulin sensitivity (Hotamisligil, 2000) and attenuated secretion of *ADIPOQ* from adipocytes (Maeda et al., 2002). In humans, *TNF-α* is not or only marginally secreted from AT in lean and healthy individuals (Mohamed-Ali et al., 1997). However, *TNF-α* is related to lipid metabolism in AT: the net action of *TNF-α* is to decrease free fatty acid uptake and lipogenesis while increasing lipolysis (Sethi and Hotamisligil, 1999). Indeed, *TNF-α* will increase production of free fatty acids and triglycerides in liver as discussed earlier (Popa et al., 2007).

In dairy cows, a reduction in plasma *TNF-α* is reported after parturition (Schoenberg et al., 2011b; Winkelman et al., 2008). Indeed, recombinant *TNF-α* induced insulin resistance in steers (Kushibiki et al., 2001) and this was also demonstrated in cows with fatty liver (Ohtsuka et al., 2001) indicating negative correlation of serum *TNF-α* with insulin sensitivity.

IL-6, another proinflammatory cytokine, is expressed in different cells i.e. immune cells, endothelial cells, and myocytes. Recently, it was shown that *IL-6* is also expressed in and released from adipocytes. In non-inflammatory conditions, nearby 25% of *IL-6* circulating in blood are estimated to origin from AT in humans (Mohamed-Ali et al., 1997). *IL-6* is closely linked to lipid metabolism (Garcia-Escobar et al., 2010) and insulin resistance (Rotter et al., 2003) beside its role in inflammation. Increased abundance of plasma *IL-6* is linked to greater lipolysis (Morisset et al., 2008). In addition, lipolytic effects of *IL-6* in a porcine adipocyte cell culture system are reported (Yang et al., 2008) similar to human AT (van Hall et al., 2003). Data are still controversial about *IL-6*. In cases of obesity and type 2 diabetes, *IL-6* increases. In contrast, there are studies suggesting beneficial effects of *IL-6* on the disease causing lipolysis without negative effect on insulin-mediated glucose transport (Ji et al., 2011).

1.2.4.1. *IL-6* and *TNF-α* in bovine AT

Both *IL-6* and *TNF-α* are proinflammatory cytokines and initiate an acute phase response (**APR**) in cows (Kushibiki et al., 2003, Nakagawa-Tosa et al., 1995, Yoshioka et al., 2002). However, limited data is available on *IL-6* and *TNF-α* expression in AT of cattle. In dairy cows, AT *TNF-α* mRNA expression is increased from 8 weeks a.p. to the day of parturition and then remains stable for the following 5 weeks p.p. (Sadri et al., 2010a). This links *TNF-α* to the process of body fat mobilization in early lactating cows and initiates a similar role for *TNF-α* in increasing lipolysis in AT as shown for monogastrics (Sethi and Hotamisligil, 1999). In AT culture samples obtained from dairy cows, *IL-6* and *TNF-α* were increased in response to lipopolysacharide (**LPS**) treatment (Mukesh et al., 2010). Thus, adipose depots of dairy cows seems immune responsive and capable of synthesizing proinflammatory cytokines in response to inflammation (Mukesh et al., 2010).

1.2.4.2. *IL-6* and *TNF-α* in bovine liver

In an experiment to test effects of repeated liver biopsies, *IL-6* and *TNF-α* mRNA were detectable in bovine liver biopsies with or without LPS stimulation. The *TNF-α* mRNA abundance was higher than the one of *IL-6* (Vels et al., 2009). Both cytokines were up-regulated after LPS vs. NaCl treatment. In liver of dairy cows, *TNF-α* mRNA exhibited a peak at the day of parturition and another peak at day 49 p.p. (Loor et al., 2005). In addition, restricted energy intake resulted in a more pronounced up-regulation of *TNF-α* (Loor et al., 2006). In consistence with data from monogastrics, treatment of cows with *TNF-α* to introduce chronic inflammation caused an increase in liver triglyceride content thus relating inflammatory pathways to the introduction of fatty liver (Bradford et al., 2009).

IL-6 up-regulates acute phase protein (**APP**) production in hepatocytes isolated from slaughtered cattle if it is added to the cell culture medium in combination with *TNF-α*. However, the presence of only one of these cytokines stimulates concentration of serum amyloid A (***SAA***), but not haptoglobin (Alsemgeest et al., 1996). *IL-6* is expressed in bovine liver Kupffer cells obtained from slaughtered calves and is up-regulated by LPS stimulation (Yoshioka et al., 1998). In microarray and qPCR data of cows with ketosis (Loor et al., 2007), *IL-6* mRNA was 3 fold up-regulated as compared to healthy cows. Network analysis revealed that *IL-6* is related to many liver specific pathways e.g.

lipoprotein metabolism and fatty acid oxidation (Loor et al., 2007). The alteration of pathways affected by *IL-6* might lead to liver lipidosis, ketosis, and insulin resistance (Loor et al., 2007). *IL-6* is thus an important molecule especially regarding the huge amount of NEFA which should be processed in the liver of dairy cows in early lactation.

1.3. Acute phase proteins

Acute phase proteins comprise more than 200 proteins that undergo marked changes in their serum concentration after infection, injury, trauma, or surgery depending on the protein. The APP include negative or positive proteins, i.e. showing a decrease or an increase in their serum concentration in response to stimuli. Albumin and transferrin are examples of negative APP. Haptoglobin, C-reactive protein, *SAA*, ceruloplasmin, fibrinogen, and alpha-1-acid glycoprotein (***AGP***) are examples of positive APP which are mainly produced by hepatocytes although extrahepatic expression was also shown (Murata et al., 2004).

The acute phase response is closely related to the immune response as an early defense mechanism in the body. Production of cytokines and chemokines by immune cells (monocytes, lymphocytes, etc.) is the first step after sensing of antigens or remaining of dead cells by these immune cells. The main proinflammatory cytokines are *IL-6* and *TNF-α*. They have numerous functions as described in the previous chapter including induction of the APR. Production of these cytokines by immune cells causes hepatocytes to produce APP (Cray et al., 2009). The APP have vast biological functions depending on the protein. Herein, the focus will be on haptoglobin and *SAA*.

1.3.1. Haptoglobin

Haptoglobin (**Hp**) is a plasma protein that binds free hemoglobin thereby inhibiting hemoglobin induced oxidative damage (Ceciliani et al., 2012). Haptoglobin comes from the Greek word *haptein* (to bind) and (hemo)globin, and was first discovered in human plasma in the late 1930s. Haptoglobin has different functions as reviewed by Ceciliani et al. (2012) i.e. limitation of iron availability for bacteria, binding hemoglobin and thus prevention of oxidative stress, anti-inflammatory, angiogenesis stimulation, and chaperone-like activity. Haptoglobin in AT might act as a monocyte chemoattractant factor (Maffei et al., 2009).

The Hp and *SAA* are the two main APP in farm animals i.e. cattle, sheep, and goat (Cray et al., 2009). In dairy cows, Hp is the most prominent APP. Although *SAA* response is

quicker than Hp (Heegaard et al., 2000), the Hp concentrations in plasma increase more in comparison to *SAA* in dairy cows (Stenfeldt et al., 2011). Measurement of APP in blood is used as diagnostic marker for diseases as summarized elsewhere (Ceciliani et al., 2012; Cray et al., 2009).

Haptoglobin was shown to be expressed in different cells i.e. leukocytes and milk somatic cells (Thielen et al., 2005) and tissues i.e. liver (Yoshioka et al., 2002), reproductive system (Lavery et al., 2004), digestive tract (Dilda et al., 2012), and mammary gland (Lai et al., 2009; Thielen et al., 2007). Liver is the main source of Hp (Lecchi et al., 2009). However, Hp expression in bovine AT has not been shown until now.

The Hp concentration in blood from healthy dairy cattle is low (< 20 μg/mL). In inflammatory situations, the Hp concentration in blood increases. For example, during an experimental model of dairy cow mastitis (Eckersall et al., 2006), serum Hp concentration increased 5 fold in comparison to a saline control. Injection of 2.5 μg/kg body weight per day recombinant *TNF-α* to lactating dairy cows (Kushibiki et al., 2003) increased serum concentrations of Hp more than 1000 fold in comparison to a saline control. Indeed, APP are related to the mild inflammatory conditions related to obesity and the consequent insulin resistance in humans (Festa et al., 2002). In dairy cows, the association between Hp concentrations in serum and in milk and the metabolic status of dairy cows peripartal was demonstrated (Hiss et al., 2009) i.e. cows having higher NEFA and BHB exhibiting higher Hp in serum.

1.3.2. Serum amyloid A

SAA is an APP belonging to a family of apolipoproteins. In cattle, pigs, horses, dogs, and cats, *SAA* is a major APP (Eckersal and Bell, 2010). In 1988, the *SAA* protein was characterized in dairy cows (Husebekk et al., 1988). Expression of *SAA* increases up to 1000 fold in response to various injuries, infection, etc. From an evolutionary point of view, *SAA* is a highly conserved protein detectable in different vertebrates. Four different *SAA* isoforms have been detected in humans and in mice. Two acute phase *SAA* (***A-SAA***) i.e. *SAA1* and *SAA2* are mainly expressed in liver (Uhlar and Whitehead, 1999). Other isoforms are *SAA3* and *SAA4*. *SAA3* is mainly expressed extrahepatically in rat and mice (Chiba et al., 2009; McDonald et al., 2001). Previously, *SAA3* was known as a pseudogene in human. In 2003, McDonald's group characterized a mammary *SAA3* (***M-SAA3***) in humans that was slightly different in size in comparison to other mammals (Larson et al., 2003). *SAA3* was shown to be secreted from mammary epithelial cells into

colostrum and milk (Molenaar et al., 2009; Eckersall et al., 2006). *SAA3* is the predominant *SAA* isoform in AT (Scheja et al., 2008). *SAA4* is a constitutively expressed *SAA* isoform (***C-SAA4***) which is moderately expressed in response to inflammatory stimuli (de Beer et al., 1996).

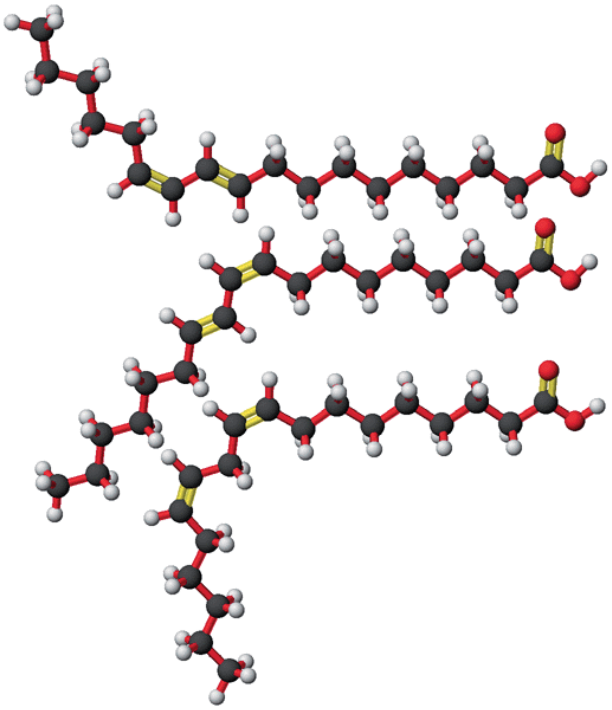
The biological functions of *SAA* are poorly understood. However, *SAA* binds to cholesterol, modulates the innate immune system, and opsonizes gram positive and negative bacteria (Ceciliani et al., 2012). *SAA* similar to the other APP is a biomarker of diseases (Eckersall and Bell, 2010). *M-SAA3* increases in cases of mastitis (Eckersall et al., 2006). In addition to inflammation and infection, *M-SAA3* increases in mid to late involution (Molenaar et al., 2009). Indeed, *M-SAA3* has antibacterial activity against *E. coli*, *Streptococcus uberis*, and *Pseudomonas aeruginosa*. Therefore, *M-SAA3* may have a role in protecting the mammary gland during mammary remodeling and infection and in neonatal calves' gastrointestinal tract as an antimicrobial against infection (Molenaar et al., 2009). *SAA3* is not only an APP but also is shown to be related to obesity and the respective insulin resistance (Lin et al., 2001); it also acts as chemoattractant in AT in which an increase in *SAA3* is linked to recruitment of monocyte-macrophages in AT (Han et al., 2007).

1.4. Conjugated linoleic acids

Conjugated linoleic acids are octadecadienoic acids (C18) containing two conjugated double bonds, separated by a single bond (Figure 7). Different CLA isomers exist; the main CLA isomers are the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 CLA isomer (Pariza et al., 2001).

1.4.1. Use of supplements containing conjugated linoleic acids

Nutritional quality is an important issue making food choice. In addition, consumers are increasingly aware of the links between diet, health, and disease prevention. Moreover, the importance of bioactive food components to enhance human health is increasingly discussed. CLA is one of the bioactive molecules present in animal derived foods. Ruminant products i.e. milk and meat contain CLA isomers. To increase the CLA concentration in these products, CLA is supplemented to the animal's diet. In addition, CLA reduces milk fat production in dairy cattle. Particularly, the *trans*-10, *cis*-12 CLA isomer is known for its milk fat reducing effect (Bauman et al., 2011).



trans-10, *cis*-12 CLA

cis-9, *trans*-11 CLA

linoleic acid (*cis*-9, *cis*-12)

Figure 7. Isomers of conjugated linoleic acids (Adapted from James Cook lab web page http://www.cook.wisc.edu/).

Studies on CLA in dairy cows are divided to diet supplementation, abomasal infusion, and intravenous infusion of individual CLA isomers, mixtures of CLA isomers, or oils rich in linoleic acid (i.e. sunflower or safflower oils). CLA infusion in comparison to dietary supplementation of CLA causes a more severe milk fat depression (**MFD**). There is a curvilinear relationship between abomasal infusion of CLA and MFD. A maximum response of 50% reduction in milk fat yield via infusion of 7.5 g/d *trans*-10, *cis*-12 CLA isomer was achieved. One-half of the maximal effect was achieved by 3.5 g/d infusion of the *trans*-10, *cis*-12 CLA. Infusion of higher amounts of CLA into the abomasum caused a weak additional MFD response (de Veth et al., 2004). Lactating cows use the majority of energy intake for milk production. The energy required for milk fat synthesis is one-half of the energy required for milk production. Therefore, the CLA induced MFD effect could be used effectively to reduce the energy requirements for milk production (de Veth et al., 2004). A meta-analysis was conducted on a data set which was compiled from 14 experiments involving abomasal infusion of CLA isomers for more than 4 days and indeed having a MFD higher than 25%. The results showed that dry matter intake decreases by 1.5 kg/d during CLA induced MFD. The dry matter intake reduction accounts for a part of the energy spared from reduced milk fat synthesis (Harvatine et al.,

2009). In early lactation, the energy spared by MFD can be used for higher yield of milk and/or protein (Bauman et al, 2008). In dairy cows, MFD is considered as a specific effect of CLA at the mammary gland; both short and long term application of CLA to induce MFD had no effect on plasma concentrations of glucose, NEFA, BHB, insulin, IGF-1, growth hormone, and *LEP* in the majority of cases (Baumgard et al., 2002; Baumgard et al., 2000; Castaneda-Gutierrez et al., 2005; de Veth et al., 2006; Perfield II et al., 2002). In conclusion, homeostatic responses seem unaltered. In monogastric animals, CLA are also known for reducing body fat. Plourde et al. (2008) summarized the results of different studies about CLA induced body fat changes in humans and in mice and stressed the discrepancies related to substantially different experimental designs used in humans as compared to animal studies: the adiposity reducing effect of CLA is consistent in mice studies. In dairy cows receiving CLA supplements, body fat seems largely unaffected as evident from various indicators for body fat content and lipolysis at least during short term treatment (Baumgard et al., 2002) or treatment including a 4 week a.p. and a 5 week p.p. interval (Kay et al., 2006). However, when comparing fat cell size in different s.c. and visceral (v.c.) fat depots from CLA treated cows vs. a control group, we recently observed a decrease of adipocyte size with CLA treatment in 5 out of 6 different depots (Akter et al., 2011) whereas fat depot mass was decreased by CLA only in the retroperitoneal fat depot at 105 DIM (von Soosten et al., 2011; Akter et al., 2011). However, reduced mammary lipid synthesis during CLA treatment is consistent with the decreased lipid synthesis and body fat effects in other models, but the cellular responses and mechanisms are quite different, as reviewed by Bauman et al. (2008). The CLA doses used to elicit reductions in body fat are much greater than the doses used to reduce milk fat synthesis in the dairy cow (about 0.5 vs. 0.05% of the diet), and the dose used to induce MFD in the cow is expected to be much lower than the effective dose required to elicit direct AT responses in monogastrics.

1.4.2. CLA supplementation and alteration of gene expression

Genes related to CLA induced MFD in the mammary gland are well documented (Bernard et al., 2008). CLA (*trans*-10, *cis*-12) induced MFD was accompanied by a dramatic reduction (> 35%) of the mRNA abundance of enzymes involved in mammary uptake and intracellular trafficking of fatty acids [lipoprotein lipase (***LPL***) and fatty acid binding protein (***FABP***)], de novo FA synthesis [acetyl-CoA carboxylase (***ACC***) and fatty acid synthase (***FAS***)], desaturation [stearoyl-CoA desaturase (***SCD***)], and esterification

[glycerol-3 phosphate acyl transfrase (***GPAT***) and acyl glycerol phosphate acyl transfrase (***AGPAT***)]. Similarly, intravenous administration of *trans*-10, *cis*-12 CLA, either from 2 to up to 6 g/day (Viswanadha et al., 2003) or 10 g/day (Harvatine & Bauman, 2006), depressed milk fat yield. In the latter study (Harvatine & Bauman, 2006), a joint decrease observed in the expression of genes involved in mammary uptake of fatty acids (*LPL*), de novo fatty acid synthesis (***FASN***), and the regulation of lipid metabolism e.g. sterol regulatory element binding transcription factor 1 (***SREBP1***), thyroid hormone responsive spot 14 (***THRSP*** or ***S14***), insulin induced gene 1 (***INSIG1***).

In s.c. fat depot of dairy cows, effects of CLA on gene expression showed that expression of genes related to lipid synthesis i.e. enzymes like *LPL*, *SCD*, *FAS*, and *FABP4* increased 4 days after abomasal infusion of 7.5 g/d *trans*-10, *cis*-12 CLA. An important nuclear receptor and regulator of lipid synthesis, *PPARγ*, was also increased due to abomasal CLA infusion (Harvatine et al., 2009).

In monogastrics, CLA are known as ligands for *PPARs* (Bensinger and Tontonoz, 2008). Supplementation with the *trans*-10, *cis*-12 CLA isomer induced severe hepatic steatosis in mice with less effect in rats and hamsters. Regardless of species, lower body adiposity is observed when hepatic steatosis happens suggesting that the liver is not able to oxidize fatty acids mobilized from AT (Vyas et al., 2012). CLA (*trans*-10, *cis*-12) reduced the cellular and secreted *ADIPOQ* protein in the murine adipocyte 3T3-L1 cell line (Miller et al., 2008) and decreased *LEP* mRNA abundance in AT from rats (Gudbrandsen et al., 2009). Analogous findings for circulating *LEP* could not be established in dairy cattle (Baumgard et al., 2002; Block et al., 2003). Dietary supplementation of an equal mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers fed to pigs had no effect on *LEPRB* immunofluorescence intensity in AT (Di Giancamillo et al., 2009) although *LEP* expression measured using Western blotting and by immunofluorescence intensity was lowered. To our knowledge, there is no information available on CLA effects on *LEPRB* expression in cattle.

1.4.3. CLA supplementation and insulin sensitivity

Using an RQUICKI index which is calculated from blood metabolites (glucose and NEFA) and insulin concentrations, insulin sensitivity in cows can be estimated (Holtenius and Holtenius, 2007). The majority of studies about CLA supplementation to cows' diets reported that the blood concentrations of insulin (Baumgard et al., 2002, Baumgard et al., 2000, Castaneda-Gutierrez et al., 2005, de Veth et al., 2006), glucose, BHB, and NEFA

are not affected (Bauman et al., 2011) . Exceptionally, Baumgard et al. (2000) and Selberg et al. (2004) reported that NEFA and IGF-1 concentrations increased in serum of cows infused abomasally with the *trans*-10, *cis*-12 CLA isomer. Long term administration of a mixture of CLA isomers (2 weeks a.p. through 20 weeks p.p.) is only reported once (Bernal-Santos et al., 2003). Unfortunately, insulin was not measured in their study. In a mouse model, an increase in insulin release capacity of islets of Langerhans in CLA (*trans*-10, *cis*-12) fed mice was reported (Poirier et al., 2005) and was explained by an increase in ß-cell mass and number. Similarly, obese men treated with *trans*-10, *cis*-12 CLA showed hyperproinsulinemia (Risérus et al., 2004). Indeed, supplying a 50:50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers increased serum insulin concentrations in women (Raff et al., 2009).

1.4.4. CLA supplementation and APP

Conjugated linoleic acids and their relation to inflammatory cell signaling were recently reviewed by Reynolds and Roche (2010) addressing isomer specific effects of CLA on different *ILs*, *TNFs*, interferon gamma. Less is known about the contribution of CLA to APP in particular Hp and *SAA*. Obese rats treated with a mixture of CLA isomers had reduced Hp serum concentrations but Hp mRNA in liver was unchanged (Noto et al., 2007). It was suggested that the anti-inflammatory effect of CLA occurs at the level of AT although the technique used by Noto et al. (2007) for mRNA quantification was not sensitive enough to detect Hp mRNA in AT. A reducing CLA effect on *TNF-α* mRNA and protein in AT was also observed (Noto et al., 2007). In another study (Silvestre et al., 2011), higher concentrations of Hp and fibrinogen (one of the APP) were observed in plasma of cows fed with safflower oil (rich in linoleic acid) vs. palm oil (mainly containing saturated fatty acids). In growing heifers, Hp was decreased by addition of a rumen protected poly unsaturated fatty acid when compared with a non-supplemented diet (Araujo et al., 2010). Other inflammatory proteins e.g. alpha-1-acid glycoprotein were not affected by CLA supplementation in piglets, although serum lysozyme and total serum immunoglobulin G were higher (Corino et al., 2002). *SAA* and pro-inflammatory cytokines i.e. *TNF-α*, *COX-2*, and *IL-1ß* were down-regulated by *cis*-9, *trans*-11 CLA vs. butter fat in hamsters (Valeille et al., 2005).

In contrast to the studies in monogastrics, majority of the factors we already discussed are scarcely investigated in dairy cattle and in some cases no information is available. In early lactation, dairy cows experience different levels of NEB and reduced insulin sensitivity.

The cases of reduced insulin sensitivity addressed in monogastrics are in animal models of obesity, type 2 diabetes, or high fat diets which cannot be conveyed to dairy cattle. Therefore, understanding the pattern of the aforementioned factors related to insulin sensitivity and inflammatory system of dairy cattle and defining the contribution of dietary supplementation with CLA to the pathophysiological status of dairy cattle is highly relevant. To be able to address these aspects, we defined our objectives in next section.