Proteomics Research in the Adipose Tissue

Fabrizio Ceciliani, Cristina Lecchi, Jeanne Bazile, and Muriel Bonnet

Abstract Adipose tissue is no longer considered only as a passive fuel reservoir, but a major endocrine organ, distributed in different depots throughout the body and actively involved in complex regulatory processes including appetite, energy expenditure, body weight, inflammation and reproduction. Proteomics has emerged as a valuable technique to characterize both cellular and secreted proteomes from adipose tissues. Adipose tissue is of major importance in farm animals: in dairy animals, it regulates energy metabolism as well as other functions. In cows and pigs, adipose tissue depots' distribution is of fundamental importance for the quality of carcasses. In this chapter, we provide a general overview of adipose tissue functions and its importance in farm animals and summarize the state of art on farm animal adipose tissue proteomics in cattle and pigs but also in chicken and in farmed fish.

Keywords Adipose tissue • Proteomics • Farm animals • Animal physiology

1 Introduction

Adipose tissue is a complex structure composed of several types of cells, including adipocytes and pre-adipocytes, macrophages, endothelial cells, fibroblasts and leucocytes. Adipose tissue historical function is to store the energy in excess, this role being already established for many years. This task is carried out by conserving the heat of the body and controlling the mobilization of lipids (Sethi and Vidal-Puig 2007). When energy is in surplus, it is efficiently deposited as neutral triglycerides in adipose tissue. When energy is required, adipocyte triglycerides are broken down into glycerol and fatty acids. The released glycerol and fatty acids are then

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transported in the blood and subsequently uptaken by the muscles, liver and other organs, orchestrating lipid distribution and whole-body energy balance (Frayn 2002). In farm animal physiology, most of the studies on adipose tissues are focused on specific mechanisms related to animal production, aiming to understand the biochemical and molecular background underlying the mobilization of lipids in lactating animals to fulfil the energy requirements of milk production or the deposition of fat in specific fat depots, to improve the quality of meat. A further role as a major player during systemic metabolic regulation has been increasingly recognized (Luo and Liu 2016). With the discovery of leptin in 1994 (Zhang et al. 1994), adipose tissue was no longer considered as a passive fuel reservoir, but a major endocrine organ, distributed in different depots throughout the body and actively involved in complex regulatory processes including appetite, energy expenditure, body weight, inflammation and reproduction by synthesizing and secreting messenger molecules which are now collectively referred to as "adipokines". Each of these depots plays an essential role in energy homeostasis as well as in endocrine regulation, at both local and systemic levels. The excessive accumulation of fat in adipose tissue causes obesity, a chronic disease that in turn drives obesity-associated metabolic disorders such as diabetes. Although not directly related to obesity, the dysregulation of adipose tissue metabolism represents an issue for farm animals as well. Adipose tissue is involved in the fine-tuning of energy turnover in dairy cows or in carcass and meat qualities in cows and pigs. Therefore, a deeper understanding of the adipose machinery regulation in rearing species can provide tools to overcome metabolic stress-related diseases and improve the quality of carcass and meat.

Among omics disciplines, transcriptomics has provided important advances in understanding the functions of adipose tissue. A major weak point of transcriptomics is that it does not provide any hint about the effective abundance of the proteins, the correlation between the abundances of mRNA and of protein being poor (Griffin et al. 2002). Integration of data derived from gene expression analyses with those from protein expression studies is therefore mandatory to draw a complete and reliable picture of the functions and the activities of adipose tissue. The proteomics of adipose tissue was recently reviewed (Sauerwein et al. 2014). In the present chapter, we will try to provide the reader with a general overview of adipose tissues and summarize the recent advances on adipose tissue proteomics, focusing on farm animals such as cattle and pigs but also chicken and fish.

2 Adipose Tissue: A Bird's-Eye View with a Proteomic Perspective

2.1 The Main Structure and Function of Adipose Tissue: White, Brown and Beige Adipose Tissue

Two main types of adipose tissue exist in mammals, based on differences in morphology, location and functions: white adipose tissue (WAT) and brown

adipose tissue (BAT) (Lizcano and Vargas 2016). WAT is the preferential site to store energy in the form of triacylglycerols during excessive energy disposal and to restore it during fasting periods. Beside this undisputed role, WAT is also involved in systemic metabolic regulation and inflammation. The main role of BAT is to dissipate chemical energy as heat via high levels of uncoupling protein 1 (UCP1), regulating hypothermia by metabolizing glucose and lipids to produce heat participating to non-shivering thermogenesis (Lizcano and Vargas 2016; Louveau et al. 2016).

Recent studies have described another type of thermogenic adipocytes known as beige/brite (brown in white) adipocytes. Beige adipocytes share morphological and functional similarities with brown ones. In BAT, cold stress or β3-adrenoceptor agonists that mimic cold stress (Barbatelli et al. 2010) stimulate UCP1 expression. Therefore, both brown and beige fat fulfil thermogenic roles. In farm animals, the presence of beige or brown-like adipocytes has been suggested in white adipose depots of fattening cattle (Asano et al. 2013) and sheep (Pope et al. 2014).

2.2 *Different Depots with Different Proteomes: Comparative Analysis of Subcutaneous and Visceral White Adipose Tissue Proteomics*

Adipose tissue develops in various anatomical sites, including the abdominal cavity, the subcutaneous districts and the musculature. They are known to display a different dynamic of growth, which is at the origin of cellular and metabolic features (Bonnet et al. 2010; Hausman et al. 2014; Louveau et al. 2016). Thus, adipose tissue depots respond differentially to rearing practices, which may have implications on the metabolic turnover of lipids and nutrients at the body level for metabolic adaptations, or on carcass qualities and valorization, as visceral fat is discarded, while subcutaneous fat is partly consumed with the muscles. To date, few studies have attempted to investigate the proteome differences among fat depots in ruminants.

A study relying on 2-DGE paired with sequencing mass spectrometry investigated the proteins in undifferentiated and differentiated preadipocytes collected from bovine omental, subcutaneous and intramuscular adipose depots, identifying differentially expressed intracellular proteins during adipogenic conversion (Rajesh et al. 2010). A total of 65 proteins were found to be differentially abundant across the three depots. The preadipocyte differentiation induced a downregulation of many structural proteins, whereas proteins associated with lipid metabolism and metabolic activity, including ubiquinol-cytochrome-c reductase complex core protein I (UQCRC1), ATP synthase D chain, superoxide dismutase (SOD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sulfotransferase 1A1 (SULT1A1), carnitine *O*-palmitoyltransferase 2 (CPT2) and heat shock protein beta 1 (HSPB1), were found to be upregulated across the three depots. Among these proteins, five

(tropomyosin alpha-4 chain, rho GDP-dissociation inhibitor 1, purine nucleoside phosphorylase, transgelin, non-muscle caldesmon), one (annexin A1) and four (glyceraldehyde-3-phosphate dehydrogenase, prohibitin, voltage-dependent anion-selective channel protein 2, voltage-dependent anion-selective channel protein 1) proteins were specifically identified during the adipogenic process of omental, subcutaneous and intramuscular cells, respectively. Using a label-free quantification LC-MS/MS, 682 proteins were identified both in subcutaneous and visceral adipose tissues of 15.5-month-old British continental steers regardless of the diet. The abundance of 51% of these proteins was modified depending on the anatomical site. Of these, 240 were known proteins and were assigned to the following top categories of molecular and cellular functions: lipid metabolism, energy production, small molecule biochemistry, post-translational modification, cellular assembly and organization, cell morphology, protein synthesis and cellular function and maintenance (Romao et al. 2013). Notably, proteins related to oxidation of lipids and fatty acids and synthesis of lipids were more abundant in visceral than subcutaneous adipose tissue, in agreement with the repeatedly observed greater metabolic activity in visceral than in subcutaneous adipose tissues. However, any of the identified proteins were reported to be specifically present in one adipose tissue depot. To date, the sole study in ruminants that reports on protein signatures that are specific to the depot location was conducted in the goat (Restelli et al. 2014), in which a LC-MS/MS approach has identified 833 proteins in at least one of the four adipose tissue deposits investigated in 1-month-old goat kids. The tissue coverage was 471 proteins identified in subcutaneous adipose tissue sampled from the base tail, 480 proteins in subcutaneous adipose tissue sampled from the sternum, 587 proteins in omental tissue and 654 proteins in perirenal adipose tissue. These proteins were assigned to four major functional categories, based on similarity of functions, namely, (1) metabolic processes (62.1% of proteins); (2) cell adhesion cytoskeleton, intramuscular transport and membrane integrity (22.2% of proteins); (3) toxic response and folding (9.5% of proteins); and (4) proteins involved in immune and inflammatory response (6.1% of proteins). Of these 39, 30, 72 and 27 proteins were identified in only one adipose depot, the subcutaneous sampled from the base tail and the sternum as well as perirenal and omental depots, respectively. For the purpose of this review, we focused on the lists of proteins specifically identified in subcutaneous, from the base tail and of visceral perirenal adipose tissue, which are the most commonly analysed, in order to identify proteins or pathways related to the depot specificity of fat accumulation. Thus, lists of proteins specifically found in subcutaneous or perirenal adipose tissues were subjected to annotations according to the gene ontology (GO) with ProteINSIDE (Kaspric et al. 2015) in order to provide the biological processes in which they are involved. The 59 (over 72) perirenal and the 35 (over 39) subcutaneous proteins were annotated by 193 and 129 significantly enriched GO terms, respectively. Of these only 16 GO terms were found in common but annotating different proteins with a different *p*-value of enrichment between lists of subcutaneous and perirenal proteins (Fig. 1). This may reveal pathways important for adipose tissue growth in 1-month-old goat kids, however, involving proteins that

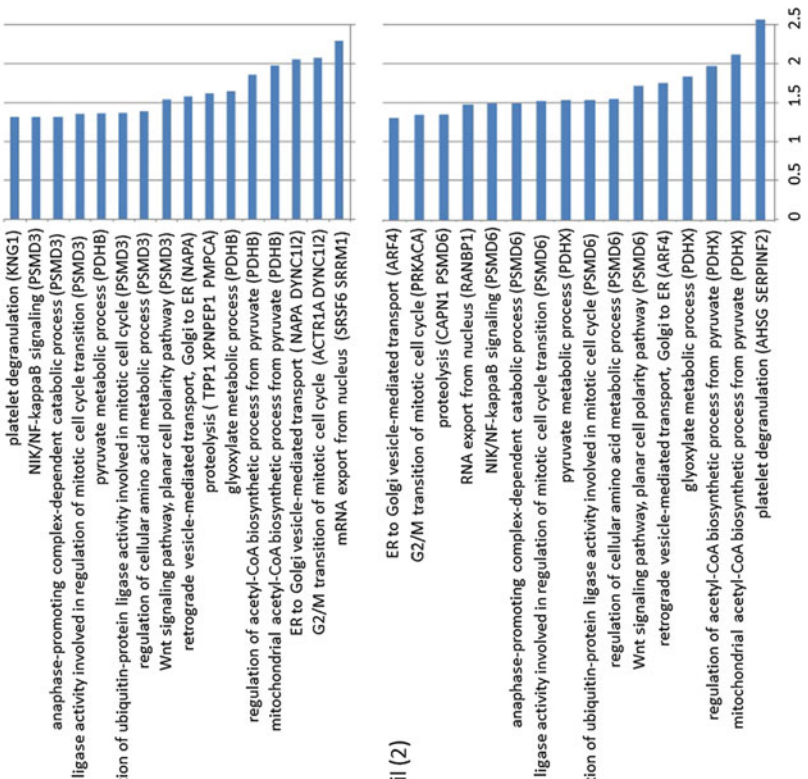

Fig. 1 Identification of 16 shared enriched GO terms ($p\text{-}value < 0.05$) of biological process category for the list of 72 proteins from the perirenal adipose tissue (1) and for the list of 39 proteins from the base tail subcutaneous adipose tissue (2) in goat. Results were obtained with ProteINSIDE, and enrichments are expressed as $-\log 10$ ($p\text{-}value$) to visually plot them on graphs, which means that a $-\log 10$ ($p\text{-}value$) of 3, 2 and 1.3 corresponds to a $p\text{-}value$ of 0.001, 0.01 and 0.05, respectively. In brackets are the gene names of the proteins annotated by GO terms

differ depending on the anatomical site of adipose tissue. The top ten of the most enriched GO terms that were specifically found for the subcutaneous proteins were related to glucose or lipid metabolism, cell proliferation and extracellular matrix organization, in line with a tissue growth both by hyperplasia and by hypertrophy (Fig. 2). For the perirenal proteins, the top ten of the most enriched GO terms were related to protein translation, rRNA and mRNA processing and DNA repair (Fig. 3). These divergent annotations may reflect the lower maturity of subcutaneous as compared to perirenal adipose tissue, in line with the known depot-specific

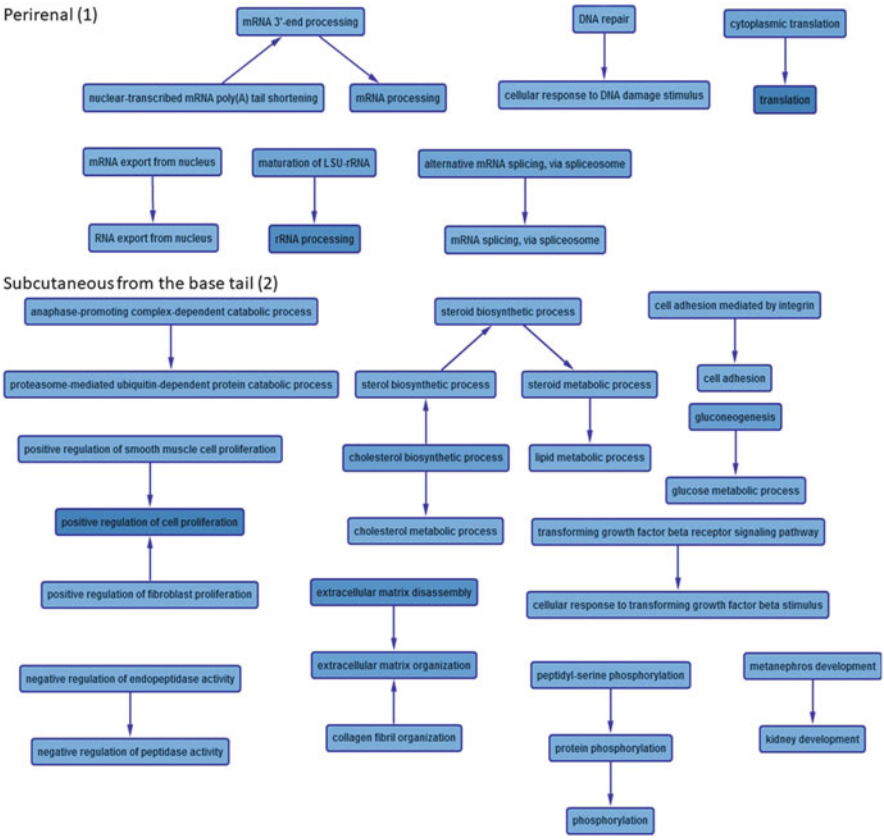

Fig. 2 Relations between the most enriched GO terms were visualized as networks provided by ProteINSIDE. The network links the most enriched GO terms ($p\text{-}value < 0.05$) in the biological process category for the list of 72 proteins (59 annotated) from the perirenal adipose tissue (1) and for the list of 39 (35 annotated) proteins from the base tail subcutaneous adipose tissue (2) in goat. The degree of colour saturation is related to the number of proteins annotated by a GO (dark and clear for high and low numbers, respectively)

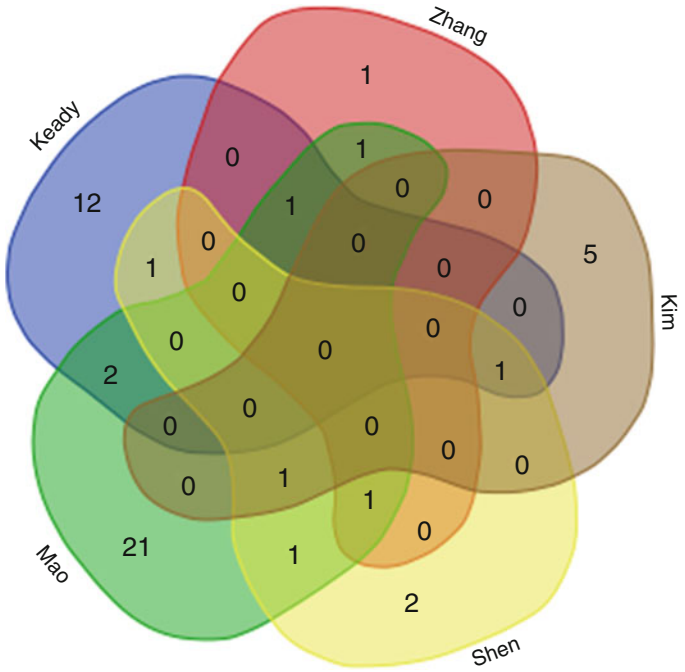

Fig. 3 Distribution and overlap of proteins identified in five proteomic analysis (Kim et al. 2009; Zhang et al. 2010; Shen et al. 2012; Keady et al. 2013; Mao et al. 2016) carried out to provide the molecular basis of marbling and to identify biomarkers. Venn diagram was proceeded with http://bioinformatics.psb.ugent.be/webtools/Venn/

growth patterns reported during the perinatal period in ruminants (Bonnet et al. 2010). Future proteomic studies might contribute to identify depot-specific proteins in order to increment available data and provide the molecular basis of depot specificity of fat accumulation for a robust data mining of the pathways or molecular functions involved.

2.3 Proteomic Analysis of Brown Adipose Tissue

Larger mammals such as ruminants are fully developed and able to thermoregulate at birth, thanks to the presence of BAT that disappears after birth and is replaced by WAT. A better knowledge of the molecular features of BAT versus WAT may help in the understanding of the balance between brown and white fat cells and is a prerequisite for a better control of thermogenesis and survival in perinatal period, as well as energy efficiency during the productive life of the ruminants. To the best of our knowledge, only one study has attempted to link the proteome to chemical composition, cellularity, histology, enzyme activities and gene expression during a

time-course analysis of the ontogeny of perirenal adipose tissue in bovine foetuses (Taga et al. 2012). Between 110 and 260 days postconception (dpc, 38 and 90% of gestation length, respectively), the increase in the weight of perirenal adipose tissue resulted from an increase in the adipocyte volume and mainly number and was accompanied by changes in the abundance of 128 proteins among the 143 proteins identified. From these data, an unexpectedly high abundance of the α-subunit of ATP synthase, a member of complex V (ATP synthase) of the respiratory chain which is normally bypassed in BAT, and of aldehyde dehydrogenases ALDH2 and ALDH9A1, has been observed at 180 dpc when compared to other foetal ages. However, these proteins were proposed as hallmark WAT in mouse (Forner et al. 2009). From 210 dpc, an increase in the mRNA abundances of UCP1 and DIO2, which are hallmarks of brown adipocytes, was observed. These results, associated with the presence of numerous unilocular white adipocytes and few brown multilocular adipocytes from 180 dpc, show that foetal ATs up to 260 dpc have molecular and cellular characteristics of WAT in addition to those of BAT (Taga et al. 2012). These results challenged the commonly accepted concept that foetal perirenal AT in cow is brown and highlighted that perirenal AT is a heterogeneous tissue made of brown and white adipocytes up to 260 dpc in bovine foetuses. This study, together with the global gene expression profiling of brown/beige to white adipose tissue during the first month of life in lambs (Basse et al. 2015), provides a useful resource to identify the molecular features of perinatal brown/beige adipose tissue relatively to those of white adipose tissues presented above.

3 Adipose Tissue in Farm Animals: Endocrinology and Immunology

With the discovery of leptin in 1994 (Zhang et al. 1994), adipose tissue has been identified as an endocrine gland secreting messenger molecules that affect a wide variety of bodily functions. From then on, numerous hormones, chemokines, cytokines as well as acute-phase proteins were discovered as adipose products and are now collectively termed as adipokines. Adipokines are involved in the dynamic control of energy metabolism. Being produced by adipose tissue, adipokines are fundamental to deliver to other tissues important information about the nutrient status of the organism, in particular to those responsible for controlling energy intake and expenditure, such as appetite regulation, energy expenditure, insulin sensitivity, glucose metabolism and fatty acid oxidation. Figure 4 presents a short list of adipokines and their main physiological roles. The human adipose tissue secretome determined by proteomic techniques was released in 2007 (Alvarez-Llamas et al. 2007). Products from all cell types present in adipose tissue were included in this first report. In fact, adipose tissue contains not only adipocytes but other cell types as well. A stromal vascular fraction of cells including preadipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells (e.g. macrophages) are also included in adipose tissue depots.

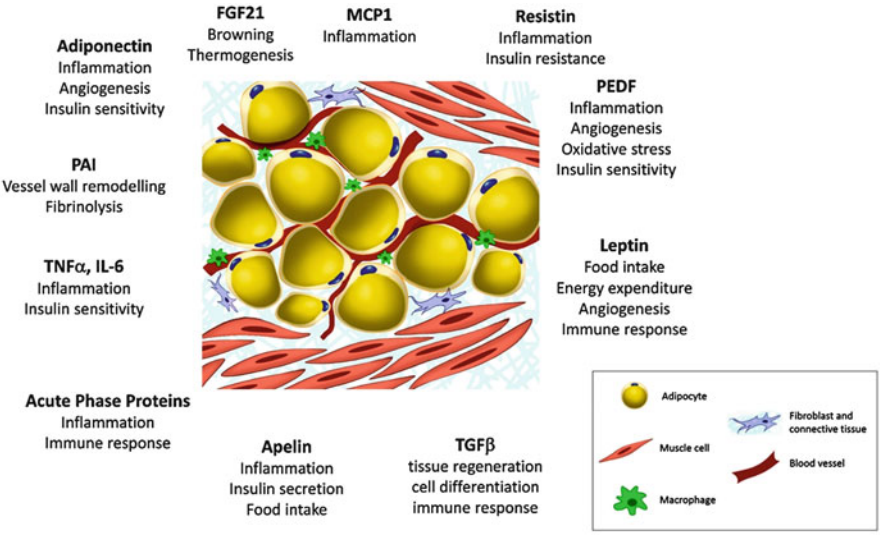

Fig. 4 The complexity of AT and physiological functions of adipokines. Beside adipocytes, adipose tissue also contains preadipocytes; fibroblasts and connective tissues; vascular endothelial cells, providing the capillary network; myocytes; and muscle cells and macrophages that increase when adipose tissue becomes inflamed. In this figure, some of the proteins that are produced by AT, and that can be found in the circulation, the so-called adipokines, are also presented. Some of their main activities are also briefly summarized. *FGF21* fibroblast growth factor 21, *IL-6* interleukin 6, *MCP1* monocyte chemoattractant protein 1, *PAI1* plasminogen activator inhibitor 1, *TNF-α* tumour necrosis factor alpha and *PEDF* pigment epithelium-derived factor. We thank Mrs Helen Arino for drawing the figure

Proteomics analysis carried out on primary human adipocyte cell culture identified 347 proteins of which 263 were predicted to be secreted (Lehr et al. 2012). The list of these which are regarded as adipokines includes not only proteins whose abundance is restricted to adipose tissue but also molecules taken up by adipose tissues that are known to be secreted from other tissues, such as cytokines and acute-phase proteins. Most of adipokines that were initially discovered and considered as adipose specific were then found to be secreted by other tissues such as skeletal muscle and are now classified as myokines. Both adipokines and myokines play major roles in exchanging information between skeletal muscle fibres and adipocytes in the framework of an endocrine cross talk (Trayhurn et al. 2011). Recently, a computational prediction of the large-scale secretome of adipose tissues and muscles in ruminant was achieved by applying algorithms to 24 publications reporting transcriptomics or proteomics results from bovine, ovine and caprine species and eight transcriptomics dataset series. In this way, 1749 proteins were proposed as secreted both by adipose tissues and muscles, and 188 and 357 proteins were predicted to be secreted either by adipose tissues or muscles, respectively (Bonnet et al. 2016).

As a consequence of the cell diversity in adipose tissue, during human obesity, macrophages forming a part of the stromal vascular fraction infiltrate adipose tissue

Table 1 Proteins identified as related to intramuscular fat deposition in at least two studies from five, in which strong differences in marbling resulted from age (Kim et al. 2009[1]; Zhang et al. 2010[2]), breed (Keady et al. 2013[3]) or marbling score at 24 (Shen et al. 2012[4]) or 30 (Mao et al. 2016[5]) months of age in bovine

Gene names	Positive or negative correlations between protein abundance and marbling
CA2	Negative[2,4,5]
MYL1	Negative[3,4] and positive[1]
HSPB1	Negative[5] and positive[2,3]
TPI1	Negative[1,5] and positive[4]
CAPZA2	Positive[3,4]
MYLPF	Negative[3,5]
MYL3	Negative[2,5]
PYGM	Negative[5] and positive[3]
PGK1	Negative[5] and positive[4]

Superscripts refer to the publications as defined in the table caption

in increasing numbers, providing important contribution to the secretory function of adipose tissue, in particular for inflammatory cytokines, such as TNF-α and IL-6 (Weisber et al. 2003). The increase in circulating levels of pro-inflammatory macrophage-derived factors during obesity induces a chronic low-grade inflammatory state leading to the development of insulin resistance and diabetes (Xu et al. 2003). It must be said that immune cells were hardly detectable in both visceral and subcutaneous adipose tissue in dairy cattle. Therefore, macrophages might not be involved in the immunity and metabolism of adipose tissue in nonobese lactating animals (Akter et al. 2012). In the previously described study on goat adipose tissue proteomics (Restelli et al. 2014), it was demonstrated that goat adipose tissue contains 51 proteins that are related to inflammatory and immune responses. The list of these proteins includes acute-phase proteins, such as C-reactive protein, ceruloplasmin and alpha-1-acid glycoprotein and galactoside-binding proteins (LGALS), such as beta-galactoside-binding lectin precursor and galactoside 3. LGALS act as agonists of platelet activation and are pro-apoptotic for immune cells, activate and increase the adhesion of neutrophil and are chemotactic for monocytes (Table 1). Whether these proteins are produced within adipose tissue or are present in adipose tissue because of their uptake from the blood remains to be investigated in ruminants.

4 When Things Turn Wrong: Adipose Tissue in Dairy Animals and Metabolic Imbalance Syndrome

Hepatic lipidosis (also known as “fatty liver disease” or “fat cow syndrome”) is a common production problem of dairy cows occurring during the critical physiologic transition from pregnancy to lactation (Bobe et al. 2004; Hammon et al.

2009). During recent decades, the genetic selection carried out on dairy cows was directed toward an increase of milk production. The result was positive, since milk production reached performance peaks unthinkable even a few years ago. This major gain did not come without serious consequences for cow health. The excessive demand for nutrients frequently ends up in a severe energetic deficit at the beginning of lactation (Mulligan and Doherty 2008; Hammon et al. 2009). The severity of this effect is increased by the need of nutrients for the foetus that is growing exponentially and the fact that feed intake does not equally increase. Animals affected by this major energy deficiency try to counteract by developing insulin resistance (IR) to spare glucose for the foetus (Bauman and Currie 1980) and rapidly mobilizing fat depots, in the attempt of providing nonesterified fatty acids (NEFA) as an energy source. Triacylglycerols are released from hepatocytes as part of lipoproteins, mostly very low-density lipoproteins (VLDL). In ruminants, the secretion of VLDL from the liver is limited as compared with other species. Consequently, the hepatic uptake of NEFA and storage in the form of triacylglycerols exceed their elimination. The storage of excess lipids in hepatocytes leads to liver damage and depressed liver functions (Geelen and Wensing 2006). A morphologic change in liver tissue occurs, characterized by the accumulation of lipid vacuoles within hepatocytes. This metabolic change is called hepatic lipidosis or liver fatty change (Cebra et al. 1997; Imhalsy et al. 2014). Considering this dual role of regulating energy storage by storing and releasing fatty acids, and acting as a major endocrine capable of influencing metabolism by secreting and regulating hormones and adipokines (Kershaw and Flier 2004), adipose tissue metabolism plays an essential role in the development of metabolism syndrome in transition dairy cows. Adipose tissue reacts to the increase of energy demand by increasing lipolysis and by regulating the development of major metabolic changes including, among the other, insulin resistance or sensitivity (Bell and Bauman 1997; Rabe et al. 2008; Loor et al. 2013).

A recent study determined the proteome of transition cow adipose tissue focusing on the relationship between insulin sensitivity (IS) and resistance (IR) of subcutaneous adipose tissue (Zachut 2015). The results showed that a number of 143 proteins out of 586 were differentially abundant in prepartum versus postpartum adipose tissue. The lipid metabolism-related functions that were significantly changed postpartum compared with prepartum include fatty acid metabolism, the esterification of lipids and oxidation of fatty acids. The proteins whose abundance was decreased in postpartum included fatty acid synthase, complement C3, annexin A1 and acyl-CoA desaturase. Finally, the study also addressed proteomic differences between IR and IS adipose tissues. Out of 586 proteins, 111 were differentially abundant between IS and IR cows. Most of them (a number of 106) were increased in IR versus IS adipose, whereas only five were decreased. As expected, the most relevant pathways differentially activated between IR and IS adipose tissue included energy-related pathways, such as gluconeogenesis and glycolysis, 14−3−3-mediated signalling, TCA cycle and ERK/MAPK signalling. The most relevant function in IR as compared to IS adipose tissue was inflammatory response, such as leukocyte migration and proliferation of T lymphocytes,

confirming the relationship between adipose tissue IR and proteins related to inflammation, and organization of actin cytoskeleton.

5 The Marbling Issue: Adipose Tissue in Beef

Understanding muscular adipogenesis and identifying a protein profile associated with intramuscular fat deposition also termed marbling are prerequisites to develop strategies to manipulate marbling in cow and pig in an effort to produce even healthier and tastier meat products for consumers. In order to provide the molecular basis of marbling and to identify biomarkers to predict the animal's ability to deposit intramuscular fat, proteomics of fat accumulation was assayed in the bovine species in five proteomic investigations mainly through 2DE followed by MS/MS analysis. The proteome of *longissimus lumborum* or *longissimus dorsi* was explored in steers, mainly of Asian breeds, showing strong differences in marbling as the result of age (Zhang et al. 2010; Kim et al. 2009), breed (Keady et al. 2013) or marbling score at 24 (Shen et al. 2012) or 30 (Mao et al. 2016) months of age that are among the major drivers of individual variations in the intramuscular fat percentage (Shingfield et al. 2013).

By merging results from these five studies, 50 unique proteins were proposed to be involved in the deposition of intramuscular fat content that are informative of the molecular basis and of the major molecular pathways involved. Among them four (Zhang et al. 2010) and seven (Kim et al. 2009) proteins were found to be related to a high intramuscular fat deposition induced by an increase in age of Korean steers. These proteins were heat shock protein beta 1 (HSPB1, upregulated), ATP synthase D chain mitochondria (ATP5H), carbonic anhydrase II (CA2), myosin light chain 3, slow-twitch muscle [MYL3, all downregulated (Zhang et al. 2010)], as well as myosin light chain 1, slow-twitch muscle A isoform (MYL1), thioredoxin-dependent peroxide reductase (PRDX3, both upregulated), actin, aortic smooth muscle (ACTA2), actin, cytoplasmic 1 (ACTB), succinate dehydrogenase [ubiquinone] flavoprotein subunit (SDHA), triosephosphate isomerase (TPI1) and zinc finger protein 323 [ZSCAN31, downregulated (Kim et al. 2009)]. These proteins are mainly involved in glycolysis/gluconeogenesis (TPI1), the oxidation of glucose and fatty acids (ATP5H) and the regulation of muscle contraction (MYL3, MYL1, ACTA2). Additionally, the most enriched GO terms provided by ProteINSIDE (Kaspric et al. 2015) for these 11 proteins are "muscle filament sliding" (MYL1 MYL3), "muscle contraction" (MYL1 ACTA2), "vascular endothelial growth factor receptor signalling pathway" (ACTB HSPB1) and "negative regulation of apoptotic process" (HSPB1 PRDX3).

Differential abundances of 7 and 28 proteins were found to be related to divergent marbling scores, in Xiangxi yellow × Angus cattle steers (Mao et al. 2016) and in Hanwoo cattles (Shen et al. 2012), respectively, three of them being reported in both breeds. The abundance of carbonic anhydrase II (CA2) was reported to be negatively related to marbling score in both breeds. However,

negative relations were reported between the abundances of triosephosphate isomerase (TPI1), phosphoglycerate kinase 1(PGK1) and the intramuscular fat content in the Xiangxi yellow × Angus cattle steers, while a positive relation was reported in Hanwoo cattle.

Differential abundances of 17 proteins were found to be related to divergent intramuscular fat content in the muscle of Belgian Blue × Holstein Friesian compared to Aberdeen Angus × Holstein Friesian (Keady et al. 2013). The top canonical pathways identified by the authors were glycolysis/gluconeogenesis (glycogen phosphorylase (PYGM), phosphoglycerate mutase 2 (PGAM2) and aldolase A (ALDOA) as greater in abundance in highly marbled Aberdeen Angus), the citric cycle (aconitase 2 (ACO2) and 2-oxoglutarate dehydrogenase (OGDH) as greater in abundance in highly marbled Aberdeen Angus), the protein kinase A signalling pathway (myosin light chain 1 (MYL1), myosin light chain, phosphorylatable (MYLPF), PYGM and troponin I (TNNI2) differing in abundance across breed), and the pentose phosphate pathway [with ALDOA greater and phosphoglucomutase (PGM1) decreased in abundance in Aberdeen Angus compared to Aberdeen Angus].

Altogether these data highlight that the main pathways related to intramuscular fat deposition in beef are metabolic pathways related to glucose, oxidative pathways, the molecular features of muscle fibres (and thus of muscle type) and pathways related to apoptosis. Some of them could be related to previous observations that glucose rather than acetate is a major precursor for lipogenesis, and hence fat accumulation, within intramuscular adipocytes (Smith and Crouse 1984) or to the repeatedly observed higher marbling in skeletal muscle of type I than II (Bonnet et al. 2007).

Of these 50 unique proteins, only 9 were related to intramuscular fat deposition in at least two publications and thus could be potential robust biomarkers to predict meat marbling (Fig. 3 and Table 1). The proteins HSPB1, MYL1, CA2 and TPI1 were declared as differentially abundant according to the intramuscular fat deposition in three studies. Of these, CA2 was always reported to be lower in abundance in muscle with high intramuscular fat content. The proteins CAPZA2, MYLPF, PYGM, MYL3 and PGK1 were declared differentially abundant according to the intramuscular fat deposition in two publications. Among them, CAPZA was always reported to be higher in abundance in muscle with high intramuscular fat content, while MYLPF and MYL3 were reported to be lower (Table 1). The abundance of HSPB1, MYL1, TPI1, PYGM and PGK1 was reported to have a positive or negative relation with marbling depending on studies. The apparent inconsistencies of these results may be explained by an increase in some pathways related to lipogenic capabilities in adipocytes of small volume that are then decreased in large adipocytes, as already observed for proteins involved in lipid synthesis (Bonnet et al. 2007; Romao et al. 2014). A variation in the abundances of HSPB1, MYL1, TPI1, PYGM and PGK1 may partially explain the result of differences depending on breed, age, nutrition and not only of marbling. To sum up, CA2, CAPZA, MYLPF and MYL3 may be proposed as robust biomarkers of

marbling in beef since their abundances were related to intramuscular fat deposition regardless of breed, age or level of marbling.

This literature review lastly shows that proteins related to marbling (Zhang et al. 2010; Kim et al. 2009; Keady et al. 2013; Shen et al. 2012; Mao et al. 2016) are different than those related to back fat thicknesses (Charolaise × Red Angus and Hereford × Angus; Zhao et al. 2010) in steers.

6 Adipose Tissue in Pigs

The pig is a major source of meat for human consumption. Knowledge of the biochemical mechanisms and understanding the biological significance of adipose tissue development in pigs are important for optimal growth efficiency and meat quality. A specific issue of porcine production is the processed meat products. The dynamic of lean and fat growth is of fundamental importance to improve the production of differentiated end products through breeding and feeding strategies. As an example, optimal adipogenesis requirement is different if the final product is fresh versus processed meat products, such as the production of regional and traditionally cured and dried high-value products, like Parma and Serrano ham (Candek-Potokar and Skrlep 2012). Meanwhile, with the background that swine physiology, genomics and nutritional requirements are very similar to that of humans, the pig has become an increasingly important animal model for human metabolic diseases and obesity (De Almeida and Bendixen 2012; Ceciliani et al. 2014). Despite these various interests, proteomic experiments to understand adipogenesis at the protein level in pig are still very limited, as compared to the information made available from genome-wide association, targeted candidate genes and large-scale transcriptomic studies (Cánovas et al. 2010; Ramayo-Caldas et al. 2012; Corominas et al. 2013; Puig-Oliveras et al. 2014; Xing et al. 2015, 2016; Shen et al. 2015; Ros-Freixedes et al. 2016; Zhang et al. 2016). Proteome research has mainly been focused on mapping the muscle proteome with postmortem modifications driving the transformation of muscle to meat, such as meat tenderness, postmortem proteolysis (Pioselli et al. 2011; Mora et al. 2015; Gallego et al. 2016), phosphoproteomics (Huang et al. 2014), water holding capacity (Di Luca et al. 2013, 2016) and meat colour traits (Lomiwes et al. 2014). Very few studies have been carried out on the pig adipose tissue proteome. The available proteomics were carried out in muscle with the aim to identify protein and pathways related to intramuscular fat deposition and in subcutaneous adipose tissue to identify pathways related to body adiposity.

Although intramuscular fat content is an important determinant of meat quality, the information related to deposition of intramuscular fat at protein and proteome levels is scarce. Differences between high and low ability for fat deposition in longissimus dorsi muscle from commercial crosses originating from Pietrain and an industrial cross originating from Duroc, Hampshire and Large White founders were identified in comparative study including both transcriptome and proteome profiles

(Liu et al. 2009). Differences for marbling were correlated to transcriptome and proteome profiles, and 40 muscular genes were identified as differently expressed between high-fat and low-fat groups, either at the mRNA level (29) or encoding proteins (12). Among them, only GSTP1 was found differently abundant at both the mRNA and protein levels. Gene ontology analysis indicated that differentially expressed genes were involved in metabolic processing, cell communication, binding and response to stimulus. The results of this study suggest that interindividual variability in intramuscular fat content might arise essentially from differences in early muscular adipogenesis that may have modified either the volume or the number of intramuscular adipocytes. A recent investigation, pairing transcriptomics with proteomics, identified several genes and proteins involved in fatty acid metabolism and intramuscular fat deposition (Yang et al. 2016). A total of 23 differentially expressed proteins were identified, several of which were potentially associated with fatty acid metabolism. In particular, adipocyte fatty acid-binding protein A, alpha-enolase isoform X1 and beta-enolase isoform X1 were more abundantly found in Chinese indigenous Shaziling pig, a fat-type line with high intramuscular fat (IMF), as compared with the Yorkshire breed, which has a leaner meat ratio. By identifying differences in breed-related protein and transcript abundance patterns between the two breeds, these data provide insights into the mechanisms of growth and development of porcine skeletal muscle and how it might influence the IMF deposition.

The main adipose tissue studied in pig was the subcutaneous. Indeed, as compared to cattle which has a major deposition of fat within visceral and intermuscular adipose tissues, pigs deposit fat mostly within subcutaneous adipose tissue. An excessive growth of subcutaneous adipose tissue decreases the gain production of pig. A characterization of subcutaneous adipose tissue proteomes of young piglets was carried out focusing on the metabolic control and acute-phase response associated with adipogenesis in lean (Large White) as compared to fat (Basque) breeds (Gondret et al. 2012). Mass spectrometry and MS/MS analyses identified 65 proteins, 57 of which were significantly different between subcutaneous adipose tissues from Basque and Large White pigs, and 12 others were expressed with different abundance. The authors demonstrated that several metabolic pathways are differentially expressed in the lean versus fat breeds including, most significantly, the pentose-phosphate pathway, with aldolase C, glucose-6-phosphate dehydrogenase and ribose 5-phosphate isomerase A; the citrate cycle with pyruvate carboxylase, dihydrolipoyl dehydrogenase and aconitase; and the pyruvate metabolism with aldolase C and dihydrolipoyl dehydrogenase; again, malic enzyme; and pyruvate carboxylase proteins. The most significant biological function affected includes lipid metabolism, which was increased in Basque breed as compared to Large White lean one. Carboxylesterase 1 was more abundant in adipose tissue of Basque breed, suggesting an increased activity of triacylglycerol synthesis and degradation aimed to protect cells against fat overload. Adipose tissue of Basque piglets also showed an increased abundance of inflammatory-related proteins, suggesting a low-grade inflammation in “fat” pigs, probably induced by an increased oxidative stress related to adipocyte differentiation. This effect closely

resembles human obesity and inflammatory metabolic syndrome status. The increased abundance of selenium-binding protein, which has ROS scavenging properties, also confirms the need of quenching ROS aiming to protect tissues from oxidative damages. To understand the impact of maternal nutrition on prenatal and offspring metabolism and body adiposity, the proteomes of subcutaneous adipose tissues in piglets born from sows fed on either low, high or normal protein diets were determined. The 2D proteome profiles between the three groups were different (Sarr et al. 2010, 2012). In details, proteins related to fatty acid metabolism and lipid transport were upregulated in piglets born from sows fed on low protein diets. Analogue transcriptome studies have confirmed that foetal programming indeed affects adiposity of pigs (Oster et al. 2011, 2012). Besides increasing our knowledge about the relationship between maternal nutrition and offspring adipose tissue development in human nutrition, these two studies also provide important information that might be implemented in improving rearing and possibly driving the practices affecting the partitioning of fat between subcutaneous and intramuscular adipose tissues.

7 Adipose Tissue in Other Species: Fish and Poultry

7.1 Adipose Tissue Proteomics in Broiler Production

Adipose tissue is regarded as a negative trait in poultry science, although it must be said that genetic pressure for rapid growth had also an increase of fat deposition as side effects (Nones et al. 2006). Proteomic analysis was carried out to obtain insights into the molecular basis of fat deposition in broiler combining 2D electrophoresis with identification by MALDI-TOF (Wang et al. 2009). Approximately 1000 protein spots were identified in adipose tissue, of which 15 proteins were shown to be differentially expressed between lean and fat chickens, most of them being involved in lipid metabolism, including adipocyte FABP, apolipoprotein A-I and acyl-coenzyme A dehydrogenase, and oxidative stress, such as HSP 27, which was downregulated, and glutathione-*S*-transferase-α and *S*-transferase-β, which were upregulated. Furthermore, members of cytoskeleton family were differentially abundant, underlying a different cytoskeleton rearrangement. These results were partially confirmed by a recent study that investigated the differences in abdominal adipose tissue proteomes between broiler lines divergently selected for abdominal fat content (NEAULF breed) (Wu et al. 2016). Thirteen proteins were found to be differentially expressed between lean and fat lines. In particular, the expression of abdominal adipose tissue Apo A-I, PPIase FKBP4 and cytokeratin otokeratin was found significantly higher in lean birds as compared with fat birds. These proteins are mainly involved in lipid metabolism, amino acid metabolism, signal transduction, energy conversion and antioxidant and cytoskeleton as well as in adipose tissue metabolism.

7.2 Fish Adipose Tissue Proteomics

Lipids are the main source of energy for fish (Weil et al. 2013). Adipose tissue is distributed in fat mainly located around the digestive tract in the abdominal cavity (visceral fat). The percentage of fat in fish may be very high (up to 25% of the body weight). Located on the ventral and dorsal area of the fish is the subcutaneous fat, which is located around the body of the fish, prominently in dorsal and ventral zones. The quality of carcass is dependent on the distribution of adipose tissue between subcutaneous and perivisceral depots, given that adipocytes are also located in muscle myosepta, a connective tissue membrane separating muscle sheets.

Only two proteomics studies have been carried out on fish adipose tissue, both of them on rainbow trout (*Oncorhynchus mykiss*). Weil et al. (2009) carried out an in vitro investigation on isolated and cultured adipocytes from subcutaneous (dorsal and ventral) and visceral fat depots. The study investigated the differentiated adipocytes from preadipocytes, while other cells that normally compose the adipose tissue depots, such as connective tissues, endothelial cells or blood cells, were excluded. Proteins were separated by means of 2D electrophoresis, and MALDI-TOF identified nine differentially abundant spots depending on differentiation stage. Of these proteins, beta actin and albumin were associated with visceral adipose tissue, whereas annexin, ATP synthase subunit-β, serum deprivation response protein and heart fatty acid-binding protein (H-FABP) were found to be more abundant in subcutaneous AT. The authors found no differences between adipocytes isolated from visceral and subcutaneous adipose tissue, although the amount of protein expressed was different for some of them. Besides proteins related to cell cultivation procedures, such as bovine serum albumin or serum deprivation response protein, the authors identified actin as the more abundant protein in visceral-derived adipocytes. Actin is involved in cytoskeleton structure, and it was found to be overexpressed during adipogenesis of intramuscular bovine adipocytes (Takenouchi et al. 2004) and is involved in cytoskeletal rearrangement as well. ATP synthase, annexin and H-FABP were found to be more abundant in subcutaneous adipocytes, suggesting that this depot is more metabolically active than visceral ones, which is in contrast with results so far demonstrated for mammalian species. The investigation was integrated with histological parameters, such as adipocyte cell diameter determination, presenting the evidence that the diameter of adipocytes derived from visceral AT was higher as compared to those derived from subcutaneous adipose tissue. A study on transcriptomics and proteomics rainbow trout liver focusing on the differences in fat allocation between visceral and muscle AT was carried out after feeding with high- and low-energy diets (Kolditz et al. 2008). The authors sorted the animals in two groups differing for adipose tissue anatomical distribution, namely, those with mainly visceral adipose tissue (lean muscle line) and mainly muscle adipose tissue (fat muscle line). H-FABP was upregulated in the fat muscle cell line when compared with the lean muscle cell line, confirming its importance in fish adipose metabolism.

GAPDH was upregulated as well, whereas apolipoprotein A-1, which is usually overexpressed in adipose tissue, was on the contrary downregulated in the liver.

8 Conclusions: New Insights into the New Knowledge Contributed by System Biology Approach to Better Understanding of Adipose Tissue in Farm Animals

Postgenomic applications in veterinary medicine, including transcriptomics, proteomics and metabolomics, are increasing exponentially. The number of omics studies carried out in farm animals pales if compared to those carried out in humans and a true system biology approach to the impact of omics in livestock are far from being fully implemented. Animal proteomics is further lagging behind those in human biology (Almeida et al. 2015). Before the full development of the "omics" revolution, the knowledge of the physiological bases of adipose tissue development and IMF deposition was obtained through independent analysis of the activity of each single protein. The study of adipose tissue carried out using proteomic approaches, in particular when applied together with a system biology approach, including also transcriptomics and metabolomics, provides an integrated network of the single elements, the knowledge of which provides, in turn, greater information than the sum of individual parts. Transcriptomics and proteomics are an evident leap forward in our understanding of basic biology of adipose tissue, integrating the information necessary to link, for example, adipose tissue and metabolic stress in dairy cows or optimal IMF deposition in cows and pigs. This information might be readily implemented into the field, on the basis that adipose tissue metabolism may be modified by changing fatty acid content through diet. Omics technologies remain quite expensive, proteomics even more. Yet, technology moves rapidly forward and the costs for omics application are constantly dropping. The goal of a $1000 genome has been almost reached, and it is expected that further drops in omic experiment costs will result in an exponential increase of proteomic studies as well.

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