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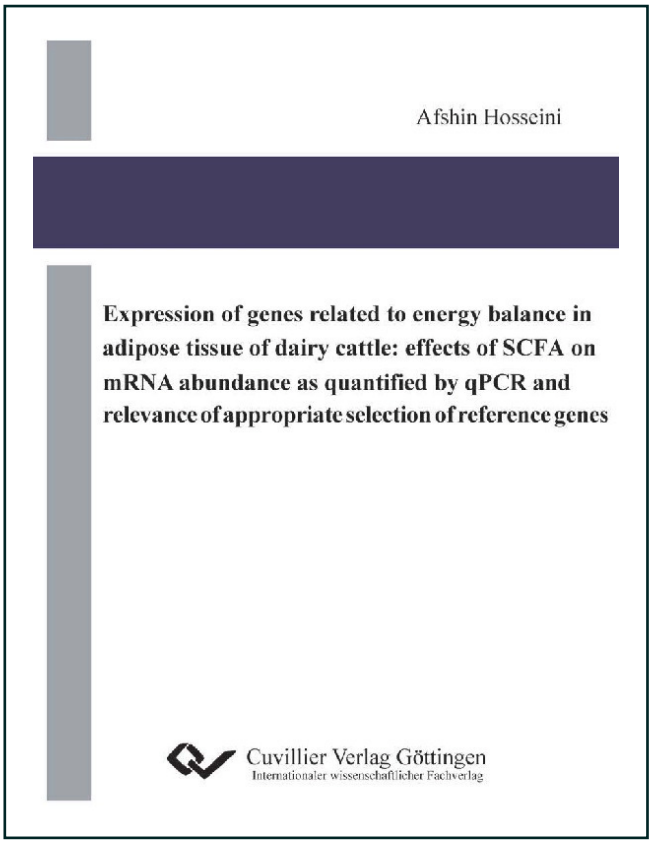
# Expression of genes related to energy balance in adipose tissue of dairy cattle: effects of SCFA on mRNA abundance as quantified by qPCR and relevance of appropriate selection

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# 1. Introduction

Today, ruminants account for almost all dairy lifestock and also contribute to about one-third of the meat production worldwide. The use of high-yielding breeds such as Holstein-Friesians has resulted in dramatic increases in milk production during the past decades. Compared to a suckler cow which would naturally produce around 4 litres of milk per day, a high yielding dairy cow will produce around 27 litres per day for a period of 10 months (305 d lactation period). After parturition, milk production peaks between wk 5 to 7 post-partum, whereas maximum feed intake is reached not until 8 to 22 wk after calving. The lag in feed intake during this period leads to a negative energy balance (Ingvartsen et al., 2000). Energy balance is defined as the difference between the energy consumed and the energy required for maintenance, growth, pregnancy, and lactation (Grummer, 2007) and is of particular importance during the transition from pregnancy to lactation (Drackley, 1999), particularly in high-yielding dairy cows. The mobilization of body stores, mainly in the form of lipids accumulated in various AT depots, to compensate the energy loss via milk can be excessive and is knowingly coupled to health disturbances, e.g. metabolic diseases and impaired immune function. Accretion of body stores occurs during the preceding pregnancy and might also reach an extent predisposing for metabolic disorders like obesity, reduced insulin sensitivity and fatty liver, which affect the health and production of dairy cattle (Ametaj et al., 2005). Positive and negative energy blance as well as their impact in relation with different factors will be discussed in further chapters. However, understanding the effect and interrelationships of both situations, i.e. energy surplus and deficit, will helps to develop concepts for improvement of dairy cattle health and performance. At the moment, there are several methods (e.g. *in vivo* and *in vitro*) to study the effects of energy surplus or deficit in dairy cattle with their advanteges and disadvanteges. The present thesis will foucus only on an *in vitro* model based on the reasons explained in the further sections.

## 1.1. Adipose tissue

Adipose tissue represents a special loose connective tissue containing lipid-loaden adipocytes and other cell types. The main focus of this study is white AT, whereas brown AT, which is almost exclusively found in neonates, will not be addressed. White AT stores lipids as trigly-

cerides and mobilizes them for systemic utilization when other tissues require energy. Besides its role as energy store, it is an active endocrine organ producing different types of hormones, cytokines and chemokines, collectively termed as adipocytokines to regulate homeostasis. Adipose tissue produces also transcriptional factors and in this way influences many aspects of energy metabolism through a network of local and systemic signals. The present study focuses on selected energy balance related genes like adiponectin, AdipoR1/2, C/EBPα, FFAR2/3, GLUT4, G-protein-coupled receptor 109A (GPR109A), IL-6, IRS-1, PPARγ2, and SREBP1 (Caimari et al., 2010; Herwig et al., 2009; Liu et al., 2003; Nilaweera et al., 2003) for which the backgrounds will be provided in the following chapters. Adipose tissue modulates energy expenditure, appetite, insulin sensitivity, endocrine and reproductive functions, bone metabolism, inflammation, and immunity (Shoelson et al., 2007).

It is localized in different places of the body. The different localizations (depots) display various functions. Subcutanous depots are located directly underneath the dermis in the subcutis (e.g. at the tail-head, withers, sternum); whereas the visceral depots reside in the abdominal and thoracic cavity as fat pads (e.g. heart, intestine, liver and kidney fat). Visceral and SC AT differ according to their structure and metabolic function. Fat accumulation in SC and visceral depots is prone to metabolic disorders in man, particularly when visceral fat deposition is abundant (Lafontan et al., 2003). However, the amount of SC AT generally exceeds the visceral fat mass by 3 to 4 times (Chowdhury et al., 1994); both depots can interact in a coordinate and compensatory manner. The intra-abdominal fat is subdivided into preperitoneal and RT depots (Shen et al., 2003). The preperitoneal depots surrounding the intestine, i.e. that the omental-mesenteric blood vessels drains into the liver via the portal circulation, whereas RP blood reaches the systemic circulation via the inferior vena cava; the release of mediators affecting insulin sensitivity from the RP AT depot will thus exert systemic, rather than specifically hepatic effects (He et al., 2008).

The cell types forming AT comprise adipocytes, which might contribute to about 35% to 70% of adipose mass in human adults, but form only 25% of the total cell population (Frühbeck, 2008). In addition to adipocytes, AT also contains preadipocytes, endothelial cells, fibroblasts, leukocytes and, most importantly, macrophages (Tilg et al., 2006; Trayhurn et al., 2004) (Fig. 1). These diverse cell types account for the remaining 75% of the total cell population; the multicellularity implies a wide range of targets for an extensive autocrine/paracrine crosstalk (Frühbeck, 2008) in AT as a local effect and/or elicits endocrine effects in different organs and thus affects energy homeostasis in organism via the aforementioned energy balance

related genes. The AT of obese individuals contains a large number of macrophages, which also are an additional source of soluble mediators in AT (Fig. 1). However, macrophages in AT of monogastrics seem to be the main source of proinflammatory cytokines like IL-6 (Galic et al., 2010), which will be in detail discussed in the next chapters. Adipose tissue contributes 30% of the IL-6 concentration in the circulation of obese individuals (Mohamed-Ali et al., 1997). The various mediators produced by adipocytes and resident macrophages might contribute to local and systemic inflammation (Tilg et al., 2006). Less is known about the infiltration and resident population of the macrophages in lactating dairy cattle during different physiological situation.

### 1.2. Energy balance and related genes

As mentioned above, negative energy balance during early lactation in dairy cows leads to an altered metabolic state by drawing on body fat reserves (Bertics et al., 1992). The depletion of AT in energy-deficit conditions is accompanied by comprehensive metabolic and endocrine changes in dairy cows during early lactation, and may result in an increased risk for many diseases and metabolic disorders like ketosis. In a situation of negative energy balance during early lactation, nonesterified fatty acids (NEFA) are released from AT by lipolysis. The oxidation of NEFA to acetyl-CoA represents the first step of energy generation for most organs. In the following steps, the activated acetyl enters the tricarboxylic acid cycle by reacting with oxaloacetate to form citrate; in a succession of reactions the acetyl is converted into carbon dioxide and energy (ATP). The availability of oxaloacetate, however, depends on an adequate supply of its precursors (e.g. pyruvate, which is the product of glucose degradation in glycolysis). Acetyl-CoA cannot enter the citric acid cycle if the concentration of oxaloacetate is lowered (e.g. when carbohydrate supply is limited and gluconeogenetic conditions develop). In fasting or diabetes, oxaloacetate is consumed to form glucose by the gluconeogenic pathway and is unavailable for condensation with Acetyl-CoA. The Acetyl-CoA is then directed to the formation of ketone bodies, i.e. acetone, BHB and acetoacetate (Fig. 3) (Berg et al., 2006). Ketone bodies may also originate from butyrate from ruminal fermentation and subsequent metabolization in the ruminal epithelium (Kristensen et al., 2000), but its concentrations in blood, milk, and urine are closer linked to lipolysis than to ruminal absorption. Therefore, BHB is used as an indicator for lipolysis in dairy cows and increased concentrations occur during energy deficit and are indicative for metabolic stress which might finally result in clin-

ical symptoms like ketosis. When the hepatic uptake of lipids exceeds the oxidation and secretion of lipids by the liver, which usually is preceded by high concentrations of plasma NEFA mobilized from AT, excess lipids are stored as triacylglycerol in the liver and are associated with decreased metabolic functions of the liver and thus open out into the fatty liver syndrom. In dairy cattle, the increased NEFA and BHB concentrations in blood are related to decreased protein and mRNA abundance of adipokines like leptin in SC AT from 10 d before parturition to 10 d thereafter (Duske et al., 2009). It is also known that BHB and butyrate inhibit adipocyte lipolysis in bovine AT *in vitro* (Metz et al., 1974). The effect of BHB and its effect on energy balance related genes will be discussed in coming chapters.

As mentioned before, surplus energy load can cause metabolic disorders like obesity, reduced insulin sensitivity and fatty liver, which in turn affect health and production of dairy cattle. Short chain fatty acids (SCFA), mainly acetate, C3 and butyrate, are known as the main energy source in ruminants. In ruminantes, most of the carbohydrates from feed stuffs are fermented to the SCFA, carbon dioxide, and methane in the rumen. The SCFA blood concentration is different between monogastrics and ruminants; besides SCFA concentrations differ in blood depending on the distance to the place of absorption, i.e. when comparing portal, hepatic and peripheral values (Bjorkman et al., 1986; Cummings et al., 1987). The peripheral concentration of acetate is between 1.2 and 2.1 mM. Acetate is the major substrate for lipogenesis and oxidation (Brockman, 2005). Propionate is efficiently extracted (80–85%) by the liver in first pass and provides the main substrate for hepatic gluconeogenesis (Baird et al., 1980). About 20% of the absorbed C3 thus reach the circulation; the periperheral C3 serum concentrations reportedly range between 0.06–0.08 mM (Bjorkman et al., 1986). Previous studies demonstrated that in contrast to acetate, C3 stimulates insulin secretion and increases glucagon concentration immediately after the infusion in dairy cattle and sheep (Bradford et al., 2006; Lee et al., 2002; Sano et al., 1995). Insulin increases leptin mRNA in bovine AT explants (Houseknecht et al., 2000). Therefore, the present thesis focuses on insulin dependent or independent effect of C3 on AT.

After the discovery of leptin as the first adipokine (Taniguchi et al., 2002; Zhang et al., 1994) our perspective about the functions of AT and adipocytes was changed. The role of adipokines like adiponectin, and resistin, but also cytokines such as IL-6, which is also secreted at high levels by the AT, became more obvious. In mouse colon, C3 elicits anti-inflammatory effects on IL-6 mRNA and protein expression (Tedelind et al., 2007), which in turn inhibit adiponectin gene expression and secretion (Fasshauer et al., 2003). In monogastrics, an exten-

sive association was shown between body mass and expression and secretion of some of energy balance related genes like leptin and adiponectin. In different physiological situations like obesity, leptin increases and thus shows an inverse correlation with adiponectin. Adiponectin and its receptors, which will be discussed in the coming chapters, influence energy homoeostasis and increase insulin sensitivity (Guerre-Millo, 2008). Decreased insulin sensitivity is defined as a pathologic state of decreased responsiveness of target tissues to normal circulating levels of insulin. Adiponectin acts as an autocrine/paracrine factor, it is involved in adipocyte lipid accumulation and differentiation, and affects other energy metabolism related genes like C/EBPα and SREBP1 expression (Fu et al., 2005), and regulates the expression of its receptors in AT (Liu et al., 2008b). Several studies demonstrated the effect of C3 on mRNA abundance of nutrient sensing receptors and adipokines in ruminant AT *in vivo* and *in vitro* (Mielenz et al., 2008; Soliman et al., 2007). The concentrations dependent SCFA silencing by demethylation (Benjamin et al., 2001) might also lead to repression of mRNA abundance of energy balance related genes as it was shown for mouse leptin promoter activity (Yokomori et al., 2002).

As mentioned before, the transcriptional pattern and the protein expression in AT under dynamic physiological regulation may change. However, it is important to note that adipokines are not all exclusively derived from AT. The cross talk between energy balance related genes in different organs such as AT, liver, skeletal muscle, and central nervous system is assumed to provide an important link between obesity, insulin sensitivity, immunity, appetite and energy balance, lipid metabolism, inflammatory disorders and acute phase response (Trayhurn et al., 2004). Under the different physiological situations, energy homeostasis depends on many factors, among which some of these factors act as nutrient sensor like FFAR2/3 (Ichimura et al., 2009) and GPR109A. The regulation of nutrient sensing receptors trough different signal transduction pathways leads to the expression of adipokines or other transcriptional factors like C/EBPα, PPARγ2, GLUT4, IRS-1, SREBP1, FABP4 (Caimari et al., 2010; Fernyhough et al., 2007), adiponectin, AdipoR1/R2, and IL-6. Most of the studies describe the regulation of protein expression but not the regulation of mRNAs like the ones for adiponectin or IL-6 (Trayhurn et al., 2004). The aforementioned energy balance related genes will be discussed in the following chapters in details.

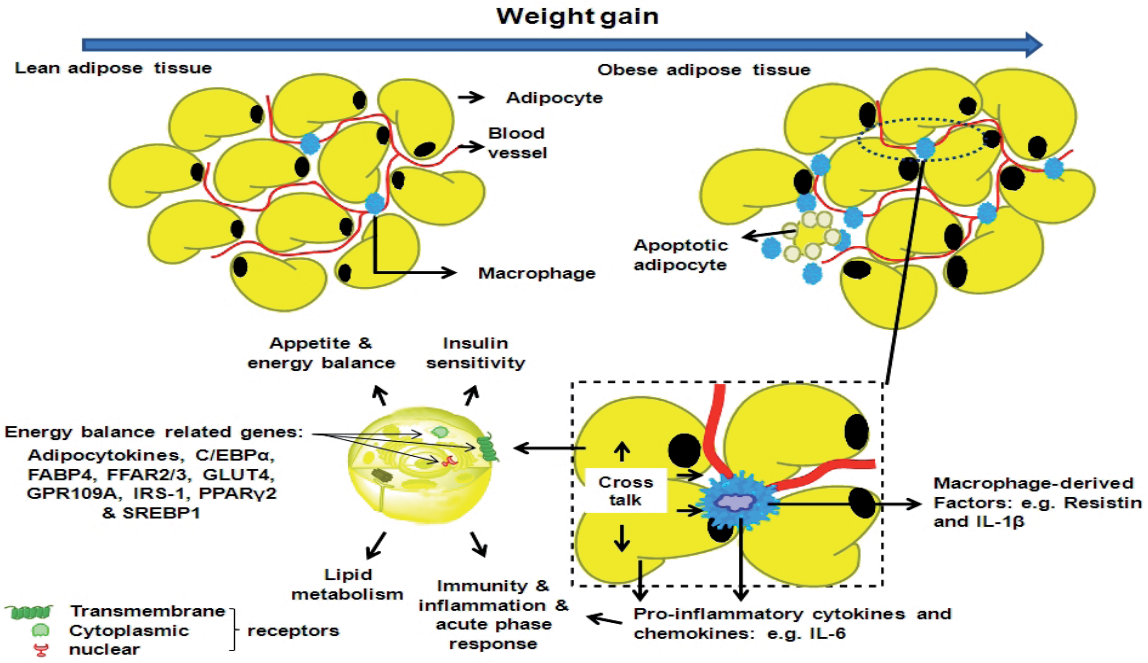


**Fig. 1. Adipose tissue: cellular components, molecules synthesized and their classified functional rules.** Expansion of the AT during weight gain leads to the recruitment of macrophages shown in monogastrics through various signals, which might include energy balance related genes produced by adipocytes. These macrophages are found mainly around apoptotic adipocytes. Energy balance related genes include C/EBPα, PPARγ2, GLUT4, IRS-1, SREBP1, FABP4, ,adipocytokines such as adiponectin, AdipoR1/2 and IL-6 and nutrient sensing receptors like FFAR2/3 and GPR109A shown as different receptors types (Modified after Tilg et al., 2006; Trayhurn et al., 2004).

### 1.2.1. Free fatty acid receptors regulate energy homeostasis

In association with the aforementioned proteins and mRNAs, the family of G-Protein coupled receptors (GPRs) is involved in fatty acid metabolism. The members of this family are involved in different physiological functions like nutritional regulation and they are of importance as pharmaceutical targets. They are localized in different organs including intestine (e.g. GPR120), adipocytes, taste buds, and lung (Ichimura et al., 2009) or in liver, heart, and skeletal muscle (e.g. GPR40). The family of GPR40 is activated by medium and long-chain fatty acids and is involved in different metabolic functions like potentiating insulin secretion, adipogenesis and lipolysis (Covington et al., 2006). In the present study, we focused on those members of this family (e.g. GPR41 and GPR43) that are activated by SCFA (Brown et al., 2005; Le Poul et al., 2003) and on one receptor that is activated by BHB and nicotinic acid, i.e. GPR109A (Gille et al., 2008).