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MOLECULAR BASIS OF THE INNATE IMMUNE RESPONSE IN RUMINANTS: FOCUS ON ADIPOSE TISSUE

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Molecular basis of the innate immune response in ruminants:

Focus on adipose tissue

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A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales.

Marie Curie.

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Abbreviations

2DE	Two dimensional electrophoresis
AGP	Alpha-1-acid glycoprotein
APP	Acute phase protein
APR	Acute phase response
AT	Adipose Tissue
BAT	Brown adipose tissue
CD	Cluster of differentiation
CID	Collision-induced dissociation
DAMPs	Damage associated molecular patterns
DC	Dendritic cell
DIGE	Differential gel electrophoresis
ECD	Electron-capture dissociation
ESI	Electrospray ionisation
ETD	Electron-transfer dissociation
FT-ICR	Fourier-transform ion cyclotron resonance
HP	Haptoglobin
IL	Interleukin
IT	Ion trap
iTRAQ	Isobaric tags for relative and absolute quantitation
LBP	LPS binding protein
LC	Liquid chromatography
LGL	Large Granular Lymphocytes
m/z	Mass/charge
MALDI	Matrix-assisted laser desorption ionisation
miRNA	microRNA
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NK cells	Natural killer cells
PAMPs	Pathogen associated molecular patterns

PRR	Pattern recognition receptor
PTM	Post translational modification
Q	Quadrupole
SAA	Serum amyloid A
SAT	Subcutaneous adipose tissue
SVF	Stromal vascular fraction
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TOF	Time-of-light
UCP	Uncoupling protein
VAT	Visceral adipose tissue
WAT	White adipose tissue

LIST OF PAPERS PRESENTED IN THE THESIS:

PAPER I (pp 38-60): UCP1 and UCP2 expression in different subcutaneous and visceral adipose tissue deposits in 30 days old goat-kids and effect of fatty acid enriched diets – Laura Restelli, Cristina Lecchi, Giancarlo Avallone, Guido Invernizzi, Giovanni Savoini, Fabrizio Ceciliani.
Submitted to *Research in Veterinary Science.*

PAPER II (pp 61-90): LC-MS/MS analysis of visceral and subcutaneous adipose tissue proteomes in young goats with focus on innate immunity and inflammation related proteins – Laura Restelli, Marius Cosmin Codrea, Giovanni Savoini, Fabrizio Ceciliani, Emoke Bendixen.
To be submitted to *Journal of Proteomics.*

PAPER III (pp 91-119): Effect of fish oil and stearic acid enriched diets on omental adipose tissue proteome in 30 days old goat-kids using iTRAQ analysis – Laura Restelli, Marius Cosmin Codrea, Guido Invernizzi, Fabrizio Ceciliani, Emoke Bendixen.
To be submitted to *Journal of Proteomics.*

OTHER PAPERS:

During my PhD, I have been involved in other side projects and reviews, investigating different aspects of the innate immune response. Part of the results have been published already or accepted for publication in peer-reviewed journals.

- Dilda F, Gioia G, Pisani L, Restelli L, Lecchi C, Albonico F, Bronzo V, Mortarino M. Ceciliani F. *Escherichia coli* lipopolysaccharides and *Staphylococcus aureus* enterotoxin B differentially modulate inflammatory microRNAs in bovine monocytes. Veterinary Journal 2012, 192(3):514-516.

- Rossi G, Capitani L, Ceciliani F, Restelli L, Paltrinieri S. Hyposialylated α1-acid glycoprotein inhibits phagocytosis of feline neutrophils. Research in Veterinary Science 2013, 95(2):465-471.

- Ceciliani F, Restelli L and Lecchi C. The Acute Phase Protein α1-Acid Glycoprotein: A Model for Altered Glycosylation During Diseases. Updates and New Perspectives (Review). Advances in Protein and Peptide Sciences 2013, 1:559-614.

- Sauerwein H, Bendixen E, Restelli L, Ceciliani F. The adipose tissue in farm animals: a proteomic approach (Review). Accepted on Current Protein and Peptide Science.

- Mavromati J, Cash P, Restelli L, Soler L. Proteomics and protein analyses of ovine and caprine body fluids: current studies and future promises (Review). Accepted on Current Protein and Peptide Science.

- Ceciliani F, Restelli L and Lecchi C. Proteomics in farm animals' models of human diseases (Review). Accepted on Proteomics: clinical application.

- (In preparation) Rahman M, Restelli L, Sauerwein H, Giudice C, Lecchi C and Ceciliani F. The acute phase proteins in bovine adipose tissue. To be submitted to The Veterinary Journal.

ABSTRACT

The main aim of this thesis is to explore new aspects of the innate immune response in ruminants, especially focusing on the role of adipose tissue. Particularly, adipose tissue was investigated in order to provide new information in a species where this tissue is very poorly characterised.

In ruminants, fat tissues play important biological roles for animal health for quality and gain in meat and milk production as well. A specific knowledge of how these pathways are controlled is of key importance for the management of animal health and from an economical perspective. Nevertheless, existing studies are mostly carried out in humans, where obesity is a major issue and little is known about ruminants.

In this thesis, the distribution of white and brown adipose tissue in several adipose deposits was investigated through UCP1 expression and general histology, showing a clear distinction between these two macroareas, with brown adipose tissue mostly present in visceral deposits. With our study, we also demonstrated the presence of brown adipose tissue in 30 days old goat kids and therefore the presence of this tissue in growing animals and not only in newborns. In addition, visceral and subcutaneous deposits were investigated with proteomic techniques, demonstrating that these two macroareas can be clearly distinguished by their proteomic profiles, but single deposits within the same macroarea do not display particular proteomic differences. Moreover, we demonstrated the involvement of adipose tissue of goat kids in inflammatory and immune response pathways, through expression of at least 27 immune related proteins, of which nine, namely ceruloplasmin, gamma fibrinogen, hemopexin, kininogen 1, lactoferrin, protein dj, thiosulfate sulfurtransferase, tumour translationally controlled 1 and valacyclovir hydrolase, were never investigated before in adipose tissue.

At a later stage, we focused our attention only on visceral adipose tissue, particularly on omentum, demonstrating that maternal diets enriched with either saturated or unsaturated fatty acid influence goat kid omentum proteome, but these influence is not confirmed at mRNA expression level. In addition, no influence of the maternal diet is showed on BAT distribution in goat kids.

PhD thesis rationale

This thesis undertakes the main aim to take a deeper look into new aspects of the innate immune response in ruminants, especially focusing on the role of adipose tissue. Adipose tissue is not only a tissue where energy is stored but is also involved in regulating several body functions, such as reproduction and inflammation. In ruminants, fat tissues play important biological roles for animal health for quality and gain in meat and milk production as well. A specific knowledge of how these pathways are controlled is of key importance for the management of animal health and thereby also for economic gain in dairy and meat production. Nevertheless, a systematic investigation of the molecular mechanisms underlying adipose tissue function has not yet been undertaken in ruminant species. Therefore, an in depth characterization of adipose tissue and an analysis on its role in inflammation and immune response prove to be of particular interest. Proteomics, being a large-scale comprehensive analysis of all the proteins in a specific cell or tissue, could of great help in understanding the molecular mechanism underlying the complex network in which adipose tissue is involved.

Several studies have been performed in human adipose tissue, where obesity has become a major issue, and therefore in rat and mouse as human model. Yet, few studies on adipose tissues' functions are available in ruminants, where obesity is not an issue, due to the controlled environment in which they live. Particularly, little information is available in goats, even though goat represents an important livestock species, both in developed and developing countries, providing not only milk and cheese, but also meat and clothing. Goats are tolerant to many diseases and parasites, easy to manage and resistant to harsh environment, therefore they represent a good model for experiments. No information about goat adipose tissues' proteome is available as well.

In the first part of the thesis a study of the distribution of different types of adipose tissue, namely white adipose tissue and brown adipose tissue, was carried out. Goat visceral and subcutaneous adipose deposits were investigated, providing for the first time a first general description of adipose tissue in young goats. The influence of maternal fatty acids-enriched diets was investigated, as well.

The second part of the thesis was focused on the possible involvement of adipose tissue of goat-kids in the innate immune response. A proteome characterization was performed in order to evaluate possible differences in subcutaneous and visceral adipose tissues depots in term of protein expression. The immune-related proteins were studied, and their expression was validated by gene expression studies.

In the third part of the thesis the possible influence of maternal diets on adipose tissue protein expression was evaluated in goat-kids. The mother were fed with different diets, including molecules that have been demonstrated to have an influence in innate immunity, and the proteome of adipose tissue of suckling goat-kids was investigated by means of quantitative proteomics.

CHAPTER 1. Principle of innate immunity and link to adaptive immunity, factors involved, inflammation and acute phase responses.

1.1 Innate immunity overview

Innate immunity is universally recognized as the initial line of defence of the body against non-self material, such as invading organisms or their products, and internal menaces. Unlike adaptive immunity, that evolved relatively recently and, therefore, is present only in higher vertebrates and jawed fish, innate immunity is considered an ancient component of the host defence system and can be found in all classes of animals and in all multicellular organisms in general (Turvey et al., 2010). Innate immunity provides a rapid immune response, within minutes or hours after infection, using a limited numbers of receptors to recognize a broad group of microorganisms. Indeed, unlike adaptive immunity that can recognize specific *non-self* antigens by clonal selection of antigen-specific lymphocytes, and provides long-lasting protection to the organism, innate immunity is an antigen independent, non-clonal, defence mechanism, and is generally considered a non-specific response. In addition, the lack of immunologic memory doesn't confer to the host a long-term innate immunity, while cells involved in adaptive immunity are able to respond more rapidly and efficiently upon subsequent exposure to an antigen (Warrington et al., 2011).

1.2 Factors involved in the innate immune response

Beside anatomical barriers, such as skin, mucus or tight junctions between cells, and physiological barriers, such as temperature, pH regulation and chemical mediators, innate immunity comprises two important categories of effectors: cellular components and humoral factors.

1.2.1 Cellular components

The principal mediators of the innate immune response are cells of hematopoietic origin. Hematopoietic cells involved in the innate immunity include cells of both myeloid and lymphoid lineages. Cells exert their functions through different mechanism, such as phagocytosis, endocytosis, production and release of signalling mediators, apoptosis induction.

Phagocytes, such as neutrophils, monocytes and macrophages, have the ability to internalize antigens and pathogens and degrade them. Dendritic cells have also the ability to phagocyte pathogenic microorganisms and, in addition, function as antigen-presenting cells, providing a link between innate and adaptive immune response. Eosinophils have an important role in the destruction of parasites and large-size targets that cannot be phagocytosed. They also fulfil a phagocytic activity. Finally, basophils and mast cells are involved in the control mechanisms regulating allergies, while natural killer (NK) cells, also called large granular lymphocytes (LGLs), recognise and destroy virus-infected cells and tumour cells (Tizard, 2000; Warrington et al., 2011). Characteristics and functions of hematopoietic cells involved in the innate immunity are listed in Table 1.1.

Interestingly, in addition to cells of hematopoietic origin, also non-hematopoietic cells, such as keratinocytes or epithelial cells of the respiratory, gastrointestinal and urinary traits are involved in innate immune reactions.

Table 1.1. Characteristics and functions of cells involved in innate immunity – (Warrington et al., 2011).

Cell	Image	% in adults	Nucleus	Functions	Lifetime	Main targets
Macrophage*		Varies	Varies	• Phagocytosis • Antigen presentation to T cells	Months – years	• Various
Neutrophil		40-75%	Multi-lobed	• Phagocytosis • Degranulation (discharge of contents of a cell)	6 hours – few days	• Bacteria • Fungi
Eosinophil		1-6%	Bi-lobed	• Degranulation • Release of enzymes, growth factors, cytokines	8-12 days (circulate for 4-5 hours)	• Parasites • Various allergic tissues
Basophil		< 1%	Bi- or tri-lobed	• Degranulation • Release of histamine, enzymes, cytokines	Lifetime uncertain; likely a few hours – few days	• Various allergic tissues
Lymphocytes (T cells)		20-40%	Deeply staining, eccentric	T helper (Th) cells (CD4+): immune response mediators Cytotoxic T cells (CD8+): cell destruction	Weeks to years	• Th cells: intracellular bacteria • Cytotoxic T cells: virus infected and tumour cells • Natural killer cells: virus-infected and tumour cells
Monocyte		2-6%	Kidney shaped	Differentiate into macrophages and dendritic cells to elicit an immune response	Hours – days	• Various

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1.2.2 Humoral factors

A large range of mediators is involved in the innate immune response, forming complex regulatory networks in which each molecule can act individually, in combination or in sequence, therefore modulating the response and its evolution. Humoral factors are involved in both sensing microorganisms and effector mechanism of the innate immune response, and can be produced by immune cells, as well as epithelial and endothelial cells. Different mediators have been identified (summarized in Table 1.2), and classified in three main families, namely complement factors, acute phase proteins (APP), and cytokines.

Table 1.2. Chemical mediators of inflammation – (Kotran et al., 1999).

Origin		Mediators	Source
cellular	Preformed mediators in secretory granules	Histamine	Mast cells, basophil, platelets
		Serotonin	platelets
		Lysosomal enzymes	Neutrophils, macrophages
	Newly synthesized	Prostaglandins	All leukocytes, platelets, endothelial cells
		Leukotrienes	All leukocytes
		Platelet-activating factor	All leukocytes, endothelial cells
		Activated oxigen species	All leukocytes
		Nitric oxide	Macrophages
		Cytokines	Macrophages, lymphocytes, endothelial cells
Liver→plasma	Factor XII (Hageman factor) activation	Kinin system (bradykinin)	
		Coagulation-fibrinolysis system	
	Complement activation	C_{3a}	
		C_{5a}	
		C_{3b}	
		C_{5b-9}	

The complement system is a group of 20 serum proteins that can interact with each other, with pathogens and with other elements of the immune system, distinguishing *self* from *non self*. Complement activation can be mediated by antibodies (classical pathway) or directly by microorganisms (alternative pathway) and has several effects on pathogens (opsonisation), leukocytes (attraction of phagocytes and induction of further release of inflammatory mediators), body cells that internalized virus or bacteria (lysis) (Tizard, 2000).

Acute phase proteins (APPs) are plasma proteins, mainly produced by liver, whose concentration increases (positive APPs) or decreases (negative APPs) of at least 50% during inflammatory reactions (Gabay and Kushner, 1999). APPs are believed to act in several aspects of inflammation and are mainly regulated by the pro-inflammatory cytokines IL1β, IL6 and TNFα.

Cytokines are proteins or peptides of low molecular mass (generally around 8-25 kDa) which modulate both innate and adaptive immune response, acting at systemic and local levels, on several different cell types, binding specific receptors on target cells. Cytokines are produced by several cell types, mainly activated leukocytes, but also by endothelium, epithelium and connective tissue cells. They may act alone or in combination to other cytokines and the same cell type usually secretes more than one at the same time. They are redundant in their biological activities, as many different cytokines have similar effects and are multifunctional, as an individual cytokine may have positive or negative regulatory actions (Kotran et al., 1999). Different families of cytokines can be distinguished, whose proteins are functionally more than structurally related (summarized in Figure 1.1).
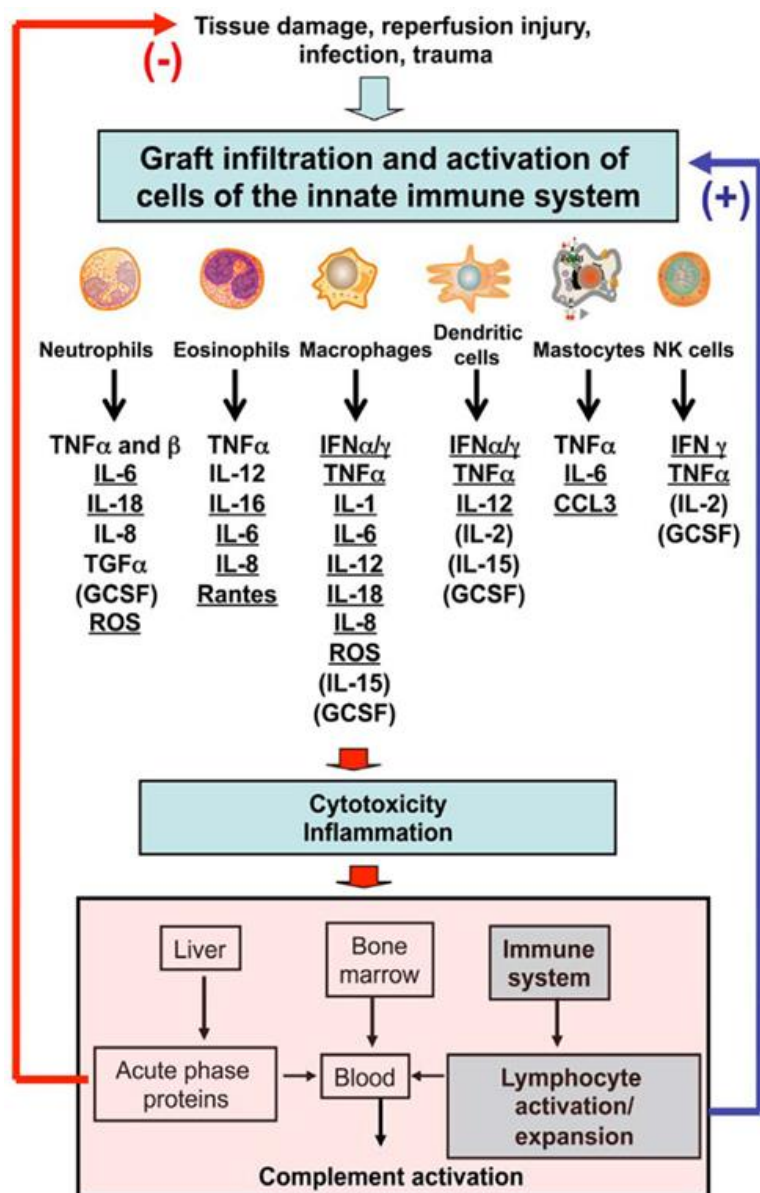
Figure 1.1. Inflammatory cytokines, sources and activities – (Kotran et al., 1999)

1.3 Local and systemic reactions to pathogens invasion: inflammation and acute phase response (APR)

1.3.1 Inflammation

One of the key aspects of the innate immunity is its ability to focus the defence mechanisms on the site of infection or tissue injury. Inflammation is a protective response aimed to resolve the infection or to repair the tissue injury, in order to return to a homeostatic state. The inflammatory response is a set of interactions among cells and soluble factors, the regulation mechanism of which has been described as a set of "stop and go" signals, determining the intensity and the length of this response.

Macrophages and dendritic cells, resident in the tissues, are responsible for the first recognition of the stimulus. This process involves highly conserved molecules, characterizing different groups of pathogens (PAMPs - pathogen associated molecular patterns) and different pattern recognition receptors (PRRs) on cells of the innate immunity. In addition, innate immunity cells can react against internal molecules which are considered as hallmark of a pathogenic situation, i.e. necrosis and other severe cellular damages. This class of molecules is known as DAMPs (Damage Associated Molecular Patterns). Damage-associated molecular pattern molecules are cell-derived and initiate and perpetuate immunity in response to trauma, ischemia, and tissue damage, either in the absence or presence of pathogenic infection (Tang et al., 2012).

After stimulus recognition, the production and secretion of inflammatory mediators, such as cytokines, chemokines, vasoactive amines, eicosanoids and products of proteolytic cascades, determine the immediate recruitment of leukocytes and plasma proteins. Elimination of the pathogen or tissue repair lead to the resolution of inflammation. On the contrary, if inflammation is not properly stimulated or well regulated, inflammation becomes chronic and it can lead to the onset of diseases (Pontieri et al., 2005).

1.3.2 Acute phase reaction (APR)

If the pathogen cannot be eliminated by local defences or the tissue repaired in a short time, the body responds by activating a large numbers of changes, acting at the systemic level and involving different organ systems. This systemic reaction to inflammation is called acute phase response (APR) (even though it refers to both acute and chronic inflammation). An APR

comprises complex endocrine, metabolic and neurological changes and it is characterized by, among others, fever, a decrease of blood cholesterol and leukocytes, the activation of the complement system and the blood coagulation system. The most evident phenomena is the over- or under-expression of a large family of structurally un-related plasma proteins, called acute phase proteins, that behave in different ways according to disease and animal species. APPs seem to be involved in pathogens' opsonisation, the identification of toxic compounds and the overall regulation of different stages of inflammation. APPs are mainly expressed by liver and the resources necessary for their production are drained from an increased catabolism of already available depots, such as lipids coming from adipose tissue. Recently, their expression has been demonstrated in other organs including among the others also bovine forestomachs and abomasum (Lecchi et al., 2009; Dilda et al., 2012). APPs in ruminants are haptoglobin (HP), serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP) and LPS binding protein (LBP) (Ceciliani et al., 2012).

1.3.3 Immune activation of the innate immune processes: the Pattern Recognition Receptors

The immune activation of the innate immunity cascades relies on recognition of evolutionary conserved traits, shared by large groups of pathogens (PAMPs) and of endogenous cell-derived signals (DAMPs), through a limited number of invariant receptors, encoded in the germ line. This strategy ensure the recognition of a wide range of pathogens in a specific way. Two are the central features of innate pattern recognition: different classes of pathogens, with completely different life cycles and different biochemical composition, are recognised by a limited number of receptors and activate relatively similar response mechanisms in the host. In addition, each class of pathogens is able to stimulate more than one type of PRRs, through different PAMPs and DAMPs, ensuring a rapid and efficient response (summarized in Figure 1.2).

PAMPs are essential for the vitality of the microorganism and distinguishable by the host from what is *self,* through pattern recognition by PRRs*.* They include lipopolysaccharides, lipoproteins, peptidoglycans, oligosaccharides, nucleic acids. DAMPs are cells-released molecules working as endogenous danger signals for the innate immune system to unscheduled cell death, to microbial invasion, and in response to stress. They include proteins located within the nucleus and the cytoplasm, the cytoplasm alone, the exosomes, the extracellular matrix and the plasma. Non-protein DAMPs can also be distinguished, such as DNA, RNA and ATP (Tang et al., 2012).

On the other hand, PRRs are involved in several aspects of the immune system and are present in plants, insects and animals. There are several different families of PRRs that can be divided into two main categories: soluble receptors, such as complement proteins, pentraxins, collectins, the LBP protein and the CD14 that can also be associated to cells, and cell-associated receptors, present on several cell types involved in the innate immunity, including leukocytes and epithelial cells. Cell associated receptors can be distinguished in intracellular, scavenger and membrane bound receptors. The major and best characterised PRRs are the Toll-like receptors (TLRs), a big family of transmembrane receptors that represent the main link between the recognition of PAMPs from bacteria, virus, fungi and protozoa to the activation of the inflammatory response (Akira et al., 2012).

In general, the activation of a PRR by PAMPs or DAMPs activates a cascade of signalling pathways that leads to the activation of gene expression and production of a wide range of signalling molecules, such as cytokines, chemokines, cell adhesion molecules and immune receptors (Basset et al., 2003; Barton, 2008; Mogensen, 2009).
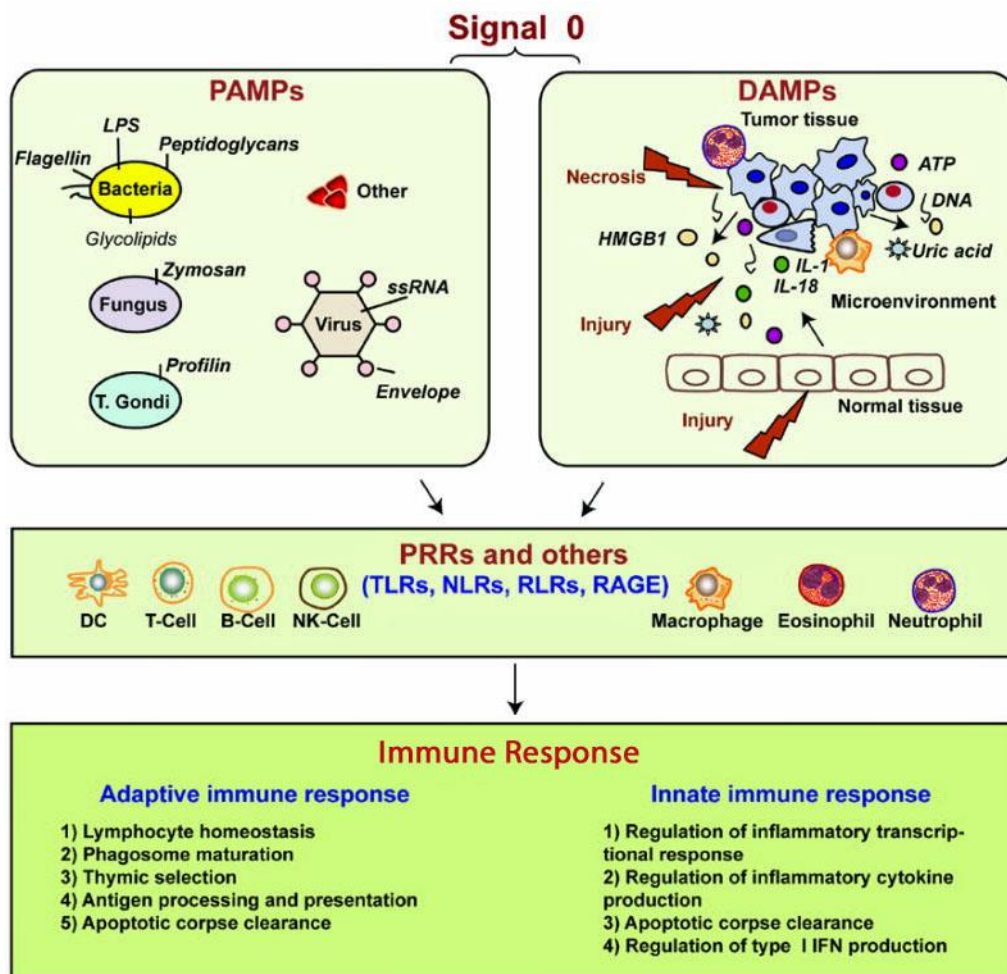
Figure 1.2. Immune response activation scheme. Different PAMPs and DAMPs can activate the immune response via interaction with PRRs – (modified from Tang et al., 2012).

1.4 Linking innate and adaptive immune responses

Innate immunity has a central role in activating the adaptive immune response. Dendritic cells play essential functions in this process when presenting antigens to T helper cells. Indeed, when encountering a pathogen, immature dendritic cells undergo developmental changes, including antigen processing and presentation, and they migrate to lymphnodes where they prime antigen specific T cells. In addition, besides direct activation of innate host-defence mechanisms after PAMPs recognition, some PRRs are involved in the activation of the adaptive immune response. Dendritic cells present on their surface most of the TLRs and different subsets of DCs present on their surface different and non-overlapping sets of TLRs, that gives to T cells the "information" regarding the type of antigen presented (Iwasaki and Medzhitov, 2004). As proposed by Medzhitov, *the basic principle of innate control of adaptive immunity is based on establishing an association between the antigens recognized by lymphocytes and the microbial products recognized by PRRs* (Medzhitov, 2007). Furthermore, it is important to remember that the differentiation of DCs is controlled by several factors, including TLR-induced cytokines.

1.5 miRNAs and the innate immune response

MicroRNAs (miRNAs) are conserved, non-coding, single strand sequences of 20-22 nucleotides that regulate post-transcriptionally gene expression by targeting the 3′-untranslated region of specific messenger RNAs, leading to translational repression or degradation of the target. miRNAs are involved in several biological processes, such as cell differentiation and maturation and have an influence on cell functions. Recently, several studies have demonstrated the role of microRNA in regulating leukocytes proliferation and different aspects of the immune responses. Indeed, as recently reviewed by Zhu (2013), several microRNAs, such as miR21, miR- 146a, miR-155, miR-196 and miR-223 are important regulators of granulocytes, NK cells and monocytes development and functions. miRNAs have a cell-specific (Merkerova et al., 2008) and species-specific (Ramkissoon et al., 2006) expression, with miR-21, miR-146 and miR-155 particularly ubiquitous. In addition, miR-9, miR-146 and miR-155 seem to have a role in the negative regulation of acute phase response.

miRNAs expression is highly regulated and seems to be strongly influenced by TLRs, with some miRNAs (for example, miR-155) highly responsive, as their expression is induced two hours

after treatment, and other miRNAs (for example, miR-21) that are induced at later times. A new trend in miRNAs biology suggests that miRNAs can, in turn, regulate TLRs signalling, targeting several aspect of this process, such as TLRs expression, signalling proteins or transcription factors. This mutual interaction is confirmed by the fact that cell stimulation by challengers, such as lipopolysaccharides, and therefore TLRs activation, induce an expression modulation of several miRNAs (Tili et al., 2007; Ceppi at al., 2009; Taganov et al., 2009).

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CHAPTER 2. Adipose tissue: general characteristics and issues, influence on animal health and meat quality.

Adipose tissue (AT) is a loose connective tissue, for a long time considered as an inert storage tissue for the excess of energy in the form of triglycerides. The identification of leptin by Zhang and co-workers in 1994 was the first demonstration that adipose tissue is also involved in regulating homeostasis (Zhang et al., 1994). Indeed, adipose tissue is an active endocrine organ, or a group of organs, regulating both metabolic and inflammatory pathways through production and secretion of signalling molecules, collectively referred to as adipokines (Lehr et al., 2012).

2.1 Brown and white adipose tissue

Adipocyte are the most represented cell type in adipose tissues. Besides adipocytes, other cell types can be found, namely preadipocytes, fibroblasts, vascular endothelial cells and different immune cells (i.e. macrophages), collectively called stromal vascular fraction (SVF).

In mammals, two types of adipose tissue can be distinguished according to function and composition: white adipose tissue (WAT) and brown adipose tissue (BAT). In both types, adipocytes are the major cellular component, but while white adipocytes have a peripheral nucleus due to the presence of one large lipid droplet (unilocular adipocytes), brown adipocytes have a central nucleus and many small lipid droplets (multilocular adipocytes), the cytoplasm is rich in mitochondria and to this is due the brown colour (Enerbäck, 2009; Cinti, 2012). WAT is the main adipose tissue that can be found in adults and its main function is to store the excess of energy as triglycerides and to make this energy available by releasing fatty acids into the blood. BAT is especially abundant in hibernating animals and newborns. Its primary function is to dissipate energy through the production of body heat, therefore its presence is important for survival in the first days of life, but also during the following days, since muscle metabolism is strongly related to that of BAT (Harwood Jr, 2012). Fatty acids from triglycerides degradation are not released into the bloodstream, but locally metabolized whereby oxidative respiration is uncoupled from the production of adenosine triphosphate by a specific protein, i.e. uncoupling protein-1 (UCP1). Exclusively expressed by BAT, UCP1, or uncoupling protein 1, belongs to a family of mitochondrial anion carrier proteins that transfer protons across the mitochondrial inner membrane, though reducing ATP synthesis and

dissipating energy as heat (Damle and Marín-García, 2010). In addition to UCP1, other uncoupling protein genes have been identified, namely UCP2, UCP3, UCP4 and UCP5 with different tissue specificity expression. They are being called "classical" or "developmentally programmed" brown adipocytes. However, brown adipocytes may appear after thermogenic stimuli at anatomical sites corresponding to white adipose tissue (WAT). This process is called the "browning" of WAT. The brown adipocytes appearing in WAT derive from precursor cells different from those in classical BAT and are closer to the white adipocyte cell lineage. The brown adipocytes appearing in WAT are often called "inducible, beige, or brite." (Giralt et al., 2013)

2.2 Visceral and subcutaneous adipose tissue

Adipose tissue is distributed as fat depots throughout the whole body. The type of adipocytes, endocrine function, lipolytic activity and response to hormones vary, according to their location. Fat depots are mainly classified as subcutaneous (SAT) or visceral (VAT) adipose tissue. In ruminants, SAT includes depots located beneath the skin, e.g. the armpit cavity, the subcutaneous areas over the sternum and the withers and the base of the tail, while VAT is located in the intra-abdominal cavities, surrounding specific organs, such as kidney and heart, or distributed among peritoneum layers, such as mesenteric and omental fat (Peinado et al., 2010). In most mammals, brown and white adipocytes are found together in both subcutaneous and visceral deposits (Cinti, 2012). Beside their topographical locations, visceral and subcutaneous adipose tissues have unique adipokines expression profiles, and different metabolic characteristics (e.g. processing excess of lipids) (Votruba and Jensen, 2007; Ibrahim et al., 2009; O'Rourke et al., 2009).

2.3 Adipose tissue in farm animals: influence on animal health and meat quality

The central role of adipose tissue in several biological processes, including metabolic control, oxidative stress and inflammation, as well as for both innate and adaptive immune response, is now well recognized. In humans, adipose tissue has a key role in obesity, while in farm animals, where obesity is not an issue, due to the controlled environment in which they live, particular focus has been given to adipose tissue's influence on animal health and meat quality. Indeed, it has been demonstrated that adipose tissue within the muscle (i.e. marbling fat) strongly

influence meat quality and composition, by affecting parameters such as tenderness, juiciness and taste (Wood et al., 2008), therefore it is clear its economic value. On the other hand, in dairy animals AT metabolism gained particular interest for its essential role in the transition period when a hormonally-controlled lipid mobilization is established in order to support milk synthesis (Shirley et al., 1973; Contreras and Sordillo, 2011). The ability of adipose tissue to regulate the wide range of biological processes ensues its capacity to produce and release adipokines. So far, several adipokines have been discovered, produced by adipose tissue (e.g. leptin, adiponectin and visfatin), but also molecules that are known to be secreted by other tissues (e.g. interleukins, APP). In humans, macrophages of the stromal vascular fraction increase in obese states and highly contribute to the secretory function of adipose tissue, especially for inflammatory cytokines, such as TNF-α and IL-6. However, in dairy cattle phagocytic cells are hardly detectable in neither visceral nor subcutaneous adipose tissues, hence the role of these immune cells in the immunologic and metabolic adaptations of adipose tissue in non-obese lactating animals has been considered to play only minor roles (Weisberg et al., 2003; Xu et al., 2003).

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CHAPTER 3: METHODS OF PROTEOMICS – Mass spectrometry, gel-based and gel-free proteomics, comparative analyses.

Proteomics is a large-scale comprehensive analysis of all the proteins in a specific cell or tissue, including studies on protein abundance, protein variations and modifications, along with their interacting partners and networks, in order to understand the complex cellular processes. The set of all protein forms expressed by a tissue or even organism, including splice isoforms and post-translational modifications (PTMs), protein interactions and activity, is called *proteome*. The study of a full proteome is of extreme interest in order to better understand the complexity of an organism, nevertheless it presents several difficulties and challenges. Indeed, despite the improvements of the recent years, it is a task that has still to be achieved in all species. The reasons are several: first of all, while the genome of an organism generally doesn't vary from cell to cell, proteins changes in different cells, different tissues and even in the same cells in response to different stimuli. Furthermore, protein identification strongly relies on the availability of sequence databases, and this has become possible only after the intense genomic revolution. At the same time, the number of proteins highly exceeds the number of genes in an organism. Finally, protein concentration range exceeds the dynamic range of any single analytical method or instrument (Domon and Aebersold, 2006).

3.1 Mass Spectrometry overview

Mass spectrometry (MS) is one of the central analytical techniques in the proteomic field, and it has increasingly become the method of choice to analyse, on a large scale, complex protein samples.

MS measures the mass-to-charge ratio (m/z) of gas-phase ionised analytes. By definition, MS consists of an ion source that converts analyte molecules into gas-phase ions, a mass analyser that separates ionised analytes on the basis of the m/z ratio, and a detector that register the number of ions at each m/z value. The data output is a spectrum reporting all the m/z ratios detected (Figure 3.1a). Bioinformatic programs, such as ProteinPilot or MASCOT are then responsible of the protein identification by matching the spectra m/z data with databases of protein sequences. This point represents one of the biggest limitation of proteomics. Indeed if

the genome of a certain species is not fully sequenced, protein identification will be only partial.

An important characteristic of MS is the use of different methodologies and components in combination, all with special strengths and weak points, rather than a single technique. Before MS analysis, sample complexity can be reduced, mainly by fractionation with liquid chromatography techniques or one- or two-dimensional gel electrophoresis.

3.1.1 Tandem Mass Spectrometry (MS/MS)

In order to determine protein or aminoacid peptide sequences and for post translational modification (PTM) analysis, tandem mass spectrometry can be applied. Tandem mass spectrometry can be performed in space, by combining different instrumentation, or in time, by instrument programmed so that the different steps are successively carried out in the same instrument. To perform tandem mass spectrometry, after the first MS analysis that gives the m/z information, some peptide ions are selected (precursor ions) and fragmented in a collision cells. The resulting product ions are then further analysed according to their m/z ration, and the MS/MS spectrum recorded (Figure 3.1b) (de Hoffmann and Stroobant, 2007).

A

Protein
fractionation/separation

⬇

Sample
Injection ⇨ **A** Ion
source ⇨ **B** Mass
analyzer ⇨ Ion
detector ⇨ Data
system

C

Mass
analyzer 1 ⇨ Reaction
region ⇨ Mass
analyzer 2

Data output
Mass spectrum

B

Precursor ion
(e.g m/z 166)

Collision-induced
dissociation (CID)

Product ion
(e.g m/z 133)

Intensity / Time
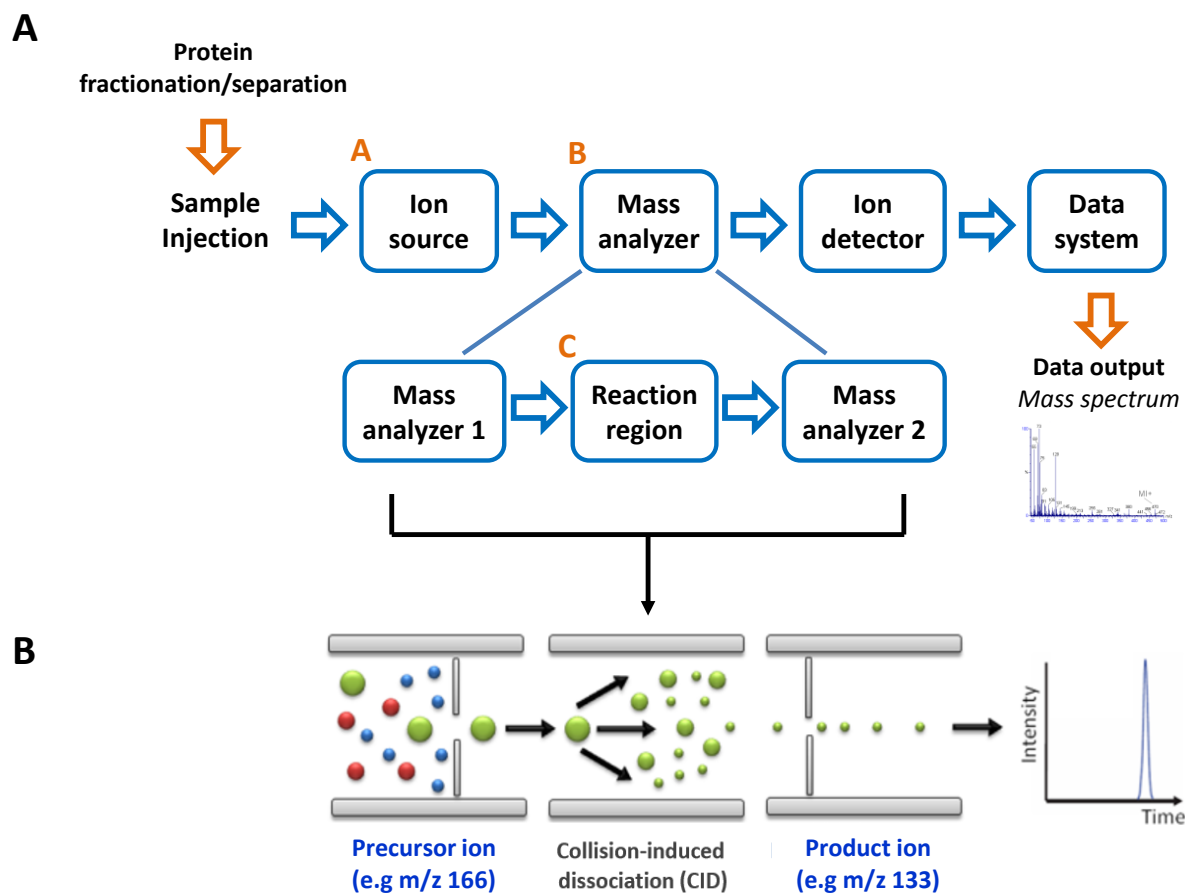
Figure 3.1. Mass Spectrometry components and workflow. A) Generic scheme of a mass spectrometer. Ion source, mass analyser and reaction region will be explained in detail in the following paragraph. B) Detail of the different steps of a tandem mass spectrometry.

3.1.2 Mass spectrometry elements

A mass spectrometer is basically composed by three elements in a vacuum atmosphere: an ionization source, a mass analyser and a detector. In this section the main types of ionization methods and mass analyser are presented.

A - ION SOURCE

There are several ionization methods that can be employed, but the most used are MALDI and ESI (Bautista-de Lucio et al., 2013).
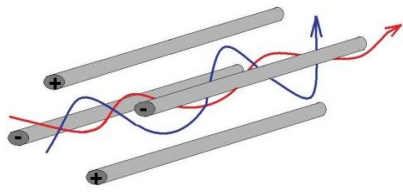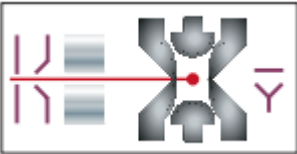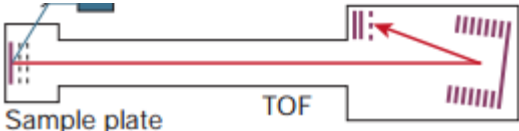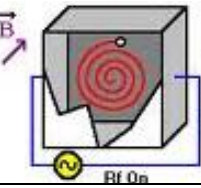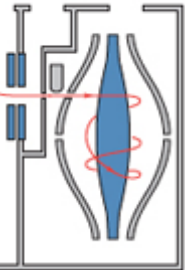
-<u>Matrix-assisted laser desorption/ionisation (MALDI)</u> uses laser pulses to sublimate and ionise analytes on a dry and crystalline matrix. Proteins/peptides are co-precipitated with an organic compound which is capable of absorbing laser light allowing the fragmentation.

-<u>Electrospray ionisation (ESI)</u> permits the ionisation when analytes are dispersed in an organic solvent spraying this mixture through a fine capillary tube that is maintained in an electric field. ESI is often coupled to liquid-based separation tools.

B - MASS ANALYSER

The mass analyser is the central part of the technology. There are different types of mass analyser, each one suitable to different scopes. The most commonly used in proteomics studies are listed in Table 3.1 together with their operating principles

Table 3.1. Main types of mass analysers and their working principles. The red line represents the stable movement of the ion within the mass analyser that will allow its detection, while the blue line represents an unstable trajectory and it means that ion will not be detected (Domon and Aebersold, 2006).

MASS ANALYZER	WORKING PRINCIPLE	SCHEME
Quadrupole (Q)	Separates ions in an electric field generated among 4 parallel poles. Ions move in a way that is directly proportional to its mass	
Ion trap (IT)	Ions are trapped for a certain time and are then analysed by MS or MS/MS according to m/z ratio.	
Time of flight (TOF)	Detects the m/z ratio of an analyte ion from its flight time through a tube of specified length and under vacuum.	
Fourier-transform ion cyclotron resonance (FT-ICR)	It is a trapping mass spectrometer that captures the ions in a high magnetic field and under high vacuum.	
Orbitrap	It is a trapping mass spectrometer in which ions orbit around a central electrode, according to their m/z ratio.	

Combinations of more than one type of analyser are often used. The key parameters on which to choose the more suitable analyser are mass accuracy, resolving power, sensitivity, dynamic range and the ability to generate MS/MS spectra. Table 3.2 lists the key parameters of the most commonly use mass analysers' combinations.

Table 3.2. Key parameters of the most commonly use mass analysers' combinations – (Domon and Aebersold, 2006).

	IT-LIT	Q-Q-ToF	ToF-ToF	FT-ICR	Q-Q-Q	QQ-LIT
Mass accuracy	Low	Good	Good	Excellent	Medium	Medium
Resolving power	Low	Good	High	Very high	Low	Low
Sensitivity (LOD)	Good		High	Medium	High	High
Dynamic range	Low	Medium	Medium	Medium	High	High
ESI	✔	✔		✔	✔	✔
MALDI	(✔)	(✔)	✔			
MS/MS capabilities	✔	✔	✔	✔	✔	✔
Additional capabilities	Seq. MS/MS				Precursor, Neutral loss, MRM	
Identification	++	++	++	+++	+	+
Quantification	+	+++	++	++	+++	+++
Throughput	+++	++	+++	++	++	++
Detection of modifications	+	+	+	+		+++

C - FRAGMENTATION METHODS

When performing tandem mass spectrometry, different fragmentation methods are available. The most commonly used is the collision-induced dissociation method. In this section the most popular are shortly presented.

-Collision-induced dissociation (CID) is the most widely used fragmentation method for MS/MS analysis. Peptides/proteins are fragmented, at the C-N bond, through internal heating by multiple collision with rare gas atoms (Figure 3.1b).

-Electron-capture (ECD) and electron-transfer (ETD) dissociation are new techniques that fragment peptides/proteins at the N-Cα bond through the capture of thermal electrons.

3.2 MS or MS/MS coupled to 2D-Gel electrophoresis

Two main branches of research can be identified. When the aim is the analysis of substantially purified proteins, a combination of two-dimensional gel electrophoresis (2DE) and mass spectrometry is used. This method is also referred to as pull-down proteomics. After extraction, proteins are separated by 2DE and stained. Spots of interest are selected, excised and digested. Peptides are then analysed by MS or MS/MS and proteins identified. Usually 2DE are associated to MALDI-MS or MALDI-MS/MS (i.e. MALDI-TOF) (Figure 3.2). This method permits the analysis of relatively simple samples and it is able to identify related proteins, such as different isoforms of the same protein. On the other hand, the dynamic range is limited and most abundant proteins are mainly identified if not eliminated previously (Aebersold and Mann, 2003; Han et al., 2008).
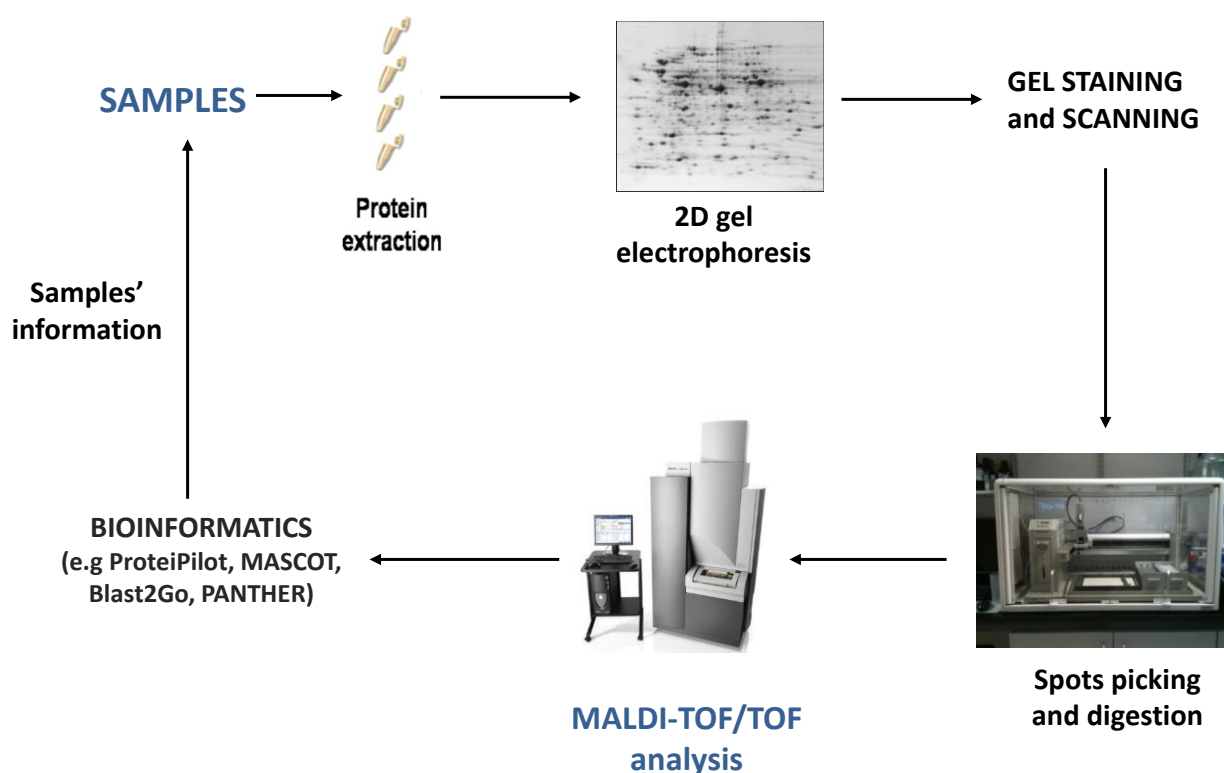
Figure 3.2. 2DE MALDI-MS/MS workflow. MALDI-TOF/TOF is the most common mass spectrometer associated to 2DE analysis.

3.3 MS or MS/MS coupled to 2D-Liquid chromatography

When the aim is the analysis of complex samples, with limited protein purification, a combination of liquid chromatography and mass spectrometry is used. This method is also referred to as shotgun proteomics. After extraction, the entire protein mixture is digested. Peptides are then fractionated using liquid chromatography in order to remove impurities and reduce sample complexity and analysed by automated MS or MS/MS. Bioinformatics is used for protein identification and analysis (Figure 3.3). As samples are in a liquid form, ESI-MS or ESI-MS/MS are often used. This technique leads to a rapid identification of complex sample mixtures, and the protein coverage is higher than with a MALDI-MS approach. On the contrary, the dynamic range is limited, there is a high redundancy in protein identification and the amount of data generated is huge and complex to analyse (Aebersold and Mann, 2003; Han et al., 2008).
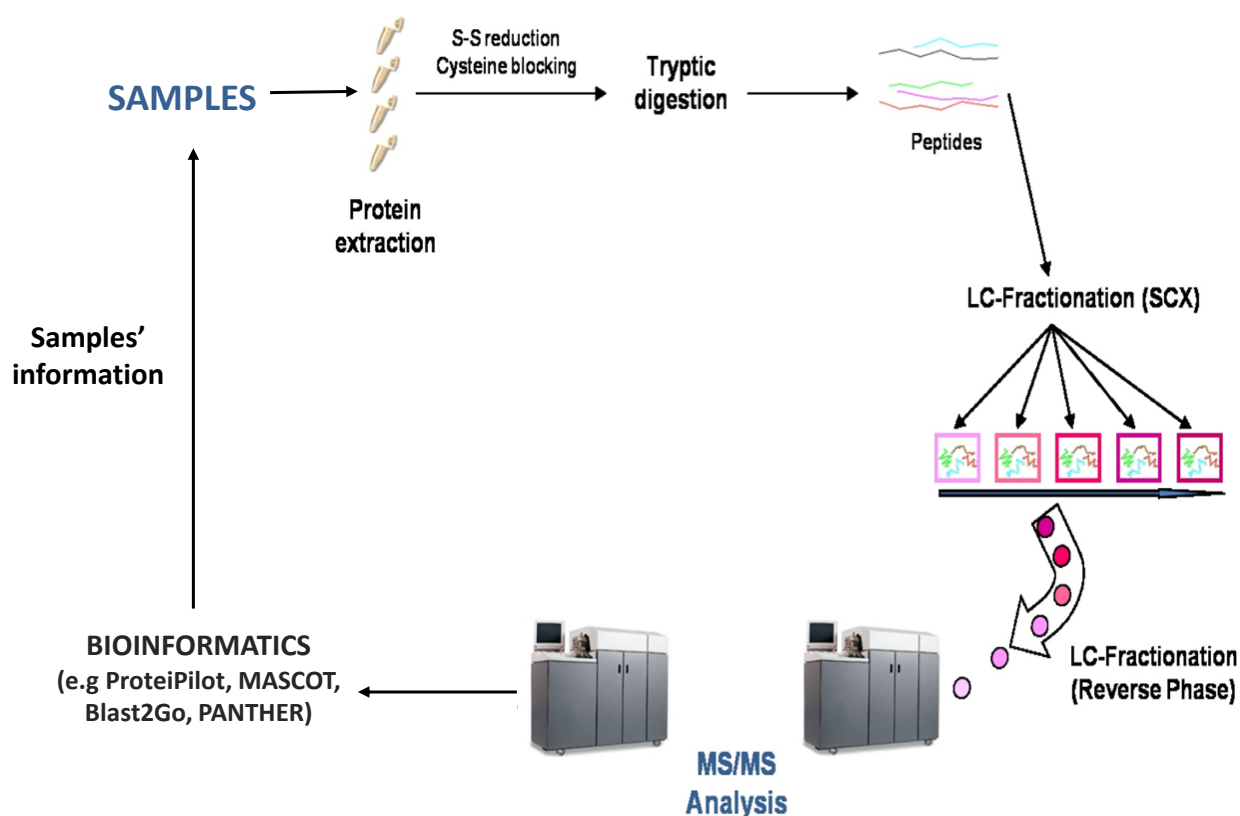
Figure 3.3. 2D-LC-MS/MS workflow.

3.4 Quantification methods

Besides its use for protein detection in a cell or tissue type and for post translational modifications identification, MS can be used to determine dynamic changes in proteins of different samples (i.e. different treated samples). Quantification methods are different, mainly divided into label free approaches and label based approaches. The first type of methods is based on spectral counting or on peptide precursor ion intensities obtained from the first mass spectrometer of an MS/MS approach. The second type of approaches is based on the assumption that the relative signal intensity measured by MS of two chemically identical analytes with different isotope composition, represents the relative abundance of the two analytes in the sample (Gstaiger and Aebersold, 2009).

3.4.1 Gel based quantification: 2D differential gel electrophoresis (2D-DIGE)

2D differential gel electrophoresis is used to separate and analyse labelled proteins, prior to their digestion to peptides. This technique is based on the use of modified cyanine fluorescent dyes to label the samples, followed by bi-dimensional SDS-PAGE that allows protein separation after the labelling and by MS or MS/MS that allows protein identification and analysis. Two differentially treated samples can be analysed at the same time, on the same gel, using different fluorescent dyes (Cy3 and Cy5). A third die (Cy2) is used to label a reference sample, which will be the same in each gel run, and will be used to compare different gel runs, reducing gel-to-gel and sample-to-sample variability. The reference sample is constituted by a mixture of equal amounts of the samples under analysis. Once run, gels are scanned at different wavelength corresponding to the fluorescent emission of each dye. On each gel, proteins-spots of two different samples can be visualized. All experimental gels are then matched together using bioinformatic software. A protein with different concentrations in different samples (e.g. treated vs non-treated) will have different fluorescent intensities. Differentially fluorescent protein-spots are then listed and individually excised. Protein identification is achieved by MS or MS/MS analysis after trypsinisation, and results are analysed by bioinformatics tools (summarised in Figure 3.4).

2D-DIGE is used to analyse proteins that differ in post-translational modification, especially phosphorylation, but allows the analysis of only a subset of identified proteins and has a low dynamic range and difficulties in identifying proteins at extreme molecular weights and isoelectric points (Coombs, 2011; Beckett, 2012).

3.4.2 Gel free quantification: isobaric tags for relative and absolute quantitation (iTRAQ)

Isobaric tags for relative and absolute quantitation can be applied to intact proteins or to peptides derived from them. Proteins can be tagged and analysed, but most commonly proteins are first digested and later peptides are differentially tagged and analysed (Figure 2.4). This technique requires instruments with relatively high resolution and capable of initial MS/MS.

After protein extraction and digestion, samples to compare (e.g. differentially treated samples) are labelled with different tags and mixed together in equivalent amounts and analysed by MS/MS previous LC separation (summarised in Figure 3.4). Up to 8 different tags can be used, that means that up to 7 differentially treated samples and a reference sample can be analysed together. The iTRAQ tags are isobaric labels that react with free amino groups, including lysine side chains and peptide N-termini. Each label has a neutral balance group to maintain an overall mass of 145Da, a peptide group that reacts with the peptides of the sample under analysis and a unique charged reporter group. During peptide fragmentation by MS/MS, the reporter groups break off producing for each sample, different ions at different m/z (114, 115, 116, 117, 118, 119, 120, and 121). The relative intensities of the reporter ions are directly proportional to the relative abundances of each peptide in the samples that are compared (Fuller and Morris, 2012).

iTRAQ is potentially able to tag every peptide and this increases protein identification confidence. In addition, the combination of different isobaric tags increases MS and MS/MS sensitivity. On the contrary, a large number of steps leads to greater variability and a variable protein solubilisation and digestion may lead to wrong quantifications (Coombs, 2011).
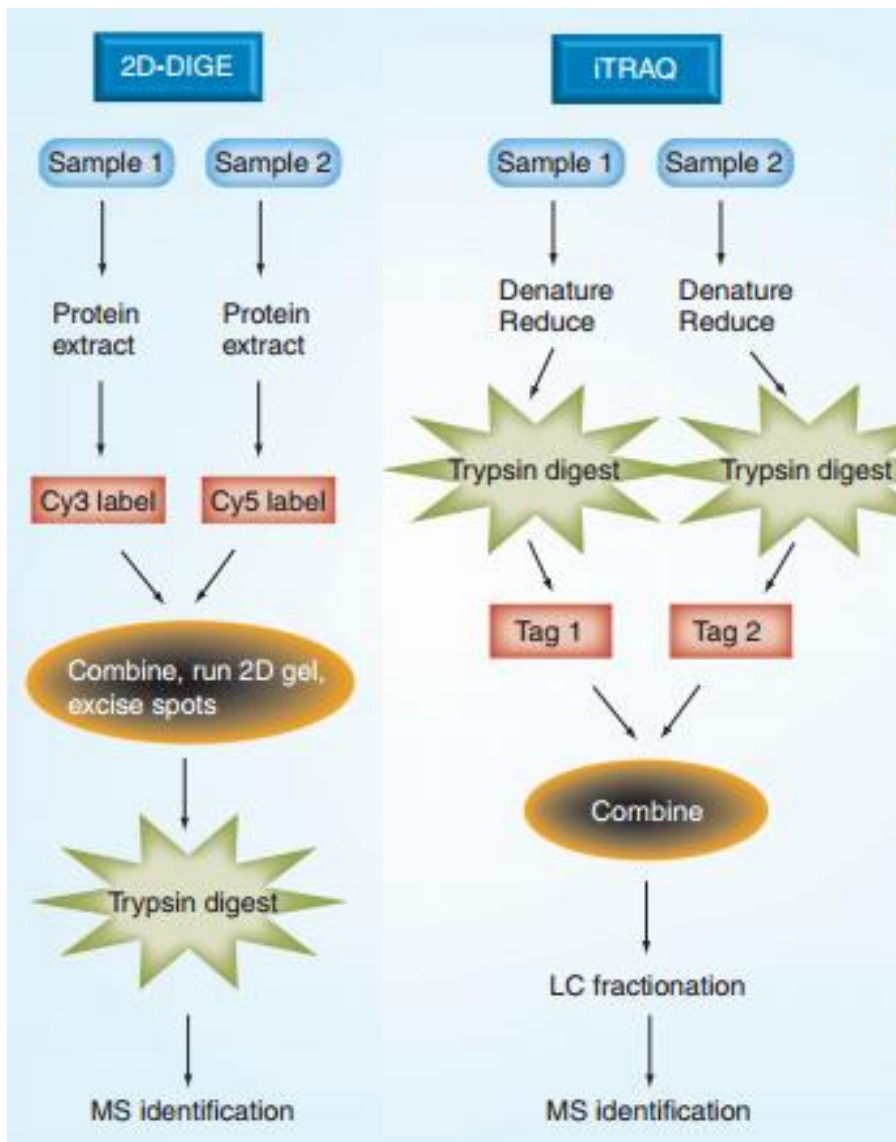
Figure 3.4. Proteomics quantification methods. 2D-DIGE and iTRAQ are two the most commonly used quantification methods in proteomics. The figure shows the workflows of the two different techniques (Coombs, 2011).

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CHAPTER 4. Experimental section: goat adipose tissues' characterization, experimental design and results.

Three aspects are of particular relevance when studing adipose tissue in farm animals:

- Adipose tissue composition in terms of white and brown deposits. Indeed, brown adipose tissue, dissipating energy through the production of body heat, is important for survival in the first days of life, but also in the adult life, since muscle metabolism is strongly related to that of BAT. Both these aspects are strongly correlated to economic aspects and therefore of particular interest.

- Behavior of different deposits in term of protein expression. Indeed, it is well known in human that adipose tissue at subcutaneous and visceral levels display unique adipokines expression profiles, and different metabolic characteristics, but also different deposits within these two macroareas can have different expression behaviours. This is of particular importance when aiming to improve animal health and production through modification of adipose tissue characteristics.

- Influence of a diet on adipose tissue. Indeed, fat is strongly related to the diet and in farm animals, where diets are strictly controlled and can be manipulated, this means adipose tissues characteristics and therefore animal products, may be influenced and controlled.

The project presented in this chapter is part of a larger one, performed by Professor Savoini and co-workers, aimed to elucidate the impact of diets enriched with saturated or unsaturated fatty acids on goat's peripartum. Hereby only the experimental parts involved in this thesis are presented (Figure 4.1).

4.1 Experimental design
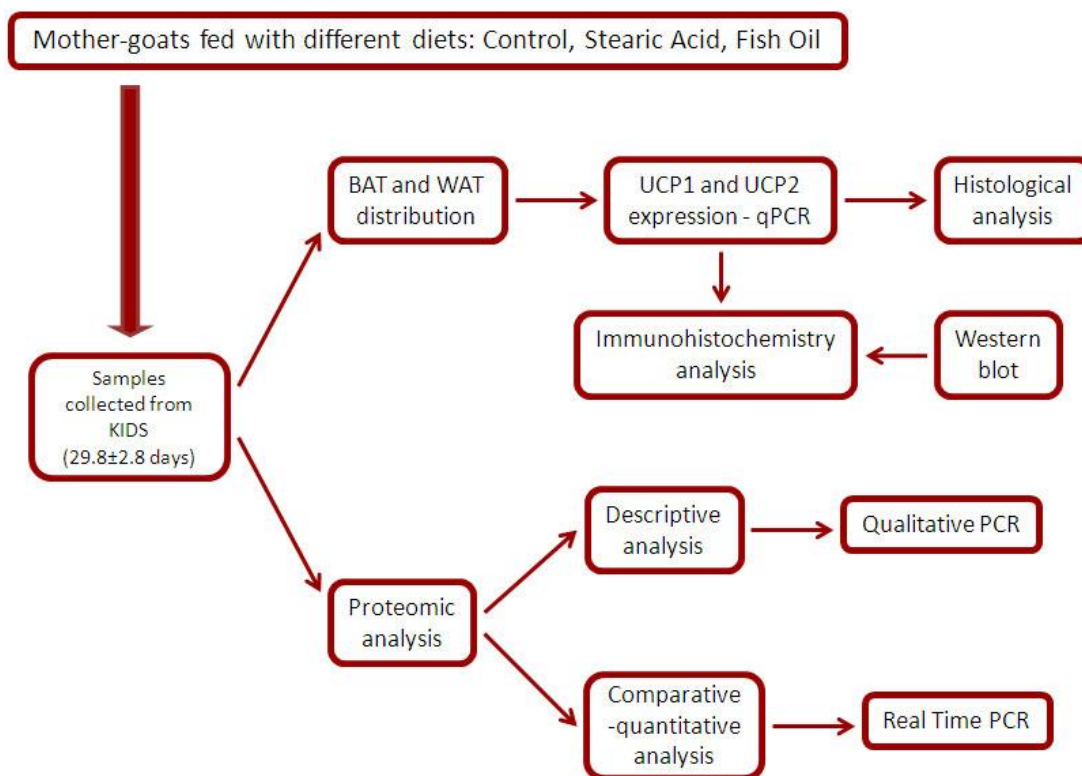
Figure 4.1. Thesis experimental design's scheme.

The experimental starting group of animals comprised 26 alpine healthy dairy goats, homogeneous for parity and milk production during the previous lactation, divided into three experimental groups, according to the administered diet. Goat were fed with either Saturated or Polyunsaturated Fatty Acids, in the form of stearic acid and fish oil, respectively. A control group, without any diet addition was also added to the experiment. Experimental diets were administered starting from two days before delivery until male kids were sacrificed (29.8 ± 2.8 days) and adjusted according to dry period or lactation period, as shown in Table 4.1.

Adipose tissue samples were collected, within 30 minutes from slaughtering, from subcutaneous and visceral adipose depots. SAT was taken from sternum, armpit cavity, base of the tail and withers, while VAT was taken from perirenal, omental and pericardial depots. Liver samples were also collected in order to use them as reference tissue during further analyses. Samples were stored according to their experimental use (i.e. RNAlater, liquid nitrogen, formalin).

Table 4.1 Experimental diets during the dry period and during lactation						
	DRY PERIOD (calculated in order to administer 30 g of fatty acids)			LACTATION (calculated in order to administer 50 g of fatty acids)		
	CTRL	FO	ST	CTRL	FO	ST
Alfalfa hay				1000	1000	1000
Mix hay	1200	1200	1200	500	500	500
Concentrate mix	600	600	600	1500	1500	1500
Corn	100	100	100	200	200	200
Vitamin E	0.072	0.072	0.072	0.080	0.080	0.080
$CaCO_3$	9	9		15	15	
Fish oil		81			135	
Calcium stearate			34			56

The distribution of WAT and BAT in different visceral and subcutaneous deposits was investigated by quantitative PCR studying the different mRNA expression of UCP1 (uncoupling protein 1 or thermogenin) and UCP2 (uncoupling protein 2). In order to confirm the results of this first part, a histological and immunohistochemistry analyses were carried out as well, preceded by antibody validation by Western blot. The influence of maternal diets was evaluated, as well, using the same techniques.

Later, a proteomic descriptive analysis was performed on control samples by two-dimensional liquid chromatography coupled with a tandem mass spectrometry system (2D-LC-MS/MS), in order to investigate the possible difference, at a proteome level, among distinct visceral and subcutaneous deposits. The ability of adipose tissue to produce factors involved in inflammatory and immune-related proteins was then investigated by qualitative PCR.

Finally, a comparative-quantitative proteomic analysis was performed by iTRAQ labelling and 2D-LC-MS/MS on omental deposits, in order to evaluate the possible influence of the maternal diet on protein and mRNA expression in visceral adipose tissue.

The proteomic studies were carried out at Aarhus University, in collaboration with Professor Emoke Bendixen by means of a COST-Short Term Scientific Mission Grant.

PAPER I

UCP1 and UCP2 expression in different subcutaneous and visceral adipose tissue deposits in 30 days old goat-kids and effect of fatty acid enriched diets.

To be submitted to *Veterinary Research Communication.*

Aim: the main aim of the present study was to determine the distribution of BAT and WAT, in different subcutaneous and visceral adipose tissue deposits of goat-kids, by means of quantitative PCR measurement of UCP1 and UCP2 gene expression and confirmation by histological and immunohistochemistry analyses. Given the background of the maternal diet's influence on newborn BAT development, we also investigated whether diets integrated with Saturated and Polyunsaturated Fatty Acids may influence the distribution of BAT and WAT.

UCP1 and UCP2 expression in different subcutaneous and visceral adipose tissue deposits in 30 days old goat kids and effect of fatty acid enriched diets.

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Abstract

Beside its importance in the first hours of life, fulfilling the heat production activity, brown adipose tissue (BAT) has also significant roles in the following stage of growth and in adults by regulating energy metabolism. Brown adipose tissue identification in adult ruminants is somehow controversial. No data is available in goats. Real Time PCR, followed by confirmation by histology and Western blot analyses was used to investigate the distribution of brown and white (WAT) adipose tissue in 30 days old kids. Given the background of the maternal diet's influence on newborn BAT development, we also investigated whether diets integrated with saturated and polyunsaturated fatty acids may influence the distribution of BAT and WAT in subcutaneous and visceral deposits. Results showed for the first time the presence of BAT in 30 days old goat-kids and the differential expression of both UCP1 and UCP2 genes between subcutaneous and visceral adipose tissues. No statistically significant differences in UCP1 or UCP2 expression were shown between control kids and kids with maternal diet enrichment. The presence of BAT in one-month goat kids is remarkable and suggests that further insights into the function of BAT in young and adult goats are worth exploring.

1. Introduction

At least two types of adipose tissue (AT) can be distinguished in mammals, according to their function and composition: white adipose tissue (WAT) and brown adipose tissue (BAT). The primary function of BAT is to dissipate energy through the production of body heat and muscle metabolism (Harwood Jr, 2012). At birth, BAT fulfils the main thermoregulatory heat production activity, which is of paramount importance for the metabolic adaptation to the extra-uterine environment. In precocious animals such as cow, sheep and goat, BAT starts developing in the fetal life and has the maximum activity in the first hours/days after birth (Shore et al., 2013). Given the thermogenic role of BAT, an efficient development of this tissue during the fetal period and the first days of life is of particular relevance for both animal health and economical gains. Indeed, the failure of BAT-controlled non shivering thermogenesis is strongly correlated to the mortality rate in offsprings as demonstrated in calves (Carstens et al., 1997), lambs (Slee, 1981) and goat kids (Mellado et al., 2000). Besides its importance in newborns, brown adipose tissue has also significant roles in adults, by regulating energy metabolism (Lee et al., 2013). The presence of BAT in adult mammals has been demonstrated in cow (Asano et al., 2013) and sheep (Henry et al., 2010), but no information is available in goat.

Differentiation between BAT and WAT can be carried out either by classical histological analysis or by quantifying the expression of Uncoupling Protein 1 (UCP1), which is an important regulator of adaptive thermogenesis in mammals and exclusively expressed in BAT (Damle and Marín-García, 2010; Mailloux and Harper, 2011). For this reason, the expression of UCP1 in adipose tissue has been unequivocally related to BAT. To the best of the authors' knowledge, only one study has been carried out in goats, showing the absence of UCP1 mRNA in newborns at ≥2.5 days (Trayhurn et al., 1993).

A relationship between maternal body condition score and maternal diets to the mortality of newborn kids has been also shown (McGregor and Butler, 2008; Skarda, 2000). Maternal diet can influence newborns' performances, especially in term of adipose tissue's development (Bispham et al., 2003) and fatty acids composition (Berthelot et al., 2012). Supplementation of Polyunsaturated Fatty Acid (PUFA) to lambs has been shown to modify fatty acid composition of adipose tissue (Bolte et al., 2002). Due to their nutraceutical activity, PUFA and Saturated Fatty Acid (SA) are often included in periparturient goat diets. PUFA can positively influence goat immune defences (Agazzi et al., 2004, Thanasak et al., 2004, Pisani et al., 2009, Lecchi et

al., 2011, Lecchi et al., 2013). Both PUFA and SA included in ewe's diets can modulate BAT development in newborn lambs up to 24h of age (Chen et al., 2007), as recently confirmed by Ojha and co-workers (2013). No information about PUFA-enriched diets and BAT distribution is available in goat.

In addition to UCP1, other uncoupling protein genes have been identified, namely UCP2, UCP3, UCP4 and UCP5, with different tissue specificity expression. UCP2 in particular is related to adipose tissue ontogeny and is apparently involved in the development of white adipose tissue characteristics after birth. Its mRNA expression has a peak at 30 days of age in sheep and can be modulated by maternal diet restriction as well (Gnanalingham et al., 2005).

The main aim of the present study was to determine the distribution of BAT and WAT, in different subcutaneous and visceral adipose tissue deposits of goat kids, by means of quantitative PCR measurement of UCP1 and UCP2 gene expression and confirmation by histological and immunohistochemistry analyses. Given the background of the maternal diet's influence on newborn BAT development, we also investigated whether diets integrated with saturated and polyunsaturated fatty acids may influence the distribution of BAT and WAT.

2. Materials and methods

The experimental protocol used in this study was approved by the ethics committee of the University of Milan (Protocol No. 5/11, 18 January 2011).

2.1 Animals, diets and tissue sampling

Adipose tissue samples were obtained from ten 29.8±2.8 day-old healthy suckling kids, which were part of a larger experiment aimed to evaluate the influence of the maternal diet on peripartum and goat kids' performances. A group of 26 multiparous Alpine goats, homogeneous for parity and milk production during the previous lactation, were fed with different diets enriched with fatty acids, either saturated (ST, 69:26 percentages ratio of stearic acid (C18:0) and palmitic acid (C16:0)) or unsaturated (FO, fish oil containing 10.22% of EPA=20:5 and 7.65 % of DHA=22:6), starting from a week before kidding until slaughtering of the kids. A third group of animals fed with a control diet without any specific diet integration was also used as control (CTRL). FO and ST goats diets were adapted for the dry period (supplemented with 30 g of fatty acids) and lactation period (supplemented with 50 g of fatty acids). Goats were housed in single boxes and kids were individually fed by their own mothers. From this larger group, ten male kids, distributed among controls (n=4) and treated (n=3+3), were randomly selected in order to be included in the present experiment. Samples were collected only from these animals. Subcutaneous fat was taken from sternum, armpit cavity, base of the tail and withers, while visceral fat was taken from perirenal, omental and pericardial depots. Samples for molecular biology analysis were snap frozen in liquid nitrogen and stored at −80°C while samples for histological and immunohistochemical analyses were fixed in 10% buffered formalin.

2.2 UCP1 and UCP2 mRNA expression

Total RNA was extracted using a commercial kit specific for all kind of tissues (RNeasy Plus Universal Mini Kit – Qiagen), and treated with DNAse (Rnase-Free Dnase Set – Qiagen). Retrotranscription was performed on 1.5 µg RNA, using the iScript cDNA Synthesis kit (Biorad) and the resulting cDNA was used as template for qualitative and quantitative PCRs. A pool was generated using 2 µl of cDNA for each sample. Qualitative PCRs were performed in 10 µl final volume with 1 µl buffer (Vivantis), 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP), 1 µM each primer and 0.025 U Taq polymerase (Vivantis). Primers are listed in Table 1.

PCRs were performed on all samples at the same conditions: 35 cycles at 96°C for 30s, 60°C for 30s, 72°C for 45s. Results were visualized on a 1.6% agarose gel stained with ethidium bromide. Quantitative Real Time PCRs were carried out in 15 µl Eva Green mix, 400 nM GAPDH, 450 nM LRP10 and 500 nM HPCAL1, UCP1 and UCP2 primers, using the iQ5 Real Time RT-PCR system (Biorad). The PCR efficiency was evaluated by creating a standard curve with 4-fold serial dilutions of the pooled cDNA. Samples were tested in duplicate and no-template reactions were performed as negative control for each target. Primers, efficiency of the reactions and R^2 are listed in Table 1. The thermal profile for each gene was 95°C for 90 s, 40 cycles at 95°C for 5 s and 60°C for 10 s; the melting curve was created running the samples at 55°C for 60 s and 80 cycles starting at 55°C up to 95°C, increasing 0.5°C each 10 s. Relative quantification of both UCP1 and UCP2 was calculated using the comparative delta-delta-Ct method (Giulietti et al., 2001), using HPCAL1 (hippocalcin-like 1), GAPDH (gliceraldehyde-3-phosphate dehydrogenase) and LRP10 (low-density lipoprotein receptor-related protein 10) as the most stable reference genes (Hosseini et al., 2010).

Table 1. List of UCPs genes under analysis and reference genes used for qPCR.						
Sequence name	Symbol	Accession number	Primer Forward (5'-3')	Primer Reverse (5'-3')	Length (bp)	Reference
Uncoupling protein 1	UCP1	X14064	TCCTGTCTTTGATCGCCTCT	GAACAGTCCATGTGCCAGTG	112	Yonezawa et al. 2009
Uncoupling protein 2	UCP2	AF127029	GCATAGGCATCCAGGAATCA	TAGGACGCTTCTGTCTCC	112	Yonezawa et al. 2008
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	NM_001034034	GGCGTGAACCACGAGAAGTATAA	CCCTCCACGATGCCAAAGT	119	Lecchi et al. 2012
Hippocalcin-like 1	HPCAL1	NM_001098964	CCATCGACTTCAGGGAGTTC	CGTCGAGGTCATACATGCTG	99	Hosseini et al. 2010
Low density lipoprotein receptor-related protein 10	LRP10	BC149232	CCAGAGGATGAGGACGATGT	ATAGGGTTGCTGTCCCTGTG	139	Hosseini et al. 2010

2.3 Antibody validation by Western Blot analysis

In order to investigate by immunohistochemistry the presence of UCP-1 in kids' adipose tissue, a rabbit polyclonal anti-UCP1 (Abcam, Ab10983) raised against human UCP1 and cross-reacting with mouse was utilized. Antibody validation was performed on samples from perirenal and pericardial kid's adipose tissue. Adipose tissue from the stifle of an 11 day-old mouse was used as positive control sample (Ringholm et al., 2013). Aliquots of 30 mg of tissue for the mouse, and 100 mg of tissue for the goats were mechanically homogenized in 6 volumes (w/v) of lysis buffer (50 mM Tris–HCl pH 7.6, 150 mM NaCl, 4% NP40, 2% Triton X100, 1% Zwitterion 3.14, 5 mM EDTA), added with a protease inhibitors cocktail (Sigma Aldrich), as previously described (Rahman et al., 2008). Briefly, after homogenization, samples were incubated on ice for 30 min. Pre-homogenized tissues were again mechanically homogenized and incubated on ice for 30 min, in order to have a better protein recover. Samples were centrifuged twice for 15 min at 14000 x g at room temperature, and the supernatant transferred to a new tube in order to remove the majority of lipids.

Proteins were separated on a 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and blotted onto nitrocellulose membranes. Before gel separation, 1 µl 2_mercaptoethanol (Sigma Aldrich) was added to each sample. Immunolabelling was performed using the rabbit polyclonal anti-UCP1 (Abcam, Ab10983) raised against human UCP1 and cross-reacting with mouse, as primary antibody (1:500 dilution for 2 h at room temperature), while an HRP-conjugated anti-rabbit (Sigma Aldrich) was used as secondary antibody (1:2000 dilution for 45 min at room temperature). Immunoreactive bands were visualized using a chemiluminescent HRP substrate (Immobilon Western Chemiluminescence substrate – Millipore).

2.4 Histological and immunohistochemical analysis

In order to evaluate the histological differences of goat subcutaneous and visceral AT, a histological analysis, with haematoxylin and eosin staining, was performed. The distribution of WAT in different AT deposits has been evaluated, as well. In addition, to confirm the presence of BAT in goat adipose tissue samples, and its higher amount in visceral deposits, an immunohistochemical analysis was carried out.

Both, histological and immunohistochemical analysis were performed on formalin fixed samples. Tissues were routinely processed and paraffin embedded. Two 4 μm thickness, serial sections from each block were mounted on glass slides and stained with haematoxylin and eosin. Pictures of 3 consecutive 20x power fields were captured. Image analysis software (Image J) was used to select areas of WAT, non-WAT and non-adipose tissue's areas. Ratios between WAT and non-WAT areas and perimeters were calculated.

Immunohistochemistry was performed using a streptavidin–biotin peroxidase complex method with the same anti-UCP1 rabbit polyclonal antibody (Ab10983, Abcam, dilution 1:200) validated by Western Blotting. Tissue sections were deparaffinised, rehydrated, reactive aldehyde groups were blocked incubating the sections with NH_4Cl 0.05M for 30 min at room temperature and endogenous peroxidase activity was quenched with 1% H_2O_2 in PBS 1X for 20 min at room temperature. Non-specific binding sites were blocked with 1% BSA (Sigma-Aldrich) in PBS 1X + Triton 0.1% for 1 h and sections were incubated overnight at 4°C with the primary antibody. Biotinylated secondary antibody was incubated for 1 h at room temperature and sections incubated with streptavidin-HRP 1:100 in PBS 1X for 10 min. The signal was detected incubating the slides, at room temperature for 6 minutes, with 3,3'-diaminobenzidine and H_2O_2 as the substrate for peroxidase (DAB Peroxidase Substrate Kit, Vector). Sections were mounted with glycerine (Sigma-Aldrich). Mouse interscapular adipose tissue was used as positive control. Primary antibody was omitted for negative controls.

2.5 Statistical analysis

All data from quantitative PCR and histological evaluation were elaborated with an analysis of variance using the statistical software SAS (SAS Inst. Inc., Cary, NC). All data were evaluated for normal distribution using the Kolmogorov–Smirnov test. Post-hoc tests were carried out on parametric data using the Tukey-Kramer method.

3. Results

3.1 mRNA expression of UCP1 and UCP2 is higher in visceral deposits

The mRNA expression of UCP1 and UCP2 was investigated in subcutaneous (sternum, armpit cavity, base of the tail and withers) and visceral (perirenal area, omentum, pericardium) adipose tissue deposits of goat kids fed the control diet. Qualitative PCRs revealed the presence of both genes in all samples under analysis (data not shown). Quantitative RT-PCR demonstrated that UCP-1, as marker of BAT, is overexpressed in a statistically significant way in visceral AT depots, as compared with subcutaneous ones ($p<0.05$) (Figure 1A). A greater expression ($p<0.05$) of UCP2 gene was detected in visceral deposits as well, as compared to subcutaneous ones (Figure 1B). Highest expression of both UCP1 and UCP2 was demonstrated in perirenal area.
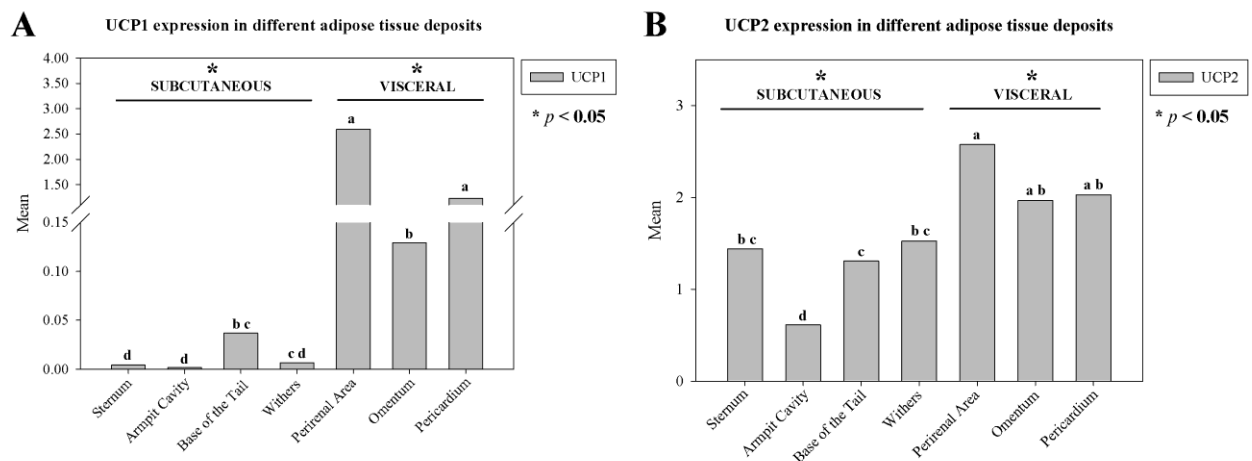
Figure 1. UCPs expression in different subcutaneous and visceral deposits. (A) UCP1 mRNA was found in all adipose tissue samples under analysis. Together with UCP2 (B), it shows a higher expression in visceral deposits than in subcutaneous' ones ($p < 0.05$).

3.2 WAT and BAT distribution in subcutaneous and visceral adipose tissue deposits

The distribution of WAT and BAT among the seven different adipose tissue deposits included in this experiment, were investigated by means of classical histology and immunohistochemistry analysis, in order to confirm gene expression results. The presence of BAT as determined by haematoxylin-eosin staining was clearly identified in all adipose tissue depots (Figure 2A). BAT was identified as containing small lipid droplets, rich cytoplasm and central nucleus, whereas white adipocytes were identified as containing one big lipid droplet, poor cytoplasm and peripheral nucleus. The different distribution pattern of the white areas between subcutaneous and visceral deposits was confirmed by statistical analysis ($p<0.05$) (Figure 2B). In order to confirm the distribution of BAT as determined by UCP-1 expression, an immunohistochemical analysis was carried out by using an anti-UCP1 primary antibody, but the staining failed to identify the protein in the tissues.
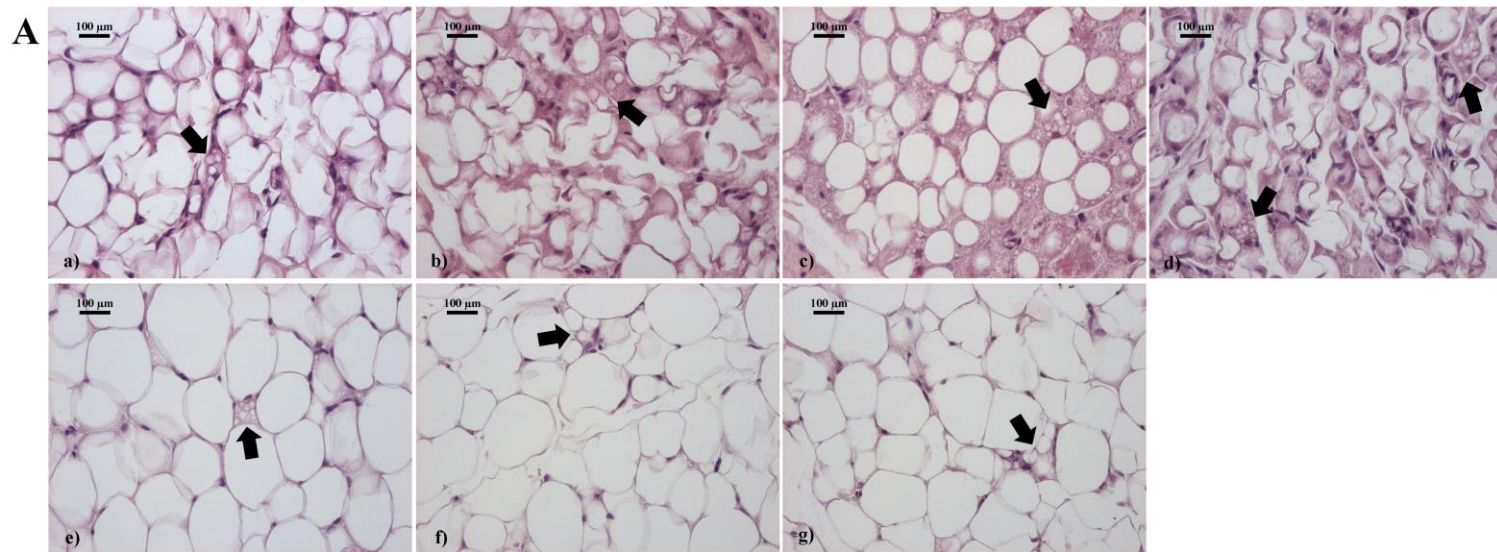
B White adipose tissue areas (WAT) in subcutaneous and visceral deposits
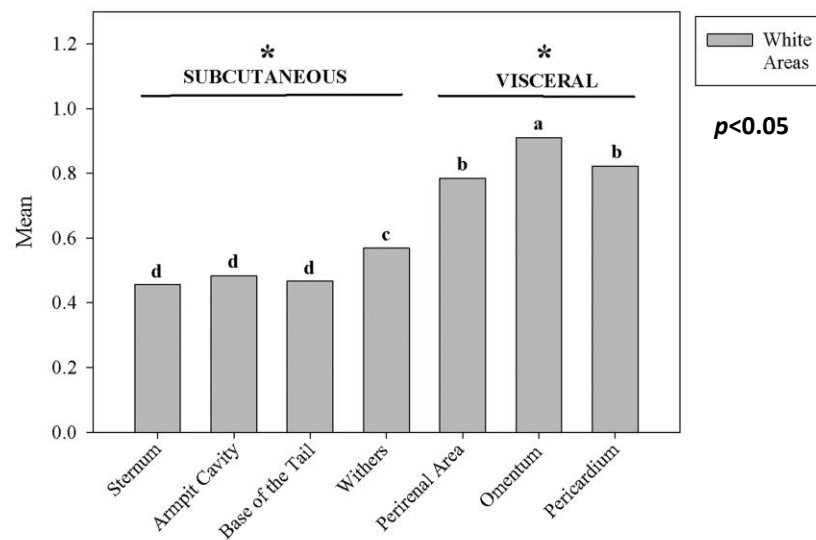
Figure 2. Histological analysis results. (A) Brown adipose tissue was identified in all tissues under analysis. White adipocytes show different characteristics in subcutaneous or visceral deposits. a) sternum, b) armpit cavity, c) base of the tail, d) withers, e) perirenal area, f) omentum, g) pericardium. (B) White areas were analyzed using Image J Pro Plus and show statistically significant differences between subcutaneous and visceral deposits ($p < 0.05$).

3.2.1 Antibody validation by Western Blot analysis

Despite the results of the immunohistochemical analysis, the Western blot analysis confirmed the ability of our antibody (Ab10983, Abcam) to identify UCP1 protein in goat adipose tissues. Antibody was tested on pericardial and omental adipose tissue, as they proved to have the higher expression of UCP1, after real-time PCR analysis. Adipose tissue from mouse stifle was used as reference sample. As shown in Figure 3, the electopherogram pattern between the two species is very similar, and the UCP1 32 kDa band is clearly visible in both adipose tissue samples, together with other bands at around 75 kDa and around 53 kDa.
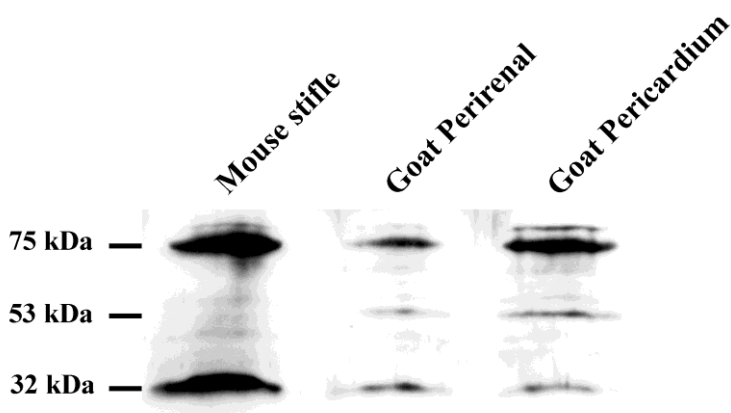
Figure 3. Antibody validation by Western blot analysis. Mouse stifle was used as positive control sample. Goat perirenal and pericardium adipose tissues show an electopherogram pattern very similar to the mouse one. UCP1 32 kDa band is clearly visible, together with other bands at around 75 kDa and around 53 kDa.

3.3 Effect of the maternal diet on UCP1 and UCP2 expression and adipose tissue' composition

In the last part of the study, the possible influence of maternal diet on BAT goat kids was investigated. Goats were fed different fatty acids enriched diets starting form a week before kidding. Either stearic acid or fish oil were used for the treatment. Gene expression of UCP1 and UCP2 genes was quantified by means of RT-PCR. As shown in Figures 4A, no statistically significant differences in the expression of either UCP1 or UCP2 genes were detected among control and fatty acid enriched diets or between the two diets. Histological analysis was also carried out and confirmed these results (Figure 4B).
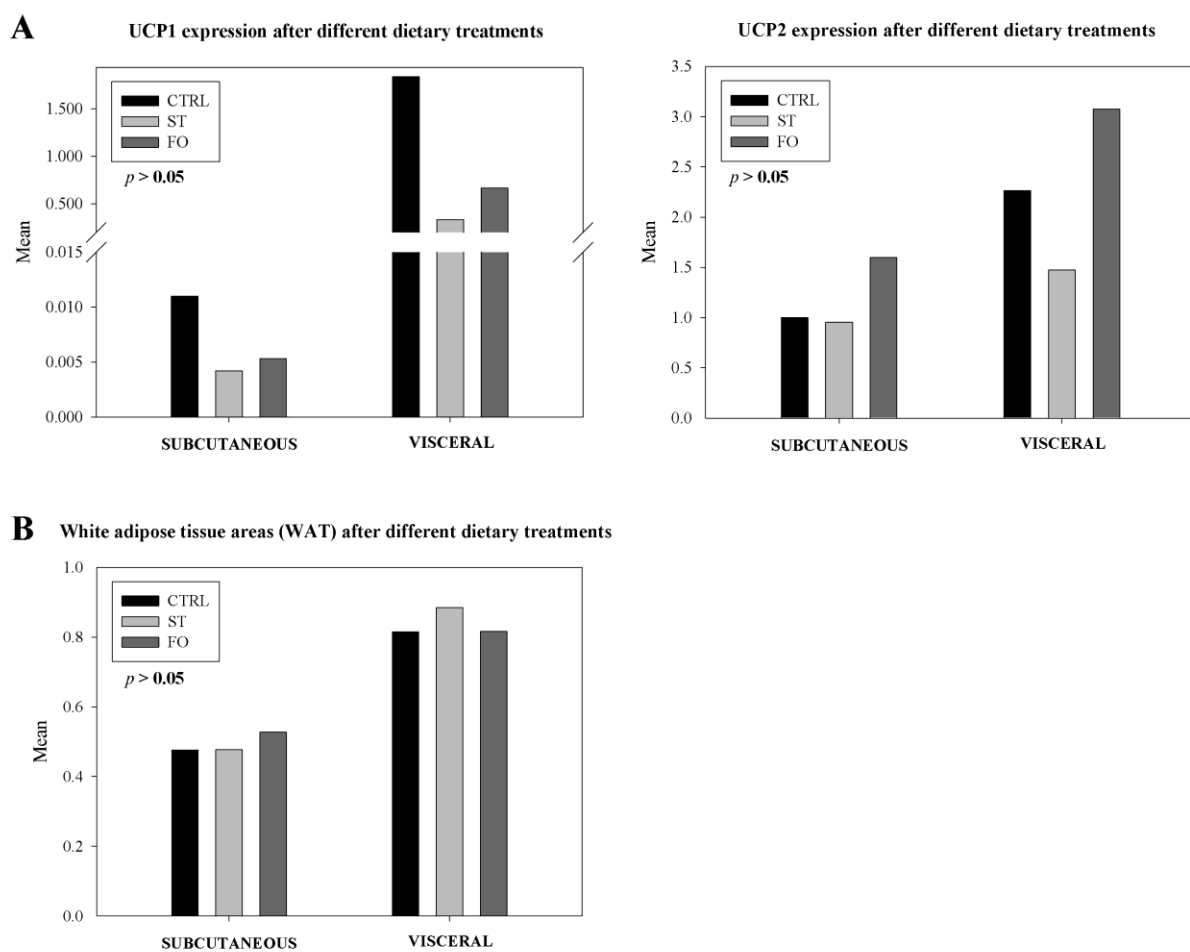
Figure 4. UCPs expression (A) and white adipose tissue distribution (B) after different maternal dietary treatments. The comparison among maternal control diet (CTRL), stearic acid (ST) and fish oil (FO) enriched diets showed no statistically significant differences in the expression of both UCP1 and UCP2 genes and in white adipocytes distribution.

4. Discussion

Of paramount importance for a newborn organism is its capability to adjust its body temperature to the new environment. Immediate energy resources are derived from the glucose released from glycogen reserves. Brown adipose tissue fulfils an essential role by releasing energy as heat during non-shivering thermogenesis. Although essential for the survival of the newborn, information about BAT distribution and activity in ruminant species are still scarce, and someway controversial. The aim of this study was to partially cover this gap in goats.

The present findings demonstrated that BAT is present in 30 days old goat kids in all subcutaneous and visceral samples included in the experiment, as determined by the expression of UCP1, confirming what described in lambs (Smith et al., 2004; Yuan et al., 2012). On the contrary, our results are in contrast with an older investigation carried out in goats showing that UCP1 was undetectable in several adipose tissue deposits in ≥2.5 days old goats-kids (Trayhurn et al., 1993), and with a recent experiment performed in lambs at 30 days of age (Pope et al., 2014), but this can be explained with the improvement of PCR techniques in the first case and with species-specific differences in the latter.

Consistent with earlier findings in sheep (Henry et al., 2010), we also demonstrated for the first time in goat that UCP1 and UCP2 genes are differentially expressed between subcutaneous and visceral adipose tissues, both genes being highly expressed in visceral (perirenal) deposits compared to subcutaneous ones. This is not surprising considering that visceral adipocytes are more metabolically active as compared to subcutaneous ones (Ibrahim, 2010) and that perirenal and omental areas are the major adipose tissue deposits in newborn lambs and goats (Clarke et al., 1997a; Skarda, 2000). The importance of perirenal tissue has also been confirmed in small ruminants by the high mortality of lambs born to light ewes, which had also less perirenal fat (Clarke et al., 1997b).

In addition, our study investigated for the first time in goat the anatomical differences between subcutaneous and visceral adipose tissue deposits, in terms of BAT and WAT distribution. The identification of brown adipocytes in sternum, base of the tail, perirenal, omental and pericardial deposits confirmed UCP1 gene expression analysis, and it is consistent with previous data in goats and other ruminant species (Trayhurn et al., 1993; Smith et al., 2004), while BAT's presence in armpit cavity and withers has never been investigated before. Quantitative gene expression of UCP2, which is commonly regarded as a marker for adipose tissue development, basically confirmed anatomical findings. Moreover, the different WAT distribution between subcutaneous

and visceral deposits supports the hypothesis that these two major adipose tissue depot groups display several anatomical and functional differences as previously suggested (Ibrahim, 2010). The detection of UCP1 presence in goat adipose tissue by immunohistochemistry gave no results. The antibody utilized was raised against human UCP-1, and reported to cross-react with mouse and rat. Yet, it has also been reported in the data sheet that the antibody may not react in humans tissues in Western blot analyses. A sequence alignment between goat and human UCP1 gave a percentage of aminoacid identity of 82.5%. Therefore, we suggest that, although the UCP1 sequences of the two species are closely related, differences in epitope conservation do occur.

Given the background of PUFA relationship with PPAR⬚, and how PPAR⬚ can influence BAT development, the second part of this study was focused on demonstrating the hypothesis that dietary supplementation with saturated or polyunsaturated fatty acids during late gestation would increase UCP1 gene expression, thus increasing the thermogenic capability of newborn kids. PUFA are regarded as strong activators of PPARγ and PPARα receptors (Hardwick et al., 2009), and treatment with PPARγ activators can induce the expression of UCP1 in humans and mice (Tiraby et al., 2003; Petrovic et al., 2010). However, fish oil did not enhance PPARα, and related hepatic genes mRNA levels in transition dairy goats (Agazzi et al., 2012). The present results extended previous experiments (Ebrahimi et al., 2013), which showed a different gene expression of PPARα, PPARγ and SCD as a consequence of different diet administration, but investigate neither UCP1 expression, nor the maternal diets influence.

Quantitative PCR data on UCP1 and UCP2 expression in adipose showed no statistically significant differences between CTRL and fatty acids supplemented kids or between FO and ST animals, thus demonstrating that feeding goats with PUFA or saturated fatty acid starting from a week before kidding up to one month after kid's birth does not influence BAT activity in the offspring. This information is therefore in contrast with other studies in rat (Priego et al., 2013), and someway different with a similar experiment carried out in sheep (Chen et al., 2007), which presented the evidence that supplementing the diets of ewes with PUFA decreased cold tolerance in newborn lams by decreasing UCP1 gene expression. It must also be said that the adipose tissue metabolism of sheep and goats have been shown to be sensibly different (Tsiplakou et al., 2011).

5. Conclusions

In conclusion, our study demonstrated for the first time the presence of brown adipose tissue in goat one month after birth as determined by the expression of UCP1 gene. We moreover showed that both brown and white adipose tissues are differently distributed among subcutaneous and visceral deposits and this fact can reflect the different functions of these two macro areas. Finally, we proved that fatty acid enriched diets administered to goats starting one week before kidding have no influence on UCP1 expression or brown and white adipose tissue distribution in suckling kids. The presence of BAT in one month goat kids is remarkable and suggests that further insights into the function of BAT in young and adult goats are worth exploring.

Acknowledgments

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PAPER II

LC-MS/MS analysis of visceral and subcutaneous adipose tissue proteomes in young goats with focus on innate immunity and inflammation related proteins.

To be submitted to *Journal of Proteomics.*

Aim: the aim of the present study was to undertake an in-depth and comparative study of the protein expression patterns of goat adipose tissues. LC-MS/MS was used to characterize and compare the proteome composition of different subcutaneous and visceral adipose deposits in goat-kids. This study presents the first adipose tissue proteome of goat. In addition, it includes an evaluation of adipose tissue involvement in inflammatory and immune-related processes, through an investigation of its protein expression ability on 35 potentially novel adipokines, of which 27 were further confirmed by RT-PCR, including 9 whose mRNA expression is observed for the first time in adipose tissues.

LC-MS/MS analysis of visceral and subcutaneous adipose tissue proteomes in young goats with focus on innate immunity and inflammation related proteins

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Abstract

The endocrine role of adipose tissue and its involvement in several physiological and pathological processes are well recognized. Studies on human, mouse and rat adipose tissues have made clear that subcutaneous and visceral deposits play different roles, which is also reflected by different protein and gene expression patterns. In ruminants, fat tissues play important biological roles not only for animal health, but also for quality and gain in meat and milk production. Yet very few studies have explored the ruminant adipose tissue proteomes. The aim of our study was to compare subcutaneous and visceral adipose tissues of goat, focusing on proteins involved in immune and inflammatory response. A 2-D LC-MS/MS approach followed by cluster analysis shows a clear distinction between subcutaneous and visceral fat tissue proteomes, and RT-PCR based analysis of 35 potential adipokines further confirmed the individual expression patterns of 27 of these, including 9 whose mRNA expression was observed for the first time in adipose tissues. This study provides a first description of adipose tissue proteomes in goat, and presents observations on novel proteins related to metabolic and inflammatory pathways.

Biological significance

The proteomic analysis of different subcutaneous and visceral adipose tissue deposits showed tissue specific differences in protein expressions of well known as well as novel adipokines. This highlights the importance of sampling site when studying adipose tissue's metabolic roles. The protein expression characteristics of adipose tissues was evaluated by quantitative RT-PCR, and confirmed that adipose tissues play a central role in controlling inflammation, detoxification and coagulation pathways, as well as regulation of body fat mobilization in dairy animals. These findings are of particular interest in farm animals where health and production traits are important for animal welfare and for economic gains.

1. Introduction

Adipose tissue (AT) has been regarded for a long time as an inert storage tissue for the excess of energy in the form of triglycerides. The identification of leptin by Zhang and co-workers in 1994 was the first demonstration that adipose tissue is also involved in regulating homeostasis [1]. Accumulating evidence highlights that adipose tissues are active endocrine organs, regulating both metabolic and inflammatory pathways through production and secretion of signalling molecules, collectively referred to as adipokines [2]. Adipokines are proteins with both local (autocrine/paracrine) and systemic (endocrine) effects, including tumour necrosis factor alpha (TNF-alpha), interleukins, leptin, resistin, plasminogen activator inhibitor-1 and adiponectin [3]. The number of potential adipokines is steadily increasing. Adipose tissue is distributed as fat depots throughout the whole body, and classified mainly as subcutaneous (SAT) and visceral (VAT) adipose tissue. In ruminants, SAT includes depots located beneath the skin, e.g. the armpit cavity, the subcutaneous areas over the sternum and the withers and the base of the tail, while VAT is located in the intra-abdominal cavities, surrounding specific organs, such as kidney and heart, or distributed among peritoneum layers, such as mesenteric and omental fat [4]. Therefore, adipose tissue should not be considered a single endocrine organ located in different region of the body, but a group of endocrine organs with location specific endocrine functions [5]. In fact, beside their topographical locations, visceral and subcutaneous adipose tissues are distinguished according to their different metabolic characteristics (e.g. processing excess of lipids) [6] and by their ability to release inflammatory cytokines [7]. In farm animals, and particularly in ruminants, the regulation of lipid metabolism is of key importance not only for animal health, but also for production of meat and milk. Nevertheless, a systematic investigation of the molecular mechanisms of adipose tissue has not yet been undertaken in these species. Transcriptome studies of adipose tissues from mice, rat, cow and sheep have shown that visceral and subcutaneous adipose tissue depots differ in mRNA abundance of adipokines, highlighting the importance of sampling site in studies of e.g. metabolic pathways in AT [8-11].

Currently, proteomic studies in ruminants are limited to *Bos taurus*, and include a comparative study of adipogenic differentiation of preadipocytes in the omental, subcutaneous and intramuscular tissues [12] and a time resolved investigation of embryonic fat deposition [13] and of intramuscular fat depots of Korean steers [14]. To the best of our knowledge, no proteomic data about adipose tissues are available in goats. The aim of the present study was to undertake an in-depth and comparative study of the protein expression patterns of goat adipose tissues. LC-

MS/MS was used to characterize and compare the proteome composition of 2 different SAT (base tail and sternum) and VAT (perirenal and omentum) from four goats. This study presents the first adipose tissue proteome of goat, and includes expression characteristics of 35 potentially novel adipokines, of which 27 were further confirmed by RT-PCR, including 9 whose mRNA expression is observed for the first time in adipose tissues.

2. Materials and methods

2.1 Animals and tissue sampling

Samples were obtained from four healthy goat-kids, Alpine breed, naturally reared by their mothers. Animals were slaughtered at the age of 30 days during routinely slaughtering procedures, and four different adipose tissues were collected from each animal. Subcutaneous fat was taken from sternum and base of the tail; visceral fat was taken from perirenal and omental depots. The four adipose tissues (sternum, base of the tail, perirenal area and omentum) were selected due to their frequent use in experimental studies on fat tissue, as also suggested by Lemor and co-workers [11]. Liver samples were also collected, and used as reference samples. Tissue samples were snap frozen in liquid nitrogen and stored at −80°C until protein and mRNA extraction and analysis.

2.2 Sample preparation and protein digestion

Protein extraction of fat and liver tissues was carried out on ice or at 4°C, as described in [15]. In short, 200 mg of each adipose tissue were homogenized in 5 μl/mg TES buffer (10 mM Tris-HCl, pH 7.6; 1 mM EDTA, 0.25 M sucrose) and centrifuged at 10000 x g, for 30 min, at 4°C. Protein concentration values of the tissue supernatants were determined by the Pierce BCA Protein Kit (VWR), using BSA as a protein standard, according to the manufacturer's manual. Hundred-twenty μg of proteins from each tissue homogenate was precipitated by adding 6 vol of ice-cold acetone. The precipitated proteins were re-suspended in 20 μl of digestion buffer (0.5 M triethylammonium bicarbonate, 0.1% SDS); cysteine residues were reduced with 2.5 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) at 60°C for 1 h, and then blocked with 10 mM methylmethanethiosulfate at room temperature, for 1 h. Samples were digested with trypsin (1:10 w/w) (AB SCIEX) at 37°C, overnight. In order to remove all the impurities that can interfere with later HPLC separation, all samples were passed through a 0.2 μm centrifuge filter (National Scientific Company) for 10 min, at 10000 x g, vacuum-dried and eventually stored at -80°C until their further analysis.

2.3 Strong Cation Exchange (SCX) liquid chromatography

The peptides were re-dissolved in 0.03% formic acid, 5% acetonitrile in water. Peptide mixtures generated from the digestion of 50 μg of protein were injected into an Agilent 1100 Series capillary HPLC equipped with a Zorbax Bio-SCX Series II, 0.8×50 mm column (Agilent Technologies) that provides peptide separation by strong cation exchange liquid chromatography. Peptides were loaded in Buffer A (0.03% formic acid and 5% acetonitrile in water) and eluted with Buffer B (0.03% formic acid, 5% acetonitrile and 1 M NaCl in water), with the following increasing NaCl concentration: 0 min 0% B; 5 min 0% B; 10 min 1.5% B; 11 min 4% B; 25.5 min 15% B; 35.5 min 50% B; 45 min 100% B; 55 min 100% B. The flow rate was 15 μl/min and fractions were collected every minute for 65 min and combined into 10 pooled samples, to achieve approximately equal peptide loads for further LC-MS/MS analyses.

2.4 LC-MS/MS

The pooled samples were de-salted and concentrated on an Agilent 1100 Series nano-flow HPLC system (Agilent Technologies), prior to be further separated by a reverse phase liquid chromatography. De-salting and concentration of the samples were carried out on an enrichment column (EASY Column – 2 cm, ID 100 μm, 5 μm, C18 - Thermo Scientific) using an isocratic pump working at 20 μl/min (0.1% formic acid and 3% acetonitrile in water). Peptides were then eluted and further separated on an analytical column (EASY Column – 10 cm, ID 75 μm, 3 μm, C18 - Thermo Scientific) with a nanoflow of 300 nl/min using a gradient of increased organic solvent (0 min 5% B; 7 min 5% B; 70 min 40% B; 73 min 95% B; 78 min 95% B; 83 min 5% B; 100 min 5% B). Buffer A contained 0.1% formic acid in water and buffer B 5% water and 0.1% formic acid in acetonitrile. The eluted peptides were sprayed through a nanospray needle (PicoTips, silica, no coating, OD 360 μm, ID 20 μm - New Objective) directly into the Q-star Elite mass spectrometer (Applied Biosystems).

2.5 Database searches

The raw spectrum files from 20 individual shotgun LC-MS/MS runs (comprising four biological replicates from each of the five tissues) were searched separately with Protein Pilot 1.0 software (Ab Sciex) using the ProGroup and Paragon algorithms for protein grouping and confidence scoring. The target database used for searching was constructed as a non-redundant union of UniProtKB Bovidae sequences (www.uniprot.org/uniprot/?query=taxonomy:9895) and

NCBI Capridae sequences (www.ncbi.nlm.nih.gov/taxonomy/?term=9963), versions as of September, 2011. Due to a limited coverage of goat genome, when LC-MS/MS data were originally annotated, we used a merged database of available bovidae genomes. This strategy was used because a very close homology between goat and cow has previously shown successful. The current coverage of the goat genome became available by the end of 2012. A repeated search of the LC-MS/MS data would likely provide a better sequence coverage, and possibly allow a larger number of significantly identified proteins. However, the identified proteins, as well as the relative expression data, will remains the same.

The reversed copy of each of the target protein sequences was appended to the list for estimating the false discovery rate (FDR) [16]. Thus, the combined database comprises 43373 forward sequences and 43373 reverse sequences. The False Discovery Rate (FDR) was estimated as the ratio of (2 x reversed sequence)/(reversed + forward sequence) in percentage. Search parameters were set with an MS tolerance of 0.15 Da and a MS/MS tolerance of 0.1 Da, and using generic modifications including deamidation of glutamine and asparagines side chains, methionine oxidation as well as methyl methanethiosulfonate modification of cysteines. Samples were SCX fractionated and analysed twice (technical replication) in order to gain higher reproducibility and proteome coverage as suggested by Chong and co-workers [17]. The two data sets from each sample were searched together in ProteinPilot (Applied Biosystems). The confidence for protein identification was selected in ProteinPilot to a protein score of 1.3, equivalent to 95% confidence and a minimum of two peptides per protein.

2.6 Cluster Analysis

To compare the proteome profiles of the tissue types, we performed a hierarchical clustering analysis using the Ward's linkage method which is an agglomerative approach based on the "error sum of squares" when merging pairs of clusters [18]. The distance measure between proteome profiles was calculated as $1-\rho_{ij}$, where ρ_{ij} is the Spearman's rank based correlation between the protein score profiles *i* and *j*. Data handling and analysis was performed using the statistical software package R (R Development Core Team).

2.6.1 Functional annotation and grouping

The open source online tool Blast2GO (http://www.blast2go.com) was used for the functional annotation of the identified proteins [19]. The default parameters were used and for the basic local alignment search tool (BLAST) protein sequences were mapped against the NCBInr.

We further narrowed the functional analysis by PANTHER classification into protein families and functional pathways in order to increase the confidence (Protein Analysis Through Evolutionary Relationship) system available at http://www.pantherdb.org [20].

2.7 Validation of proteomic results by qualitative PCR

Qualitative PCRs of selected genes, involved in immunity and inflammatory pathways, was used to confirm protein expression data. Total RNA was extracted from the same tissue samples as those used for proteome analyses. Extraction was made using a commercial kit (RNeasy Plus Universal Mini Kit - Qiagen), and DNAse treatment was carried out with RNase-Free DNase Set (Qiagen); the RNA concentration in each sample was quantified using a NanoDrop ND-1000 UV-spectrophotometer. 1 μg RNA was retrotranscribed using the iScript cDNA Synthesis kit (Biorad). The resulting cDNA was amplified by means of a qualitative PCR, performed in 10 μl final volume, containing 1 μl buffer (Vivantis), 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP), 1 μM each primer and 0.025 U Taq polymerase (Vivantis). Selected genes and primers are listed in Table 1. PCRs were performed on all the samples, under the same conditions: 34 cycles at 96°C for 30s, 60°C for 30s, 72°C for 45s. Results were visualized on a 1.6% agarose gel stained with ethidium bromide.

Table 1 - Inflammation and innate immunity related proteins selected for mRNA expression analysis, accession numbers, primers and length of the amplified fragments				
Sequence name	Accession number	Primer Forward (5'-3')	Primer Reverse (5'-3')	Length (bp)
Adiponectin	[11]	CTGGAGAGAAGGGAGAGAAAGGAG	TGGGTACATTGGGAACAGTG	204
Adipose most abundant gene transcript 2	XM_005699317.1	TCAGCAAGTGGTGGATCAGG	AGAGGCCTGGTTAGCAGTCT	103
Alpha 1 acid glycoprotein	[21]	GCATAGGCATCCAGGAATCA	TAGGACGCTTCTGTCTCC	112
Alpha 1 b glycoprotein precursor	NM_001046243.2	GTGGACCATGTCTGCTTGGG	CTGGGGTCAAAGAACGTCGC	90
Alpha 2 hs glycoprotein precursor	NM_173984.3	TTCCAGTCCAGATTCCGCC	TCGGCATTGAAGGTAGCCAG	123
Alpha 2 macroglobulin precursor	NM_001109795.1	GCAACACAGTCTGGTCTCCT	CCATGTATTGCGGTTTTCCAGA	138
Alpha crystallin b chain	NM_174290.2	GCTTCTACTTCCCTGAGCCC	GTCCTTCTCCAGACGCATCTCT	102
Beta galactoside-binding lectin precursor	NM_175782.1	GGGCAAAGACGACAACAACC	TGCATACCTCCACGACACTT	164
Ceruloplasmin	NM_001256556.1	GAGCATGAAGGGGCCATTTATC	GCTGTCTTCCTCACCAGG	130
Complement component 1, q subcomponent binding protein	NM_001034527.2	AGTGCGGAAAGTTGCTGGAGA	GAGCTCCACCAATTCATCGGC	415
Complement component 3	NM_001040469.2	GGGAACCCCATGTATTCCATGAT	GACCGAGACCTGAATAGTCCC	108
Complement component 4a	NM_001166485.1	GCTGAGGAGCGAGAATCCAG	AGGTCAGCTTCTCCAGGTCA	142
Fibrinogen alpha chain	NM_001033626.1	TGAGATCCTGAGGCGCAAAG	TGTCCACCTCCAATCGTTTCAT	104
Fibrinogen beta chain	NM_001142917	GACAACGACGGCTGGAAAAC	ACGCTCCACCCCAGTAGTAT	124
Fibrinogen gamma chain	NM_173911	TGCCAATAAGGGGGCCAAAG	GACTGCCATCAAGCCTCTTCT	134
Galactoside 3	NM_001102341.2	GGGAGTATTTGAGGCGCAGAG	CGTCTGCCATTTTCCCGAC	114
Gli pathogenesis related 2	NM_001076112.2	ATGGGCAAATCAGCCTCCAA	TCTTGCAGAGCTTCAGTGGG	100
Hemopexin precursor	NM_001034612.2	TACGATGGCTCAACCGCTAC	TGTGCATGGCCTCTGTTAGG	139
Immunoglobulin gamma 1 heavy chain	X62916.1	CCCCGAAAGTCTACCCTCTG	CTGCTGAGAGAGTACAGCCC	187
Immunoglobulin gamma 2 heavy chain constant region	EF564267.1	GGACTGGAGGGAAGGAGTTC	CTGGAGATGGTCCTCACGAT	76
Immunoglobulin lambda light chain	AY835588.1	TACAACATCGTTTTCGGCGG	TCGCTGATGAGACACACCAC	137
Kininogen 1, transcript variant 1	NM_175774.3	GTCCCTTTCAAGTGGTGATACTGG	AAAGCCTGGTGGGTGGTTGT	134
Lactoferrin	NM_180998.2	TCCAGACTCTGTGCCTTGTG	TGTTCTCCCAGACTGTGTCG	160
Macrophage migration inhibitory factor	NM_001033608.1	CCCGGACAGGATCTACATCAAC	CGTCTCCACACCGTTTATTGC	200
Plasminogen precursor	NM_173951.2	CATAAGGCGGTGGTGTTCCT	GGAATGCCCTGCAAACGAAG	187
Protein dj 1	NM_001015572.1	TAAGGTCACCGTTGCAGGTC	CAGCAGCGGACTCGGATAAA	170
Retinol binding protein 4	NM_001040475.2	TTCGACAAGGCTCGCTTCG	CTGCACACACGTCCCAGTTA	172
Selenium binding protein 1	NM_001046048.1	GCAGCATGGCTACCAAATGT	CGGTAAATGCAGGGCAGGTA	106
Serum albumin precursor	NM_180992.2	TGGCACAATGAAGTGGGTGA	TCACTCTTGTGTGTATCTCGACG	96
Thioredoxin	NM_173968.3	ACGTGGATGACTGCCAGGAT	AGTCAAATGCATGCCAACCT	57
Thiosulfate sulfurtransferase	NM_177489.3	GATGCAGTAGGACTGGACTCG	ATGAGGGGCTTTGTGAGGTC	149
Transforming growth factor beta 68kda	NM_001205402.1	CAGCCCTGCCACTCTCTAAC	CAGGGAGTCCAGCACTTCAG	182
Tumor translationally controlled 1	NM_001014388.1	GGGCTGCAGAACAAATCAAGCACA	ACACCATCCTCACGGTAGTCCAAT	118
Valacyclovir hydrolase like	NM_001045918.1	ACGGTACCCCTCGTATGTCA	GCTTCCAGAGGCTTTCTCGT	135
Zinc alpha 2 glycoprotein precursor	NM_001034331.1	CACCTGGACCGACAAGAGTC	TGGGTAGAAGTCGTAGGCCA	99

3. Results and Discussion

In order to study protein expression characteristics of subcutaneous (SAT) and visceral (VAT) adipose deposits of goat, we carried out a proteome mapping of adipose tissues sampled at 4 different locations on the goat carcass, namely base of the tail and sternum (subcutaneous adipose tissues), omentum and perirenal (visceral adipose tissues). Likewise, liver tissues from the same animals were mapped in parallel in order to present a reference point to common adipose tissue characteristics. The 2-D LC-MS/MS based proteome profiling of these tissues allowed the identification of a matching number of proteins across the different tissues with mean=506 and SD=45.2 proteins. This confirms that the protein extraction protocols which are well established for extraction of liver [22] and muscle [23] proteins, are indeed efficient for extraction of adipose tissue as well, despite the high lipid contents of these tissues.

3.1 Protein identification

In total, 1351 protein groups were identified across the five tissues (all peptide information are listed in Supplementary Table 1). Of these, 919 proteins were observed in at least 2 biological replicates and with at least 2 unique peptides (Supplementary Tables 2, 3, 4 and 5). Two proteins were matched to decoy (reversed) protein sequences, which gives a fraction of incorrect assignments of (2 x reversed sequence)/(reversed + forward sequence)=4/919=0.004, equivalent to FDR=0.43%. The tissue coverage was: 518 proteins (liver), 476 (base tail), 456 (sternum), 507 (omentum) and 574 (perirenal area). The 158 liver-specific proteins (Supplementary Table 2) were excluded from further investigation, because these were not within the focus of this study on adipose tissue proteomes. Hence, the 761 proteins (919 - 158) that were observed in at least one type of adipose tissue deposit were included in our comparative and descriptive analysis. The distribution of the identified proteins among the four adipose tissues is presented as Venn diagram in Figure 1. 301 proteins were found to be globally present in all adipose tissues types (listed in Supplementary Table 3), whereas 123 and 204 proteins were identified only in subcutaneous or visceral adipose tissues, respectively (listed in Supplementary Table 4), of these 33, 48, 38 and 95 proteins were uniquely detected in either sternal, tail, omental and perirenal AT deposits, respectively (listed in Supplementary Table 4). Finally, Supplementary Table 5 shows the 133 proteins that do not display a particular tissue or macro-area specificity.
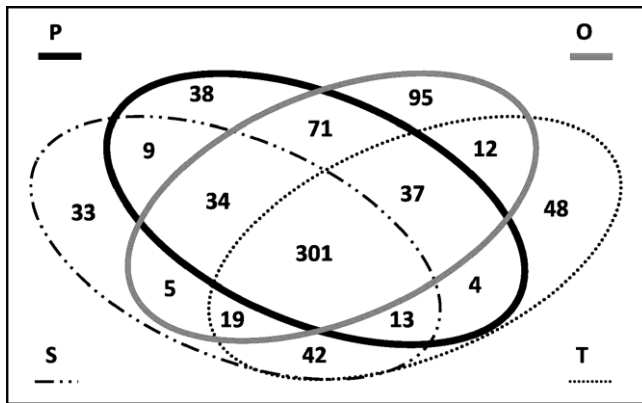
Figure 1. Venn diagram. Distribution and overlap of proteins identified in the four adipose tissue deposits (irrespective of their presence in liver). P=perirenal, O=omentum, S=sternum, T=tail.

3.2 Comparison of the proteome maps

A dendrogram grouping the five different tissues according to their protein identification profiles was generated by hierarchical clustering using the expression data from all 20 separate LC-MS/MS studies (Figure 2). This comparison clearly shows 3 distinct groups of protein expressions profiles. Liver samples are included here for graphical clarity. The significant similarity of the 4 biological replicate samples and the noticeable differences of protein expression patterns between subcutaneous and visceral adipose tissues result in a clearly distinct grouping of the proteomes. Likewise, adipose tissues and liver were clearly distinguishable from each other by their proteome patterns. The cluster patterns clearly demonstrate the close similarities between the two subcutaneous tissues (sternum and tail) and likewise, the proteome characteristics of the two visceral adipose tissues (omentum and perirenal) are closely related to each other. These results confirm previous studies in rat, where the effect of high fat diets on gene expression and secretion of adiponectin was found to be specific to the anatomical location of the adipose tissue depot [24]. In studies of human adipose tissues, it was observed that proteomes and metabolic profiles are significantly different between omental and subcutaneous adipose tissue [25].
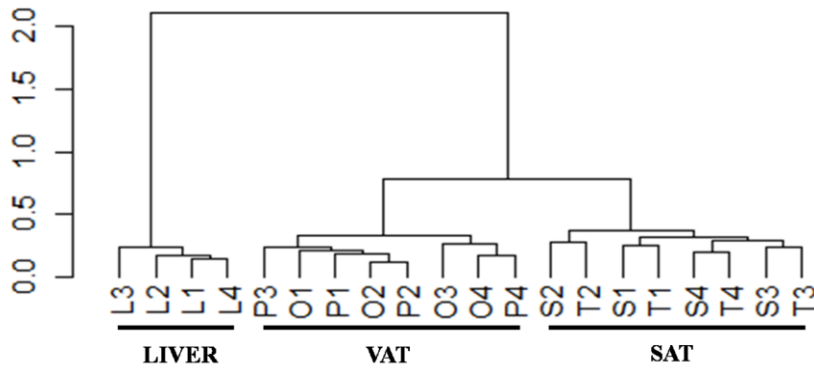
Figure 2. Hierarchical clustering analysis. The distance measure between proteome profiles was calculated as 1-ρ_{ij}, where ρ_{ij} is the Spearman's rank based correlation between the protein score profiles i and j. Liver, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) proteomes are clearly distinguishable

3.3 Functional grouping of adipose tissue proteins

Blast2GO retrieved 2360 unique Biological Processes GO terms and 1068 unique Molecular Function GO terms associated with our protein dataset. The generic Blast2GO annotation was subsequently reduced to PANTHER functional terms for a clearer segregation of the proteins in four major categories, based on similarity of functions, namely proteins involved in metabolic processes (G1), proteins involved in cell adhesion, cytoskeleton, intracellular transport and membrane integrity (G2), proteins involved in toxic response and folding (G3) and proteins involved in immune and inflammatory response (G4) (Figure 3).

3.3.1 Group 1: proteins involved in metabolic processes

As expected, a high percentage (63.8%) of the proteins detected in adipose tissues is involved in carbohydrate processing and metabolic pathways (485 out of 761). As seen in Figure 4a and 4b, this group of proteins dominate both VAT and SAT tissues, although significantly more in VAT (73.5%) than in SAT (54.5%). Moreover, the considerably higher percentage of G1 proteins uniquely found in perirenal and omental adipose tissue (67.4 and 73.7%, respectively), compared with sternal and tail adipose tissues (42.4% and 60.4%, respectively) further confirms the importance of VAT in metabolic homeostasis (Figures 4c-d-e-f). Despite the fact that our observations were made on young goat kids (30 days), the observed proteome profiles are well in line with previous transcriptome studies of lactating goat adipose tissues [26]. However, a few remarkable exceptions were observed, including the presence of lipoprotein lipase in young but not in adult animal tissues. This difference may be related to animal age, although it should also be noted that proteome and transcriptome profiles cannot be directly correlated [27].

3.3.2 Group 2: proteins involved in cell adhesion, cytoskeleton, intracellular transport and membrane integrity

Structural proteins represent 20.4% of the observed adipose tissue proteome (155 out of 761 proteins observed in total), hence must be regarded as a dominant group of proteins in all the studied adipose tissues. Figures 4a and 4b show that these proteins are more abundant in visceral AT than subcutaneous AT (30.1% and 19.6%, respectively), and particularly so for sternum deposits (48.5%), if compared to other tissues (Figures 4c-d-e-f). Structural proteins, including actin and annexin family members, are involved in cell architecture and mobility. A relative overexpression of actin has been reported to be related to cytoskeleton rearrangement, and to intracellular accumulation of lipid droplets during adipogenesis in bovine intramuscular preadipocytes [28], while overexpression of annexin 1 has previously been associated with bovine fat accumulation [29]. Considering that our study was carried out on young goats, where adipogenesis is actively ongoing, the presence of these two proteins is not surprising.

3.3.3 Group 3: proteins involved in toxic response and folding

Proteins involved in antioxidant and detoxifying defences are of particular importance in adipose tissues, where the high metabolic activity is associated with high levels of oxidative stress and thereby with a continuous generation of potentially toxic metabolites. Furukawa and co-workers observed that adipogenesis and adipocyte differentiation are associated with increased levels of reactive oxygen species [30], while Pessler-Cohen and colleagues showed that the formation of free radicals inhibits fat cell formation [31]. In our studies, 71 out of 761 (9.3%) adipose tissue proteins relate to this group (Figure 3). G3 proteins include families such as HSP, chaperons and peroxiredoxins, but also selenium binding protein, which is directly responsible for quenching Reactive Oxygen Species (ROS) [32]. Several Heat Shock Proteins were found across the four adipose tissues, including HS27Kda1, HSP70 8, HSP70 9, HSP90b1, HSP98 alpha heat responsive 12, HSP70 4, SP B6, and clusterin. HSP are intracellular chaperons, controlling protein folding [33] also shown to be involved in adipose tissue differentiation [34] and metabolism [25]. Peroxiredoxins are peroxidases whose main function is to protect proteins from ROS induced oxidative damage [35]. In the studied goat adipose tissues, we have observed five of the six peroxiredoxins that have so far been identified in various mammalian cells, with the only exception of Prx-4. Moreover, the oxidation-reduction cycles of peroxiredoxins have been shown to be involved in fine tuning for circadian rhythms [36]. The identification of several members of peroxiredoxin family in adipose tissue supports the involvement of adipose tissue depots as key regulator of circadian rhythms, and in body activity synchronization to environmental conditions and challenges.

3.3.4 Group 4: proteins involved in immune and inflammatory response

Adipokines, or adipocytokines, are cytokines secreted by adipose tissue, hence their primary role is believed to be the modulation of inflammatory and immune responses. Included in this family there are also other proteins whose roles are not directly related to inflammation, such as adiponectin, leptin, and resistin, which may more properly be refereed as adipose derived hormones. Our proteome study of goat AT revealed that 49 proteins out of 761 (6.4%) (Figure 3) are related to inflammatory and immune response. The list of inflammatory and immune-related proteins found in goat kid proteome is presented in Supplementary Table 6. In contrast with non-

ruminants, where adipocytes are often intermingled with immune cells, which are believed to be the principal producers of inflammatory regulators in human species [37], the number of white cells located within adipose tissue of ruminants has been found to be very limited, at least in cattle [38], suggesting that adipocytes are actively involved in the production of immune related proteins.
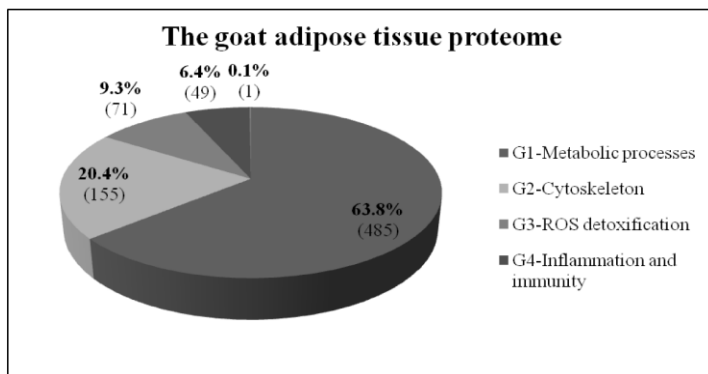
Figure 3. The goat adipose tissue proteome. Proteins are sorted in four different groups according to their functions. Most of the proteins are mainly involved in metabolic processes. Only protein noxp20-like has an unknown function.
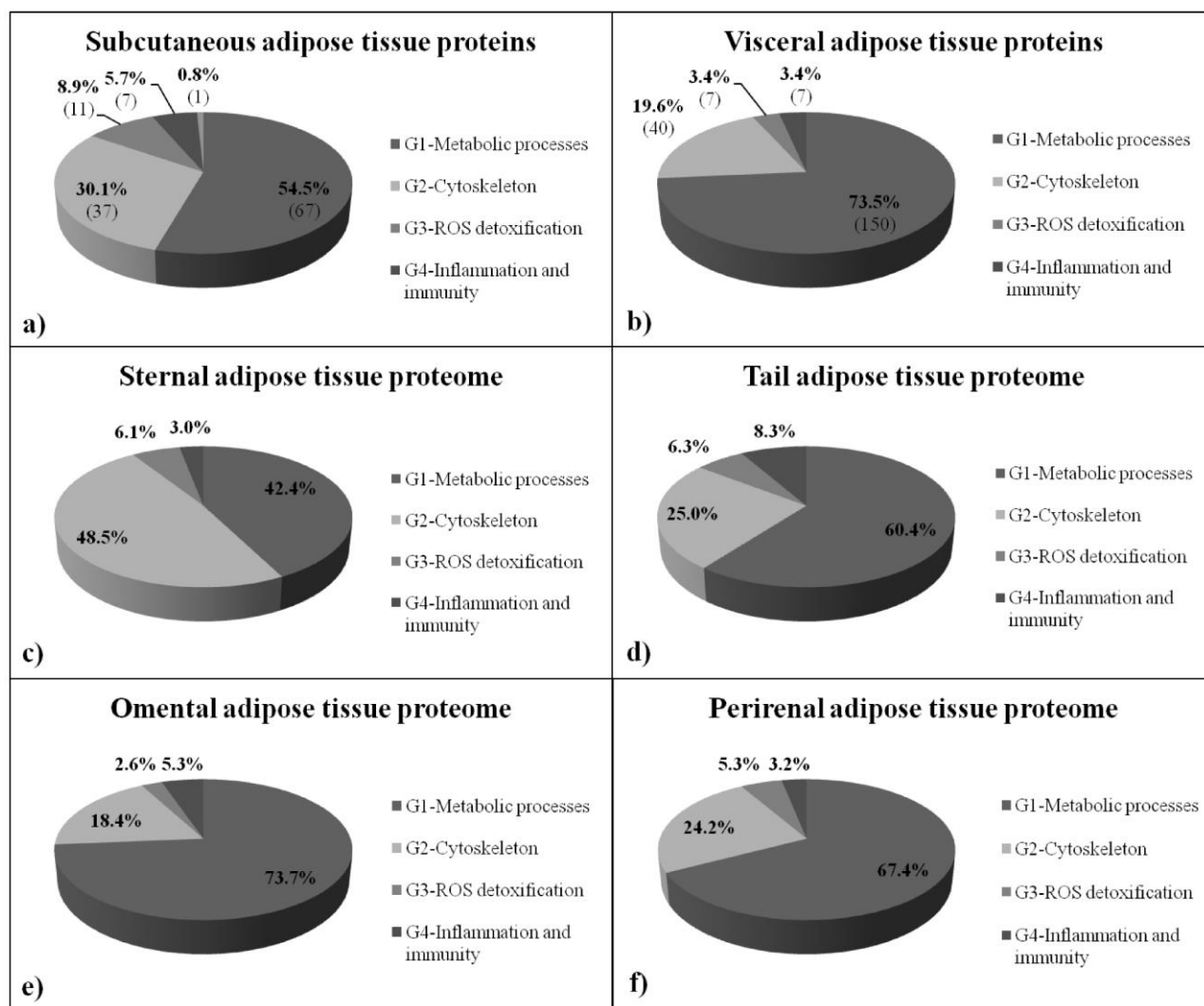
Figure 4. Proteome characteristics of different adipose tissues. Classification of the proteins present only in subcutaneous (a) or visceral (b) adipose tissue, sternum (c), tail (d), omental (e) or perirenal (f) deposits. Comparatively, there is a larger percentage of non-G1 proteins in subcutaneous deposits. In sternal deposits most of the proteins belongs to G2 (48.5%), while G4 proteins are mostly present in tail (8.3%). The percentage of G3 proteins is noticeably low in omentum (2.6%), compared to the other deposits.

3.4 Assessment of adipose tissue's protein expression capability: focus on the identification of novel adipokines

The intact adipose tissue (AT) features an intense capillary network surrounding each adipocytes. Consequently, the finding of a protein by proteomic techniques does not confirm per se its expression by AT, since, several proteins are expressed by liver, and delivered to tissues through blood. These proteins may then be detected in extrahepatic tissues as well. Therefore, the effective capability of AT to produce proteins was eventually confirmed by qualitative RT-PCR through detection of their mRNAs. The mRNA expression of 35 of the 49 proteins belonging to the group of inflammatory and immune-related proteins was investigated. Results are summarized in Table 2. Six genes out of 35 were not found expressed in adipose tissues, suggesting that these proteins are delivered to adipose tissues through blood. These include immunoglobulin lambda light chain (IgLL), immunoglobulin gamma 1 heavy chain (IGHG1), immunoglobulin gamma 2 heavy chain constant region (IGHG2), plasminogen precursor, fibrinogen alpha, fibrinogen beta. No studies have investigated their mRNA expression in adipose tissue to date.

Adipose tissue is involved in the regulation of platelet functions, fibrinolysis and coagulation processes [39]. The presence of fibrinogen gamma in goat samples confirms previous studies in human visceral and subcutaneous adipose tissue [40;41], but to the best of our knowledge, fibrinogen gamma's mRNA expression by AT has never been reported before. Also the mRNA expression of kininogen-1 has never been investigated, but the relation between kinin B1 and B2 receptors and AT is well known [42]. Other circulating proteins that have been identified by proteomics and validated by gene expression studies include albumin, involved in the transportation of fatty acids into adipose tissue and muscles [43;44], complement component 3 (C3) and complement component 4A (C4a) [45;46].

The important role of adipose tissue in the immune response is demonstrated by the mRNA expression of galactoside-binding proteins (LGALS), such as beta galactoside-binding lectin precursor and galactoside-3, and Acute Phase Proteins (APP), namely ceruloplasmin and alpha-1-acid glycoprotein. LGALS act as agonists of platelet activation [47] and are involved in the promotion of immune cells apoptosis [48], neutrophil activation, adhesion and opsonisation and chemoattraction of monocytes [49]. Their mRNA expression confirms previous experiments in human subcutaneous and mice epididymal fat, where they have been correlated to diabetes [50]

and obesity [49], respectively. APP family includes structurally un-related proteins, expressed by liver after systemic inflammatory challenge [51]. The expression of some of them by AT has been recently demonstrated in farm animal species, including cow, horses and pigs [52-54]. Serum ceruloplasmin level is associated with obesity in humans [55] and mice [56], but its mRNA expression in adipose tissue has never been investigated. To the best of our knowledge, this is the first report of the expression of ceruloplasmin by adipose tissue, even in non-pathological condition, partially explaining why the serum concentration of the protein is elevated in obese IL-6 KO mice as well [56]. Tumour protein translationally-controlled 1 is a ubiquitously expressed protein in all eukaryotes, but never identified in adipose tissue. It is overexpressed in many tumors and correlated to several biological processes such as cell growth, protein degradation and apoptosis [57]. Its expression has been already demonstrated in goat mammary gland [58], but not in adipose tissue.

Beside their involvement in oxidative stress [59], protein dj-1 and thioredoxin are also involved in the regulation of innate immunity, being protein-dj upregulated by Natural Killer cells after challenging with Substance P [60], and thioredoxin overexpressed in sheep following experimental infection with *Teladorsagia circumcincta* [61]. Thioredoxin mRNA expression has been positively correlated with obesity in human [62], while the presence of protein dj-1 in adipose tissue has already been demonstrated in rats [63] and humans [64], but not its mRNA expression. Valacyclovir hydrolase-like (also called biphenyl hydrolase-like) and thiosulfate sulfurtransferase have also been correlated to detoxification processes. The first is a serine hydrolase catalysing the hydrolytic activation of the antiviral prodrug valacyclovir [65] and expressed in several tissues in humans [66]; the latter is involved in the formation of iron-sulphur complexes and cyanide detoxification and recently identified in several tissues in rabbits [67]. This is the first study in which the expression of these two proteins by adipose tissue has been demonstrated.

Particular focus has been given in the last years to the alpha(B)-crystallin, a structural protein of the ocular lens, also dampening several inflammatory pathways in both immune system and central nervous system (CNS) [68], and involved in several immune diseases including human amyloidosis [69] and chronic neuroinflammation [70]. Alpha(B)-crystallin was found expressed in

different tissues types [71], including both visceral and subcutaneous depots in humans [41] Its mRNA expression was previously reported in rats [72], but never in goats.

The adipokines that we found for the first time in goat adipose tissues, and validated by mRNA expression include also lactoferrin and hemopexin, two proteins related to both inflammation and iron transport. It should be noted that the potential adipokine role of lactoferrin was recently studied by in vitro experiments on 3T3-L1 fibroblast derived adipocyte cell lines, and mouse or human adipose tissues; its role in adipocyte physiology suggested [73]. Also hemopexin was found in adipose tissue, but its presence was attributed to the presence of blood [74]. On the contrary, we demonstrated here that adipose tissue may produce hemopexin mRNA, and therefore may be considered as an additional source of the protein, which can be found also in blood.

Finally, although no previous studies have investigated its expression in goat, it is not surprising that adipose most abundant gene 2 (APM2) is expressed by adipose tissues, since this recently discovered protein was found at high levels in white adipose tissue of obese subjects, and it is probably involved in adipogenesis in human [75].

Table 2 – mRNA expression of inflammation and innate immunity-related proteins in goat-kid adipose tissue. Qualitative RT-PCR results: (P) presence, (A) absence.				
Sequence name	Sternum	Base of the tail	Perirenal area	Omentum
Adiponectin	P	P	P	P
Adipose most abundant gene transcript 2	P	P	P	P
Alpha 1 acid glycoprotein	P	P	P	P
Alpha 1 b glycoprotein precursor	P	P	P	P
Alpha 2 hs glycoprotein precursor	P	P	P	P
Alpha 2 macroglobulin precursor	P	P	P	P
Alpha crystallin b chain	P	P	P	P
Beta galactoside-binding lectin precursor	P	P	P	P
Ceruloplasmin	P	P	P	P
Complement component 1, q subcomponent binding protein	P	P	P	A
Complement component 3	P	P	P	P
Complement component 4a	P	P	P	P
Fibrinogen alpha chain	A	A	A	A
Fibrinogen beta chain	A	A	A	A
Fibrinogen gamma chain	P	P	P	P
Galactoside 3	P	P	P	P
Gli pathogenesis related 2	P	P	P	P
Hemopexin precursor	P	P	P	P
Immunoglobulin gamma 1 heavy chain	A	A	A	A
Immunoglobulin gamma 2 heavy chain constant region	A	A	A	A
Immunoglobulin lambda light chain	A	A	A	A
Kininogen 1, transcript variant 1	P	P	P	P
Lactoferrin	P	P	P	P
Macrophage migration inhibitory factor	P	P	P	P
Plasminogen precursor	A	A	A	A
Protein dj 1	P	P	A	P
Retinol binding protein 4	P	P	P	P
Selenium binding protein 1	P	P	P	P
Serum albumin precursor	P	P	P	P
Thioredoxin	P	P	P	P
Thiosulfate sulfurtransferase	P	P	P	P
Transforming growth factor beta 68kda	P	P	P	P
Tumour translationally controlled 1	P	P	P	P
Valacyclovir hydrolase like	P	P	P	P
Zinc alpha 2 glycoprotein precursor	P	P	P	P

4. Conclusions

It has become increasingly clear that adipose tissue plays important roles in a wide range of biological processes, including metabolic control, oxidative stress and inflammation, as well as for both innate and adaptive immune response, but in depth knowledge of the molecular characteristics of different adipose tissues is not yet well covered. To the best of our knowledge, we have here presented the first comparison of subcutaneous and visceral fat deposits proteomes in goat. The global proteome mapping allowed the identification of 761 proteins, and the majority of these were specifically detected in either subcutaneous or visceral adipose tissue deposits, allowing a clear distinction between the proteomes of these two major types of adipose tissue deposits. These observations are well in line with previous studies of rat and human adipose tissues. Furthermore, the validation at gene expression level of proteins involved in inflammation and immune response demonstrated that adipose tissue expresses at least 27 factors involved in these pathways. These include 9 host response-related proteins that have not been previously recognized as adipokines, namely ceruloplasmin, gamma fibrinogen, hemopexin, kininogen 1, lactoferrin, protein dj, thiosulfate sulfurtransferase, tumour translationally controlled 1 and valacyclovir hydrolase, although some of these have previously been identified in human and/or mouse adipose tissue. Our findings provide further insight in the immunological competence of adipose tissues. The ability of fat tissues to produce signalling factors plays a key role in animal health, and particularly for dairy animals, where lipid mobilization is hormonally controlled during the transition from pregnancy to lactation. Little is so far known about the molecular mechanisms underlying fat reserves mobilization and milk production in goats, but specific knowledge of how these pathways are controlled is of key importance for the management of animal health and thereby also for economic gain in dairy production.

Acknowledgments

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PAPER III

Effect of fish oil or stearic acid enriched diets on omental adipose tissue proteome in 30 days old goat-kids using iTRAQ analysis.

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Aim: the aim of the present study was to perform a comparative investigation of visceral adipose tissue proteomes in goat-kids with different high-fat fed mothers. A quantitative 2D-LC-MS/MS analysis was performed, using iTRAQ labelling, followed by Real Time PCR, in order to evaluate the possible influence of fish oil or stearic acid enriched diets on kid's omentum proteome and on adipose tissue protein expression.

Effect of fish oil or stearic acid enriched diets on omental adipose tissue proteome in 30 days old goat kids using iTRAQ analysis.

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Abstract

Both saturated and unsaturated fatty acids can modulate animal health by interacting at different levels with the immune system. It has been demonstrated that adipose tissue protein expression can be modulated by fatty acid diet enrichment and that maternal diets can influence adipogenesis in lambs. Although proteomics could be of great help in understanding the relationship between maternal diet and kids' adipose tissues, studies have been performed only in humans and rodents. In order to evaluate the relationship of saturated and unsaturated enriched diets on goat kids' adipose protein expression, lactating goats were fed with different high-fat diets, and omental adipose tissue proteomes of goat kids was determined by quantitative iTRAQ 2D-LC-MS/MS analysis and validated by qualitative mRNA gene expression analysis. Quantitative mRNA expression of selected significant proteins involved in innate immunity was also carried out by Real Time PCR. In this study, we demonstrated that kids' omental proteome can be modified by maternal diet enrichments with either saturated or unsaturated fatty acids, the feeding with fish oil inducing changes in a higher number of proteins when compared to stearic acid. Finally we demonstrated that proteome changes are not confirmed by mRNA expression changes.

Biological significance

In our proteomic analysis of kids' omental adipose tissue, we showed that adipose tissue proteome can be modulated by different maternal dietary treatments. This result points out the possibility of intervening on kids' health and meat quality with an indirect action on maternal diet. In farm animals this opportunity is of a special interest, also considering that the maternal diet can influence embryo survival, subsequent kids' reproductive ability, but also unsaturated fatty acid status of the neonate with consequent influence on the economic gain. In addition, a combined proteomic and quantitative PCR analyses demonstrated that changes at proteomic levels not always reflect changes in gene expression, highlighting the importance of combining different techniques when characterising a tissue.

1. Introduction

The involvement of adipose tissue (AT) in several physiological and pathological processes, such as appetite regulation, reproduction, and inflammatory and immune response, is well recognized (for a very recent and comprehensive review please see [1]). Therefore, while in humans adipose tissue has a key role in obesity, in farm animals, where obesity is not an issue, due to the controlled environment in which they live, particular focus has been given to adipose tissue's influence on animal health and meat quality.

The adipose tissue content of meat products has an impact on both the economic value for producers and the nutrition and health of ready meat consumers. Beef producers need to produce beef cattle with a moderate amount of adipose tissue in the right adipose depots i.e. marbling fat. Marbling fat is defined as intramuscular adipose tissue, and has been shown to play an important role in the eating quality and composition of meat [2;3], therefore it is clear its economic value. On the other hand, in dairy animals AT metabolism gained particular interest for its essential role in the transition period when a hormonally-controlled lipid mobilization is established in order to support milk synthesis [4;5]. The active role of adipose tissue in regulating the wide range of body functions is explicated by its ability to produce and secrete adipokines. Adipokines are signalling molecules with endocrine, autocrine or paracrine functions, secreted in response to stimulus coming from the hormone system and the central nervous system [6]. Taking into account the profound relationship between body fat reserves and food, it is not surprising that adipose tissue's transcriptomic profile can be modified by diets or feed deprivation. As demonstrated in goats, 48 h of feed deprivation alter the expression profile of several genes in omental and perirenal AT deposits, the omental fat depots being more sensitive to feed deprivation than perirenal areas [7]. In addition, Ebrahimi and coworkers [8] demonstrated that linseed oil supplementation to Boer goats' diet leads to changes in fatty acid profile of subcutaneous adipose tissue and expression of genes related to fat metabolism such as PPARα, PPARγ and stearoyl-CoA desaturase. No proteomics studies are available in goat species.

Distinct fat sources in diets have distinct effects on adipose tissue, as demonstrated by Thering et al. [9], which investigated the effect of fish and soybean oils or saturated lipids enriched diets on lipogenic and adipogenic gene expression in cow's tail-head adipose tissue. Fish oil is particularly rich in eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6,

n-3) that can positively influence animal health due to their involvement in innate immune pathways [10;11]. On the other hand, Bueno et al. [12] demonstrated that diets enriched with coconut oil or lard, both rich in saturated fatty acids, can modify the pro-inflammatory environment of white adipose tissue in rats, by upregulating haptoglobin expression.

Adipose tissues originate during early life from mesenchymal stem cells, which can differentiate into adipocytes, osteoblasts, chondrocytes, and myoblasts [13]. It has been demonstrated that a diet based on milk or milk replacer can influence meat quality and fat composition of suckling kids [14]. It is still unknown how mother diets can influence newborn adipose tissue through milk. In addition, fish oil enriched diets increases the amount of n-3 PUFAs in colostrum and mature milk in pregnant dairy goats [15], and a specific involvement in fetal and neonatal development has been recognized for DHA [16]. Although proteomics could be of great help in understanding the relationship between maternal diet and kids' adipose tissues' characteristics, mainly transcriptomic studies have been carried out to date. Beside those driven in human species, the few existing proteomic experiments have been performed in rodents, aimed to evaluate the effect of high fat diets on the protein expression of insulin target tissues in mice [17] or on the expression of adipose tissue proteins between obesity-susceptible and obese-resistant rats [18]. In farm animals, few information are available, among which, the demonstration of the influence of maternal diets on adipose tissue proteome in newborn pigs [19]. To the best of our knowledge, no proteomic data are available in goats.

In the present study, we therefore performed a comparative investigation of visceral adipose tissue proteomes of goat-kids with different high-fat fed mothers. A quantitative 2D-LC-MS/MS analysis was carried out, using iTRAQ labelling, in order to evaluate the possible influence of fish oil or stearic acid enriched diets on kids' omentum protein expression. mRNA expression of selected significant proteins was also evaluated by quantitative PCR.

2. Materials and methods

The experimental protocol used in this study was approved by the ethics committee of the University of Milan (Protocol No. 5/11, 18 January 2011).

2.1 Animals, diets and tissue sampling

Adipose tissue samples were obtained from ten 29.8±2.8 day-old healthy suckling kids, which were part of a larger experiment aimed to evaluate the influence of the maternal diet on peripartum and goat kids' performances. A group of 26 multiparous Alpine goats, homogeneous for parity and milk production during the previous lactation, were fed with different diets enriched with fatty acids, either saturated (ST, 69:26 percentages ratio of stearic acid (C18:0) and palmitic acid (C16:0)) or unsaturated (FO, fish oil containing 10.22% of EPA=20:5 and 7.65 % of DHA=22:6), starting from a week before kidding until slaughtering of the kids. A third group of animals fed with a control diet without any specific diet integration was also used as control (CTRL). FO and ST goats diets were adapted for the dry period (supplemented with 30 g of fatty acids) and lactation period (supplemented with 50 g of fatty acids). Goats were housed in single boxes and kids were individually fed by their own mothers. From this larger group, twelve male kids, equally distributed among maternal control diet (CTRL-Kid=4), stearic acid (ST-Kid=4) and fish oil (FO-Kid=4) treated samples, were randomly selected in order to be included in the present experiment. Samples were obtained from omental region. Tissue samples for both molecular biology and proteomic analysis were snap frozen in liquid nitrogen and stored at –80°C for further analysis.

2.2 Sample preparation and protein digestion

In addition to the 12 omentum samples, a reference sample was created by pooling equal amounts of the four controls. The reference sample was divided into four identical aliquots, one for each iTRAQ run and the 16 samples (12 experimental samples + 4 reference samples) were processed together through all the processes allowing the comparison of multiple iTRAQ runs.

Protein extraction procedures were carried out on ice or at 4°C as described previously [20] . 200 mg of each adipose tissue were homogenized in 5 µl/mg TES buffer (10 mM Tris-HCl, pH 7.6; 1 mM EDTA, 0.25 M sucrose) and centrifuged at 10000 x g, for 30 min, at 4°C. Protein concentration values of the tissue supernatants were determined by the Pierce BCA Protein Kit (VWR), using BSA as a protein standard, according to the manufacturer's manual. Hundred-twenty

μg of proteins from each tissue homogenate was precipitated by adding 6 vol of ice-cold acetone. The precipitated proteins were re-suspended in 20 μl of digestion buffer (0.5 M triethylammonium bicarbonate, 0.1% SDS); cysteine residues were reduced with 2.5 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) at 60°C for 1 h, and then blocked with 10 mM methylmethanethiosulfate at room temperature, for 1 h. Samples were digested with trypsin (1:10 w/w) (AB SCIEX) at 37°C, overnight.

2.3 iTRAQ (Isobaric Tag for Relative and Absolute Quantitation) labelling

iTRAQ labelling was performed according to the manufacturer's instructions (Applied Biosystems). Four independent iTRAQ runs were performed. Reference samples were labelled with reagent 114, control samples were labelled with reagent 115, fish oil treated samples were labelled with reagent 116, stearic acid treated samples were labelled with reagent 117 (as shown in Table 1). Each isobaric tagging reagent was added directly to the peptide mixture and incubated at room temperature for one hour. The 16 samples were then combined in 1:1:1:1 ratios into four tubes, each containing a common reference sample, a control and two treated samples (one stearic acid and one fish oil). In order to remove all the impurities that can interfere with later HPLC separation, all samples were passed through a 0.2μm centrifuge filter (National Scientific Company) for 10 min at 10000 x g, vacuum-dried and eventually stored at -80°C until further analysis.

Table 1. iTRAQ labelling scheme

| iTRAQ runs | iTRAQ labelling and reporter ions | | | |
	114	115	116	117
1	Ref[1]	C_1	FO_1	ST_1
2	Ref[1]	C_2	FO_2	ST_2
3	Ref[1]	C_3	FO_3	ST_3
4	Ref[1]	C_4	FO_4	ST_4

[1]reference sample was created by pooling equal amounts of the four control samples

2.4 2D-LC-MS/MS analysis

2.4.1 Strong Cation Exchange (SCX) liquid chromatography

The peptides were re-dissolved in 0.03% formic acid, 5% acetonitrile in water. Peptides mixture generated from the digestion of 50 µg of protein were injected into an Agilent 1100 Series capillary HPLC equipped with a Zorbax Bio-SCX Series II, 0.8×50 mm column (Agilent Technologies) that provides peptide separation by strong cation exchange liquid chromatography.

Peptides were eluted with a gradient of increasing NaCl (0 min 0% B; 5 min 0% B; 10 min 1.5% B; 11 min 4% B; 25.5 min 15% B; 35.5 min 50% B; 45 min 100% B; 55 min 100% B). Buffer A contained 0.03% formic acid and 5% acetonitrile in water, buffer B contained 0.03% formic acid, 5% acetonitrile and 1 M NaCl in water. The flow rate was 15 µl/min and fractions were collected every minute for 65 minutes and then combined according to their peptide loads into 10 pooled samples to achieve approximately equal peptide loads for further LC-MS/MS analyses.

2.4.2 LC-MS/MS

The pooled samples were de-salted and concentrated on Agilent 1100 Series nano-flow HPLC system (Agilent Technologies), prior to be further separated by reverse phase liquid chromatography. De-salting and concentration of the samples were carried out on an enrichment column (EASY Column, 2cm, ID 100µm, 5µm, C18 - Thermo Scientific) using an isocratic pump working at 20 µl/min (0.1% formic acid and 3% acetonitrile in water). Peptides were then eluted and further separated on an analytical column (EASY Column, 10cm, ID 75µm, 3µm, C18 - Thermo Scientific) with a nanoflow of 300nl/min, using a gradient of increased organic solvent (0 min 5% B; 7 min 5% B; 70 min 40% B; 73 min 95% B; 78 min 95% B; 83 min 5% B; 100 min 5% B). Buffer A containing 0.1% formic acid in water and buffer B containing 5% water and 0.1% FA in acetonitrile. The eluted peptides were sprayed through nanospray needle (PicoTip®, silica, no coating, OD 360µm, ID 20µm - New Objective) directly into the Q-star Elite mass spectrometer (Applied Biosystems).

2.5 Database searches and statistical analysis

The raw spectrum files from 20 individual shotgun LC-MS/MS runs (comprising four biological replicates from each of the five tissues) were searched separately with Protein Pilot 1.0 software (Ab Sciex) using the ProGroup and Paragon algorithms for protein grouping and

confidence scoring. The target database used for searching was constructed as a non-redundant union of UniProtKB Bovidae sequences (www.uniprot.org/uniprot/?query=taxonomy:9895) and NCBI Capridae sequences (www.ncbi.nlm.nih.gov/taxonomy/?term=9963). The reversed copy of each of the target protein sequences was appended to the list for estimating the false discovery rate (FDR) [16]. The False Discovery Rate (FDR) was estimated as the ratio of (2 x reversed sequence)/(reversed + forward sequence) in percentage. Search parameters were set with an MS tolerance of 0.15 Da and a MS/MS tolerance of 0.1 Da, and using generic modifications including deamidation of glutamine and asparagines side chains, methionine oxidation as well as methyl methanethiosulfonate modification of cysteines. Samples were SCX fractionated and analyzed twice (technical replication) in order to gain higher reproducibility and proteome coverage as suggested by Chong and coworkers [21]. The two data sets from each sample were searched together in ProteinPilot (Applied Biosystems). The confidence for protein identification was selected in ProteinPilot to a protein score of 1.3, equivalent to 95% confidence and a minimum of two peptides per protein.

Data handling and analysis was performed using the statistical software package R (R Development Core Team). Statistical analysis was carried out using the one-way ANOVA, while diets comparison was performed using the Tukey option within the MIXED procedure of SAS (SAS Institute, 2008).

2.5.1 Functional annotation and grouping

The open source online tool Blast2GO (http://www.blast2go.com) was used for the functional annotation of the identified proteins [22]. The default parameters were used and for the basic local alignment search tool (BLAST) protein sequences were mapped against the NCBInr. We further narrowed the functional analysis by PANTHER classification into protein families and functional pathways in order to increase the confidence (Protein Analysis Through Evolutionary Relationship) system available at http://www.pantherdb.org [23].

2.6 Validation of the proteomic results by Real Time PCR

Total RNA was extracted from the same tissues used for the proteomic analysis, stored at -80°C, by means of a commercial kit specific for all kind of tissues (RNeasy Plus Universal Mini Kit - Qiagen), a DNAse treatment was also carried out (RNase-Free DNase Set - Qiagen); the RNA concentration in each sample was quantified by NanoDrop ND-1000 UV-spectrophotometer. 1 µg RNA was retrotrancribed using the iScript cDNA Synthesis kit (Biorad). The resulting cDNA was used as template for qualitative and quantitative PCR reactions. In case of absence of goat sequences primers were designed on bovine sequences. The same primers were used in qualitative and quantitative PCR (primers sequences, accession numbers and length of the amplified fragments are listed Table 2). A pool of cDNA from liver of all the 12 animals was created in order to use it as positive control in the qualitative PCRs and as reference sample for the Real Time PCRs.

In order to confirm the mRNA expression of the selected proteins by omental adipose tissue, qualitative PCRs were performed and samples were amplified in 10 µl final volume, containing 1 µl buffer (Vivantis), 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP), 1 µM each primer and 0.025 U Taq polymerase (Vivantis). No-template reactions were performed as negative control for each target. PCRs were carried out on all the samples at the same conditions: 34 cycles at 96°C for 30 s, 60°C for 30 s, 72°C for 45 s. Results were visualized on 1.6% agarose gel stained with ethidium bromide.

Real Time PCRs were performed in 12 µl Eva Green mix, 250 nM YWHAG and CD36 primers, 300 nM AGP, PRDX6 and SERPINA1 primers, 350 nM HPCAL1 primers, 400 nM GAPDH and LRP10 primers, using the ECO^{TM} Real Time PCR system (Illumina). Samples were tested in duplicate and no-template reactions were performed as negative control for each target. The PCR efficiency was evaluated by creating a standard curve with 1:3 serial dilutions of the liver pool. The thermal profile for each gene was 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 10 s and 60°C for 30 s; the melting curve was created running the samples at 55°C for 5 s and 80 cycles starting at 55°C up to 95°C, increasing 0.5°C each 5 s. Relative quantification was calculated using the comparative delta-delta-Ct method [24] and GAPDH, HPCAL1 and LRP10 as the most stable reference genes [25].

2.6.1 Statistical analysis on qPCR data

All data from quantitative PCR and histological evaluation were elaborated with an analysis of variance using the statistical software SAS (SAS Inst. Inc., Cary, NC). All data were evaluated for normal distribution using the Kolmogorov–Smirnov test. Post-hoc tests were carried out on parametric data using the Tukey-Kramer method.

Sequence name	Symbol	Accession number	Primer Forward (5'-3')	Primer Reverse (5'-3')	Lenght (bp)	PCR efficiency and R^2
14-3-3 protein gamma	YWHAG	NM_174793.2	TCCTGTCTTTGATCGCCTCT	GAACAGTCCATGTGCCAGTG	112	99.35 0.991
Alpha-1-acid glycoprotein	AGP	[26]	GCATAGGCATCCAGGAATCA	TAGGACGCTTCTGTCTCC	112	106.86 0.996
Coagulation factor XIII, A chain	F13A1	NM_001167894	AAGTGGATCACCACACCGAC	TGTGGGGTCATAGGGACGAT	104	- -
Extracellular superoxide dismutase	EC-SOD	EU559622.1	CTCTGTGCCTCTCTGCTCCT	TCATCTCCTGCCAGATCTCC	136	91.16 0.991
Peroxiredoxin 5	PRDX5	NM_174749.2	GCCCCGATTAAGGTTGGAGAT	CACCGATTTATTCCCAATGC	100	102.77 0.997
Peroxiredoxin 6	PRDX6	NM_174643.1	CTGACGGCAGAAAAGAGGGT	TGCCAGATGGGAGCTCTTTG	139	104.67 0.998
Plasma proteinase inhibitor fragment	SERPINA1	NM_173882.2	GGTGTCCAAGGCTCTCCAC	AGGGTCTGTTGAACTCGACG	125	99.32 0.997
Platelet glycoprotein 4	CD36	JF690773	CACCGATTTATTCCCAATGC	GCAGGGACACTGATGAGGAT	120	109.83 0.994
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	NM_001034034	GGCGTGAACCACGAGAAGTATAA	CCCTCCACGATGCCAAAGT	119	100.2 0.994
Hippocalcin-like 1	HPCAL1	[25]	CCATCGACTTCAGGGAGTTC	CGTCGAGGTCATACATGCTG	99	102.23 0.995
LDL receptor-related protein10	LRP10	[25]	CCAGAGGATGAGGACGATGT	ATAGGGTTGCTGTCCCTGTG	139	101.35 0.995

Table 2. Selected proteins for mRNA expression analysis and housekeeping genes. Accession numbers, primers sequences and length of the amplified fragments are also show. In the last column Real Time PCR efficiency and R^2 are presented.

3. Results and discussion

In this study, we investigated the possible influence of the maternal diet on adipose tissue's protein expression in goat kids. Kids were divided into three groups (n=4) according to the different maternal diets (CTRL, ST, FO), and slaughtered at 30 days. The iTRAQ 2-D LC-MS/MS based approach was used to compare omental adipose tissue's proteomes allowing the identification of differential expressed proteins. After proteomic analyses, the mRNA expression of selected genes was quantified on the same samples.

3.1 Protein identification

Four iTRAQ runs were performed, in which the identified proteins were 837, 749, 724, 802, respectively, with high confidence and coverage despite the high amount of lipids in the samples that can interfere with LC-MS/MS analysis. Indeed, in all experiments, the average unused ProtScore was 8.41 and the average protein sequence coverage was 32.8%. Three proteins were matched to decoy (reversed) protein sequences, which gives a fraction of incorrect assignments of (2 x reversed sequence)/(reversed + forward sequence)=3/635=0.0047. In total, 635 unique proteins with at least two unique peptides were identified and quantified (listed in Supplementary Table 1).

3.2 Differential expressed proteins by iTRAQ analysis and functional grouping

The evaluation of the effect that the maternal diet has on kids' performances, rather than the study of a direct effect of the diet on the animal, is of particular interest in farm animals where animal health and economical gain are strictly correlated. Polyunsaturated Fatty Acid (PUFA), for example, can positively influence animal health due to their involvement in innate immune pathways [10;11].

In order to identify the differentially expressed proteins in animals whose mothers were fed with high fed diets, we compared the proteomes of control animals (CTRL-Kid) and animals whose mothers were fed with either stearic acid (ST-Kid) or fish oil (FO-Kid) enriched diets. A protein was considered differentially expressed when the protein fold change was above 1.3 and the *p*_value below 0.05. In total, 41 proteins were found differentially expressed in a statistically significant way (listed in Table 3). Of these, 9 proteins were differentially expressed in ST-Kid

samples compared to CTRL-Kid samples, while 19 proteins were differentially expressed in FO-Kid samples compared to CTRL-Kid samples. In addition, the proteome differences between samples from the two dietary treatments were compared and 25 proteins were found differentially expressed. Three proteins out of 41 samples showed an ANOVA $p\_$value below 0.05 even though they don't show any statistically significant differences among diets, therefore further analysis were focused only on the remaining 38 proteins. The functional grouping of the 38 differentially expressed proteins, according to Biological Process and Molecular Function, was performed using Blast2GO. The generic Blast2GO annotation was subsequently reduced to PANTHER functional terms for a clearer segregation of the proteins in four major categories, based on similarity of functions, namely proteins involved in metabolic processes; proteins involved in cell adhesion, cytoskeleton, intracellular transport and membrane integrity; proteins involved in toxic response and folding and proteins involved in immune and inflammatory response (Table 3).

Thirty one out of out of 38 proteins whose expression was modified by mother-feeding with different fatty acid diets are involved in metabolic processes (n=20) or cell adhesion and molecular transport (n=11). Five proteins are involved in toxic response and two proteins are involved in immune and inflammatory processes.

Table 3. List of the proteins showing statistical significant fold changes and relative p-values. Unused and total protein score, % of sequence coverage and accession numbers are shown. In the last column the functional group is presented.

N	Unused	Total	% Cov	Accession Number	Sequence Description	FO vs CTRL (Ctrl=1)		ST vs CTRL (Ctrl=1)		ST vs FO (FO=1)		Function
						Quantity	P_value	Quantity	P_value	Quantity	P_value	
3	80.2	80.2	78.97	gi|145226795|gb|ABP48145.1|	vimentin	1.19*	0.042	1.03	0.821	0.87	0.089	G2
28	29.47	29.47	90.15	gi|167900488|ref|NP_001108139.1|	fatty acid binding protein 4	1.06	0.623	0.83	0.057	0.78*	0.018	G1
39	23.8	23.8	68.02	tr|A7Z057|A7Z057_BOVIN	14-3-3 protein gamma	1.07	0.414	0.91	0.232	0.85*	0.042	G1
59	19.44	19.44	42.88	sp|P20000|ALDH2_BOVIN	aldehyde dehydrogenase 2 family	1.11*	0.016	1.00	0.995	0.90*	0.015	G1
89	15.57	18.73	48.74	sp|P05786|K2C8_BOVIN	keratin 8	0.67	0.052	1.11	0.719	1.66*	0.020	G2
93	15.14	20.18	50.98	gi|71153780|sp|P62262.1|1433E_SHEEP	tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation epsilon polypeptide	1.01	0.961	0.83*	0.033	0.82*	0.024	G1
112	13.24	14.55	15.8	tr|E1BKX7|E1BKX7_BOVIN	filamin-b isoform 2	0.95	0.485	0.87*	0.027	0.91	0.122	G2
125	11.77	11.77	48.62	sp|P08728|K1C19_BOVIN	keratin 19	0.74*	0.040	1.06	0.835	1.43*	0.020	G2
148	10.06	10.07	62.5	sp|O77834|PRDX6_BOVIN	peroxiredoxin 6	0.86*	0.024	0.93	0.247	1.08	0.223	G3
153	9.97	10.01	23.1	sp|Q05443|LUM_BOVIN	lumican	0.74*	0.027	0.83	0.145	1.12	0.417	G2
166	9.61	9.61	41.64	tr|F1N1N0|F1N1N0_BOVIN	heterogeneous nuclear ribonucleoproteins a2 b1-like	0.91	0.324	1.11	0.240	1.21*	0.033	G1
167	9.58	9.59	32.85	sp|Q17QJ1|ACSF2_BOVIN	acyl- synthetase family member mitochondrial	1.19	0.098	0.95	0.769	0.80*	0.041	G1
172	9.3	9.3	32.21	sp|O46629|ECHB_BOVIN	hydroxyacyl-coenzyme a dehydrogenase 3-ketoacyl-coenzyme a thiolase enoyl-	1.08	0.310	0.9	0.189	0.83*	0.025	G1

					coenzyme a hydratase (trifunctional protein) beta subunit								
202	7.62	7.62	26.85	sp|Q3MHL4|SAHH_BOVIN	s-adenosylhomocysteine hydrolase	0.77*	0.038	0.84	0.137	1.08	0.588		G1
236	6.8	6.8	26.43	tr|F1MWR3|F1MWR3_BOVIN	electron-transfer alpha polypeptide	0.96	0.451	0.85*	0.009	0.89*	0.037		G1
238	6.61	6.61	42.39	sp|P62833|RAP1A_BOVIN	ras-related protein rap-1a	1	0.998	0.82*	0.010	0.83*	0.011		G1
259	5.84	5.84	36.18	sp|Q5E9F5|TAGL2_BOVIN	transgelin 2	0.79*	0.016	1.13	0.166	1.43*	0.002		G2
263	5.75	5.99	31.78	gi|338163311|gb|AEI74425.1|	platelet glycoprotein 4	1.23*	0.045	0.97	0.860	0.79*	0.024		G2
269	5.64	5.64	23.06	tr|F1MWR8|F1MWR8_BOVIN	t-complex protein 1 subunit eta	1.13*	0.033	1.06	0.292	0.94	0.265		G3
274	5.35	5.35	13.88	sp|Q3SWX8|RBBP7_BOVIN	retinoblastoma binding protein7	0.96	0.850	1.29*	0.047	1.34*	0.030		G1
282	5.24	5.31	47.69	sp|P80311|PPIB_BOVIN	peptidylprolyl isomerase b (cyclophilin b)	1.21*	0.036	1.12	0.189	0.93	0.431		G3
292	4.92	9.41	26.08	tr|B0JYK4|B0JYK4_BOVIN	camp-dependent protein kinase type ii-beta regulatory subunit	1.1	0.268	0.87	0.112	0.79*	0.014		G1
300	4.76	4.76	18.14	tr|E1BB91|E1BB91_BOVIN	collagen alpha-3 chain	0.93	0.070	1.04	0.289	1.13*	0.017		G2
334	4.27	4.27	18.85	gi|171198344|gb|ACB45430.1|	extracellular superoxide dismutase	0.77*	0.043	0.98	0.970	1.28	0.058		G3
392	3.71	3.71	17.81	gi|339522297|gb|AEJ84313.1|	peroxiredoxin 5	1.1	0.426	0.84	0.092	0.76*	0.018		G3
416	3.41	3.41	25.74	gi|197725615|gb|ACH73011.1|	alpha-1-acid glycoprotein	2.65*	0.009	2.20*	0.020	0.83	0.547		G4
460	3.06	3.06	32.76	tr|E3SAZ8|E3SAZ8_BOVIN	nucleophosmin (nucleolar	0.79*	0.007	1.07	0.360	1.35*	0.002		G1

					phosphoprotein numatrin)							
497	2.8	2.84	29.84	tr|F1N3H1|F1N3H1_BOVIN	calumenin	0.84*	0.002	0.88*	0.012	1.05	0.253	G2
579	2.32	2.32	14.63	tr|E1B819|E1B819_BOVIN	copine iii	0.78*	0.011	0.97	0.755	1.25*	0.018	G2
582	2.3	2.3	16.08	sp|Q5EA79|GALM_BOVIN	aldose-1-epimerase	0.70*	0.049	0.72	0.066	1.03	0.943	G1
585	2.26	2.26	32.65	tr|Q3SX47|Q3SX47_BOVIN	heterogeneous nuclear ribonucleoprotein c (c1 c2)	0.9	0.256	1.1	0.335	1.22*	0.037	G1
587	2.25	3.8	27.66	tr|F1N6N3|F1N6N3_BOVIN	eh-domain containing 1	0.86*	0.017	0.98	0.831	1.13*	0.033	G2
595	2.01	2.04	9.97	tr|F1MW44|F1MW44_BOVIN	coagulation factor a1 polypeptide	0.81*	0.005	1.15*	0.026	1.41*	0.001	G1
597	2.01	2.01	30.31	tr|F1MF48|F1MF48_BOVIN	hydroxysteroid dehydrogenase-like protein 2	0.87	0.065	0.84*	0.030	0.96	0.655	G1
618	2.1	2.1	22.88	tr|A5D9H5|A5D9H5_BOVIN	heterogeneous nuclear ribonucleoprotein d	0.82	0.075	1.03	0.934	1.24*	0.049	G1
740	1.76	1.76	70.41	sp|P25417|CYTB_BOVIN	cystatin b	0.89*	0.005	0.94	0.108	1.07	0.076	G1
749	1.72	2.58	65	tr|Q7M371|Q7M371_SHEEP	plasma proteinase inhibitor	1.24	0.093	0.95	0.833	0.76*	0.045	G4
766	1.7	1.7	18.35	tr|E1BJX1|E1BJX1_BOVIN	ribosomal protein l30	0.86	0.145	1.18	0.120	1.38*	0.016	G1

* Statistically significant changes (p-value<0.05)

3.3 Proteins differentially expressed in adipose tissue of both experimental groups (ST-kids and FO-kids)

Of the 38 proteins whose presence was found to be modified after FO or ST treatment, only three, namely calumenin, coagulation factor a1 polypeptide and alpha-1-acid glycoprotein (AGP) were found to be modified after both treatments. The concentration of the first two was decreased as compared with controls, whereas that of AGP is increased. Alpha-1-acid glycoprotein (AGP) is of particular interest as, in our study, it increases its levels > 2-folds in both FO-Kid and ST-Kid. Our results are confirmed by a previous experiment in mice that demonstrated a selective increase in AGP levels in adipose tissue of obese animals in order to suppress an excess of inflammation and protect adipose tissue from metabolic dysfunctions [27]. AGP is an immunocalin with basically anti-inflammatory functions [28]. Its presence in adipose tissue has been already identified in pig, and its concentration increases with obesity [29]. The anti-inflammatory activity of FO has been established long time ago and it is now well known [30]. PUFA may induce an anti-inflammatory activity in several ways, by modify the plasma membrane of cells thus inducing the production, when needed, of anti-inflammatory mediators [31] or directly reduce the excess, and damages, of inflammation via PPAR interaction [32]. On the background of these results we may not rule out the possibility that one of the anti-inflammatory mechanisms triggered by PUFA also includes the expression of an anti-inflammatory protein, namely AGP. Remains to be determined the biological significance of the increased presence of AGP also in ST-kids.

The presence of coagulation factor XIII alpha chain (coagulation factor a1 polypeptide) was also found to be modified in both ST- and FO-kids. Interesting, but not well documented is the role of adipose tissue in coagulation processes. As reviewed by Faber and co-workers [33], adipose tissue functions as a site of production of tissue factors during obesity, promoting a pro-coagulant state demonstrated in human and mouse. In our experiment coagulation factor VIII alpha chain increases in a statistically significant way in ST-Kid, while it decreases in FO-Kid. This result is of particular relevance as fish oil has been demonstrated to increase bleeding time and to reduce platelet aggregation, as reviewed by Stranger and co-workers [34].

3.4 Proteins differentially expressed in FO-kids only

Sixteen proteins were differentially expressed in kids whose mother were fed with FO – enriched diets. Five of them were overexpressed whereas the other were underexpressed. Of the five overexpressed, we targeted our attention to vimentin and platelet glycoprotein 4 (CD36).

Vimentin is a fundamental component of cells and has been associated to pathological states influenced by diets. Indeed, vimentin is a type III intermediate filament protein, that was found significantly regulated in the omental fat of obese individuals compared to non-obese ones [35] and after caloric restriction [36]. Vimentin participates in fat droplets organization, and it is remarkable how we have already found that in vitro treatment with PUFA such as EPA and DHA, may modify the number of lipid droplets per cell, at least in goat monocytes [36]. In our experiments vimentin increases in a statistically significant way in FO-kids when compared to controls, but it is not influenced by stearic acid addition to the maternal diets. In has already been demonstrated that platelet glycoprotein 4 or CD36 plays different adipose-related roles, including uptake of long-chain fatty acids and oxidation of low-density lipoproteins and its mRNA levels increase after fish oil administration [37]. In addition, CD36 is strongly regulated by transcription factors belonging to the PPAR family and PUFAs are regarded as strong activators of PPARγ and PPARα receptors [38]. This previous studies are in line with our experiment where CD36 levels increased in FO-kids. In addition, we demonstrated that maternal stearic acid administration doesn't influence goat-kid CD36 levels.

Proteins involved in antioxidant and detoxifying defences are of particular importance in adipose tissues, where the high metabolic activity is associated with high levels of oxidative stress and thereby with a continuous generation of potentially toxic metabolites. Recently, Long and colleagues [39] demonstrated that high fed diet influence, in adipose tissue, the expression of genes linked to antioxidant biology, such as peroxiredoxin 3, and this mechanism is deposit-dependent. In our study most of the diet-influenced proteins (five out of thirty eight) is involved in toxic response and folding. Peroxiredoxin 6 in particular, which was downregulated after FO addition in our study, has already been identified in human adipose tissue where its expression decreases in obese patients [40]. Proteins belonging to this group are mostly influenced by FO enrichment, but not by ST enriched diets. The major influence of FO on antioxidant enzymes is well explained by the fact that antioxidant enzymes regulate polyunsaturated fatty acids' peroxidation and therefore lipid aldehydes formation [41].

3.4 Proteins differentially expressed in ST-kids only

Six proteins were differentially expressed in kids whose mother were fed with ST-enriched diets. Of them, only one was overexpressed, the retinoblastoma binding protein 7 (RBP7), whereas the other five were underexpressed. The retinoblastoma binding protein 7 is a ubiquitously

expressed nuclear protein whose main function is to regulate cell proliferation. The precise role of RBP7 is unknown, as well as also its relationship with adipose tissue. It must be noted that proteins belonging to RBP families have been found to be involved in the differentiation processes of adipose tissue [42;43] and therefore we may speculate that one of the cause of the differential expression of RBP7 in ST-kids may be due to the differentiation activity of ST upon adipose tissue of young animals.

3.5 Proteins differentially expressed between ST-kids and FO-kids

The last group of proteins belongs to those which were differentially expressed between kids whose mothers were fed with ST-enriched diets and others whose mother were fed with FO-enriched diets. This group included 14-3-3 protein gamma, a regulatory protein that binds many functionally different signalling molecules. It has never been studied in goat adipose tissue samples, but its presence in the omental samples confirms previous reports in humans, where it was found to be increased in obese patients, probably due to the adipose tissue metabolic changes induced by obesity [40]. The involvement of adipose tissue in antioxidant and detoxifying defences is again confirmed by the identification of peroxiredoxin 5. This protein was downregulated in ST treatment when compared to FO ones. The influence of high fat diets on peroxiredoxin 5 expression has been shown previously [44].

3.4 mRNA expression of selected proteins is not influenced by maternal high fat diet administration

The finding of a protein by proteomic techniques does not confirm per se its expression by adipose tissue. Several proteins can be expressed by liver and then delivered to adipose tissues through blood thanks to the presence of an intense capillary network in this tissues. Therefore, the effective influence of maternal enriched diets on adipose tissue protein expression was eventually confirmed by quantitative PCR through detection of their mRNAs. Following their involvement in inflammation, immune and anti-oxidant defences, eight proteins were selected for the validation of the proteomics data, namely 14-3-3 protein gamma, alpha-1-acid glycoprotein, coagulation factor VIII alpha chain, extracellular superoxide dismutase, peroxiredoxin 5, peroxiredoxin 6, plasma proteinase inhibitor, platelet glycoprotein 4 (Table 2).

mRNA expression levels of coagulation factor VIII alpha chain were undetectable after Real Time PCR analysis, suggesting this protein is delivered to adipose tissues through blood. Plasma

proteinase inhibitor mRNA was detected by quantitative PCR, although in a very low concentration and, even so, not influenced by maternal high fat diet administration.

For what concerns the other selected targets, all of them were shown to be expressed by adipose tissue, as evidenced by the finding of their respective mRNA.

Overall, after statistical analysis, none of the selected proteins proved to be regulated at the mRNA level by maternal high fat diet administration (Figure 1). This further confirms that the correlation between DNA levels and actual protein expression is poor, and integration between the two techniques, genomics and proteomics, is required. Regarding the protein CD36, the present findings are in contrast with a very recent report [45] suggesting that CD36 gene expression is downregulated in bovine mammary gland by a concentrate-based diet that is supplemented with sunflower oil, which is particularly rich in monounsaturated (MUFA)/polyunsaturated (PUFA) acids (mostly oleic acid (omega-9) and linoleic acid (omega-6) and upregulated in caprine liver by including a rapidly degradable starch in the diet.
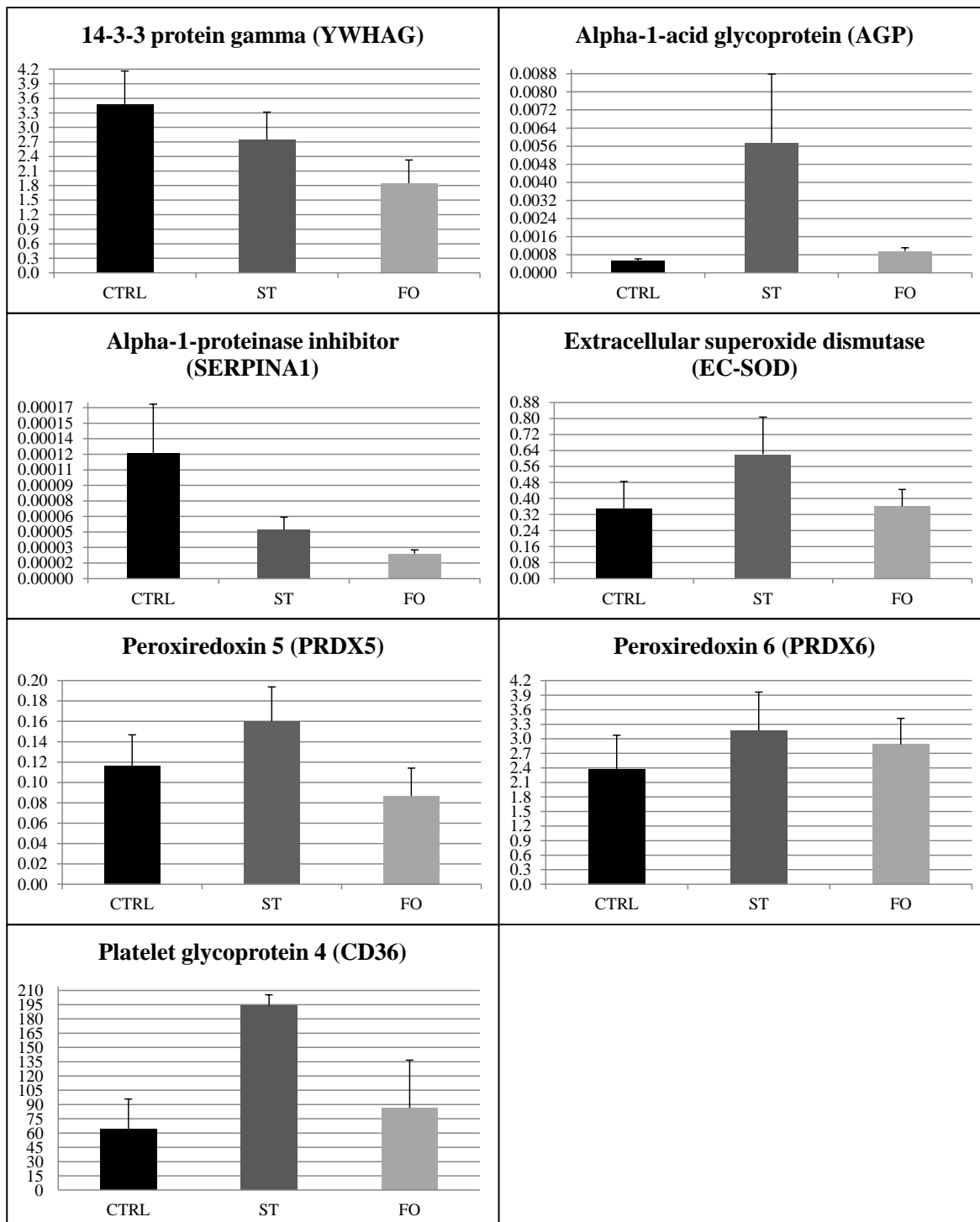
Figure 1. Real Time PCR analysis results. Graphs show the expression profiles of seven selected genes in omentum compared to liver samples (=1) in CTRL-Kid, ST-Kid and FO-Kid. Comparison of the mRNA expression profiles of the three groups show no statistically significant differences. Error standard bars are showed.

4. Conclusions

The relationship between diets and adipose tissue is already well known in humans as obesity is one of the major issues of the Western population. In farm animals, particular focus has been given to adipose tissue's influence on animal health and meat quality. Both these aspects are highly correlated to the diet type administered to animals. In the present experiment we report the first proteomic analysis of goat visceral adipose tissue after maternal diet enrichment with different fatty acids. The analysis was carried out by 2D-LC-MS/MS and iTRAQ labelling on omentum samples of goat kids. In this experiment we demonstrated that kids' omental proteome can be modified by maternal diet enrichments with either saturated or unsaturated fatty acids. Fish oil induces changes in a higher number of proteins when compared to stearic acid. Finally we demonstrated that proteome changes are not confirmed by mRNA expression changes. The influence of maternal diet on kids' proteome, even if not confirmed by gene expression changes is noteworthy and suggest that further insights are worth exploring.

Acknowledgments

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Final remarks and possible future developments

In ruminants, fat tissues play important biological roles not only for animal health, but also for quality and gain in meat and milk production. A specific knowledge of how these pathways are controlled is of key importance for the management of animal health and thereby also for economic gain in dairy and meat production. Proteomics, being a large-scale comprehensive analysis of all the proteins in a specific cell or tissue, is of great help in understanding the molecular mechanism underlying the complex network in which adipose tissue is involved.

The aim of the experiments presented in this thesis, we investigated several aspects of the complex endocrine network of organs called adipose tissue. We especially investigated its composition, its distribution and its protein expression abilities.

With the experiments presented in this thesis, we demonstrated for the first time that UCP1, exclusively expressed by brown adipose tissue, is differentially expressed in subcutaneous and visceral deposits in goats and this indicates a different distribution of brown (and therefore white) adipose tissue in these two macroareas. In addition, different deposits within the same macroareas (i.e. subcutaneous or visceral), do not display a different expression of UCP1. We, therefore, demonstrated, for the first time, the presence of brown adipose tissue in goat kids at 30 days of age, indicating that this tissue is present in growing goat and not only in the first days of life. The presence of BAT in one month goat kids is remarkable and suggests that further insights into the function of BAT in young and adult goats are worth exploring.

Furthermore, we hereby presented the first proteomic study on goat adipose tissue in which we demonstrated that subcutaneous and visceral deposits can be distinguished by their proteome profile, while different deposits within the same macroarea do not display any particular proteome difference. Since adipose tissue has an intense capillary network, proteins can be produced by other organs and carried to adipose tissue through blood. In this thesis we demonstrated that goat adipose tissue produce at least 27 proteins involved in inflammatory and immune related processes, confirming the involvement of fat tissues in these processes. In addition, we demonstrated for the first time, the expression by adipose tissue of 9 proteins involved in inflammatory and immune processes, namely ceruloplasmin, gamma fibrinogen, hemopexin, kininogen 1, lactoferrin, protein dj, thiosulfate sulfurtransferase, tumour translationally controlled 1 and valacyclovir hydrolase. Our findings provide further insight in the

immunological competence of adipose tissues. The ability of fat tissues to produce signalling factors plays a key role in animal health, and particularly for dairy animals, where lipid mobilization is hormonally controlled during the transition from pregnancy to lactation. Little is so far known about these molecular mechanisms and further investigation are of great interest also from an economical point of view.

Finally, we investigated the influence of maternal diets enriched with either saturated or unsaturated fatty acids on omental adipose tissue in term of brown adipose tissue distribution and protein expression in goat kids. We demonstrated that maternal diets seem not to influence UCP1 expression and therefore brown adipose tissue distribution, but have an influence on at least 38 proteins identified in omentum adipose tissue by proteomic techniques. Yet, these results seem not to be confirmed at mRNA expression level. The modification of goat kid proteome by maternal diet is of particular interest, even if not confirmed by mRNA expression analysis and further studies on subcutaneous deposits are worthy.

SUPPLEMENTARY MATERIAL TO PAPERS:

PAPER II

Supplementary Table 2 – Proteins identified in liver only		
Protein name	Accession Number	Protein Score
17-beta-hydroxysteroid dehydrogenase type 6-like	tr|F1MG20|F1MG20_BOVIN	1.60
2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase	tr|F1MIY5|F1MIY5_BOVIN	3.15
3 -phosphoadenosine 5 -phosphosulfate synthase 2	tr|Q0VC88|Q0VC88_BOVIN	1.47
3-hydroxy-3-methylglutaryl-coenzyme a synthase 2	gi|291010688|gb|ADD71721.1|	26.85
3-hydroxyanthranilate -dioxygenase	sp|Q0VCA8|3HAO_BOVIN	14.71
40s ribosomal protein s27	sp|Q2KHT7|RS27_BOVIN	1.52
4-hydroxyphenylpyruvate dioxygenase	sp|Q5EA20|HPPD_BOVIN	15.16
a chain crystallographic studies of the catalytic mechanism of the neutral form of fructose-bisphosphatase	gi|20141075|sp|P09199.2|F16P1_SHEEP	16.38
abhydrolase domain-containing protein 14b	sp|A7YY28|ABHEB_BOVIN	3.30
acyl- dehydrogenase family member 11	tr|Q0P5G8|Q0P5G8_BOVIN	1.35
acyl- synthetase short-chain family member mitochondrial	tr|F1MQV8|F1MQV8_BOVIN	3.10
acyl-coenzyme an amino acid n-acyltransferase 2-like	tr|F1MBX4|F1MBX4_BOVIN	6.89
acyl-coenzyme a dehydrogenase member 10	tr|E1BE95|E1BE95_BOVIN	4.00
acyl-coenzyme a dehydrogenase member 9	tr|Q3MHJ6|Q3MHJ6_BOVIN	2.01
acyl-coenzyme a synthetase mitochondrial	tr|A7MBE6|A7MBE6_BOVIN	8.50
adenosine kinase	tr|Q17R18|Q17R18_BOVIN	1.51
alanine--glyoxylate aminotransferase mitochondrial	tr|F1MLG7|F1MLG7_BOVIN	8.52
alcohol dehydrogenase 1c (class i) gamma polypeptide	tr|Q2T9S5|Q2T9S5_BOVIN	4.59
alcohol dehydrogenase 4	tr|F1MFZ4|F1MFZ4_BOVIN	3.89
alcohol dehydrogenase 5 (class iii) chi polypeptide	tr|F1N5F8|F1N5F8_BOVIN	2.02

alcohol dehydrogenase 6	tr|F1MZN9|F1MZN9_BOVIN	14.00
aldehyde dehydrogenase 1 member l1	tr|A7YY67|A7YY67_BOVIN	23.83
aldehyde dehydrogenase family 8 member a1 isoform 1	tr|F1MN79|F1MN79_BOVIN	12.06
aldehyde oxidase	tr|F1MRY9|F1MRY9_BOVIN	15.24
aminoadipate-semialdehyde synthase	tr|F1MY13|F1MY13_BOVIN	2.70
arginase-1	gi|239916450|gb|ACS34711.1|	16.02
argininosuccinate synthetase 1	gi|284157309|gb|ADB79800.1|	21.16
arylacetamide deacetylase	sp|Q0P5B7|AAAD_BOVIN	1.74
asialoglycoprotein receptor 1	tr|Q32KM0|Q32KM0_BOVIN	2.00
betaine-homocysteine s-methyltransferase 1	sp|Q5I597|BHMT1_BOVIN	17.07
beta-ureidopropionase	tr|A7MBE8|A7MBE8_BOVIN	3.64
bifunctional atp-dependent dihydroxyacetone kinase fad-amp lyase	tr|A0JN77|A0JN77_BOVIN	4.22
carbamoyl-phosphate synthase	tr|F1ML89|F1ML89_BOVIN	113.10
carbohydrate kinase domain-containing protein isoform 2	tr|E1BNQ4|E1BNQ4_BOVIN	2.74
carboxylesterase 2 (liver)	tr|Q3T0R6|Q3T0R6_BOVIN	2.74
carboxylesterase 2-like	tr|F1MI11|F1MI11_BOVIN	3.54
catechol o-methyltransferase	tr|F1MMK0|F1MMK0_BOVIN	2.04
cathepsin z	tr|F1MW68|F1MW68_BOVIN	1.53
chromosome 10 open reading frame 65	sp|Q0P5I5|HOGA1_BOVIN	2.00
cystathionine beta-synthase	tr|A7MBF8|A7MBF8_BOVIN	2.30
cystathionine gamma-lyase	tr|F1MKF5|F1MKF5_BOVIN	3.40
cytochrome family subfamily polypeptide 13	gi|325305977|gb|ADZ11093.1|	2.77
cytochrome p450	gi|2493370|sp|Q29496.1|CP3AO_SHEEP	4.04
cytochrome p450 2d6	gi|325305975|gb|ADZ11092.1|	5.79
cytochrome p450 2d6	tr|Q2KJJ2|Q2KJJ2_BOVIN	2.02
cytochrome p450 2e1	gi|325305979|gb|ADZ11094.1|	6.43
cytosolic beta-glucosidase	tr|F1MNT6|F1MNT6_BOVIN	4.43
dehydrogenase reductase sdr family member 4	sp|Q8SPU8|DHRS4_BOVIN	2.85
dehydrogenase reductase sdr family member 7	tr|Q24K14|Q24K14_BOVIN	2.31

dicarbonyl l-xylulose reductase	tr|A5PJR3|A5PJR3_BOVIN	5.91
dihydropyrimidinase	tr|E1BFN6|E1BFN6_BOVIN	9.42
dihydropyrimidine dehydrogenase	sp|Q28007|DPYD_BOVIN	10.45
dimethylaniline monooxygenase	sp|Q8HYJ9|FMO3_BOVIN	3.23
elastin microfibril interfacer 1	tr|E1BLS8|E1BLS8_BOVIN	3.42
enoyl coenzyme a hydratase domain containing 3	tr|E1BLR8|E1BLR8_BOVIN	2.80
enoyl- hydratase domain-containing protein mitochondrial-like isoform 1	sp|Q2TBT3|ECHD2_BOVIN	2.00
ester hydrolase c11orf54 homolog	sp|Q2HJH3|CK054_BOVIN	3.22
farnesyl pyrophosphate synthase	tr|Q2NL07|Q2NL07_BOVIN	2.74
fatty acid-binding liver	sp|P80425|FABPL_BOVIN	7.08
fatty aldehyde dehydrogenase	tr|A6QQT4|A6QQT4_BOVIN	6.80
fk506 binding protein 25kda	sp|P26884|FKBP3_BOVIN	1.52
formiminotransferase cyclodeaminase	tr|F1MNA6|F1MNA6_BOVIN	4.81
fumarylacetoacetate hydrolase domain containing 2a	tr|F1MLX0|F1MLX0_BOVIN	3.48
galactokinase 1	sp|A6H768|GALK1_BOVIN	15.09
galactose-1-phosphate uridylyltransferase	tr|Q58CX1|Q58CX1_BOVIN	4.00
gamma-butyrobetaine dioxygenase	tr|F1N4U6|F1N4U6_BOVIN	3.74
general vesicular transport factor p115	sp|P41541|USO1_BOVIN	5.50
glutaredoxin -like	gi|187937000|ref|NP_001120760.1|	3.72
glutathione s- theta 3	tr|Q0VCS8|Q0VCS8_BOVIN	4.77
glutathione s-transferase kappa 1	tr|Q2KIW8|Q2KIW8_BOVIN	2.02
glutathione s-transferase m4	tr|A1A4L7|A1A4L7_BOVIN	8.75
glutathione s-transferase theta 1	sp|Q2NL00|GSTT1_BOVIN	4.37
glutathione transferase zeta 1	tr|Q2KIM6|Q2KIM6_BOVIN	4.01
glycine amidinotransferase (l-arginine:glycine amidinotransferase)	sp|Q2HJ74|GATM_BOVIN	25.25
glycine dehydrogenase	tr|E1BJQ1|E1BJQ1_BOVIN	2.13
glycine n-acyltransferase	sp|Q2KIR7|GLYAT_BOVIN	19.22
glycine n-phenylacetyltransferase-like	tr|F1N1X6|F1N1X6_BOVIN	2.12
glycogen debranching enzyme	tr|F1MHT1|F1MHT1_BOVIN	19.05

guanidinoacetate n-methyltransferase	sp|Q2TBQ3|GAMT_BOVIN	4.41
haloacid dehalogenase-like hydrolase domain containing 3	sp|Q5E9D6|HDHD3_BOVIN	2.69
heterogeneous nuclear ribonucleoprotein a1	tr|E1BKB5|E1BKB5_BOVIN	2.46
hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	tr|F1MM13|F1MM13_BOVIN	2.25
high density lipoprotein binding protein	tr|F1MT25|F1MT25_BOVIN	2.08
homogentisate -dioxygenase	tr|B8YB76|B8YB76_BOVIN	16.02
hydroxyacid oxidase 1	tr|E1BC79|E1BC79_BOVIN	7.27
hydroxypyruvate isomerase-like isoform 1	tr|E1BAZ4|E1BAZ4_BOVIN	2.68
inorganic pyrophosphatase mitochondrial	tr|Q2KIV7|Q2KIV7_BOVIN	1.58
isovaleryl coenzyme a dehydrogenase	sp|Q3SZI8|IVD_BOVIN	4.47
keratin 18	tr|A6H7D3|A6H7D3_BOVIN	10.16
ketohexokinase-like isoform 1	tr|Q0II75|Q0II75_BOVIN	2.05
lambda-crystallin homolog	tr|F1MUQ0|F1MUQ0_BOVIN	1.40
l-arginine:glycine amidinotransferase	gi|47934919|gb|AAT39898.1|	2.72
macro domain-containing protein 1	sp|Q2KHU5|MACD1_BOVIN	2.00
malonyl- mitochondrial	tr|A5PJC5|A5PJC5_BOVIN	2.00
member of ras oncogene family	sp|P62833|RAP1A_BOVIN	3.07
methylmalonyl coenzyme a mutase	sp|Q9GK13|MUTA_BOVIN	6.36
methylthioadenosine phosphorylase	tr|Q3MHF7|Q3MHF7_BOVIN	4.65
methyltransferase-like protein 7b-like	tr|E1BGA1|E1BGA1_BOVIN	1.52
mevalonate kinase	tr|Q5EAA2|Q5EAA2_BOVIN	2.80
microsomal triglyceride transfer protein	tr|A7MBC6|A7MBC6_BOVIN	1.34
monoamine oxidase b	tr|F1MG79|F1MG79_BOVIN	4.70
nad kinase domain-containing protein 1 isoform 1	tr|F1MV80|F1MV80_BOVIN	5.23
nicotinamide n-methyltransferase	tr|E1BEK0|E1BEK0_BOVIN	3.49
nicotinate-nucleotide pyrophosphorylase	tr|F1MHB8|F1MHB8_BOVIN	7.40
nipsnap homolog 1 (elegans)	tr|Q0P5K8|Q0P5K8_BOVIN	8.65
nucleobindin 2	tr|Q0IIH5|Q0IIH5_BOVIN	1.71
ornithine carbamoyltransferase	gi|284157311|gb|ADB79801.1|	13.08

paraoxonase 3	tr|Q1JQD3|Q1JQD3_BOVIN	2.09
peroxisomal acyl-coenzyme a oxidase 1 isoform 2	sp|Q3SZP5|ACOX1_BOVIN	3.91
peroxisomal acyl-coenzyme a oxidase 2	tr|F1MRF5|F1MRF5_BOVIN	10.32
peroxisomal bifunctional enzyme	tr|E1BMH4|E1BMH4_BOVIN	25.32
peroxisomal -dienoyl- reductase	tr|F1MKM5|F1MKM5_BOVIN	1.64
phenazine biosynthesis-like domain-containing protein	sp|Q2HJF4|PBLD_BOVIN	1.36
phenylalanine hydroxylase	sp|Q2KIH7|PH4H_BOVIN	2.97
phosphoenolpyruvate cytosolic	gi|116874239|gb|ABK30806.1|	2.00
phosphoenolpyruvate cytosolic	tr|Q2KIE8|Q2KIE8_BOVIN	4.91
phosphoserine aminotransferase 1	tr|F1MNK2|F1MNK2_BOVIN	6.29
phytanoyl- 2-hydroxylase	sp|O18778|PAHX_BOVIN	4.50
prostaglandin e synthase 3	sp|Q3ZBF7|TEBP_BOVIN	2.18
proteasome (macropain) activator subunit 1 (pa28 alpha)	tr|Q2KJE7|Q2KJE7_BOVIN	2.45
proteasome activator complex subunit 2-like	gi|165940938|gb|ABY75314.1|	2.00
protein transport protein sec31a isoform 1	tr|E1BMP2|E1BMP2_BOVIN	3.54
pterin-4-alpha-carbinolamine dehydratase	sp|Q3ZBD3|PHS_BOVIN	4.00
pyruvate liver and rbc	gi|74273340|gb|ABA01338.1|	6.59
quinone oxidoreductase-like protein 2-like	sp|A6QQF5|QORL2_BOVIN	2.16
ras gtpase-activating-like protein iqgap2	tr|F1MTR1|F1MTR1_BOVIN	14.35
ras-related protein rab-10	tr|A6QLS9|A6QLS9_BOVIN	1.70
regucalcin	gi|195972815|ref|NP_001124407.1|	25.12
ribosomal protein l12	sp|P61284|RL12_BOVIN	2.30
ribosomal protein l7	sp|Q58DT1|RL7_BOVIN	4.55
ribosomal protein l9	tr|E1BCK0|E1BCK0_BOVIN	1.40
ribosomal protein s13	sp|Q56JX8|RS13_BOVIN	1.34
s-adenosylmethionine synthase isoform type-1	sp|Q2KJC6|METK1_BOVIN	1.38
sec14-like protein 3	tr|E1B8H8|E1B8H8_BOVIN	2.52
sec14p-like protein tap3	tr|F1MVY9|F1MVY9_BOVIN	3.04
selenocysteine lyase	tr|F1MQ48|F1MQ48_BOVIN	4.03

serine hydroxymethyltransferase 1	gi|1707994|sp|P35623.3|GLYC_SHEEP	16.73
serine hydroxymethyltransferase 2	sp|Q3SZ20|GLYM_BOVIN	4.00
serine--pyruvate mitochondrial-like	tr|A7MBF1|A7MBF1_BOVIN	5.42
slc9a3r2 protein	tr|Q2YDP5|Q2YDP5_BOVIN	2.00
sorting nexin 3	sp|Q1RMH8|SNX3_BOVIN	1.52
succinic semialdehyde dehydrogenase precursor	tr|E1BDP3|E1BDP3_BOVIN	2.91
sulfotransferase 1c4	tr|E1BJ78|E1BJ78_BOVIN	2.05
sulfotransferase dehydroepiandrosterone - member 1	tr|E1BG19|E1BG19_BOVIN	10.81
sulfotransferase dehydroepiandrosterone - member 1	tr|F1N0S6|F1N0S6_BOVIN	3.14
tetratricopeptide repeat protein 36-like	sp|Q3SZV0|TTC36_BOVIN	2.00
tetratricopeptide repeat protein 38	tr|Q0V7L6|Q0V7L6_BOVIN	1.60
thimet oligopeptidase	sp|Q1JPJ8|THOP1_BOVIN	2.94
thioredoxin domain-containing protein 17	tr|Q95M40|Q95M40_BOVIN	1.46
threonyl-trna cytoplasmic	sp|Q3ZBV8|SYTC_BOVIN	3.70
treacher collins-franceschetti syndrome 1	tr|E1BHU1|E1BHU1_BOVIN	1.40
tyrosyl-trna synthetase	tr|F1MHM5|F1MHM5_BOVIN	2.23
udp glucuronosyltransferase 2 family-like	tr|Q0II94|Q0II94_BOVIN	4.14
udp glucuronosyltransferase 2b10-like isoform 1	tr|E1BJK3|E1BJK3_BOVIN	4.30
udp-glucose dehydrogenase	sp|P12378|UGDH_BOVIN	22.75
uridine phosphorylase 1	tr|F1N0Y0|F1N0Y0_BOVIN	4.41
urocanase domain containing 1	tr|E1BD93|E1BD93_BOVIN	7.08
xylulose kinase	sp|Q3SYZ6|XYLB_BOVIN	3.46

Supplementary Table 3 - Proteins identified in all adipose tissue deposits

Protein name	Accession Number	Protein Score	Function
Omentum, Perirenal, Sternum, Tail			
26s proteasome non-atpase regulatory subunit 4	tr|Q58D01|Q58D01_BOVIN	2.20	G1
3-oxoacid transferase 1	tr|Q24JZ7|Q24JZ7_BOVIN	12.48	G1
acetyl- carboxylase 1	sp|Q28559-3|ACACA_SHEEP	43.74	G1
acyl- synthetase short-chain family member 2	tr|A7YWF1|A7YWF1_BOVIN	13.09	G1
acyl-protein thioesterase 1-like	sp|Q3MHR0|LYPA1_BOVIN	1.87	G1
adipose most abundant gene transcript 2	tr|Q2NKR5|Q2NKR5_BOVIN	2.00	G4
adp atp translocase 2-like	gi|186886456|gb|ACC93604.1|	2.02	G1
alpha-1-acid glycoprotein	gi|197725615|gb|ACH73011.1|	7.87	G4
alpha-crystallin b chain	sp|P02510|CRYAB_BOVIN	2.37	G4
annexin a8	sp|Q95L54|ANXA8_BOVIN	1.52	G2
apolipoprotein a-iv	tr|F1N3Q7|F1N3Q7_BOVIN	4.38	G1
atp synthase subunit mitochondrial precursor	tr|Q862C2|Q862C2_BOVIN	5.54	G1
b-cell receptor-associated protein 31	tr|Q5E9F1|Q5E9F1_BOVIN	6.32	G2
beta-galactoside-binding lectin precursor	gi|47779226|gb|AAT38511.1|	6.10	G4
calcium-regulated heat stable protein 1	tr|Q2NKU4|Q2NKU4_BOVIN	1.40	G1
calpastatin isoform ii	gi|293627945|gb|ADE58451.1|	3.55	G1
camp-dependent protein kinase type ii-alpha regulatory subunit	sp|P00515|KAP2_BOVIN	2.72	G3
caveolin 1	gi|114573506|gb|ABI75290.1|	4.11	G2
clusterin	tr|F1MWI1|F1MWI1_BOVIN	3.00	G2
copine iii	tr|E1B819|E1B819_BOVIN	2.17	G2
creatine brain	tr|F1MYI3|F1MYI3_BOVIN	9.30	G2
cysteine and glycine-rich protein 1	sp|Q3MHY1|CSRP1_BOVIN	2.02	G1
cytochrome b5 reductase 3	sp|P07514|NB5R3_BOVIN	4.25	G1
dihydropyrimidinase-related protein 3	tr|A7MBI5|A7MBI5_BOVIN	9.13	G1
eh-domain containing 2	tr|Q2KJ47|Q2KJ47_BOVIN	9.62	G2

endopin 1b	sp|Q3ZEJ6|SPA33_BOVIN	3.35	G1
fascin	tr|Q3MHK9|Q3MHK9_BOVIN	4.15	G2
fatty acid binding protein 4	gi|167900488|ref|NP_001108139.1|	20.94	G1
fatty acid binding protein 5	gi|78557716|gb|ABB46354.1|	11.60	G1
gdp-l-fucose synthase	tr|Q2KIT8|Q2KIT8_BOVIN	2.00	G1
gelsolin isoform b	gi|327346104|gb|AEA50998.1|	3.00	G2
glucose-6-phosphate dehydrogenase	gi|87244605|gb|ABD34655.1|	1.96	G1
heat shock 70 kda protein 4	tr|E1BBY7|E1BBY7_BOVIN	1.90	G3
heat shock protein beta-6	sp|Q148F8|HSPB6_BOVIN	1.57	G3
hemopexin precursor	sp|Q3SZV7|HEMO_BOVIN	1.89	G4
histidine triad nucleotide-binding protein 1	tr|F1MJD5|F1MJD5_BOVIN	1.84	G1
hsp90 co-chaperone cdc37	sp|Q5EAC6|CDC37_BOVIN	2.08	G3
immunoglobulin v lambda chain	gi|2766665|gb|AAB95466.1|	2.64	G4
inter-alpha-trypsin inhibitor heavy chain h2	tr|F1MNW4|F1MNW4_BOVIN	3.29	G1
isochorismatase domain containing 1	sp|A6QLY4|ISOC1_BOVIN	3.76	G1
isocitrate dehydrogenase 3 alpha (NAD)	tr|Q148J8|Q148J8_BOVIN	5.57	G1
isopentenyl-diphosphate delta-isomerase 1	sp|Q1LZ95-2|IDI1_BOVIN	2.28	G1
lactoferrin	tr|Q2HJF0|Q2HJF0_BOVIN	7.22	G4
laminin subunit alpha-4 isoform 2	tr|F1MYX9|F1MYX9_BOVIN	11.33	G2
leucine-rich alpha-2-glycoprotein 1	tr|Q2KIF2|Q2KIF2_BOVIN	1.70	G1
long-chain-fatty-acid-- ligase 1	tr|Q0VCZ8|Q0VCZ8_BOVIN	20.12	G1
l-threonine 3- mitochondrial-like	sp|Q2KIR8|TDH_BOVIN	2.59	G1
lumican	sp|Q05443|LUM_BOVIN	1.31	G2
macrophage migration inhibitory factor	gi|77744696|gb|ABB02309.1|	4.00	G4
microtubule-associated protein 4	sp|P36225-3|MAP4_BOVIN	4.29	G2
myotrophin	tr|Q862U9|Q862U9_BOVIN	2.00	G2
myristoylated alanine-rich c-kinase substrate	tr|F1N2N5|F1N2N5_BOVIN	2.25	G1
oxoglutarate (alpha-ketoglutarate) dehydrogenase	sp|Q148N0|ODO1_BOVIN	2.61	G1
perilipin 4	tr|F1MNM7|F1MNM7_BOVIN	20.15	G1

perilipin-1	gi|166157508|ref|NP_001107245.1|	29.75	G1
peroxisomal biogenesis factor 19	sp|Q3SZD1|PEX19_BOVIN	1.70	G1
phosphoacetylglucosamine mutase	tr|Q2KIQ1|Q2KIQ1_BOVIN	2.00	G1
phosphoprotein enriched in astrocytes 15	tr|Q0VCY8|Q0VCY8_BOVIN	3.40	G2
phosphoserine phosphatase	gi|182636736|gb|ACB97626.1|	4.68	G1
polymerase i and transcript release factor	tr|E1BNE7|E1BNE7_BOVIN	7.72	G1
progesterone receptor membrane component 2	tr|A5PJQ6|A5PJQ6_BOVIN	2.02	G1
programmed cell death 6-interacting protein	tr|E1BKM4|E1BKM4_BOVIN	2.00	G1
proteasome (macropain) beta 6	sp|Q3MHN0|PSB6_BOVIN	2.47	G1
proteasome subunit alpha type-5	sp|Q5E987|PSA5_BOVIN	4.40	G1
proteasome subunit alpha type-6	gi|222154129|gb|ACM47241.1|	2.17	G1
protein fam49b-like isoform 2	sp|Q2KJI3|FA49B_BOVIN	2.70	G4
protein phosphatase 1 regulatory subunit 1b	sp|P07516|PPR1B_BOVIN	2.00	G1
protein s100-a10	sp|P60902|S10AA_BOVIN	4.00	G1
pyruvate dehydrogenase alpha 1	sp|A7MB35|ODPA_BOVIN	3.02	G1
reticulocalbin ef-hand calcium binding domain	sp|Q2KJ39|RCN3_BOVIN	4.19	G1
s100 calcium binding protein a11	tr|Q862H7|Q862H7_BOVIN	1.46	G1
s100 calcium binding protein a4	sp|P35466|S10A4_BOVIN	2.11	G1
septin 7	tr|F1MIH2|F1MIH2_BOVIN	2.44	G2
serum albumin precursor	tr|Q3I349|Q3I349_BOSIN	2.02	G2
serum deprivation-response protein	gi|339522395|gb|AEJ84362.1|	12.29	G1
serum deprivation-response protein	tr|Q17QZ6|Q17QZ6_BOVIN	2.28	G1
small ubiquitin-related modifier 3-like	sp|Q17QV3|SUMO3_BOVIN	2.00	G3
stathmin 1 oncoprotein 18	sp|Q3T0C7|STMN1_BOVIN	2.00	G1
synuclein gamma	sp|Q9NZ50|SYUG_BOVIN	3.15	G1
t-complex protein 1 subunit zeta	tr|F1MGX0|F1MGX0_BOVIN	3.10	G3
transaldolase 1	gi|165875541|gb|ABY68598.1|	4.00	G1
vacuolar protein sorting-associated protein 35	sp|Q2HJG5|VPS35_BOVIN	2.96	G2
vimentin	gi|145226795|gb|ABP48145.1|	67.93	G2

Liver, Omentum, Perirenal, Sternum, Tail			
10 kda heat shock mitochondrial-like	sp|P61603|CH10_BOVIN	10.83	G3
14-3-3 protein beta alpha	sp|P68251-2|1433B_SHEEP	3.33	G1
14-3-3 protein gamma	tr|A7Z057|A7Z057_BOVIN	2.54	G1
26s protease regulatory subunit 8	sp|P62194|PRS8_BOVIN	2.27	G1
2-amino-3-ketobutyrate coenzyme a mitochondrial	sp|Q0P5L8|KBL_BOVIN	8.20	G1
3-hydroxyacyl- dehydrogenase type-2-like isoform 1	sp|O02691|HCD2_BOVIN	15.70	G1
3-hydroxyisobutyrate dehydrogenase	sp|Q2HJD7|3HIDH_BOVIN	6.97	G1
3-ketoacyl- mitochondrial	sp|Q3T0R7|THIM_BOVIN	38.37	G1
40s ribosomal protein s19-like	tr|E1BHA5|E1BHA5_BOVIN	4.04	G1
4-trimethylaminobutyraldehyde dehydrogenase	tr|F1N2L9|F1N2L9_BOVIN	15.41	G1
60 kda heat shock mitochondrial	tr|F1MUZ9|F1MUZ9_BOVIN	43.66	G3
78 kda glucose-regulated protein precursor	sp|Q0VCX2|GRP78_BOVIN	39.76	G3
[a] a chain elaborate manifold of short hydrogen bond arrays mediating binding of active site-directed serine protease inhibitors	tr|F1N5M0|F1N5M0_BOVIN	28.24	G4
acetyl- mitochondrial	sp|Q29RZ0|THIL_BOVIN	24.18	G1
aconitase mitochondrial	sp|P20004|ACON_BOVIN	10.50	G1
actin cytoplasmic 1	gi|46397336|sp|P60713.1|ACTB_SHEEP	30.27	G2
acyl-binding protein	sp|P07107|ACBP_BOVIN	2.57	G1
acyl-coenzyme a long chain	tr|Q08D92|Q08D92_BOVIN	4.45	G1
adenylate cyclase-associated protein 1	tr|A6QLB7|A6QLB7_BOVIN	4.01	G1
adenylate kinase 2	sp|P08166|KAD2_BOVIN	7.66	G1
ahnak nucleoprotein isoform 1	tr|F1MCK2|F1MCK2_BOVIN	4.75	G1
alcohol dehydrogenase	sp|Q3ZCJ2|AK1A1_BOVIN	10.15	G1
aldehyde dehydrogenase 2 family	sp|P20000|ALDH2_BOVIN	28.94	G1
aldehyde dehydrogenase 7 member a1	sp|Q2KJC9|AL7A1_BOVIN	16.47	G1
aldolase fructose-bisphosphate	tr|A6QLL8|A6QLL8_BOVIN	10.87	G1
aldose 1-epimerase	sp|Q5EA79|GALM_BOVIN	7.33	G1
alpha 4	sp|A5D7D1|ACTN4_BOVIN	10.11	G2

alpha-1-antitrypsin	gi|112890|sp|P12725.1|A1AT_SHEEP	3.30	G4
alpha-1b-glycoprotein precursor	sp|Q2KJF1|A1BG_BOVIN	2.97	G4
alpha-2-hs-glycoprotein precursor	gi|231469|sp|P29701.1|FETUA_SHEEP	12.14	G4
alpha-2-macroglobulin precursor	tr|E1BJW0|E1BJW0_BOVIN	3.14	G4
aminoacylase 1	tr|F1MR63|F1MR63_BOVIN	8.90	G1
annexin a1	tr|F1N650|F1N650_BOVIN	4.04	G2
annexin a2	gi|148876772|sp|A2SW69.1|ANXA2_SHEEP	4.02	G2
annexin a4	sp|P13214|ANXA4_BOVIN	11.96	G2
annexin a5	tr|A1L5B6|A1L5B6_BOVIN	16.31	G2
annexin a6	sp|P79134|ANXA6_BOVIN	38.37	G2
antithrombin-iii precursor	gi|416622|sp|P32262.1|ANT3_SHEEP	1.70	G1
apolipoprotein a-i	sp|P15497|APOA1_BOVIN	12.45	G1
aspartate cytoplasmic	gi|339522139|gb|AEJ84234.1|	3.85	G1
aspartate mitochondrial	sp|P12344|AATM_BOVIN	15.53	G1
aspartyl aminopeptidase	tr|F1MX45|F1MX45_BOVIN	2.00	G1
atp h+ mitochondrial f1 alpha subunit cardiac muscle	sp|P19483|ATPA_BOVIN	8.23	G1
atp h+ mitochondrial f1 beta polypeptide	sp|P00829|ATPB_BOVIN	6.78	G1
beta-2-microglobulin precursor	gi|145207023|gb|ABP37876.1|	2.39	G4
calpain-2 catalytic subunit	gi|163914410|ref|NP_001106288.1|	3.72	G1
calreticulin	sp|P52193|CALR_BOVIN	23.97	G3
calumenin	tr|F1N3H1|F1N3H1_BOVIN	4.00	G2
capping protein (actin filament) muscle z- beta	sp|P79136|CAPZB_BOVIN	3.26	G1
carbonic anhydrase ii	gi|118582300|sp|P00922.2|CAH2_SHEEP	2.01	G1
carbonyl reductase	tr|Q3T0T9|Q3T0T9_BOVIN	8.52	G1
carboxymethylenebutenolidase homolog	tr|F1N2I5|F1N2I5_BOVIN	11.51	G1
catalase	gi|242200439|gb|ACS88258.1|	74.61	G3
cathepsin d	gi|18203300|sp|Q9MZS8.1|CATD_SHEEP	3.62	G1
ceruloplasmin	gi|307742663|emb|CBJ23824.1|	4.97	G4
clathrin heavy chain 1	sp|P49951|CLH1_BOVIN	8.79	G2

cofilin 1 (non-muscle)	gi|54035753|sp|Q6B7M7.3|COF1_SHEEP	4.74	G2
complement component 3	sp|Q2UVX4|CO3_BOVIN	5.87	G4
cystatin b	gi|1706279|sp|Q10994.1|CYTB_SHEEP	2.06	G1
cytoplasmic aconitate hydratase	sp|Q0VCU1|ACOC_BOVIN	12.37	G1
cytosol aminopeptidase	sp|P00727|AMPL_BOVIN	17.00	G1
cytosolic non-specific dipeptidase	sp|Q3ZC84|CNDP2_BOVIN	15.94	G1
delta-aminolevulinic acid dehydratase	tr|Q2KIL3|Q2KIL3_BOVIN	15.96	G1
dihydrolipoamide dehydrogenase	tr|F1N206|F1N206_BOVIN	4.94	G1
dihydrolipoamide s-succinyltransferase (e2 component of 2-oxo-glutarate complex)	tr|F1MEQ3|F1MEQ3_BOVIN	5.19	G1
dihydropyrimidinase-related protein 2	sp|O02675|DPYL2_BOVIN	1.97	G1
electron transfer flavoprotein subunit beta	sp|Q2TBV3|ETFB_BOVIN	8.45	G1
electron-transfer- alpha polypeptide	tr|F1MWR3|F1MWR3_BOVIN	16.96	G1
elongation factor 1-delta	gi|57164211|ref|NP_001009449.1|	7.30	G1
elongation factor 2	sp|Q3SYU2|EF2_BOVIN	14.76	G1
endoplasmic reticulum resident protein 29-like	sp|P81623|ERP29_BOVIN	3.73	G2
endoplasmin precursor	sp|Q95M18|ENPL_BOVIN	22.49	G3
enolase	tr|F1MB08|F1MB08_BOVIN	29.01	G1
enoyl coenzyme a short mitochondrial	sp|Q58DM8|ECHM_BOVIN	8.04	G1
epoxide hydrolase microsomal	gi|327199784|gb|AEA36045.1|	12.54	G1
eukaryotic initiation factor 4a-i	sp|Q3SZ54|IF4A1_BOVIN	5.36	G1
eukaryotic translation elongation factor 1 alpha 1	sp|P68103|EF1A1_BOVIN	12.07	G1
eukaryotic translation elongation factor 1 gamma	tr|Q1JPA2|Q1JPA2_BOVIN	10.17	G1
eukaryotic translation initiation factor 5a	gi|261244964|ref|NP_001159665.1|	3.82	G1
extracellular superoxide dismutase	gi|171198344|gb|ACB45430.1|	6.00	G3
fatty acid synthase	gi|78214939|gb|ABB36643.1|	2.12	G1
fibrinogen gamma (partial)	gi|1916272|gb|AAB51261.1|	2.00	G4
filamin alpha (actin binding protein 280)	tr|F1N169|F1N169_BOVIN	2.00	G2
filamin beta isoform 4	tr|E1BKX7|E1BKX7_BOVIN	8.31	G2
flavin reductase	sp|P52556|BLVRB_BOVIN	8.00	G1

fumarate hydratase	gi|240849257|ref|NP_001155363.1|	15.48	G1
gdp dissociation inhibitor 2	gi|240849297|ref|NP_001155337.1|	3.44	G1
glucose-6-phosphate isomerase	tr|F1MD19|F1MD19_BOVIN	12.68	G1
glutathione peroxidase 1	sp|P00435|GPX1_BOVIN	1.50	G3
glutathione s-transferase a1	gi|168693445|ref|NP_001108238.1|	23.68	G3
glutathione s-transferase mu 1	tr|A5PKM0|A5PKM0_BOVIN	10.81	G3
glutathione s-transferase omega-1-like	tr|E1BJ08|E1BJ08_BOVIN	8.21	G3
glutathione s-transferase pi	sp|P28801|GSTP1_BOVIN	9.97	G3
glutathione s-transferase p-like	gi|84029298|sp|Q9TTY8.2|GSTP1_CAPHI	1.33	G3
glyceraldehyde-3-phosphate dehydrogenase	gi|298676425|ref|NP_001177319.1|	13.12	G1
glycerol-3-phosphate dehydrogenase	sp|Q5EA88|GPDA_BOVIN	11.75	G1
heat shock 27kda protein 1	tr|E1BEL7|E1BEL7_BOVIN	12.69	G3
heat shock 70kda protein 8	sp|P19120|HSP7C_BOVIN	33.16	G3
heat shock 70kda protein 9	sp|Q3ZCH0|GRP75_BOVIN	23.69	G3
heat shock protein	gi|238801231|gb|ACR56335.1|	20.53	G3
heat shock protein 90kda alpha class b member 1	sp|Q76LV1|HS90B_BOVIN	12.06	G3
heat shock protein hsp 90-alpha	gi|115503919|gb|ABI99473.1|	33.29	G3
heat-responsive protein 12	gi|47606778|sp|P80601.3|UK114_CAPHI	5.03	G3
hemoglobin alpha globin chain	gi|302425108|sp|P0CH25.1|HBA1_CAPHI	42.26	G2
hemoglobin beta fetal	gi|122540|sp|P02077.1|HBBA_CAPHI	8.01	G2
hemoglobin beta globin chain	gi|164136|gb|AAA30914.1|	25.21	G2
hemoglobin beta subunit a	gi|122545|sp|P02082.1|HBBF_CAPHI	20.55	G2
heterogeneous nuclear ribonucleoprotein f	sp|Q5E9J1|HNRPF_BOVIN	5.30	G1
heterogeneous nuclear ribonucleoprotein k	gi|291358560|gb|ADD96765.1|	8.07	G1
heterogeneous nuclear ribonucleoprotein u	tr|A2VDN7|A2VDN7_BOVIN	3.71	G1
heterogeneous nuclear ribonucleoproteins a2 b1-like	tr|F1N1N0|F1N1N0_BOVIN	11.87	G1
histidine triad nucleotide binding protein 2	sp|Q8SQ21|HINT2_BOVIN	3.32	G1
histone cluster h1e	sp|P02253|H11_BOVIN	2.60	G1
hsc70-interacting protein	tr|A7E3S8|A7E3S8_BOVIN	5.91	G3

hydroxyacyl-coenzyme a dehydrogenase 3-ketoacyl-coenzyme a thiolase enoyl-coenzyme a hydratase (trifunctional protein) beta subunit	tr|A5D9E7|A5D9E7_BOVIN	2.15	G1
hydroxysteroid (17-beta) dehydrogenase 4	tr|Q0IIL6|Q0IIL6_BOVIN	16.93	G1
immunoglobulin gamma-1 heavy chain	gi|388235|emb|CAA49451.1|	4.77	G4
immunoglobulin lambda light chain	gi|61378762|gb|AAX45027.1|	6.65	G4
isocitrate dehydrogenase	tr|F1MLK0|F1MLK0_BOVIN	20.96	G1
isocitrate dehydrogenase (NADP)	sp|Q04467|IDHP_BOVIN	8.02	G1
karyopherin beta 1	tr|E1BFV0|E1BFV0_BOVIN	4.41	G2
lactate dehydrogenase b	tr|A0FH35|A0FH35_BOSMU	16.09	G1
leukocyte elastase inhibitor	gi|297660644|gb|ADI49847.1|	1.72	G1
l-lactate dehydrogenase a chain	gi|269204793|gb|ACZ28899.1|	1.63	G1
malate cytoplasmic	sp|Q3T145|MDHC_BOVIN	9.62	G1
malate dehydrogenase nad	sp|Q32LG3|MDHM_BOVIN	23.87	G1
malic enzyme nadp(+)- cytosolic	gi|207028179|ref|NP_001128692.1|	5.30	G1
medium-chain acyl- dehydrogenase	sp|Q3SZB4|ACADM_BOVIN	14.60	G1
member ras oncogene family	tr|F1MYU3|F1MYU3_BOVIN	6.30	G1
membrane primary amine oxidase	tr|F1MCC1|F1MCC1_BOVIN	4.12	G1
methylmalonate-semialdehyde dehydrogenase	tr|F1N7K8|F1N7K8_BOVIN	22.72	G1
mitochondrial malate dehydrogenase nad	gi|7274396|gb|AAF44753.1|	2.00	G1
myosin light polypeptide 6	sp|P60661|MYL6_BOVIN	3.05	G2
neutral alpha-glucosidase ab	tr|F1N6Y1|F1N6Y1_BOVIN	5.43	G1
non-muscle caldesmon	tr|Q8HYY3|Q8HYY3_BOVIN	8.09	G2
non-specific lipid-transfer protein	tr|F1MV74|F1MV74_BOVIN	8.43	G1
nucleoporin 133kda	tr|E1BG41|E1BG41_BOVIN	5.41	G1
nucleoside diphosphate kinase b	tr|F1MPL4|F1MPL4_BOVIN	4.43	G1
peptidylprolyl isomerase a (cyclophilin a)	sp|P62935|PPIA_BOVIN	11.97	G3
peroxiredoxin 1	sp|Q5E947|PRDX1_BOVIN	2.40	G3
peroxiredoxin 2	gi|261244978|ref|NP_001159672.1|	2.18	G3
peroxiredoxin 3	sp|P35705|PRDX3_BOVIN	7.77	G3

peroxiredoxin 6	sp|O77834|PRDX6_BOVIN	8.29	G3
phosphatidylethanolamine-binding protein 1-like	sp|P13696|PEBP1_BOVIN	16.96	G1
phosphoenolpyruvate carboxykinase	tr|F1MDS3|F1MDS3_BOVIN	22.34	G1
phosphoglucomutase 1	tr|F1N1X7|F1N1X7_BOVIN	11.50	G1
phosphogluconate dehydrogenase	gi|157835675|pdb|2PGD|A	4.42	G1
phosphoglycerate kinase 1	gi|215983082|ref|NP_001135988.1|	19.40	G1
phosphoglycerate mutase 1	sp|Q3SZ62|PGAM1_BOVIN	19.70	G1
plastin-3 isoform 1	tr|F1MSB7|F1MSB7_BOVIN	18.68	G2
polyadenylate-binding protein 1	sp|P61286|PABP1_BOVIN	8.14	G1
profilin 1	tr|E1BHJ0|E1BHJ0_BOVIN	9.19	G2
programmed cell death 5	sp|Q2HJH9|PDCD5_BOVIN	2.04	G1
prostaglandin reductase 1	tr|F1N2W0|F1N2W0_BOVIN	4.75	G1
proteasome (macropain) 26s 6	tr|F1MLV1|F1MLV1_BOVIN	2.28	G1
proteasome (macropain) 26s non- 2	sp|P56701|PSMD2_BOVIN	6.00	G1
proteasome (macropain) alpha 1	sp|Q3T0X5|PSA1_BOVIN	4.13	G1
proteasome subunit alpha type-2-like	sp|Q3T0Y5|PSA2_BOVIN	4.00	G1
proteasome subunit alpha type-7	sp|Q3ZBG0|PSA7_BOVIN	2.79	G1
protein disulfide isomerase family member 4	gi|78499369|gb|ABB45719.1|	4.60	G3
protein disulfide isomerase family member 4	tr|F1MEN8|F1MEN8_BOVIN	10.46	G3
protein disulfide-isomerase a3 precursor	gi|251823897|ref|NP_001156517.1|	35.62	G3
protein disulfide-isomerase a6 precursor	tr|A6QNL5|A6QNL5_BOVIN	16.01	G3
protein disulfide-isomerase precursor	tr|A6H7J6|A6H7J6_BOVIN	34.08	G3
protein dj-1	sp|Q5E946|PARK7_BOVIN	7.42	G4
protein transport protein sec23a	sp|A2VDL8|SC23A_BOVIN	5.05	G1
pyridoxal kinase	gi|7387989|sp|P82197.1|PDXK_SHEEP	3.52	G1
pyruvate muscle	tr|Q3ZC87|Q3ZC87_BOVIN	2.98	G1
quinone oxidoreductase	sp|O97764|QOR_BOVIN	8.70	G1
rad23-like b	sp|Q29RK4|RD23B_BOVIN	3.53	G1
ras homolog gene member a	gi|240849361|ref|NP_001155347.1|	2.80	G1

reticulocalbin-1 precursor	tr|A4IF88|A4IF88_BOVIN	1.84	G2
retinal dehydrogenase 1	gi|1706388|sp|P51977.2|AL1A1_SHEEP	13.52	G1
retinol binding protein plasma	sp|P18902|RET4_BOVIN	2.29	G4
rho gdp-dissociation inhibitor 1	tr|Q7M2Q9|Q7M2Q9_BOVIN	5.52	G1
ribonuclease inhibitor	tr|Q3SZN8|Q3SZN8_BOVIN	4.80	G1
ribosomal p0	tr|Q862I9|Q862I9_BOVIN	3.77	G1
ribosomal protein s28	sp|Q56JX6|RS28_BOVIN	2.82	G1
ribosomal protein sa	gi|298108676|gb|ADI56590.1|	2.00	G1
s-adenosylhomocysteine hydrolase	sp|Q3MHL4|SAHH_BOVIN	6.99	G1
selenium-binding protein 1	sp|Q2KJ32|SBP1_BOVIN	12.63	G4
serine threonine-protein phosphatase 2a 65 kda regulatory subunit a alpha isoform	tr|A5D973|A5D973_BOVIN	5.78	G1
serotransferrin precursor	tr|B8R1K3|B8R1K3_BOSMU	13.25	G1
serpin h1 precursor	sp|Q2KJH6|SERPH_BOVIN	3.65	G1
serum albumin precursor	gi|193085052|gb|ACF10391.1|	63.17	G4
spectrin alpha brain isoform 1	tr|E1BFB0|E1BFB0_BOVIN	15.99	G2
stress-induced-phosphoprotein 1	sp|Q3ZBZ8|STIP1_BOVIN	8.40	G3
succinyl-CoA ligase a chain, mithocondrial	tr|A5D9G3|A5D9G3_BOVIN	8.35	G1
succinyl-CoA ligase b chain, mithocondrial-like	tr|F1MZ38|F1MZ38_BOVIN	9.11	G1
superoxide dismutase (Cu-Zn)	gi|75061021|sp|Q5FB29.3|SODC_CAPHI	7.75	G3
superoxide dismutase Mn	gi|242200441|gb|ACS88259.1|	4.01	G3
talin 1	tr|F1MDH3|F1MDH3_BOVIN	19.13	G2
t-complex protein 1 subunit alpha	gi|165940912|gb|ABY75301.1|	2.05	G3
t-complex protein 1 subunit beta	sp|Q3ZBH0|TCPB_BOVIN	6.91	G3
t-complex protein 1 subunit epsilon	tr|F1MWD3|F1MWD3_BOVIN	3.68	G3
t-complex protein 1 subunit gamma	sp|Q3T0K2|TCPG_BOVIN	9.56	G3
t-complex protein 1 subunit theta	sp|Q3ZCI9|TCPQ_BOVIN	4.87	G3
thioredoxin	gi|1729950|sp|P50413.2|THIO_SHEEP	6.63	G4
thioredoxin domain containing 5	tr|F1MKS3|F1MKS3_BOVIN	2.00	G3
thiosulfate sulfurtransferase	sp|P00586|THTR_BOVIN	7.44	G4

thymosin beta 4	gi|315620165|gb|ADU52989.1|	7.21	G2
transaldolase	sp|Q2TBL6|TALDO_BOVIN	2.10	G1
transgelin	sp|Q9TS87|TAGL_BOVIN	2.15	G2
transgelin 2	sp|Q5E9F5|TAGL2_BOVIN	3.01	G2
transitional endoplasmic reticulum atpase	sp|Q3ZBT1|TERA_BOVIN	15.22	G1
transketolase	tr|A5PJ79|A5PJ79_BOVIN	3.36	G1
triosephosphate isomerase 1	sp|Q5E956|TPIS_BOVIN	31.48	G1
tropomyosin alpha-1 chain	gi|187607525|ref|NP_001119823.1|	11.59	G2
tubulin alpha-4a chain	gi|251823917|ref|NP_001156527.1|	5.87	G2
tubulin beta 5 chain	sp|Q2KJD0|TBB5_BOVIN	13.63	G2
tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation epsilon polypeptide	gi|71153780|sp|P62262.1|1433E_SHEEP	4.52	G1
tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation theta polypeptide	sp|Q3SZI4|1433T_BOVIN	3.25	G1
tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation zeta polypeptide	gi|112696|sp|P29361.1|1433Z_SHEEP	9.11	G1
ubiquitin c	gi|302595880|sp|P0CG55.1|UBB_SHEEP	3.00	G1
ubiquitin-activating enzyme e1	tr|F1MP69|F1MP69_BOVIN	8.76	G1
ubiquitin-conjugating enzyme e2 l3	tr|F1MC72|F1MC72_BOVIN	2.00	G1
ump-cmp kinase	sp|Q2KIW9|KCY_BOVIN	9.01	G1
vinculin isoform 1	tr|F1N789|F1N789_BOVIN	4.21	G2

[a] *A chain elaborate manifold of short hydrogen bond arrays mediating binding of active site-directed serine protease inhibitors* revealed to be identical to immunoglobulin gamma 2 heavy chain constant region's mRNA sequence.

Supplementary Table 4 - Proteins identified in subcutaneous or visceral adipose tissue deposits only			
Protein name	**Accession Number**	**Protein Score**	**Function**
Only visceral deposits			
Omentum, Perirenal			
1-acylglycerol-3-phosphate o-acyltransferase abhd5	gi|157278608|ref|NP_001098403.1|	2.00	G1
40s ribosomal protein s14	tr|F1MZF3|F1MZF3_BOVIN	2.00	G1
60s ribosomal protein l26-like	sp|P61257|RL26_BOVIN	1.47	G1
acyl- synthetase family member mitochondrial	sp|Q17QJ1|ACSF2_BOVIN	4.08	G1
adiponectin	sp|Q3Y5Z3|ADIPO_BOVIN	2.00	G4
aspartyl asparaginyl beta-hydroxylase	sp|Q28056|ASPH_BOVIN	8.05	G1
atp citrate lyase	gi|121957977|sp|Q2TCH3.1|ACLY_SHEEP	27.35	G1
atp synthase subunit mitochondrial-like	gi|218783550|ref|NP_001136363.1|	4.51	G1
basal cell adhesion molecule	sp|Q9MZ08|BCAM_BOVIN	2.64	G2
basigin precursor	tr|Q3ZBX0|Q3ZBX0_BOVIN	2.37	G1
camp-dependent protein kinase type ii-beta regulatory subunit	tr|B0JYK4|B0JYK4_BOVIN	6.66	G1
capz-interacting protein	tr|F1MMB8|F1MMB8_BOVIN	2.00	G1
cytochrome b-c1 complex subunit mitochondrial-like	gi|339521899|gb|AEJ84114.1|	4.01	G1
cytochrome c oxidase subunit mitochondrial-like	sp|P13184|CX7A2_BOVIN	2.00	G1
cytochrome c oxidase subunit vb	sp|P00428|COX5B_BOVIN	4.15	G1
dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase mitochondrial	tr|F1N690|F1N690_BOVIN	2.51	G1
dynactin subunit 1 isoform 3	tr|F1MIC9|F1MIC9_BOVIN	3.46	G2
fibrinogen alpha chain	gi|85701330|sp|P68214.1|FIBA_SHEEP	2.00	G4
heavy chain non-muscle	tr|F1MQ37|F1MQ37_BOVIN	15.89	G2
heparan sulfate proteoglycan 2	tr|F1MER7|F1MER7_BOVIN	3.20	G2
heterogeneous nuclear ribonucleoprotein r	tr|A3KMV6|A3KMV6_BOVIN	1.57	G1
hexokinase 1	tr|F1MIQ6|F1MIQ6_BOVIN	2.09	G1
histone h2a type 1-like	tr|F2Z4I6|F2Z4I6_BOVIN	6.72	G1
hormone-sensitive lipase	gi|161784136|gb|ABX79556.1|	2.19	G1

isocitrate dehydrogenase (NAD) subunit b	sp|O77784|IDH3B_BOVIN	3.30	G1
marcks-like 1	sp|Q0VBZ9|MRP_BOVIN	2.00	G1
mitochondrial ribosomal protein l12	tr|A5PJ86|A5PJ86_BOVIN	1.33	G1
myosin-ic isoform a	tr|F1MSC5|F1MSC5_BOVIN	7.18	G2
NADH dehydrogenase (ubiquinone) Fe-S protein 7	gi|147744666|gb|ABQ51200.1|	2.00	G1
nascent polypeptide-associated complex subunit alpha	sp|Q5E9A1|NACA_BOVIN	4.00	G1
nidogen 1	tr|F1MWN3|F1MWN3_BOVIN	3.68	G2
nsfl1 cofactor	sp|Q3SZC4|NSF1C_BOVIN	2.00	G1
nucleolin	tr|E1B8K6|E1B8K6_BOVIN	3.70	G1
platelet glycoprotein 4	gi|338163311|gb|AEI74425.1|	3.06	G2
pyruvate dehydrogenase e1 component subunit beta, mitochondrial	sp|P11966|ODPB_BOVIN	7.25	G1
redox-regulatory protein pamm isoform 1 precursor	sp|Q3ZBK2|CJ058_BOVIN	1.76	G3
ribosomal protein s2	sp|O18789|RS2_BOVIN	2.00	G1
ribosomal protein s3	tr|Q862V8|Q862V8_BOVIN	2.30	G1
splicing arginine serine-rich 1	sp|Q0VCY7|SRSF1_BOVIN	3.59	G1
synaptic 2	sp|Q3ZCD7|TECR_BOVIN	2.00	G1
thymopoietin beta	tr|F1MCA8|F1MCA8_BOVIN	2.00	G1
type alpha 1	tr|F1MUC5|F1MUC5_BOVIN	3.68	G2
ubiquinone biosynthesis protein mitochondrial-like	sp|Q2NL34|COQ9_BOVIN	4.09	G1
Liver, Omentum, Perirenal			
40s ribosomal protein s5	sp|Q5E988|RS5_BOVIN	2.02	G1
60s acidic ribosomal protein p1	tr|E1BCL5|E1BCL5_BOVIN	2.00	G1
60s ribosomal protein l31-like	sp|Q56JX3|RL31_BOVIN	2.00	G1
acyl-coenzyme a synthetase mitochondrial 1	tr|F1MPP7|F1MPP7_BOVIN	30.35	G1
adenylate kinase 3	sp|P08760|KAD3_BOVIN	2.33	G1
apolipoprotein c-iii	sp|P19035|APOC3_BOVIN	2.00	G1
calmodulin	gi|75072157|sp|Q6YNX6.3|CALM_SHEEP	4.28	G1
carnitine o-acetyltransferase	tr|Q08DN5|Q08DN5_BOVIN	1.40	G1
cytochrome b5	gi|339521981|gb|AEJ84155.1|	4.14	G1
enoyl coenzyme a hydratase peroxisomal	tr|Q3T172|Q3T172_BOVIN	2.25	G1

ethylmalonic encephalopathy 1	sp|Q3T094|ETHE1_BOVIN	9.44	G1
glutamate dehydrogenase 1	tr|F1MTM8|F1MTM8_BOVIN	48.18	G1
glutaryl-coenzyme a dehydrogenase	sp|Q2KHZ9|GCDH_BOVIN	5.62	G1
heme-binding protein 1-like	sp|Q148C9|HEBP1_BOVIN	6.60	G1
heterogeneous nuclear ribonucleoprotein d	tr|A6H6Y0|A6H6Y0_BOVIN	5.01	G1
histone h2b type 2-e-like	tr|A5D7N2|A5D7N2_BOVIN	1.74	G1
hydroxyacyl-coenzyme a dehydrogenase	tr|Q2KJC5|Q2KJC5_BOVIN	3.71	G1
hypoxanthine phosphoribosyltransferase 1	sp|Q3SZ18|HPRT_BOVIN	6.14	G1
mitochondrial short-chain specific acyl- dehydrogenase	tr|F1MVL2|F1MVL2_BOVIN	12.76	G1
nucleophosmin-like isoform 1	tr|E3SAZ8|E3SAZ8_BOVIN	2.92	G1
parathymosin	tr|A6QPM9|A6QPM9_BOVIN	3.61	G1
propionyl coenzyme a beta polypeptide	sp|Q2TBR0|PCCB_BOVIN	25.25	G1
protein kinase c substrate 80k-h	sp|Q28034|GLU2B_BOVIN	5.91	G1
ribosomal protein s3a	tr|E1BD16|E1BD16_BOVIN	2.00	G1
synaptic vesicle membrane protein vat-1 homolog	tr|F1MUP9|F1MUP9_BOVIN	2.57	G1
transmembrane emp24 domain-containing protein 10-like	tr|F1N3K8|F1N3K8_BOVIN	2.00	G2
vesicle-associated membrane protein-associated protein b	sp|A2VDZ9|VAPB_BOVIN	4.80	G2
y box binding protein 1	gi|148225376|ref|NP_001087255.1|	3.42	G1
Omentum			
60s ribosomal protein l29-like	sp|Q58DW3|RL29_BOVIN	3.33	G1
atp synthase subunit mitochondrial precursor	sp|P05630|ATPD_BOVIN	2.48	G1
bucentaur	gi|209869972|dbj|BAG75458.1|	1.57	G2
cytochrome b-c1 complex subunit mitochondrial precursor	sp|P31800|QCR1_BOVIN	2.00	G1
cytochrome p-450	tr|Q4JHN7|Q4JHN7_BOVIN	1.40	G1
desmin	tr|E1BLN2|E1BLN2_BOVIN	4.00	G2
diablo homolog	tr|Q2NL36|Q2NL36_BOVIN	1.70	G1
fusion (involved in t(12 16) in malignant liposarcoma)	tr|F1N198|F1N198_BOVIN	4.00	G1
gli pathogenesis-related 2	tr|Q0VCH9|Q0VCH9_BOVIN	2.00	G4
guanine nucleotide-binding protein g g g subunit gamma-2-like	sp|P63212|GBG2_BOVIN	1.46	G1

keratin 19	sp|P08728|K1C19_BOVIN	5.45	G2
mitochondrial cytochrome c oxidase subunit va	sp|P00426|COX5A_BOVIN	4.44	G1
mitochondrial nadh-ubiquinone oxidoreductase 75 kda subunit	sp|P15690|NDUS1_BOVIN	1.54	G1
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7	sp|Q05752|NDUA7_BOVIN	2.00	G1
plasminogen precursor	tr|F1MTM6|F1MTM6_BOVIN	2.00	G4
poly binding protein 2	tr|Q3SYT9|Q3SYT9_BOVIN	1.42	G1
proteasome inhibitor pi31 subunit	sp|Q3SX30|PSMF1_BOVIN	1.40	G1
ribosomal protein s11	sp|Q3T0V4|RS11_BOVIN	1.52	G1
ribosomal protein s18	gi|206558173|sp|A5JST6.1|RS18_CAPHI	2.49	G1
ribosomal protein s21	sp|Q32PB8|RS21_BOVIN	3.71	G1
secernin 1	sp|P83939|SCRN1_BOVIN	2.00	G1
signal transducer and activator of transcription 5b	sp|Q9TUM3|STA5B_BOVIN	1.84	G1
splicing arginine serine-rich 3	gi|339521945|gb|AEJ84137.1|	1.74	G1
thymosin beta-10	sp|P21752|TYB10_BOVIN	1.76	G1
tubulin-specific chaperone a	sp|P48427|TBCA_BOVIN	2.60	G3
ubiquinol-cytochrome c reductase binding protein	sp|P00129|QCR7_BOVIN	2.04	G1
voltage-dependent anion channel 1	tr|F1MIN1|F1MIN1_BOVIN	2.38	G2
Liver, Omentum			
acetyl- cytosolic	tr|Q17QI3|Q17QI3_BOVIN	13.90	G1
aldehyde dehydrogenase 4 member a1	sp|A7YWE4|AL4A1_BOVIN	11.92	G1
esterase d formylglutathione hydrolase	sp|Q08E20|ESTD_BOVIN	7.70	G1
keratin 8	sp|P05786|K2C8_BOVIN	13.61	G1
na(+) h(+) exchange regulatory cofactor nhe-rf1	sp|Q3SZK8|NHRF1_BOVIN	5.10	G2
phosphoglycerate dehydrogenase	sp|Q5EAD2|SERA_BOVIN	10.14	G1
ras-related protein rab-2a	tr|F1MIR4|F1MIR4_BOVIN	2.00	G1
ribosomal protein l23a-like	tr|E1BII3|E1BII3_BOVIN	4.06	G1
ribosomal protein s15	sp|Q56K10|RS15_BOVIN	2.00	G1
scaffold attachment factor b2	tr|E1BDE7|E1BDE7_BOVIN	2.00	G2
staphylococcal nuclease domain-containing protein 1	sp|Q863B3|SND1_BOVIN	1.89	G1

Perirenal			
1-acylglycerol-3-phosphate o-acyltransferase 3	tr|Q32LK4|Q32LK4_BOVIN	2.54	G1
28 kda heat- and acid-stable phospho	tr|F1MNS8|F1MNS8_BOVIN	2.00	G1
40s ribosomal protein s16	sp|Q3T0X6|RS16_BOVIN	4.68	G1
55 kda erythrocyte membrane protein	sp|Q17QN6|EM55_BOVIN	1.49	G2
actin-related protein 2	tr|F1MRG7|F1MRG7_BOVIN	2.00	G2
acyl- synthetase short-chain family member 1	tr|Q9BEA3|Q9BEA3_BOVIN	2.05	G1
acyl- thioesterase 9	sp|Q3SWX2|ACOT9_BOVIN	2.04	G1
adducin 1	tr|E1BHK2|E1BHK2_BOVIN	2.00	G3
adipocyte plasma membrane-associated	tr|F1MQX2|F1MQX2_BOVIN	2.20	G1
alpha-parvin-like	tr|F1MK74|F1MK74_BOVIN	2.00	G2
alpha-soluble nsf attachment protein	tr|A5D7S0|A5D7S0_BOVIN	2.00	G2
arp1 actin-related protein 1 homolog centractin alpha	tr|Q3MHY9|Q3MHY9_BOVIN	2.00	G2
aspartoacylase	tr|Q2KI21|Q2KI21_BOVIN	1.52	G1
band -like protein 3	tr|E1B7S3|E1B7S3_BOVIN	2.11	G2
cadherin-13 precursor	tr|F1N200|F1N200_BOVIN	2.00	G2
calcium binding protein p22	sp|Q3SYS6|CHP1_BOVIN	3.89	G3
caseinolytic atp- proteolytic subunit homolog (coli)	sp|Q2KHU4|CLPP_BOVIN	3.40	G1
clathrin light chain a	sp|P04973|CLCA_BOVIN	1.72	G2
complement component 4a (rodgers blood group)	tr|F1MVK1|F1MVK1_BOVIN	5.85	G4
complement component q subcomponent binding protein	sp|Q3T0B6|C1QBP_BOVIN	1.41	G4
dynein cytoplasmic 1 intermediate chain 2	tr|F1N7H7|F1N7H7_BOVIN	2.01	G2
eh-domain containing 1	tr|F1N6N3|F1N6N3_BOVIN	3.27	G2
enoyl- hydratase domain-containing protein 1-like	tr|F1MDK4|F1MDK4_BOVIN	2.00	G1
estradiol 17-beta-dehydrogenase 12	tr|A6H7H3|A6H7H3_BOVIN	2.04	G1
eukaryotic translation initiation factor 2 subunit 1	gi|339522347|gb|AEJ84338.1|	2.00	G1
eukaryotic translation initiation factor 4b	tr|Q3MHP6|Q3MHP6_BOVIN	2.00	G1
fetuin b	sp|Q58D62|FETUB_BOVIN	2.00	G1
gnas complex locus	sp|P04896-2|GNAS2_BOVIN	2.01	G1
heat shock 70 kda protein 12a	tr|F1MJ70|F1MJ70_BOVIN	2.35	G3

high-mobility group box 1	sp|P10103|HMGB1_BOVIN	2.35	G1
integrin beta-1 precursor	gi|215275342|sp|B0FYY4.1|ITB1_SHEEP	2.12	G2
kininogen-1 isoform 1	sp|P01044|KNG1_BOVIN	2.00	G4
kynurenine--oxoglutarate transaminase 3 isoform 1	sp|Q0P5G4|KAT3_BOVIN	2.00	G1
laminin subunit beta-2	tr|E1BDK6|E1BDK6_BOVIN	3.06	G2
lysosomal-associated membrane protein 1	sp|Q05204|LAMP1_BOVIN	1.40	G2
matrin-3	tr|F1MXI4|F1MXI4_BOVIN	2.04	G1
methylthioribose-1-phosphate isomerase	sp|Q2NL31|MTNA_BOVIN	2.00	G1
mitochondrial lon protease-like protein	sp|Q59HJ6|LONM_BOVIN	4.41	G1
mitochondrial-processing peptidase subunit beta-like	tr|E1B941|E1B941_BOVIN	1.54	G1
monocarboxylate transporter 2	tr|Q2EF45|Q2EF45_BOVIN	1.70	G2
monoglyceride lipase	tr|F1MC02|F1MC02_BOVIN	2.70	G1
myosin light chain smooth muscle	sp|Q28824|MYLK_BOVIN	2.01	G2
nfu1 iron-sulfur cluster scaffold homolog (cerevisiae)	tr|Q2KJF3|Q2KJF3_BOVIN	2.00	G2
non-histone chromosomal protein hmg-17-like	tr|F2Z4H2|F2Z4H2_BOVIN	6.01	G1
pdz and lim domain 1	tr|A6H7E3|A6H7E3_BOVIN	2.00	G2
peptidase (mitochondrial processing) alpha	sp|Q0P5M8|MPPA_BOVIN	2.74	G1
prefoldin subunit 2	tr|Q862M6|Q862M6_BOVIN	2.02	G3
proteasome (macropain) 26s non- 3	sp|Q2KJ46|PSMD3_BOVIN	2.14	G1
proteolipid protein 2	sp|Q6Y1E2|PLP2_BOVIN	2.00	G1
pyruvate dehydrogenase beta	tr|D3JWS9|D3JWS9_BUBBU	2.00	G1
ras-like protein rab-11B	gi|339521883|gb|AEJ84106.1|	2.53	G1
related ras viral (r-ras) oncogene homolog 2	tr|A5PKL2|A5PKL2_BOVIN	2.00	G1
ribosomal protein l10a	sp|Q5E9E6|RL10A_BOVIN	1.47	G1
ribosomal protein l22	tr|F1N301|F1N301_BOVIN	2.00	G1
ribosomal protein l36a-like	tr|Q3ZCJ0|Q3ZCJ0_BOVIN	1.70	G1
ribosomal protein l4	sp|Q58DW0|RL4_BOVIN	1.79	G1
ribosomal protein l7a	sp|Q2TBQ5|RL7A_BOVIN	1.93	G1
serine arginine repetitive matrix 1	tr|E1BP16|E1BP16_BOVIN	2.00	G1
serum albumin precursor	gi|113582|sp|P14639.1|ALBU_SHEEP	1.70	G2

splicing arginine serine-rich 6	tr|F1MXY9|F1MXY9_BOVIN	2.60	G1
splicing proline- and glutamine-rich	tr|E1BQ37|E1BQ37_BOVIN	2.30	G1
transcription elongation factor b polypeptide 2-like	tr|Q3SZ32|Q3SZ32_BOVIN	1.46	G1
tripeptidyl-peptidase 1 precursor	tr|F1MK08|F1MK08_BOVIN	3.10	G1
tumor protein d52	tr|Q3ZCA8|Q3ZCA8_BOVIN	2.00	G1
tumor protein d54	tr|Q3SYU8|Q3SYU8_BOVIN	2.02	G1
ubiquinol-cytochrome c rieske iron-sulfur polypeptide 1	sp|P13272|UCRI_BOVIN	1.70	G1
vacuolar protein sorting-associated protein 26a	sp|Q0VD53|VP26A_BOVIN	2.00	G2
vacuolar protein sorting-associated protein 29-like isoform 2	sp|Q3T0M0|VPS29_BOVIN	2.00	G2
vacuolar protein-sorting-associated protein 25	sp|Q5E9A6|VPS25_BOVIN	1.70	G2
von hippel-lindau binding protein 1	sp|Q2TBX2|PFD3_BOVIN	2.05	G3
xaa-pro aminopeptidase 1	sp|Q1JPJ2|XPP1_BOVIN	2.19	G1
[a] zinc finger CCCH domain-containing protein 15	REV_sp|Q1RMM1|ZC3HF_BOVIN	1.70	G1
Liver, Perirenal			
3-hydroxymethyl-3-methylglutaryl-coenzyme a lyase	sp|Q29448|HMGCL_BOVIN	2.61	G1
acylamino-acid-releasing enzyme	tr|F1N7B4|F1N7B4_BOVIN	4.80	G1
alpha-methylacyl- racemase	tr|Q148I9|Q148I9_BOVIN	2.60	G1
c-1-tetrahydrofolate cytoplasmic	tr|A4FUD0|A4FUD0_BOVIN	26.86	G1
coatomer subunit alpha isoform 1	sp|Q27954|COPA_BOVIN	1.60	G2
far upstream element-binding protein 2	tr|F1MHR6|F1MHR6_BOVIN	2.00	G1
fumarylacetoacetate hydrolase	tr|F1MYZ7|F1MYZ7_BOVIN	3.75	G1
glycine cleavage system h mitochondrial-like	tr|E1BDC8|E1BDC8_BOVIN	2.68	G1
nucleobindin 1	sp|Q0P569|NUCB1_BOVIN	1.74	G1
propionyl- carboxylase alpha mitochondrial	tr|A4FV90|A4FV90_BOVIN	16.32	G1
ribosomal protein l13	tr|Q3SZG7|Q3SZG7_BOVIN	1.46	G1
ribosomal protein l18	tr|Q0QEV5|Q0QEV5_BOVIN	2.35	G1
ribosomal protein l24	tr|Q862D6|Q862D6_BOVIN	2.00	G1
ribosomal protein l28	sp|Q3T0L7|RL28_BOVIN	2.00	G1
ribosomal protein l6-like	gi|165940892|gb|ABY75291.1|	2.04	G1
ribosomal protein l8	sp|Q3T0S6|RL8_BOVIN	3.06	G1

ribosomal protein s25	gi|51316657|sp|Q6Q311.1|RS25_SHEEP	3.09	G1
ribosomal protein s4	tr|A2VE06|A2VE06_BOVIN	2.82	G1
ribosomal protein s6	gi|267850626|gb|ACY82396.1|	1.35	G1
sar1 gene homolog a (cerevisiae) isoform cra_b	sp|Q3T0D7|SAR1A_BOVIN	3.12	G2
seryl-trna synthetase	gi|165940924|gb|ABY75307.1|	2.01	G1
short branched chain specific acyl- mitochondrial	sp|Q5EAD4|ACDSB_BOVIN	14.62	G1
ubiquitin-conjugating enzyme e2n	gi|251823923|ref|NP_001156530.1|	1.70	G1
Only subcutaneous deposits			
Sternum, Tail			
3 heavy chain constant region	gi|147744654|gb|ABQ51194.1|	2.00	G4
6-phosphogluconolactonase	tr|F1MM83|F1MM83_BOVIN	3.79	G1
actin-related protein 2 3 complex subunit 5	sp|Q3SYX9|ARPC5_BOVIN	2.00	G2
acyl-coenzyme a synthetase mitochondrial-like	tr|F1MES1|F1MES1_BOVIN	1.55	G1
annexin a7	sp|P20072|ANXA7_BOVIN	1.33	G2
branched chain aminotransferase mitochondrial	gi|4105815|gb|AAD02563.1|	3.76	G1
collagen alpha-1 chain precursor	tr|F1MGW0|F1MGW0_BOVIN	3.83	G2
dcc-interacting protein 13-alpha	tr|A5PKI0|A5PKI0_BOVIN	2.00	G2
fibronectin precursor	tr|B8Y9S9|B8Y9S9_BOVIN	7.62	G2
fk506 binding protein 10	sp|Q2HJ89|FKB10_BOVIN	2.06	G3
fk506 binding protein 63 kda	sp|Q2KJC8|FKBP9_BOVIN	2.17	G3
galactoside- 3	tr|A6QLZ0|A6QLZ0_BOVIN	4.82	G4
glutathione synthetase	sp|Q5EAC2|GSHB_BOVIN	3.12	G3
hexosaminidase a (alpha polypeptide)	gi|187607461|ref|NP_001119815.1|	2.08	G1
inter-alpha-trypsin inhibitor heavy chain h3	tr|F1MME9|F1MME9_BOVIN	1.72	G1
low quality protein: collagen alpha-3 chain-like	tr|E1BB91|E1BB91_BOVIN	3.36	G2
lysosomal alpha-glucosidase precursor	sp|Q9MYM4|LYAG_BOVIN	1.89	G1
nuclear distribution gene c homolog (nidulans)	sp|Q17QG2|NUDC_BOVIN	2.11	G1
ornithine mitochondrial	tr|F1MYG0|F1MYG0_BOVIN	2.03	G1
osteoblast specific factor	tr|Q2KJC7|Q2KJC7_BOVIN	24.22	G2

peptidyl-prolyl cis-trans isomerase fkbp4	tr|F1MU79|F1MU79_BOVIN	4.45	G3
proteasome (macropain) 26s 1	tr|Q5E9D7|Q5E9D7_BOVIN	4.23	G1
proteasome (macropain) beta 1	sp|Q2TBX6|PSB1_BOVIN	4.49	G1
protein kinase delta binding protein	sp|A4FV37|PRDBP_BOVIN	2.43	G1
protein noxp20-like	tr|E1BFB9|E1BFB9_BOVIN	2.00	unannotated
sh3 domain-binding glutamic acid-rich-like protein	sp|Q58DU7|SH3L1_BOVIN	2.82	G1
ubiquitin carboxyl-terminal hydrolase 14	tr|F1MJV5|F1MJV5_BOVIN	2.01	G1
ubiquitin-conjugating enzyme e2 variant 2	sp|Q3SZ43|UB2V2_BOVIN	2.18	G1
wd repeat domain 1	tr|F1MNZ1|F1MNZ1_BOVIN	1.40	G2
Liver, Sternum, Tail			
1,4-alpha-glucan branching enzyme 1	tr|B1PK18|B1PK18_BOVIN	5.31	G1
adenylate kinase 3-like 1	sp|Q0VCP1|KAD4_BOVIN	3.83	G1
aldo-keto reductase family member a3 (aflatoxin aldehyde reductase)	tr|F1N6I4|F1N6I4_BOVIN	12.31	G1
archain 1	sp|P53619|COPD_BOVIN	1.67	G2
catalase	tr|F1MBH1|F1MBH1_BOVIN	3.45	G3
dihydrodiol dehydrogenase 3	tr|E1BP71|E1BP71_BOVIN	11.74	G1
epoxide hydrolase cytoplasmic	tr|Q17QK4|Q17QK4_BOVIN	8.51	G1
n-acylneuraminate cytidylyltransferase	sp|Q3SZM5|NEUA_BOVIN	2.00	G1
pyridoxine 5 -phosphate oxidase	sp|Q5E9K3|PNPO_BOVIN	7.91	G1
pyrophosphatase 1	gi|339522181|gb|AEJ84255.1|	2.10	G1
sorbitol dehydrogenase	gi|330689592|pdb|3QE3|A	17.20	G1
sulfotransferase 1a1-like isoform 1	tr|F4YD34|F4YD34_BUBBU	5.22	G1
t-complex protein 1 subunit delta	tr|F1N0E5|F1N0E5_BOVIN	2.01	G3
Sternum			
6-phosphofructokinase liver type	tr|F1MIV8|F1MIV8_BOVIN	4.00	G1
actin-related protein 2 3 complex subunit 3-like	sp|Q3T035|ARPC3_BOVIN	1.52	G2
adenosine deaminase	tr|A6H7A2|A6H7A2_BOVIN	2.31	G1
adhesion regulating molecule 1	sp|A1L5A6|ADRM1_BOVIN	2.00	G2
alanyl-trna cytoplasmic	tr|A6QLT9|A6QLT9_BOVIN	1.34	G1

charged multivesicular body protein 4b-like	tr|Q08E32|Q08E32_BOVIN	2.01	G2
coatomer subunit beta	sp|A0JN39|COPB_BOVIN	2.00	G2
collagen alpha-1 chain precursor	tr|F1MXS8|F1MXS8_BOVIN	3.65	G2
copine i	gi|240849253|ref|NP_001155362.1|	1.40	G2
fibulin 5	tr|Q2KJ89|Q2KJ89_BOVIN	2.92	G2
fibulin 5-like	tr|E1BEB4|E1BEB4_BOVIN	4.00	G2
galectin 9 short isoform	gi|57163983|ref|NP_001009251.1|	2.01	G2
glutathione peroxidase 7	sp|A6QLY2|GPX7_BOVIN	1.53	G3
haloacid dehalogenase-like hydrolase domain-containing protein 2-like	sp|Q3ZCH9|HDHD2_BOVIN	2.00	G1
hemoglobin beta	gi|86129753|gb|ABC86528.1|	2.80	G2
hexosaminidase b	tr|F2Z4G2|F2Z4G2_BOVIN	2.00	G1
iq motif containing gtpase activating protein 1	tr|F1MC48|F1MC48_BOVIN	3.03	G2
m1-type pyruvate kinase	tr|B3IVN4|B3IVN4_BOVIN	1.40	G1
periplakin	tr|F1N2K8|F1N2K8_BOVIN	2.11	G2
phosphomannomutase 2	sp|Q3SZJ9|PMM2_BOVIN	2.61	G1
prolyl 3-hydroxylase 1	tr|A4FUY3|A4FUY3_BOVIN	2.10	G1
proteasome (macropain) beta 4	sp|Q3T108|PSB4_BOVIN	1.82	G1
proteasome subunit alpha type-3	sp|Q58DU5|PSA3_BOVIN	2.00	G1
protein hp-25 homolog 1-like	sp|Q2KIT0|HP20_BOVIN	2.00	G3
septin-2	tr|F1MLX3|F1MLX3_BOVIN	2.09	G2
solute carrier family 25 (mitochondrial carrier citrate transporter) member 1	sp|P79110|TXTP_BOVIN	4.00	G2
s-phase kinase-associated protein 1	gi|339522013|gb|AEJ84171.1|	2.00	G1
transforming growth beta- 68kda	tr|F1MBS3|F1MBS3_BOVIN	2.00	G4
ubiquilin 3	tr|Q32KL3|Q32KL3_BOVIN	1.70	G1
versican core protein precursor	tr|F1MZ85|F1MZ85_BOVIN	4.98	G2
Liver, Sternum			
actin binding 1a	sp|Q92176|COR1A_BOVIN	2.22	G2
fructose-bisphosphate aldolase b	gi|1703242	2.01	G1
glyoxalase domain containing 4	tr|A7MBI6|A7MBI6_BOVIN	5.92	G1

Tail			
26s proteasome non-atpase regulatory subunit 6	tr|F1MXE4|F1MXE4_BOVIN	4.36	G1
a chain human camp-dependent protein kinase in complex with an inhibitor	sp|P00517|KAPCA_BOVIN	1.68	G1
a chain structures of glycogen phosphorylase-inhibitor complexes and the implications for structure-based drug design	gi|14916625|sp|O18751.3|PYGM_SHEEP	9.63	G1
adp-ribosylation factor 4	gi|242247463|ref|NP_001156018.1|	2.22	G2
alpha globin chain	sp|P09423|HBA_BISBO	2.38	G2
alpha-2-antiplasmin precursor	sp|P28800|A2AP_BOVIN	2.13	G1
alpha-2-hs-glycoprotein precursor	sp|P12763|FETUA_BOVIN	4.00	G4
beta-enolase-like isoform 1	sp|Q3ZC09|ENOB_BOVIN	17.47	G1
bifunctional purine biosynthesis protein purh	sp|Q0VCK0|PUR9_BOVIN	1.62	G1
calpain-1 catalytic subunit	sp|Q27970|CAN1_BOVIN	2.00	G1
cellular nucleic acid-binding protein	sp|Q3T0Q6|CNBP_BOVIN	1.70	G1
cysteine sulfinic acid decarboxylase	tr|E1BP42|E1BP42_BOVIN	1.90	G1
fibrillin-1 precursor	tr|F1N4K8|F1N4K8_BOVIN	1.72	G2
fibulin 1	tr|F1MYN5|F1MYN5_BOVIN	2.15	G2
filamin-c isoform 1	tr|E1BE25|E1BE25_BOVIN	15.50	G2
gdp-mannose pyrophosphorylase isoform cra_a	tr|E1BEN4|E1BEN4_BOVIN	2.00	G1
glioblastoma amplified sequence	tr|Q3SWX4|Q3SWX4_BOVIN	1.52	G2
heterogeneous nuclear ribonucleoprotein g	tr|D3JUI8|D3JUI8_BOVIN	2.00	G1
lysophosphatidic acid phosphatase type 6	tr|A6H757|A6H757_BOVIN	2.59	G1
mannose-1-phosphate guanyltransferase beta	tr|F1N7H5|F1N7H5_BOVIN	3.54	G1
mevalonate decarboxylase	sp|Q0P570|MVD1_BOVIN	2.38	G1
microfibrillar-associated protein 5 precursor	sp|Q28022|MFAP5_BOVIN	1.70	G2
mid1-interacting protein 1	tr|Q08E46|Q08E46_BOVIN	3.87	G1
myoglobin	gi|322518672|sp|B7U9B5.2|MYG_CAPHI	10.51	G2
phosphoglycerate mutase 2	tr|F1N2F2|F1N2F2_BOVIN	2.16	G1
phosphopantothenate--cysteine ligase-like	tr|F1MFB8|F1MFB8_BOVIN	2.00	G1
platelet-activating factor acetylhydrolase ib subunit beta	sp|P68401|PA1B2_BOVIN	2.08	G1
procollagen- 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase) alpha polypeptide i	tr|A6QL77|A6QL77_BOVIN	2.01	G3

protein phosphatase catalytic alpha isoform	sp|Q3T0E7|PP1A_BOVIN	2.00	G1
pyruvate dehydrogenase component x	sp|P22439|ODPX_BOVIN	2.09	G1
ran-specific gtpase-activating protein	sp|Q3T0M7|RANG_BOVIN	1.70	G2
small subunit 1	sp|P13135|CPNS1_BOVIN	2.00	G1
solute carrier family 25 (mitochondrial carrier phosphate carrier) member 3	tr|F1MR58|F1MR58_BOVIN	2.27	G1
tensin 1	tr|E1BNT5|E1BNT5_BOVIN	2.01	G2
thiosulfate sulfurtransferase rhodanese-like domain-containing protein 1-like	tr|F1MW03|F1MW03_BOVIN	1.70	G1
tropomodulin 3	tr|Q2KJH0|Q2KJH0_BOVIN	3.70	G2
valacyclovir hydrolase-like	tr|Q2KIX6|Q2KIX6_BOVIN	2.00	G4
zinc-alpha-2-glycoprotein precursor	sp|Q3ZCH5|ZA2G_BOVIN	1.70	G4
zyxin	tr|Q08DQ6|Q08DQ6_BOVIN	2.00	G2
Liver, Tail			
acyl- dehydrogenase family member 10	tr|E1BGS2|E1BGS2_BOVIN	13.66	G1
beta-lactamase-like protein 2	tr|F1N2H3|F1N2H3_BOVIN	2.17	G4
carbonic anhydrase 3	sp|Q3SZX4|CAH3_BOVIN	10.93	G1
coproporphyrinogen oxidase	tr|E1BKY9|E1BKY9_BOVIN	11.74	G1
glutathione s-transferase mu 3	tr|Q2KIV8|Q2KIV8_BOVIN	1.35	G3
methionine adenosyltransferase alpha	tr|A7E3T7|A7E3T7_BOVIN	4.90	G1
mitochondrial import receptor subunit tom70	tr|Q08E34|Q08E34_BOVIN	4.94	G3
nicotinate phosphoribosyltransferase	tr|F1N3B0|F1N3B0_BOVIN	4.51	G1
sulfotransferase 1c4	tr|Q58CV8|Q58CV8_BOVIN	8.18	G1

ᵃ Reversed sequence

Supplementary Table 5 - Proteins identified in both subcutaneous and visceral adipose tissue without tissue or macro-area specificity			
Protein name	**Accession Number**	**Protein Score**	**Function**
Omentum, Sternum			
capping protein (actin filament) gelsolin-like	sp|Q865V6|CAPG_BOVIN	2.32	G2
cytoskeleton-associated protein 4	tr|F1ME65|F1ME65_BOVIN	4.01	G2
pigment epithelium-derived factor	gi|213021132|ref|NP_001132919.1|	1.83	G1
proteasome subunit beta type-5	sp|Q32KL2|PSB5_BOVIN	2.00	G1
protein s100-a13	sp|P79342|S10AD_BOVIN	1.76	G1
serine threonine-protein phosphatase 2a catalytic subunit alpha isoform	tr|Q58D70|Q58D70_BOVIN	2.35	G1
small glutamine-rich tetratricopeptide repeat-containing protein alpha	sp|Q32LM2|SGTA_BOVIN	2.04	G3
Liver, Omentum, Sternum			
collagen alpha-1 chain	tr|E1BA17|E1BA17_BOVIN	3.78	G2
omega-amidase nit2	tr|F1MJ59|F1MJ59_BOVIN	2.00	G1
Omentum, Tail			
26s proteasome non-atpase regulatory subunit 7	tr|A1L5B1|A1L5B1_BOVIN	2.00	G1
adenylate kinase 1	tr|E1BDU3|E1BDU3_BOVIN	1.71	G1
tryptophanyl-trna isoform cra_a	sp|P17248|SYWC_BOVIN	2.00	G1
Liver, Omentum, Tail			
polypyrimidine tract binding protein 1	sp|Q8WN55|PTBP1_BOVIN	2.19	G1
Omentum, Sternum, Tail			
capping protein (actin filament) muscle z- alpha 2	tr|F1N4V7|F1N4V7_BOVIN	3.23	G2
fibrinogen beta chain	tr|A6QPX7|A6QPX7_BOVIN	1.84	G2
peptidase d	tr|F1N3W7|F1N3W7_BOVIN	3.22	G1
prolyl endopeptidase	tr|A5D7C6|A5D7C6_BOVIN	1.70	G1
sh3 domain binding glutamic acid-rich protein like 3	sp|Q3ZCL8|SH3L3_BOVIN	2.13	G1
Liver, Omentum, Sternum, Tail			

apolipoprotein a-i binding protein	sp|Q6QRN6|AIBP_BOVIN	3.51	G1
dipeptidyl-peptidase 3	tr|F2Z4F5|F2Z4F5_BOVIN	1.60	G1
fibrinogen gamma chain	tr|Q3SZZ9|Q3SZZ9_BOVIN	2.33	G4
glyoxylate reductase hydroxypyruvate reductase	tr|F1MB84|F1MB84_BOVIN	3.11	G1
hydroxyacylglutathione mitochondrial-like	sp|Q3B7M2|GLO2_BOVIN	2.14	G1
liver carboxylesterase-like isoform 1	tr|Q0VCI3|Q0VCI3_BOVIN	7.57	G4
mercaptopyruvate sulfurtransferase	tr|Q3MHG3|Q3MHG3_BOVIN	11.10	G1
protein ndrg2	sp|Q3ZBA8|NDRG2_BOVIN	4.01	G1
Omentum, Perirenal, Sternum			
1,4-alpha-glucan branching enzyme 1	tr|E1BBH3|E1BBH3_BOVIN	2.00	G1
40s ribosomal protein s20	sp|Q3ZBH8|RS20_BOVIN	3.52	G1
[a] abnormal spindle-like microcephaly-associated protein homolog	REV_gi|60391794|sp|P62297.1|ASPM_SHEEP	2.66	G2
activated rna polymerase ii transcriptional coactivator p15-like	tr|A7YWC6|A7YWC6_BOVIN	1.40	G1
acyl- thioesterase 1	tr|F1N234|F1N234_BOVIN	2.19	G1
atp h+ mitochondrial f1 gamma polypeptide 1	sp|P05631|ATPG_BOVIN	3.68	G1
complement component c3d	gi|12649541|gb|AAB92374.2|	2.96	G4
cytochrome c-1	tr|F1MNZ2|F1MNZ2_BOVIN	4.77	G1
group-specific component (vitamin d binding protein)	tr|F1N5M2|F1N5M2_BOVIN	6.10	G1
lamin a c	tr|Q3SZI2|Q3SZI2_BOVIN	14.77	G2
laminin subunit beta-1	tr|F1MNT4|F1MNT4_BOVIN	8.18	G2
methylcrotonoyl- carboxylase beta mitochondrial	tr|E1BPP6|E1BPP6_BOVIN	2.00	G1
nidogen-2 precursor	tr|F1MF97|F1MF97_BOVIN	2.25	G2
peroxisomal acyl-coenzyme a oxidase 3	tr|A4IFA4|A4IFA4_BOVIN	6.84	G3
plasma proteinase inhibitor	tr|Q7M371|Q7M371_SHEEP	1.78	G4
reticulon isoform cra_f	tr|F1N405|F1N405_BOVIN	2.09	G2
serotransferrin precursor	gi|2318026|gb|AAB66468.1_SHEEP	2.00	G2
thioredoxin-like 1	tr|Q0II43|Q0II43_BOVIN	2.03	G1
vesicle-trafficking protein sec22b-like	tr|Q3T0L9|Q3T0L9_BOVIN	5.10	G2

Liver, Omentum, Perirenal, Sternum			
argininosuccinate lyase	gi|239916452|gb|ACS34712.1|	24.19	G1
chloride intracellular channel 4	tr|F1MT96|F1MT96_BOVIN	4.00	G2
es1 protein mitochondrial-like isoform 1	gi|242247519|ref|NP_001156032.1|	2.82	G1
eukaryotic translation elongation factor 1 beta 2	sp|Q5E983|EF1B_BOVIN	4.00	G1
heterogeneous nuclear ribonucleoprotein a b	tr|Q3ZC44|Q3ZC44_BOVIN	4.00	G1
histone h4	tr|E1BK94|E1BK94_BOVIN	3.68	G1
hydroxysteroid dehydrogenase-like protein 2	tr|F1MF48|F1MF48_BOVIN	4.52	G1
peptidylprolyl isomerase b (cyclophilin b)	sp|P80311|PPIB_BOVIN	8.63	G3
plectin isoform 1hij	tr|E1BF59|E1BF59_BOVIN	1.67	G2
prothymosin alpha	sp|P01252|PTMA_BOVIN	6.14	G1
pyruvate carboxylase	sp|Q29RK2|PYC_BOVIN	29.63	G1
radixin	tr|F1N0R3|F1N0R3_BOVIN	6.28	G2
ribosomal protein l5	sp|Q58DW5|RL5_BOVIN	2.00	G1
ribosome-binding protein 1	tr|F1MAW9|F1MAW9_BOVIN	16.14	G1
spectrin beta brain 1	tr|F1MYC9|F1MYC9_BOVIN	14.52	G2
Omentum, Perirenal, Tail			
26s protease regulatory subunit 6a	tr|F1MWE0|F1MWE0_BOVIN	1.88	G1
26s protease regulatory subunit 7	sp|Q5E9F9|PRS7_BOVIN	3.41	G1
3-hydroxybutyrate type 1	sp|Q02337|BDH_BOVIN	3.59	G1
alpha-2-macroglobulin receptor-associated protein precursor	tr|Q148K7|Q148K7_BOVIN	2.59	G4
annexin a3	tr|F1MWQ2|F1MWQ2_BOVIN	3.60	G2
apoptosis-inducing factor mitochondrial	tr|E1BJA2|E1BJA2_BOVIN	5.19	G4
citrate synthase	sp|Q29RK1|CISY_BOVIN	4.10	G1
coiled-coil domain-containing protein 79	tr|E1BFE5|E1BFE5_BOVIN	2.00	G1
complement factor b	gi|148645283|gb|ABR01165.1|	1.70	G4
decorin	gi|327199782|gb|AEA36044.1|	1.40	G2
dynactin 2	sp|Q3ZCF0|DCTN2_BOVIN	2.34	G2

guanine nucleotide-binding protein g g g subunit beta-1	tr|A7E3V7|A7E3V7_BOVIN	2.00	G1
laminin subunit gamma-1	tr|F1MD77|F1MD77_BOVIN	5.84	G2
lim and sh3 domain protein 1	sp|Q3B7M5|LASP1_BOVIN	2.00	G2
mitochondrial succinyl- ligase	tr|F1MGC0|F1MGC0_BOVIN	2.04	G1
osteoglycin	gi|119712147|gb|ABL96619.1|	1.71	G1
poly -binding protein 1	sp|Q5E9A3|PCBP1_BOVIN	2.65	G1
t-complex protein 1 subunit eta	tr|F1MWR8|F1MWR8_BOVIN	2.04	G3
thyroid hormone-inducible hepatic protein	tr|Q690M9|Q690M9_BOVIN	1.40	G1
tumor translationally-controlled 1	tr|Q862L1|Q862L1_BOVIN	2.00	G4
Liver, Omentum, Perirenal, Tail			
3-ketoacyl- mitochondrial	gi|302124928|gb|ADK93976.1|	3.41	G1
60s acidic ribosomal protein p2	sp|P42899|RLA2_BOVIN	6.00	G1
aminoacyl trna synthase complex-interacting multifunctional protein 1	tr|Q3ZBX5|Q3ZBX5_BOVIN	2.80	G4
brain membrane attached signal protein 1	sp|P80724|BASP1_BOVIN	2.81	G2
carnitine palmitoyltransferase 2	tr|F1N1M7|F1N1M7_BOVIN	6.00	G1
cell division control protein 42 homolog isoform 1	tr|F1N5L2|F1N5L2_BOVIN	1.74	G2
coatomer protein subunit beta 2 (beta prime)	sp|P35605|COPB2_BOVIN	2.00	G2
copper transport protein atox1	gi|7531050|sp|Q9XT28.1|ATOX1_SHEEP	2.21	G2
cytochrome c	tr|Q3LUG8|Q3LUG8_BUBBU	1.40	G1
decr1	tr|F1N5J8|F1N5J8_BOVIN	9.31	G1
dimethylarginine dimethylaminohydrolase 2	sp|Q3SX44|DDAH2_BOVIN	2.00	G2
dodecenoyl-coenzyme a delta isomerase (trans-enoyl-coenzyme a isomerase)	tr|Q2NL38|Q2NL38_BOVIN	3.25	G1
elongation factor mitochondrial-like	sp|P49410|EFTU_BOVIN	6.07	G1
heterogeneous nuclear ribonucleoprotein a3	tr|E1BEG2|E1BEG2_BOVIN	3.04	G2
peptidyl-prolyl cis-trans isomerase fkbp1a	tr|Q3ZCG6|Q3ZCG6_BOVIN	4.00	G3
trifunctional enzyme subunit mitochondrial	gi|211063449|ref|NP_001129962.1|	14.19	G1
very-long-chain acyl- dehydrogenase	sp|P48818|ACADV_BOVIN	14.06	G1
Perirenal, Sternum			

eukaryotic translation initiation factor x-chromosomal	gi|223633892|ref|NP_001138651.1|	2.00	G1
leukotriene a-4 hydrolase	sp|Q3SZH7|LKHA4_BOVIN	4.00	G1
ras-related protein rab-7a	tr|F1MJQ1|F1MJQ1_BOVIN	3.79	G1
ribosomal protein s17	tr|F1MSH2|F1MSH2_BOVIN	2.00	G1
Liver, Perirenal, Sternum			
ribosomal protein s10	gi|339521935|gb|AEJ84132.1|	2.00	G1
Perirenal, Tail			
28s ribosomal protein mitochondrial-like	sp|P82908|RT36_BOVIN	1.43	G1
atp-dependent rna helicase ddx3x	tr|D3IVZ3|D3IVZ3_BOVIN	2.00	G1
disks large homolog 1 isoform 1	tr|F1MNQ3|F1MNQ3_BOVIN	2.00	G1
eukaryotic translation initiation factor 3 subunit i	sp|Q5E966|EIF3I_BOVIN	1.52	G1
four and a half lim domains 1	tr|F1MR86|F1MR86_BOVIN	2.10	G2
ras-related c3 botulinum toxin substrate 1 (rho small gtp binding protein rac1)	gi|240849265|ref|NP_001155328.1|	1.70	G1
ras-related protein rab-14	tr|F1MY39|F1MY39_BOVIN	2.24	G1
Liver, Perirenal, Tail			
acetyl-coenzyme a acyltransferase 1	tr|Q3ZC41|Q3ZC41_BOVIN	24.31	G1
acyl carrier mitochondrial-like	sp|P52505|ACPM_BOVIN	2.00	G1
dihydropteridine reductase	sp|Q3T0Z7|DHPR_BOVIN	4.52	G1
enoyl-CoA mitochondrial precursor	gi|146395421|ABQ28660.1|	1.70	G1
proteasome (macropain) 26s non- 11	sp|Q2KI42|PSD11_BOVIN	2.96	G1
Perirenal, Sternum, Tail			
26s proteasome non-atpase regulatory subunit 1	tr|A7MBA2|A7MBA2_BOVIN	1.41	G1
acetyl- carboxylase 1	gi|229610179|gb|ACQ83619.1|	2.00	G1
aminopeptidase puromycin sensitive	tr|E1BP91|E1BP91_BOVIN	2.89	G1
cop9 signalosome complex subunit 4	sp|Q3SZA0|CSN4_BOVIN	2.00	G1
creatine muscle	sp|Q9XSC6|KCRM_BOVIN	4.04	G2
glutamine synthetase	tr|F1MDB3|F1MDB3_BOVIN	4.85	G1

peptidyl-prolyl cis-trans isomerase d	sp|P26882|PPID_BOVIN	2.17	G3
platelet-activating factor acetylhydrolase ib subunit alpha	tr|F1MJ51|F1MJ51_BOVIN	2.00	G1
proteasome (macropain) beta 2	sp|Q5E9K0|PSB2_BOVIN	4.18	G1
protein-l-isoaspartate(d-aspartate) o-methyltransferase	sp|P15246|PIMT_BOVIN	3.52	G3
ran	gi|240849365|ref|NP_001155348.1|	3.45	G1
serpine1 mrna binding protein 1	tr|F1MFK6|F1MFK6_BOVIN	2.00	G1
ubiquitin specific peptidase 5 (isopeptidase t)	tr|F1N3P2|F1N3P2_BOVIN	1.68	G1
valyl-trna synthetase	tr|E1BLV6|E1BLV6_BOVIN	2.14	G1
Liver, Perirenal, Sternum, Tail			
glycogen liver form	gi|62900665|sp|Q5MIB5.3|PYGL_SHEEP	14.41	G1
glyoxalase i	tr|F1MUW8|F1MUW8_BOVIN	3.25	G1
peroxiredoxin 5	gi|339522297|gb|AEJ84313.1|	2.26	G1
tubulin-tyrosine ligase-like protein 12	tr|Q08E58|Q08E58_BOVIN	2.53	G3
udp-glucose pyrophosphorylase 2	sp|Q07130|UGPA_BOVIN	13.54	G1

ᵃ Reversed sequence

Supplementary Table 6 – Proteins related to inflammatory and innate immune response (G4)		
Protein name	**Accession Number**	**Protein Score**
Omentum, Perirenal, Sternum, Tail		
adipose most abundant gene transcript 2	tr|Q2NKR5|Q2NKR5_BOVIN	2.00
alpha-1-acid glycoprotein	gi|197725615|gb|ACH73011.1|	7.87
alpha-crystallin b chain	sp|P02510|CRYAB_BOVIN	2.37
beta-galactoside-binding lectin precursor	gi|47779226|gb|AAT38511.1|	6.10
hemopexin precursor	sp|Q3SZV7|HEMO_BOVIN	1.89
immunoglobulin v lambda chain	gi|2766665|gb|AAB95466.1|	2.64
lactoferrin	tr|Q2HJF0|Q2HJF0_BOVIN	7.22
macrophage migration inhibitory factor	gi|77744696|gb|ABB02309.1|	4.00
protein fam49b-like isoform 2	sp|Q2KJI3|FA49B_BOVIN	2.70
Liver, Omentum, Perirenal, Sternum, Tail		
[a] a chain elaborate manifold of short hydrogen bond arrays mediating binding of active site-directed serine protease inhibitors	tr|F1N5M0|F1N5M0_BOVIN	28.24
alpha-1-antitrypsin	gi|112890|sp|P12725.1|A1AT_SHEEP	3.30
alpha-1b-glycoprotein precursor	sp|Q2KJF1|A1BG_BOVIN	2.97
alpha-2-hs-glycoprotein precursor	gi|231469|sp|P29701.1|FETUA_SHEEP	12.14
alpha-2-macroglobulin precursor	tr|E1BJW0|E1BJW0_BOVIN	3.14
beta-2-microglobulin precursor	gi|145207023|gb|ABP37876.1|	2.39
ceruloplasmin	gi|307742663|emb|CBJ23824.1|	4.97
complement component 3	sp|Q2UVX4|CO3_BOVIN	5.87
fibrinogen gamma (partial)	gi|1916272|gb|AAB51261.1|	2.00
immunoglobulin gamma-1 chain	gi|388235|emb|CAA49451.1|	4.77
immunoglobulin lambda light chain	gi|61378762|gb|AAX45027.1|	6.65
protein dj-1	sp|Q5E946|PARK7_BOVIN	7.42
retinol binding protein plasma	sp|P18902|RET4_BOVIN	2.29
selenium-binding protein 1	sp|Q2KJ32|SBP1_BOVIN	12.63

serum albumin precursor	gi|193085052|gb|ACF10391.1|	63.17
thioredoxin	gi|1729950|sp|P50413.2|THIO_SHEEP	6.63
thiosulfate sulfurtransferase	sp|P00586|THTR_BOVIN	7.44
Omentum, Perirenal		
adiponectin	sp|Q3Y5Z3|ADIPO_BOVIN	2.00
fibrinogen alpha chain	gi|85701330|sp|P68214.1|FIBA_SHEEP	2.00
Omentum		
gli pathogenesis-related 2	tr|Q0VCH9|Q0VCH9_BOVIN	2.00
plasminogen precursor	tr|F1MTM6|F1MTM6_BOVIN	2.00
Perirenal		
complement component 4a (rodgers blood group)	tr|F1MVK1|F1MVK1_BOVIN	5.85
complement component 1, q subcomponent binding protein	sp|Q3T0B6|C1QBP_BOVIN	1.41
kininogen-1 isoform 1	sp|P01044|KNG1_BOVIN	2.00
Sternum, Tail		
3 heavy chain constant region	gi|147744654|gb|ABQ51194.1|	2.00
galactoside- 3	tr|A6QLZ0|A6QLZ0_BOVIN	4.82
Sternum		
transforming growth beta- 68kda	tr|F1MBS3|F1MBS3_BOVIN	2.00
Tail		
alpha-2-hs-glycoprotein precursor	sp|P12763|FETUA_BOVIN	4.00
valacyclovir hydrolase-like	tr|Q2KIX6|Q2KIX6_BOVIN	2.00
zinc-alpha-2-glycoprotein precursor	sp|Q3ZCH5|ZA2G_BOVIN	1.70
Liver, Tail		
beta-lactamase-like protein 2	tr|F1N2H3|F1N2H3_BOVIN	2.17
Liver, Omentum, Sternum, Tail		
fibrinogen gamma chain	tr|Q3SZZ9|Q3SZZ9_BOVIN	2.33
liver carboxylesterase-like isoform 1	tr|Q0VCI3|Q0VCI3_BOVIN	7.57
Omentum, Perirenal, Sternum		
complement component c3d	gi|12649541|gb|AAB92374.2|	2.96

plasma proteinase inhibitor	tr|Q7M371|Q7M371_SHEEP	1.78
Omentum, Perirenal, Tail		
alpha-2-macroglobulin receptor-associated protein precursor	tr|Q148K7|Q148K7_BOVIN	2.59
apoptosis-inducing factor mitochondrial	tr|E1BJA2|E1BJA2_BOVIN	5.19
complement factor b	gi|148645283|gb|ABR01165.1|	1.70
tumor translationally-controlled 1	tr|Q862L1|Q862L1_BOVIN	2.00
Liver, Omentum, Perirenal, Tail		
aminoacyl trna synthase complex-interacting multifunctional protein 1	tr|Q3ZBX5|Q3ZBX5_BOVIN	2.80

[a] *A chain elaborate manifold of short hydrogen bond arrays mediating binding of active site-directed serine protease inhibitors* revealed to be identical to immunoglobulin gamma 2 heavy chain constant region's mRNA sequence.

PAPER III

N	Unused	Total	% Cov	Accession Number	Sequence Description	FO vs CTRL (Ctrl=1)	ST vs CTRL (Ctrl=1)	ST vs FO (FO=1)
				Supplementary Table 1. List of the proteins identified and quantified in goat kid omentum after iTRAQ 2D-LC-MS/MS. Protein fold changes in FO-Kid and ST-Kid compared to CTRL-Kid are shown as well as protein fold changes in ST-Kid compared to FO-Kid.				
1	163.19	163.19	59.43	gi|148841334|gb|ABI95140.2|	fatty acid synthase	0.87	1.08	1.25
2	137.06	137.06	94.00	gi|193085052|gb|ACF10391.1|	serum albumin precursor	1.02	0.83	0.81
3	80.20	80.20	78.97	gi|145226795|gb|ABP48145.1|	vimentin	1.19*	1.03	0.87
4	61.06	61.08	38.72	tr|E1BFB0|E1BFB0_BOVIN	spectrin alpha brain isoform 1	1.01	1.01	1.00
5	55.73	55.73	66.76	tr|B8R1K3|B8R1K3_BOSMU	serotransferrin precursor	0.97	0.81	0.83
6	49.86	49.86	55.29	tr|F1N789|F1N789_BOVIN	vinculin isoform 2	1.00	1.02	1.02
7	44.84	44.84	63.23	sp|P19120|HSP7C_BOVIN	heat shock 70kda protein 8	1.03	1.02	0.99
8	44.78	44.78	35.25	sp|Q28559-3|ACACA_SHEEP	acetyl- carboxylase 1	0.95	1.17	1.23
9	41.89	41.89	59.14	sp|P79134|ANXA6_BOVIN	annexin a6	1.04	0.95	0.91
10	41.46	41.46	72.53	gi|46397336|sp|P60713.1|ACTB_SHEEP	cytoplasmic 1	1.00	0.99	0.99
11	41.17	41.17	84.25	tr|F1MLK0|F1MLK0_BOVIN	isocitrate dehydrogenase	1.12	1.00	0.90
12	38.55	38.55	86.69	tr|F1N5M0|F1N5M0_BOVIN	a chain elaborate manifold of short hydrogen bond arrays mediating binding of active site-directed serine protease inhibitors	0.88	0.97	1.11
13	38.47	38.47	47.51	tr|A5PJ79|A5PJ79_BOVIN	transketolase	0.86	0.92	1.07
14	36.49	36.49	48.93	tr|Q0VCZ8|Q0VCZ8_BOVIN	long-chain-fatty-acid-ligase 1	1.03	0.97	0.94
15	36.11	36.11	73.75	gi|148876772|sp|A2SW69.1|ANXA2_SHEEP	annexin a2	1.10	0.98	0.90
16	34.97	34.97	34.27	tr|F1MDH3|F1MDH3_BOVIN	talin 1	1.06	1.08	1.01
17	34.69	34.69	53.29	tr|A4IFB3|A4IFB3_BOVIN	perilipin-1	1.14	0.98	0.86
18	34.23	34.23	51.02	gi|115503919|gb|ABI99473.1|	heat shock protein hsp 90-alpha	1.01	0.95	0.94
19	34.05	34.05	72.29	sp|Q5E956|TPIS_BOVIN	triosephosphate isomerase 1	0.98	0.94	0.97
20	33.97	33.97	69.90	gi|251823897|ref|NP_001156517.1|	protein disulfide-isomerase a3 precursor	0.99	1.05	1.06
21	33.74	33.75	62.94	tr|A6H7J6|A6H7J6_BOVIN	protein disulfide-isomerase precursor	0.93	0.98	1.05
22	32.71	32.71	68.13	tr|A6QLL8|A6QLL8_BOVIN	aldolase fructose-bisphosphate	1.00	1.05	1.06
23	31.59	32.02	41.79	sp|Q95M18|ENPL_BOVIN	endoplasmin precursor	0.95	1.00	1.05

24	31.24	31.24	36.85	sp|Q3ZBT1|TERA_BOVIN	transitional endoplasmic reticulum atpase	1.01	0.98	0.96
25	30.91	30.91	49.38	tr|F1MDS3|F1MDS3_BOVIN	phosphoenolpyruvate carboxykinase	0.89	0.96	1.08
26	30.10	30.10	55.71	gi|242200439|gb|ACS88258.1|	catalase	1.01	0.98	0.97
27	29.83	30.05	68.11	gi|215983082|ref|NP_001135988.1|	phosphoglycerate kinase 1	1.01	0.94	0.93
28	29.47	29.47	90.15	gi|167900488|ref|NP_001108139.1|	fatty acid binding protein 4	1.06	0.83	0.78*
29	28.36	28.36	46.54	sp|P20004|ACON_BOVIN	aconitase mitochondrial	0.91	0.90	0.99
30	28.28	28.28	49.86	sp|Q5EA88|GPDA_BOVIN	glycerol-3-phosphate dehydrogenase	1.01	1.10	1.09
31	27.31	27.95	54.05	sp|Q3ZCH0|GRP75_BOVIN	heat shock 70kda protein 9	0.94	1.02	1.08
32	26.83	26.83	47.21	sp|P00727|AMPL_BOVIN	cytosol aminopeptidase	0.90	1.06	1.18
33	26.78	26.83	49.81	sp|P15497|APOA1_BOVIN	apolipoprotein a-i	1.01	1.01	0.99
34	26.53	26.53	72.19	sp|Q32LG3|MDHM_BOVIN	malate dehydrogenase nad	0.95	0.98	1.03
35	26.49	26.49	58.48	sp|P48644|AL1A1_BOVIN	retinal dehydrogenase 1	1.01	0.88	0.87
36	26.32	26.32	62.67	sp|Q9XSJ4|ENOA_BOVIN	enolase	0.96	0.94	0.98
37	24.54	24.54	50.79	tr|F1MUZ9|F1MUZ9_BOVIN	60 kda heat shock mitochondrial	1.09	1.02	0.94
38	24.36	24.36	58.29	tr|F1N6S9|F1N6S9_BOVIN	neuroblast differentiation-associated protein ahnak-like	1.00	1.02	1.02
39	23.80	23.80	68.02	tr|A7Z057|A7Z057_BOVIN	14-3-3 protein gamma	1.07	0.91	0.85*
40	23.55	23.55	24.84	tr|F1N169|F1N169_BOVIN	filamin alpha (actin binding protein 280)	1.01	1.02	1.01
41	23.46	23.46	34.62	sp|Q3SYU2|EF2_BOVIN	elongation factor 2	0.98	1.03	1.05
42	23.23	23.23	58.52	sp|P00829|ATPB_BOVIN	atp h+ mitochondrial f1 beta polypeptide	1.00	0.99	0.99
43	22.58	29.79	58.66	gi|343432731|gb|AEM24982.1|	heat shock protein	1.03	1.03	0.99
44	22.35	22.35	61.98	sp|Q3T145|MDHC_BOVIN	malate cytoplasmic	1.01	0.88	0.87
45	22.08	22.08	89.63	sp|P62935|PPIA_BOVIN	peptidylprolyl isomerase a (cyclophilin a)	0.99	0.93	0.94
46	21.77	21.77	68.90	sp|Q3SZ62|PGAM1_BOVIN	phosphoglycerate mutase 1	1.00	0.99	0.98
47	21.66	21.67	26.03	tr|F1MYC9|F1MYC9_BOVIN	spectrin beta brain 1	1.04	1.06	1.01
48	21.63	21.63	84.51	gi|302425109|sp|P0CH26.1|HBA2_CAPHI	ii alpha globin	1.42	1.16	0.82
49	21.29	24.35	50.69	sp|Q0VCX2|GRP78_BOVIN	78 kda glucose-regulated protein precursor	0.95	0.99	1.04
50	20.63	20.63	45.88	gi|231469|sp|P29701.1|FETUA_SHEEP	alpha-2-hs-glycoprotein precursor	0.94	0.79	0.84
51	20.24	20.27	27.22	tr|F1MQ37|F1MQ37_BOVIN	heavy chain non-muscle	1.14	1.15	1.01
52	20.15	20.15	26.70	gi|121957977|sp|Q2TCH3.1|ACLY_SHEEP	atp citrate lyase	1.07	1.32	1.23
53	20.10	20.10	73.04	tr|E1BEL7|E1BEL7_BOVIN	heat shock 27kda protein 1	1.07	1.10	1.03
54	20.05	20.05	34.68	sp|Q2KJD0|TBB5_BOVIN	tubulin beta-5 chain	1.38	1.13	0.82

55	19.99	19.99	56.81	tr|A3KN02|A3KN02_BOVIN	histone -like	0.73	1.06	1.44
56	19.87	19.87	39.30	sp|A5D7D1|ACTN4_BOVIN	alpha-actinin-4	1.02	1.01	0.99
57	19.80	19.96	44.31	tr|B0JYN3|B0JYN3_BOVIN	lactate dehydrogenase b	0.94	0.91	0.97
58	19.80	19.80	34.70	tr|F1N1I6|F1N1I6_BOVIN	gelsolin (finnish type)	0.92	0.89	0.96
59	19.44	19.44	42.88	sp|P20000|ALDH2_BOVIN	aldehyde dehydrogenase 2 family	1.11*	1.00	0.90*
60	19.23	19.24	42.68	tr|A1L5B6|A1L5B6_BOVIN	annexin a5	0.87	0.98	1.13
61	18.86	19.38	60.97	tr|F1MNM7|F1MNM7_BOVIN	low quality protein: perilipin-4	1.13	1.00	0.88
62	18.60	18.60	49.58	sp|Q2KJ32|SBP1_BOVIN	selenium binding protein 1	1.01	0.88	0.88
63	18.36	18.36	47.48	tr|A5D7J6|A5D7J6_BOVIN	calreticulin	0.90	0.96	1.06
64	18.15	18.15	82.22	sp|P55052|FABP5_BOVIN	fatty acid binding protein 5	1.02	0.82	0.80
65	18.04	18.04	24.50	sp|Q2UVX4|CO3_BOVIN	complement component 3	1.06	1.17	1.11
66	17.84	17.84	41.81	sp|Q3SZB4|ACADM_BOVIN	medium-chain acyl- dehydrogenase	0.98	1.00	1.03
67	17.79	17.94	48.43	sp|Q3ZBZ8|STIP1_BOVIN	stress-induced-phosphoprotein 1	0.89	0.99	1.11
68	17.67	17.68	30.81	sp|Q29RK2|PYC_BOVIN	pyruvate carboxylase	1.01	0.95	0.94
69	17.61	17.61	42.42	sp|P68103|EF1A1_BOVIN	eukaryotic translation elongation factor 1 alpha 1	0.92	1.12	1.21
70	17.54	17.54	45.21	sp|P19483|ATPA_BOVIN	atp h+ mitochondrial f1 alpha subunit cardiac muscle	1.02	1.00	0.98
71	17.46	17.46	97.24	gi|122545|sp|P02082.1|HBBF_CAPHI	hemoglobin beta	1.05	0.96	0.92
72	17.24	17.24	49.87	sp|Q3T0R7|THIM_BOVIN	3-ketoacyl- mitochondrial	0.86	0.84	0.98
73	17.23	17.26	37.52	tr|F1MD19|F1MD19_BOVIN	glucose-6-phosphate isomerase	0.95	0.91	0.96
74	16.99	17.54	51.04	sp|Q2TBL6|TALDO_BOVIN	transaldolase	0.95	1.05	1.11
75	16.72	16.72	32.78	gi|157835675|pdb|2PGD|A	phosphogluconate dehydrogenase	0.95	1.08	1.14
76	16.70	16.70	39.15	tr|F1N1X7|F1N1X7_BOVIN	phosphoglucomutase 1	0.95	0.96	1.01
77	16.57	16.61	70.16	sp|Q5KR47-2|TPM3_BOVIN	tropomyosin 3	0.99	0.99	1.01
78	16.48	16.48	47.92	tr|F1MZW0|F1MZW0_BOVIN	moesin	1.02	1.04	1.02
79	16.45	16.45	52.05	tr|E1BNE7|E1BNE7_BOVIN	polymerase i and transcript release factor	1.17	0.91	0.78
80	16.37	16.37	35.91	sp|P00515|KAP2_BOVIN	camp-dependent protein kinase type ii-alpha regulatory subunit	1.07	0.88	0.82
81	16.20	16.22	39.41	tr|Q2KJ47|Q2KJ47_BOVIN	eh-domain containing 2	1.23	1.12	0.91
82	15.96	15.96	24.16	tr|F1MTM8|F1MTM8_BOVIN	glutamate dehydrogenase 1	1.08	1.07	1.00
83	15.94	15.94	34.81	sp|P81947|TBA1B_BOVIN	tubulin alpha-1b chain	1.17	1.12	0.95

84	15.85	28.67	47.93	sp|Q76LV1|HS90B_BOVIN	heat shock protein 90kda alpha class b member 1	1.08	1.04	0.96
85	15.85	15.86	30.05	tr|F1MD77|F1MD77_BOVIN	laminin subunit gamma-1	1.12	0.97	0.87
86	15.80	15.80	46.63	gi|112890|sp|P12725.1|A1AT_SHEEP	alpha-1-antitrypsin	0.88	0.98	1.11
87	15.78	15.80	47.11	sp|P46193|ANXA1_BOVIN	annexin a1	0.88	0.98	1.12
88	15.64	15.64	69.12	gi|47779226|gb|AAT38511.1|	beta-galactoside-binding lectin precursor	1.03	1.05	1.02
89	15.57	18.73	48.74	sp|P05786|K2C8_BOVIN	keratin 8	0.67	1.11	1.66*
90	15.56	15.58	60.52	tr|F1MWD3|F1MWD3_BOVIN	t-complex protein 1 subunit epsilon	1.11	1.01	0.91
91	15.42	15.42	49.34	tr|A5PKM0|A5PKM0_BOVIN	glutathione s-transferase mu 1	0.97	0.92	0.95
92	15.41	15.42	25.43	sp|Q7SIH1|A2MG_BOVIN	alpha-2-macroglobulin precursor	1.00	1.03	1.03
93	15.14	20.18	50.98	gi|71153780|sp|P62262.1|1433E_SHEEP	tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation epsilon polypeptide	1.01	0.83*	0.82*
94	15.08	15.11	22.04	tr|F1MNT4|F1MNT4_BOVIN	laminin subunit beta-1	1.14	0.97	0.85
95	14.98	14.98	42.77	sp|Q3ZCJ2|AK1A1_BOVIN	alcohol dehydrogenase	1.02	0.97	0.95
96	14.69	14.69	33.41	sp|Q29RZ0|THIL_BOVIN	acetyl- mitochondrial	1.06	1.13	1.07
97	14.49	14.49	88.77	sp|P13696|PEBP1_BOVIN	phosphatidylethanolamine-binding protein 1	0.97	0.96	0.99
98	14.30	14.30	33.15	tr|F1MV74|F1MV74_BOVIN	non-specific lipid-transfer protein	1.04	1.04	1.00
99	14.25	14.25	22.81	tr|F1MWN3|F1MWN3_BOVIN	nidogen 1	1.13	1.00	0.88
100	14.02	14.02	51.88	tr|A6QNL5|A6QNL5_BOVIN	protein disulfide-isomerase a6 precursor	1.02	1.01	0.99
101	13.93	13.93	54.95	gi|298676425|ref|NP_001177319.1|	glyceraldehyde-3-phosphate dehydrogenase	1.03	1.11	1.08
102	13.83	13.84	58.20	sp|Q5E946|PARK7_BOVIN	protein dj-1	0.99	0.94	0.95
103	13.78	18.34	45.15	tr|F1MWC2|F1MWC2_BOVIN	l-lactate dehydrogenase a chain	1.04	0.93	0.89
104	13.74	13.74	18.90	tr|F1MPU0|F1MPU0_BOVIN	clathrin heavy chain 1	0.97	1.05	1.09
105	13.68	13.68	22.83	gi|388235|emb|CAA49451.1|	immunoglobulin gamma-1 chain	1.02	0.59	0.58
106	13.61	13.61	27.78	sp|Q0VCU1|ACOC_BOVIN	cytoplasmic aconitate hydratase	0.87	1.09	1.25
107	13.42	13.42	50.00	sp|Q2KJH6|SERPH_BOVIN	serpin h1 precursor	0.90	1.06	1.18
108	13.41	13.41	34.60	gi|211063449|ref|NP_001129962.1|	trifunctional enzyme subunit mitochondrial	1.03	0.89	0.87
109	13.38	13.38	40.63	sp|A7E3Q8|PLST_BOVIN	plastin-3 isoform 1	0.97	0.95	0.98
110	13.35	13.35	35.34	gi|61378762|gb|AAX45027.1|	immunoglobulin lambda light chain	0.95	0.59	0.63
111	13.27	13.27	33.12	tr|Q17QZ6|Q17QZ6_BOVIN	serum deprivation-response protein	1.05	1.00	0.96
112	13.24	14.55	15.80	tr|E1BKX7|E1BKX7_BOVIN	filamin-b isoform 2	0.95	0.87*	0.91
113	13.23	13.23	52.26	sp|Q5E947|PRDX1_BOVIN	peroxiredoxin 1	0.95	1.02	1.07

114	12.98	13.09	61.62	gi|261244978|ref|NP_001159672.1|	peroxiredoxin 2	1.09	0.97	0.89
115	12.57	12.57	39.81	tr|F1N206|F1N206_BOVIN	dihydrolipoamide dehydrogenase	1.05	0.99	0.94
116	12.54	12.54	29.74	sp|Q3ZCI9|TCPQ_BOVIN	t-complex protein 1 subunit theta	1.03	1.02	0.99
117	12.48	12.48	38.70	tr|F1MYG5|F1MYG5_BOVIN	lamin a c	0.88	0.94	1.07
118	12.33	15.62	93.66	gi|164136|gb|AAA30914.1|	beta globin	1.17	1.29	1.11
119	12.28	12.28	35.01	tr|F1N7K8|F1N7K8_BOVIN	methylmalonate-semialdehyde dehydrogenase	0.99	0.94	0.95
120	12.13	12.13	43.89	tr|F1MDC1|F1MDC1_BOVIN	ribosome-binding protein 1	0.93	1.08	1.15
121	12.04	12.04	17.91	tr|F1N6Y1|F1N6Y1_BOVIN	neutral alpha-glucosidase ab	0.97	0.88	0.91
122	12.04	12.04	15.57	gi|163914410|ref|NP_001106288.1|	calpain-2 catalytic subunit	0.95	1.02	1.07
123	11.87	11.87	30.77	tr|Q24JZ7|Q24JZ7_BOVIN	3-oxoacid transferase 1	0.86	0.82	0.96
124	11.84	11.84	29.05	gi|327199784|gb|AEA36045.1|	epoxide hydrolase microsomal	1.23	1.00	0.81
125	11.77	11.77	48.62	sp|P08728|K1C19_BOVIN	keratin 19	0.74*	1.06	1.43*
126	11.68	11.68	60.95	gi|84029298|sp|Q9TTY8.2|GSTP1_CAPHI	glutathione s-transferase p	1.02	0.92	0.90
127	11.60	11.60	29.82	tr|Q3MHK9|Q3MHK9_BOVIN	fascin	0.92	1.04	1.13
128	11.38	11.41	85.06	sp|P07107|ACBP_BOVIN	acyl- -binding protein	0.89	0.85	0.96
129	11.25	11.25	44.59	sp|Q3T0K2|TCPG_BOVIN	t-complex protein 1 subunit gamma	1.10	1.02	0.94
130	11.24	11.42	27.74	sp|A2I7N1|SPA35_BOVIN	endopin 1b	1.33	1.06	0.80
131	11.24	11.24	78.43	sp|P61603|CH10_BOVIN	10 kda heat shock mitochondrial-like	0.94	1.01	1.08
132	11.23	11.23	42.50	tr|F1MIZ0|F1MIZ0_BOVIN	pyruvate muscle	0.83	0.86	1.03
133	11.18	11.18	50.20	tr|Q5E9F1|Q5E9F1_BOVIN	b-cell receptor-associated protein 31	1.21	1.02	0.84
134	11.17	11.17	28.10	tr|E1BD43|E1BD43_BOVIN	membrane primary amine oxidase	1.06	0.96	0.91
135	11.07	11.08	29.76	tr|Q0IIL6|Q0IIL6_BOVIN	hydroxysteroid (17-beta) dehydrogenase 4	0.92	1.00	1.08
136	11.07	11.07	79.55	gi|315620165|gb|ADU52989.1|	thymosin beta 4	0.92	0.90	0.97
137	10.89	10.89	45.93	sp|Q02337|BDH_BOVIN	3-hydroxybutyrate type 1	1.02	1.01	0.99
138	10.82	18.54	32.15	tr|F1MNY7|F1MNY7_BOVIN	alpha-actinin-1-like isoform 3	1.10	1.12	1.03
139	10.62	10.62	38.02	sp|Q3T0X5|PSA1_BOVIN	proteasome (macropain) alpha 1	0.96	0.93	0.97
140	10.52	10.52	43.78	sp|Q2HJH2|RAB1B_BOVIN	ras-related protein rab-1b	1.09	1.03	0.94
141	10.49	10.49	46.71	tr|A1A4N9|A1A4N9_BOVIN	nucleoside diphosphate kinase a	1.03	1.05	1.02
142	10.48	10.48	25.71	sp|Q3SZV7|HEMO_BOVIN	hemopexin precursor	1.07	0.87	0.82
143	10.33	10.33	62.05	gi|54035753|sp|Q6B7M7.3|COF1_SHEEP	cofilin 1 (non-muscle)	0.98	0.99	1.01
144	10.29	10.34	24.57	tr|F1MSC5|F1MSC5_BOVIN	myosin-ic-like isoform 2	1.06	0.98	0.92
145	10.21	10.21	51.24	sp|Q9TS87|TAGL_BOVIN	transgelin	1.16	1.05	0.90

146	10.16	10.16	50.59	sp|Q2TBV3|ETFB_BOVIN	electron transfer flavoprotein subunit beta	1.02	0.88	0.86
147	10.08	10.08	43.80	tr|Q3T172|Q3T172_BOVIN	enoyl coenzyme a hydratase peroxisomal	0.90	1.06	1.17
148	10.06	10.07	62.50	sp|O77834|PRDX6_BOVIN	peroxiredoxin 6	0.86*	0.93	1.08
149	10.03	10.03	19.10	tr|F1MYX9|F1MYX9_BOVIN	laminin subunit alpha-4 isoform 2	1.09	0.94	0.86
150	10.01	10.01	35.81	sp|P12344|AATM_BOVIN	aspartate mitochondrial	0.81	0.94	1.16
151	10.01	10.01	33.82	tr|F1MZ38|F1MZ38_BOVIN	succinyl- ligase	1.04	0.92	0.89
152	10.00	10.00	38.25	tr|F1MN74|F1MN74_BOVIN	isocitrate dehydrogenase	0.95	1.02	1.08
153	9.97	10.01	23.10	sp|Q05443|LUM_BOVIN	lumican	0.74*	0.83	1.12
154	9.94	10.12	14.79	tr|E1BA17|E1BA17_BOVIN	collagen alpha-1 chain	0.78	0.89	1.15
155	9.92	9.93	30.11	sp|P11179|ODO2_BOVIN	dihydrolipoamide s-succinyltransferase (e2 component of 2-oxo-glutarate complex)	1.12	1.01	0.91
156	9.92	9.92	44.71	tr|Q148D3|Q148D3_BOVIN	fumarate hydratase	0.95	1.00	1.05
157	9.91	9.91	34.03	tr|A5D9G3|A5D9G3_BOVIN	succinyl- ligase	0.81	1.03	1.27
158	9.90	9.90	33.71	gi|75062591|sp|Q6B3Y2.1|CAV1_SHEEP	caveolin 1	1.13	1.05	0.92
159	9.88	10.00	57.14	gi|1729950|sp|P50413.2|THIO_SHEEP	thioredoxin	1.00	0.98	0.98
160	9.76	11.76	19.88	tr|F1MF97|F1MF97_BOVIN	nidogen-2 precursor	1.09	0.91	0.83
161	9.75	9.76	30.08	gi|62900665|sp|Q5MIB5.3|PYGL_SHEEP	glycogen liver form	0.88	0.97	1.11
162	9.68	9.68	37.88	sp|Q56JV9|RS3A_BOVIN	ribosomal protein s3a	1.09	0.98	0.90
163	9.66	9.81	33.43	sp|Q5E9A3|PCBP1_BOVIN	poly -binding protein 1	0.89	1.02	1.15
164	9.64	9.65	33.81	tr|A7YWF1|A7YWF1_BOVIN	acyl- synthetase short-chain family member 2	1.02	1.07	1.05
165	9.63	9.63	69.54	sp|P60661|MYL6_BOVIN	myosin light polypeptide 6	1.05	0.99	0.94
166	9.61	9.61	41.64	tr|F1N1N0|F1N1N0_BOVIN	heterogeneous nuclear ribonucleoproteins a2 b1-like	0.91	1.11	1.21*
167	9.58	9.59	32.85	sp|Q17QJ1|ACSF2_BOVIN	acyl- synthetase family member mitochondrial	1.19	0.95	0.80*
168	9.55	9.59	25.24	tr|F1MGX0|F1MGX0_BOVIN	t-complex protein 1 subunit zeta	1.15	1.06	0.92
169	9.44	9.46	32.96	sp|P49410|EFTU_BOVIN	elongation factor mitochondrial-like	1.01	1.02	1.01
170	9.38	9.39	40.51	sp|A7MB35|ODPA_BOVIN	pyruvate dehydrogenase alpha 1	1.03	0.94	0.91
171	9.32	9.33	21.81	tr|F1MHJ8|F1MHJ8_BOVIN	aldehyde dehydrogenase 7 member a1	0.91	1.03	1.13
172	9.30	9.30	32.21	sp|O46629|ECHB_BOVIN	hydroxyacyl-coenzyme a dehydrogenase 3-ketoacyl-coenzyme a thiolase enoyl-coenzyme a hydratase (trifunctional protein) beta subunit	1.08	0.90	0.83*
173	9.30	9.30	52.14	sp|P02584|PROF1_BOVIN	profilin 1	0.84	0.88	1.04

174	9.25	9.25	28.76	sp|Q29RK1|CISY_BOVIN	citrate synthase	1.01	0.98	0.97
175	9.18	9.27	21.60	sp|Q148N0|ODO1_BOVIN	oxoglutarate (alpha-ketoglutarate) dehydrogenase	1.06	1.03	0.97
176	9.01	9.01	36.36	sp|O02675|DPYL2_BOVIN	dihydropyrimidinase-related protein 2	0.98	1.01	1.02
177	8.99	8.99	33.33	sp|Q3MHN5|VTDB_BOVIN	group-specific component (vitamin d binding protein)	1.00	0.99	0.99
178	8.96	8.96	41.23	tr|Q1JPA2|Q1JPA2_BOVIN	eukaryotic translation elongation factor 1 gamma	1.27	1.07	0.85
179	8.83	10.83	29.94	tr|F1N2L9|F1N2L9_BOVIN	4-trimethylaminobutyraldehyde dehydrogenase	0.98	0.98	1.00
180	8.78	8.78	20.16	tr|F1MNR8|F1MNR8_BOVIN	hormone-sensitive lipase	1.13	0.95	0.84
181	8.68	8.68	44.67	sp|Q3ZBH0|TCPB_BOVIN	t-complex protein 1 subunit beta	1.07	1.07	0.99
182	8.61	8.61	27.74	gi|416622|sp|P32262.1|ANT3_SHEEP	antithrombin-iii precursor	0.89	0.83	0.92
183	8.55	8.55	14.42	tr|F1N076|F1N076_BOVIN	ceruloplasmin	1.06	1.11	1.05
184	8.44	8.44	29.87	tr|F1MIN1|F1MIN1_BOVIN	voltage-dependent anion channel 1	1.00	1.12	1.12
185	8.39	8.39	15.51	tr|F1N210|F1N210_BOVIN	tensin 1	1.02	0.95	0.93
186	8.37	13.51	51.02	sp|P63103|1433Z_BOVIN	tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation zeta polypeptide	1.07	0.90	0.84
187	8.37	8.42	36.84	tr|F1N3Q7|F1N3Q7_BOVIN	apolipoprotein a-iv	1.02	1.06	1.03
188	8.32	8.32	16.43	tr|F1N3W7|F1N3W7_BOVIN	peptidase d	1.07	0.83	0.77
189	8.30	8.30	20.56	sp|Q28056|ASPH_BOVIN	aspartyl asparaginyl beta-hydroxylase	1.16	1.05	0.90
190	8.19	8.34	24.35	sp|Q3T0D0|HNRPK_BOVIN	heterogeneous nuclear ribonucleoprotein k	0.88	0.99	1.13
191	8.11	8.13	24.62	sp|Q2KJB7|CPT2_BOVIN	carnitine palmitoyltransferase 2	1.03	0.92	0.90
192	8.07	8.08	71.03	sp|Q32PD5|RS19_BOVIN	40s ribosomal protein s19-like	1.03	1.08	1.04
193	7.97	14.29	79.31	gi|86129745|gb|ABC86524.1|	hemoglobin beta	1.46	1.26	0.87
194	7.96	7.97	33.95	tr|Q08D92|Q08D92_BOVIN	acyl-coenzyme a long chain	0.89	0.90	1.01
195	7.88	7.88	33.45	sp|Q58DM8|ECHM_BOVIN	enoyl coenzyme a short mitochondrial	0.91	0.95	1.05
196	7.86	7.86	21.96	tr|Q2KIQ1|Q2KIQ1_BOVIN	phosphoacetylglucosamine mutase	0.99	0.93	0.94
197	7.85	7.85	26.65	tr|F1MYG0|F1MYG0_BOVIN	ornithine mitochondrial	0.95	0.98	1.04
198	7.76	7.76	26.54	sp|Q3SZ65|IF4A2_BOVIN	eukaryotic initiation factor 4a-ii	1.02	0.96	0.94
199	7.68	8.74	28.50	tr|E1BEG2|E1BEG2_BOVIN	heterogeneous nuclear ribonucleoprotein a3	1.07	1.11	1.04
200	7.67	7.67	29.44	tr|F1MNG3|F1MNG3_BOVIN	ras-related c3 botulinum toxin substrate 1 (rho small gtp binding protein rac1)	1.00	1.01	1.01

201	7.63	7.63	22.66	sp|Q2KJF1|A1BG_BOVIN	alpha-1b-glycoprotein precursor	1.04	1.08	1.04
202	7.62	7.62	26.85	sp|Q3MHL4|SAHH_BOVIN	s-adenosylhomocysteine hydrolase	0.77*	0.84	1.08
203	7.60	7.61	21.36	gi|87244605|gb|ABD34655.1|	glucose-6-phosphate dehydrogenase	1.03	1.18	1.15
204	7.59	7.59	22.89	sp|Q5EAC6|CDC37_BOVIN	hsp90 co-chaperone cdc37	1.07	1.10	1.04
205	7.57	7.57	19.54	sp|P04896|GNAS2_BOVIN	gnas complex locus	1.06	0.90	0.85
206	7.54	7.56	27.93	tr|F1MDB3|F1MDB3_BOVIN	glutamine synthetase	1.06	0.98	0.92
207	7.54	7.54	66.02	tr|E1BLC2|E1BLC2_BOVIN	histone h4-like	1.32	1.09	0.83
208	7.53	7.55	17.42	tr|C7AR56|C7AR56_LITWA	integrin beta-1 precursor	0.94	0.99	1.05
209	7.51	7.51	23.58	sp|P61286|PABP1_BOVIN	polyadenylate-binding protein 1	1.06	1.06	1.00
210	7.51	7.51	36.52	sp|Q3MHR0|LYPA1_BOVIN	acyl-protein thioesterase 1	0.92	0.92	1.00
211	7.44	7.44	36.18	gi|75061021|sp|Q5FB29.3|SODC_CAPHI	superoxide dismutase	1.01	0.92	0.91
212	7.43	7.43	33.72	sp|P81623|ERP29_BOVIN	endoplasmic reticulum protein 29	0.98	0.93	0.95
213	7.36	7.36	13.15	tr|F1MER7|F1MER7_BOVIN	heparan sulfate proteoglycan 2	0.90	0.94	1.04
214	7.35	7.57	39.26	sp|P05631|ATPG_BOVIN	atp h+ mitochondrial f1 gamma polypeptide 1	1.06	1.02	0.96
215	7.23	7.24	31.14	sp|P22439|ODPX_BOVIN	pyruvate dehydrogenase protein x mitochondrial isoform 1	0.93	0.96	1.03
216	7.23	7.23	38.60	sp|P79136-2|CAPZB_BOVIN	capping protein (actin filament) muscle z- beta	1.00	0.97	0.97
217	7.22	7.22	16.85	sp|Q1JPJ2|XPP1_BOVIN	xaa-pro aminopeptidase 1	0.97	0.86	0.88
218	7.22	7.22	20.28	tr|Q3T0T9|Q3T0T9_BOVIN	carbonyl reductase	1.19	0.80	0.67
219	7.21	7.21	15.26	tr|F1MNW4|F1MNW4_BOVIN	inter-alpha-trypsin inhibitor heavy chain h2	0.95	1.07	1.13
220	7.19	7.19	35.37	gi|222154129|gb|ACM47241.1|	proteasome subunit alpha type-6	0.94	0.89	0.95
221	7.18	7.18	35.07	sp|Q32L40|TCPA_BOVIN	t-complex protein 1 subunit alpha	1.12	1.07	0.95
222	7.14	7.23	36.78	tr|F1N5J8|F1N5J8_BOVIN	decr1	1.10	1.06	0.96
223	7.10	7.10	24.45	tr|F1ME65|F1ME65_BOVIN	cytoskeleton-associated protein 4	0.83	1.04	1.25
224	7.07	7.08	18.65	tr|F1MZV1|F1MZV1_BOVIN	hexokinase 1	0.91	1.06	1.17
225	7.05	7.05	36.27	sp|P19803|GDIR1_BOVIN	rho gdp dissociation inhibitor alpha	0.93	0.96	1.03
226	6.99	7.01	43.64	tr|A6QPX7|A6QPX7_BOVIN	fibrinogen beta chain	0.88	1.26	1.43
227	6.98	6.98	22.14	sp|Q28034|GLU2B_BOVIN	protein kinase c substrate 80k-h	0.95	0.92	0.97
228	6.95	6.95	35.42	tr|Q3ZBX0|Q3ZBX0_BOVIN	basigin precursor	0.99	0.99	0.99
229	6.92	6.92	20.87	tr|F1MP69|F1MP69_BOVIN	ubiquitin-activating enzyme e1	1.10	1.06	0.96
230	6.89	6.89	24.76	sp|P13214|ANXA4_BOVIN	annexin a4	0.95	0.91	0.96
231	6.85	6.92	40.22	sp|P50397|GDIB_BOVIN	gdp dissociation inhibitor 2	0.99	0.96	0.97

232	6.84	6.84	52.61	tr|F1MIR4|F1MIR4_BOVIN	ras-related protein rab-2a	0.90	0.98	1.08
234	6.61	6.61	26.23	sp|P18902|RET4_BOVIN	retinol binding protein plasma	0.90	0.75	0.84
234	6.82	6.82	23.60	tr|A5D973|A5D973_BOVIN	serine threonine-protein phosphatase 2a 65 kda regulatory subunit a alpha isoform	1.07	1.03	0.96
235	6.82	6.82	33.59	sp|P48818|ACADV_BOVIN	very-long-chain acyl- dehydrogenase	1.06	0.99	0.93
236	6.80	6.80	26.43	tr|F1MWR3|F1MWR3_BOVIN	electron-transfer- alpha polypeptide	0.96	0.85*	0.89*
237	6.64	6.64	15.81	tr|Q2KIL3|Q2KIL3_BOVIN	delta-aminolevulinic acid dehydratase	0.94	0.78	0.83
238	6.61	6.61	42.39	sp|P62833|RAP1A_BOVIN	ras-related protein rap-1a	1.00	0.82*	0.83*
239	6.60	6.65	28.09	sp|P33097|AATC_BOVIN	aspartate cytoplasmic	0.89	0.92	1.03
240	6.57	6.57	29.83	tr|Q08E18|Q08E18_BOVIN	hepatoma-derived growth factor	0.72	0.96	1.34
241	6.43	6.43	17.66	tr|E1BP91|E1BP91_BOVIN	aminopeptidase puromycin sensitive	0.90	0.91	1.01
242	6.38	6.38	35.99	tr|F1N338|F1N338_BOVIN	hydroxyacyl-coenzyme a dehydrogenase	1.14	1.11	0.98
243	6.27	6.51	37.03	sp|P26882|PPID_BOVIN	peptidyl-prolyl cis-trans isomerase d	1.06	1.02	0.96
243	6.36	6.36	31.87	tr|F1MXK5|F1MXK5_BOVIN	propionyl coenzyme a beta polypeptide	0.99	1.01	1.02
244	6.34	6.34	21.99	sp|P08166|KAD2_BOVIN	adenylate kinase 2	1.06	0.97	0.92
245	6.24	6.24	34.34	tr|Q3MHG3|Q3MHG3_BOVIN	mercaptopyruvate sulfurtransferase	1.00	1.00	1.00
246	6.22	11.42	55.99	sp|Q5KR48-2|TPM2_BOVIN	tropomyosin 2	1.06	1.09	1.03
248	6.12	6.12	20.62	tr|F1MPP7|F1MPP7_BOVIN	acyl-coenzyme a synthetase mitochondrial	0.99	0.91	0.92
249	6.10	6.10	31.47	tr|Q3T0L9|Q3T0L9_BOVIN	vesicle-trafficking protein sec22b-like	0.98	1.20	1.22
250	6.09	6.10	52.32	sp|Q56JX8|RS13_BOVIN	ribosomal protein s13	0.96	1.00	1.04
251	6.09	6.09	20.91	tr|F1N234|F1N234_BOVIN	acyl- thioesterase 1	0.99	0.92	0.93
252	6.07	13.51	51.42	tr|F1MNP5|F1MNP5_BOVIN	14-3-3 protein beta alpha	1.05	1.01	0.96
253	6.06	6.06	23.98	tr|F1N550|F1N550_BOVIN	proteasome (macropain) 26s 1	1.08	0.96	0.90
254	6.04	6.04	15.32	tr|Q2KIF2|Q2KIF2_BOVIN	leucine-rich alpha-2-glycoprotein 1	0.98	1.10	1.13
255	6.00	6.00	22.06	tr|A5PKL2|A5PKL2_BOVIN	related ras viral (r-ras) oncogene homolog 2	0.86	0.82	0.95
256	5.99	6.00	21.12	tr|F1MKS3|F1MKS3_BOVIN	thioredoxin domain containing 5	0.94	0.95	1.01
257	5.95	5.95	60.81	tr|F1MHA4|F1MHA4_BOVIN	calmodulin	1.05	1.04	0.99
258	5.84	5.84	13.48	tr|F1MUC5|F1MUC5_BOVIN	collagen alpha-1 chain	1.06	1.07	1.00
259	5.84	5.84	36.18	sp|Q5E9F5|TAGL2_BOVIN	transgelin 2	0.79*	1.13	1.43*
260	5.81	5.81	48.81	tr|F1MK30|F1MK30_BOVIN	ribosomal protein l13	1.04	1.21	1.16
262	5.78	5.78	29.62	sp|Q58DW0|RL4_BOVIN	ribosomal protein l4	1.19	1.23	1.03
263	5.75	5.99	31.78	gi|338163311|gb|AEI74425.1|	platelet glycoprotein 4	1.23*	0.97	0.79*

264	5.75	5.75	20.36	tr|F1MNZ1|F1MNZ1_BOVIN	wd repeat domain 1	1.00	1.01	1.01
265	5.73	5.79	24.47	tr|A4IF88|A4IF88_BOVIN	reticulocalbin-1 precursor	0.70	0.97	1.38
266	5.72	5.72	28.74	sp|P02465|CO1A2_BOVIN	collagen alpha-2 chain precursor	0.92	1.20	1.30
267	5.71	5.71	8.15	tr|Q08DN5|Q08DN5_BOVIN	carnitine o-acetyltransferase	1.05	0.97	0.93
268	5.70	5.70	25.21	gi|18203300|sp|Q9MZS8.1|CATD_SHEEP	cathepsin d	1.03	0.85	0.82
269	5.64	5.64	23.06	tr|F1MWR8|F1MWR8_BOVIN	t-complex protein 1 subunit eta	1.13*	1.06	0.94
270	5.64	5.64	17.09	sp|Q2HJG5|VPS35_BOVIN	vacuolar protein sorting-associated protein 35	1.10	0.95	0.86
271	5.59	5.59	48.48	gi|147744664|gb|ABQ51199.1|	ribosomal protein s10	0.99	1.05	1.06
272	5.53	5.53	61.54	tr|Q3LUG8|Q3LUG8_BUBBU	cytochrome c	1.01	1.03	1.02
273	5.49	5.49	32.68	tr|F1MFQ2|F1MFQ2_BOVIN	udp-glucose pyrophosphorylase 2	1.02	0.97	0.95
274	5.35	5.35	13.88	sp|Q3SWX8|RBBP7_BOVIN	retinoblastoma binding protein 7	0.96	1.29*	1.34*
275	5.34	17.21	58.69	tr|F1MCK2|F1MCK2_BOVIN	neuroblast differentiation-associated protein ahnak-like	0.87	1.00	1.15
276	5.34	5.34	36.67	gi|327199782|gb|AEA36044.1|	decorin	0.95	0.99	1.04
277	5.34	5.34	38.40	gi|51316657|sp|Q6Q311.1|RS25_SHEEP	ribosomal protein s25	0.94	1.00	1.06
278	5.30	5.33	24.77	sp|Q95L54|ANXA8_BOVIN	annexin a8	0.90	1.00	1.11
279	5.27	5.27	16.75	tr|A5PJE3|A5PJE3_BOVIN	fibrinogen alpha chain	0.88	1.40	1.59
280	5.26	7.71	26.21	tr|Q2HJF0|Q2HJF0_BOVIN	inhibitor of carbonic anhydrase-like	1.08	0.97	0.90
281	5.26	5.26	14.85	tr|E1BLV6|E1BLV6_BOVIN	valyl-trna synthetase	0.95	1.02	1.07
282	5.24	5.31	47.69	sp|P80311|PPIB_BOVIN	peptidylprolyl isomerase b (cyclophilin b)	1.21*	1.12	0.93
283	5.23	5.23	36.57	sp|P00171|CYB5_BOVIN	cytochrome b5	1.15	0.85	0.74
284	5.20	5.20	48.68	gi|206558173|sp|A5JST6.1|RS18_CAPHI	ribosomal protein s18	1.01	1.05	1.04
285	5.12	5.12	42.62	sp|Q32PA8|CK067_BOVIN	upf0366 protein c11orf67 homolog isoform 1	1.05	1.08	1.03
286	5.11	5.11	18.45	sp|Q2HJD7|3HIDH_BOVIN	3-hydroxyisobutyrate dehydrogenase	0.94	0.87	0.92
287	5.02	5.02	51.34	tr|F1N0W3|F1N0W3_BOVIN	ribosomal protein l17	1.13	1.21	1.06
288	4.98	4.98	28.60	sp|Q6Q137|SEPT7_BOVIN	septin 7	0.94	1.00	1.06
290	4.95	4.95	16.60	tr|E1BH06|E1BH06_BOVIN	complement component 4a (rodgers blood group)	0.97	1.09	1.12
291	4.94	4.94	46.63	sp|Q3MHY1|CSRP1_BOVIN	cysteine and glycine-rich protein 1	1.29	1.04	0.81
292	4.92	9.41	26.08	tr|B0JYK4|B0JYK4_BOVIN	camp-dependent protein kinase type ii-beta regulatory subunit	1.10	0.87	0.79*
293	4.92	4.92	26.90	tr|E1BAM7|E1BAM7_BOVIN	40s ribosomal protein s23	1.04	1.08	1.04

294	4.87	4.87	26.26	sp|P00586|THTR_BOVIN	thiosulfate sulfurtransferase	0.86	1.05	1.21
296	4.80	5.47	29.59	sp|Q2KIW9|KCY_BOVIN	ump-cmp kinase	1.06	1.01	0.96
297	4.80	4.80	18.54	tr|F1N2W0|F1N2W0_BOVIN	prostaglandin reductase 1	1.10	1.01	0.92
298	4.80	4.80	33.33	tr|Q2KII5|Q2KII5_BOVIN	histone h2b type 2-e-like	1.30	1.08	0.83
299	4.79	7.62	25.66	sp|Q04467|IDHP_BOVIN	isocitrate dehydrogenase	0.99	1.02	1.03
300	4.76	4.76	18.14	tr|E1BB91|E1BB91_BOVIN	collagen alpha-3 chain	0.93	1.04	1.13*
301	4.75	4.75	17.95	gi|7387989|sp|P82197.1|PDXK_SHEEP	pyridoxal kinase	1.01	0.92	0.91
302	4.69	4.69	39.39	sp|Q58DW5|RL5_BOVIN	ribosomal protein l5	1.06	1.10	1.04
303	4.68	8.38	45.11	tr|F1MLW7|F1MLW7_BOVIN	immunoglobulin lambda-like polypeptide 1	1.20	0.76	0.64
304	4.68	4.69	21.76	gi|240849361|ref|NP_001155347.1|	ras homolog gene member a	1.03	0.83	0.81
305	4.66	4.82	41.15	sp|P02453|CO1A1_BOVIN	collagen alpha-1 chain precursor	0.82	0.95	1.16
306	4.65	4.67	24.50	sp|P02722|ADT1_BOVIN	adp atp translocase 1	0.99	1.24	1.25
307	4.64	4.64	15.44	tr|E1BGN2|E1BGN2_BOVIN	uv excision repair protein rad23 homolog b	0.89	0.92	1.03
308	4.60	4.60	31.28	sp|Q3T169|RS3_BOVIN	ribosomal protein s3	0.97	0.97	1.01
309	4.59	4.61	14.32	sp|P56701|PSMD2_BOVIN	proteasome (macropain) 26s non- 2	0.93	1.16	1.25
310	4.59	4.59	14.03	tr|E1B8K6|E1B8K6_BOVIN	nucleolin	0.99	1.05	1.06
311	4.56	4.56	16.45	gi|297660644|gb|ADI49847.1|	leukocyte elastase inhibitor	0.98	1.10	1.11
312	4.55	4.55	11.97	sp|Q28085|CFAH_BOVIN	complement factor h	1.07	1.08	1.01
313	4.54	4.54	16.67	tr|F1MVL2|F1MVL2_BOVIN	mitochondrial short-chain specific acyl-dehydrogenase	1.00	0.94	0.95
314	4.51	4.51	67.24	sp|O18789|RS2_BOVIN	ribosomal protein s2	1.02	1.02	1.00
315	4.51	4.51	33.03	sp|Q2T9X2|TCPD_BOVIN	t-complex protein 1 subunit delta	0.96	1.06	1.10
316	4.51	4.51	28.46	tr|A7E3S8|A7E3S8_BOVIN	heat shock 70kd protein binding protein	1.00	1.01	1.02
317	4.50	4.50	10.88	sp|Q5EAD4|ACDSB_BOVIN	short branched chain specific acyl-mitochondrial	0.99	1.12	1.13
318	4.42	4.42	30.00	sp|P31800|QCR1_BOVIN	cytochrome b-c1 complex subunit mitochondrial precursor	0.99	1.06	1.07
318	4.49	4.49	34.29	sp|A5D989|EF1D_BOVIN	eukaryotic translation elongation factor 1delta	1.10	1.16	1.05
319	4.46	4.46	16.03	tr|E1BBY7|E1BBY7_BOVIN	heat shock 70 kda protein 4	0.92	1.00	1.08
321	4.45	4.45	39.06	tr|F1MY44|F1MY44_BOVIN	heterogeneous nuclear ribonucleoprotein m	1.07	1.15	1.08
322	4.41	4.41	27.69	sp|A4FV37|PRDBP_BOVIN	protein kinase delta binding protein	1.05	0.98	0.93
323	4.41	4.41	12.71	tr|E1BHQ9|E1BHQ9_BOVIN	cell surface glycoprotein muc18	1.17	0.89	0.76

324	4.38	4.38	30.87	tr|F1MT96|F1MT96_BOVIN	chloride intracellular channel 4	0.96	0.96	0.99
325	4.36	4.36	61.29	sp|Q3ZCL8|SH3L3_BOVIN	sh3 domain binding glutamic acid-rich protein like 3	0.87	0.96	1.11
326	4.33	4.33	15.09	tr|F1MU79|F1MU79_BOVIN	peptidyl-prolyl cis-trans isomerase fkbp4	1.03	1.02	0.99
327	4.32	4.32	60.47	sp|P00429|CX6B1_BOVIN	cytochrome c oxidase subunit 6b1-like	1.08	1.31	1.21
328	4.31	4.31	22.66	sp|P13619|AT5F1_BOVIN	atp synthase subunit mitochondrial-like	1.21	1.08	0.89
330	4.29	4.30	30.87	tr|F1MFK6|F1MFK6_BOVIN	serpine1 mrna binding protein 1	0.86	1.12	1.30
331	4.29	4.29	18.21	gi|207028179|ref|NP_001128692.1|	malic enzyme nadp(+)- cytosolic	0.80	0.78	0.97
332	4.29	4.29	21.74	sp|Q3SWX2|ACOT9_BOVIN	acyl- thioesterase 9	1.06	1.01	0.95
333	4.28	4.28	25.00	tr|Q58D01|Q58D01_BOVIN	26s proteasome non-atpase regulatory subunit 4	0.92	1.03	1.12
334	4.27	4.27	18.85	gi|171198344|gb|ACB45430.1|	extracellular superoxide dismutase	0.77*	0.98	1.28
335	4.26	4.26	18.23	sp|Q2KIR8|TDH_BOVIN	l-threonine 3- mitochondrial-like	0.98	1.23	1.26
336	4.24	4.24	16.28	gi|239916452|gb|ACS34712.1|	argininosuccinate lyase	1.10	1.00	0.91
338	4.22	4.22	67.01	sp|P60902|S10AA_BOVIN	protein s100-a10	0.99	1.18	1.19
339	4.22	4.22	24.62	sp|Q29448|HMGCL_BOVIN	3-hydroxymethyl-3-methylglutaryl-coenzyme a lyase	1.01	1.00	0.99
340	4.21	4.21	15.16	tr|F1MUP9|F1MUP9_BOVIN	synaptic vesicle membrane protein vat-1 homolog	0.92	0.91	0.99
341	4.20	4.20	8.89	gi|213021132|ref|NP_001132919.1|	pigment epithelium-derived factor	0.98	0.92	0.93
342	4.19	4.30	29.88	sp|Q5E987|PSA5_BOVIN	proteasome subunit alpha type-5	0.96	0.97	1.01
345	4.16	4.16	25.87	sp|Q3ZCH9|HDHD2_BOVIN	haloacid dehalogenase-like hydrolase domain-containing protein 2-like	0.92	0.84	0.91
346	4.16	4.16	22.73	sp|A4FUA8|CAZA1_BOVIN	capping protein (actin filament) muscle z- alpha 1	0.95	0.95	0.99
347	4.16	4.16	16.73	sp|P35705|PRDX3_BOVIN	peroxiredoxin 3	0.92	0.94	1.02
348	4.15	4.15	21.36	gi|20141075|sp|P09199.2|F16P1_SHEEP	fructose-16-bisphosphatase 1	1.08	1.13	1.04
349	4.13	4.13	34.15	sp|P00435|GPX1_BOVIN	glutathione peroxidase 1	0.94	0.89	0.95
350	4.11	4.42	19.41	sp|Q2KHZ9|GCDH_BOVIN	glutaryl-coenzyme a dehydrogenase	0.89	0.85	0.95
351	4.11	4.11	16.49	tr|F1MDR3|F1MDR3_BOVIN	cytosolic non-specific dipeptidase	1.00	1.01	1.01
352	4.09	4.09	31.90	sp|P04975-2|CLCB_BOVIN	clathrin light chain b	0.95	0.95	0.99
354	4.07	4.07	25.34	gi|209869972|dbj|BAG75458.1|	craniofacial development protein 2-like	0.89	1.04	1.17

355	4.01	18.66	59.08	gi|302124928|gb|ADK93976.1|	3-ketoacyl- mitochondrial	1.00	0.95	0.95
356	4.07	4.07	16.90	gi|218783558|ref|NP_001136367.1|	solute carrier family 25 (mitochondrial carrier phosphate carrier) member 3	1.18	1.37	1.16
357	4.07	4.07	20.12	tr|F1N2N5|F1N2N5_BOVIN	myristoylated alanine-rich c-kinase substrate	0.88	1.06	1.20
357	4.01	4.01	20.53	sp|Q8WN55|PTBP1_BOVIN	polypyrimidine tract binding protein 1	0.83	0.89	1.06
359	4.04	4.04	39.22	sp|Q58DU5|PSA3_BOVIN	proteasome subunit alpha type-3	1.00	0.91	0.91
360	4.04	4.04	26.97	sp|Q58D31|DHSO_BOVIN	sorbitol dehydrogenase	1.04	0.94	0.90
361	4.03	4.03	51.38	sp|P01096|ATIF1_BOVIN	atpase inhibitory factor 1	1.03	1.08	1.06
362	4.02	14.30	60.36	gi|165875541|gb|ABY68598.1|	transaldolase 1	1.00	0.89	0.89
363	4.02	4.02	11.14	tr|F1MGY7|F1MGY7_BOVIN	nadph-cytochrome p450 reductase	1.04	1.10	1.06
364	4.02	4.02	15.43	tr|Q32LK4|Q32LK4_BOVIN	1-acyl-sn-glycerol-3-phosphate acyltransferase gamma	1.06	1.01	0.95
365	4.00	4.00	51.95	gi|1916272|gb|AAB51261.1|	gamma fibrinogen	0.85	1.42	1.66
365	4.02	4.02	23.11	sp|Q3T108|PSB4_BOVIN	proteasome (macropain) beta 4	0.94	0.89	0.95
366	4.01	4.08	57.94	tr|F1MJD5|F1MJD5_BOVIN	histidine triad nucleotide-binding protein 1	1.01	1.00	0.99
367	4.01	4.01	27.94	tr|Q3ZCG6|Q3ZCG6_BOVIN	fk506 binding protein 12kda	1.05	0.91	0.87
368	4.01	4.01	25.74	sp|P35466|S10A4_BOVIN	s100 calcium binding protein a4	0.59	1.08	1.85
369	4.01	4.01	30.89	sp|Q3MHM7|RL35_BOVIN	ribosomal protein l35	0.81	0.96	1.18
371	4.00	4.02	10.98	tr|A7E3V7|A7E3V7_BOVIN	guanine nucleotide-binding protein g g g subunit beta-1	1.18	1.15	0.98
372	4.00	4.00	18.86	tr|Q3SZN8|Q3SZN8_BOVIN	ribonuclease inhibitor	1.03	1.05	1.02
373	4.00	4.00	29.32	tr|Q5U7H2|Q5U7H2_BOVIN	ribosomal protein l15	1.11	1.24	1.12
374	4.00	4.00	26.96	gi|118403290|ref|NP_001072123.1|	macrophage migration inhibitory factor	0.95	0.97	1.03
375	3.92	4.20	27.64	tr|F1MG70|F1MG70_BOVIN	26s protease regulatory subunit 6b	1.00	1.20	1.21
376	3.92	4.00	19.11	tr|A6QLT9|A6QLT9_BOVIN	alanyl-trna cytoplasmic	0.94	0.99	1.06
377	3.91	3.91	26.58	tr|F1N690|F1N690_BOVIN	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase mitochondrial	0.99	0.96	0.96
378	3.86	3.86	20.14	tr|F1MU66|F1MU66_BOVIN	cytochrome b-c1 complex subunit mitochondrial-like	0.92	0.92	0.99
379	3.85	3.85	9.15	sp|Q9XTA2|PPCE_BOVIN	prolyl endopeptidase	0.94	0.91	0.97
380	3.83	4.42	16.59	tr|F1MGC0|F1MGC0_BOVIN	mitochondrial succinyl- ligase	1.02	0.86	0.84

381	3.78	3.78	29.35	tr|Q8HYY3|Q8HYY3_BOVIN	non-muscle caldesmon	0.93	0.99	1.06
382	3.78	3.78	30.62	tr|F1MM83|F1MM83_BOVIN	6-phosphogluconolactonase	0.92	0.95	1.03
383	3.77	3.77	21.55	tr|E1B8P9|E1B8P9_BOVIN	mitogen-activated protein kinase 3	0.97	0.99	1.02
384	3.76	3.76	31.43	sp|P02510|CRYAB_BOVIN	alpha-crystallin b chain	1.23	1.16	0.94
385	3.76	3.76	29.65	sp|Q5E936|TXD12_BOVIN	thioredoxin domain containing 12 (endoplasmic reticulum)	1.01	1.08	1.07
386	3.74	59.61	76.19	tr|F1MEQ0|F1MEQ0_BOVIN	serum albumin precursor	0.86	0.89	1.03
387	3.74	3.75	22.55	tr|F1MEN8|F1MEN8_BOVIN	protein disulfide isomerase family member 4	0.86	0.93	1.09
388	3.74	3.74	28.87	sp|Q3MHN0|PSB6_BOVIN	proteasome (macropain) beta 6	1.12	0.94	0.83
389	3.73	3.74	33.33	sp|Q76I81|RS12_BOVIN	ribosomal protein s12	1.10	1.06	0.96
391	3.71	3.71	14.61	tr|Q3T0N2|Q3T0N2_BOVIN	lysyl-trna synthetase	0.93	1.07	1.15
392	3.71	3.71	17.81	gi|339522297|gb|AEJ84313.1|	peroxiredoxin 5	1.10	0.84	0.76*
393	3.70	3.70	35.89	sp|Q1LZ95-2|IDI1_BOVIN	isopentenyl-diphosphate delta-isomerase 1	0.83	1.06	1.28
394	3.63	3.63	21.93	tr|F1MBW7|F1MBW7_BOVIN	sh3 domain-binding glutamic acid-rich-like protein	0.89	0.80	0.90
395	3.68	3.68	35.27	sp|Q2TBX6|PSB1_BOVIN	proteasome (macropain) beta 1	0.90	1.06	1.17
396	3.67	7.79	78.69	gi|2318026|gb|AAB66468.1|	serotransferrin precursor	0.88	0.92	1.05
397	3.65	3.65	30.27	sp|Q3ZCF0|DCTN2_BOVIN	dynactin 2	0.98	1.03	1.05
398	3.59	3.59	23.03	tr|F1N5F2|F1N5F2_BOVIN	branched chain keto acid dehydrogenase alpha polypeptide	0.83	0.81	0.98
400	3.61	3.61	16.54	sp|Q9GMB8|SYSC_BOVIN	seryl-trna synthetase	0.92	0.85	0.92
401	3.59	3.59	35.68	sp|P13621|ATPO_BOVIN	atp h+ mitochondrial f1 o subunit	1.33	1.12	0.85
402	3.58	3.58	41.73	gi|89628429|gb|ABD77505.1|	ribosomal protein l34	1.21	1.19	0.99
403	3.58	3.58	23.84	sp|Q5E984|TCTP_BOVIN	tumor translationally-controlled 1	0.88	1.02	1.15
409	3.47	3.55	58.82	sp|Q3ZBH8|RS20_BOVIN	40s ribosomal protein s20	0.96	1.04	1.08
410	3.47	3.47	19.92	tr|F1N3P2|F1N3P2_BOVIN	ubiquitin carboxyl-terminal hydrolase 5 isoform 2	1.08	1.10	1.02
412	3.45	3.45	45.31	tr|F1N2I5|F1N2I5_BOVIN	carboxymethylenebutenolidase homolog	1.03	0.95	0.93
413	3.45	3.45	40.84	tr|B6VAP7|B6VAP7_BOVIN	cell division control protein 42 homolog isoform 1	0.98	0.96	0.98
415	3.41	3.41	12.43	tr|E1BFV0|E1BFV0_BOVIN	karyopherin beta 1	1.02	1.06	1.04
416	3.41	3.41	25.74	gi|197725615|gb|ACH73011.1|	alpha-1-acid glycoprotein	2.65*	2.20*	0.83

417	3.41	3.41	33.33	sp|Q0VCY1|VAPA_BOVIN	vamp-associated protein a	1.36	1.06	0.78
419	3.40	55.52	66.90	sp|Q29443|TRFE_BOVIN	serotransferrin precursor	1.05	0.80	0.76
421	3.40	3.41	15.57	tr|Q0II43|Q0II43_BOVIN	thioredoxin-like 1	0.83	0.88	1.06
422	3.38	3.50	28.29	tr|F1MCT8|F1MCT8_BOVIN	syncrip protein	1.02	1.07	1.04
423	3.37	6.10	50.67	tr|Q2KIV8|Q2KIV8_BOVIN	glutathione s-transferase mu 3	1.01	0.99	0.98
424	3.37	3.45	16.19	tr|E1BPX9|E1BPX9_BOVIN	mitochondrial leucine-rich ppr motif-containing protein	1.05	1.12	1.06
426	3.36	3.36	19.69	sp|Q3SWY2|ILK_BOVIN	integrin-linked protein kinase	1.18	1.04	0.88
427	3.35	3.35	39.00	sp|P11966|ODPB_BOVIN	pyruvate dehydrogenase beta	1.06	1.05	0.99
428	3.35	3.35	32.11	gi|119712147|gb|ABL96619.1|	osteoglycin	0.93	0.97	1.04
428	3.24	3.24	32.35	gi|7531050|sp|Q9XT28.1|ATOX1_SHEEP	copper transport protein atox1	1.20	1.25	1.05
429	3.22	6.11	28.88	tr|A6QM09|A6QM09_BOVIN	uncharacterized protein	0.98	0.45	0.46
429	3.33	13.76	53.52	gi|187607525|ref|NP_001119823.1|	tropomyosin alpha-1 chain	0.92	1.04	1.13
430	3.33	3.41	24.25	sp|Q5E9F9|PRS7_BOVIN	26s protease regulatory subunit 7	0.98	1.14	1.16
431	3.32	3.38	30.63	gi|256665379|gb|ACV04835.1|	superoxide dismutase	1.02	0.97	0.96
432	3.31	3.31	21.70	tr|A2VDN7|A2VDN7_BOVIN	heterogeneous nuclear ribonucleoprotein u	0.93	1.08	1.16
433	3.31	3.31	11.14	tr|F1MQG3|F1MQG3_BOVIN	peroxisomal acyl-coenzyme a oxidase 3	0.97	1.18	1.22
435	3.31	3.31	6.86	tr|E1B9D1|E1B9D1_BOVIN	rac-beta serine threonine-protein kinase	0.91	1.03	1.13
436	3.30	3.32	18.85	sp|Q3SZ20|GLYM_BOVIN	serine hydroxymethyltransferase 2	1.00	1.07	1.07
437	3.25	3.26	34.16	tr|Q3ZBX5|Q3ZBX5_BOVIN	aminoacyl trna synthase complex-interacting multifunctional protein 1	1.00	0.98	0.98
438	3.24	3.24	28.87	sp|A6QLG5|RS9_BOVIN	ribosomal protein s9	0.91	1.09	1.19
439	3.24	3.24	36.13	tr|F1N477|F1N477_BOVIN	coactosin-like 1	0.79	0.89	1.13
440	3.23	3.23	28.70	sp|Q0VCP1|KAD4_BOVIN	adenylate kinase 3-like 1	0.83	1.05	1.26
441	3.22	156.45	59.23	gi|78214939|gb|ABB36643.1|	fatty acid synthase	0.99	1.07	1.08
441	3.13	3.41	12.01	REV_tr|F1N757|F1N757_BOVIN	uncharacterized protein	0.95	0.96	1.01
442	3.22	3.22	32.48	tr|Q3T0U3|Q3T0U3_BOVIN	es1 protein mitochondrial-like isoform 1	1.12	0.99	0.88
443	3.21	8.19	35.66	tr|E1BKB5|E1BKB5_BOVIN	heterogeneous nuclear ribonucleoprotein a1	1.01	1.07	1.06
445	3.15	3.15	29.92	tr|D3JUI8|D3JUI8_BOVIN	heterogeneous nuclear ribonucleoprotein g	0.93	0.93	1.01
447	3.12	3.12	17.73	tr|E1BIB4|E1BIB4_BOVIN	heterogeneous nuclear ribonucleoprotein l	0.89	1.07	1.19
448	3.11	3.11	35.71	tr|E1BG13|E1BG13_BOVIN	ribosomal protein s6	0.99	1.03	1.04
449	3.10	3.10	19.21	gi|215983084|ref|NP_001135989.1|	camp-dependent protein kinase type i-alpha	0.90	0.96	1.06

					regulatory subunit			
450	3.10	3.10	25.00	tr|F1MUW8|F1MUW8_BOVIN	glyoxalase i	1.17	1.04	0.89
452	3.10	3.10	16.33	tr|Q2NKU4|Q2NKU4_BOVIN	calcium-regulated heat stable protein 1	0.85	1.08	1.28
454	3.08	3.09	18.81	sp|P13213|SPRC_BOVIN	secreted cysteine-rich	1.04	1.18	1.14
455	3.08	3.08	23.13	tr|F1MWE0|F1MWE0_BOVIN	26s protease regulatory subunit 6a	0.92	1.02	1.11
456	3.08	3.08	19.46	sp|Q3T0S6|RL8_BOVIN	ribosomal protein l8	1.07	1.11	1.04
457	3.07	3.13	13.07	tr|E1BDK6|E1BDK6_BOVIN	laminin subunit beta-2	1.07	0.98	0.92
458	3.07	3.07	34.09	sp|Q3ZCK3|BPNT1_BOVIN	3 (2) -bisphosphate nucleotidase 1	0.76	0.80	1.05
459	3.06	24.93	51.84	tr|Q58DT9|Q58DT9_BOVIN	alpha smooth aorta	0.98	1.11	1.13
459	2.86	13.49	39.60	gi|339521995|gb|AEJ84162.1|	atp h+ mitochondrial f1 alpha subunit cardiac muscle	1.06	1.11	1.04
460	3.06	3.06	32.76	tr|E3SAZ8|E3SAZ8_BOVIN	nucleophosmin (nucleolar phosphoprotein numatrin)	0.79*	1.07	1.35*
461	2.83	2.83	36.05	sp|Q3SZ52|UB2V1_BOVIN	ubiquitin-conjugating enzyme e2 variant 1	1.12	0.96	0.86
462	3.05	3.05	26.25	tr|F1N0N6|F1N0N6_BOVIN	upf0587 protein c1orf123 homolog	1.02	0.93	0.91
463	3.04	3.05	14.82	tr|F1MAZ1|F1MAZ1_BOVIN	microtubule-associated protein 4	0.96	1.03	1.07
465	3.01	3.01	31.42	tr|A6QLB7|A6QLB7_BOVIN	adenylate cyclase-associated protein 1	0.99	0.96	0.97
466	3.01	3.01	21.15	tr|A6H7H3|A6H7H3_BOVIN	estradiol 17-beta-dehydrogenase 12	1.06	0.85	0.80
466	2.81	2.81	22.22	tr|F1N5W9|F1N5W9_BOVIN	guanine nucleotide binding protein (g protein) beta polypeptide 2-like 1	0.97	1.05	1.08
467	3.00	3.00	31.33	sp|Q4U5R3|PSME1_BOVIN	proteasome (macropain) activator subunit 1 (pa28 alpha)	0.84	1.00	1.19
468	2.98	3.09	18.32	tr|Q9XSX1|Q9XSX1_BOVIN	calpastatin	0.86	1.00	1.17
472	2.97	2.97	21.62	tr|A7MB21|A7MB21_BOVIN	fermitin family homolog 2	1.06	0.95	0.90
473	2.97	2.97	43.20	sp|Q2HJH9|PDCD5_BOVIN	programmed cell death 5	1.01	1.01	1.00
475	2.97	2.97	86.59	gi|147744642|gb|ABQ51188.1|	nadh dehydrogenase	1.11	1.10	0.99
476	2.95	3.70	20.41	sp|P68002|VDAC2_BOVIN	voltage-dependent anion channel 2	1.01	1.12	1.11
477	2.94	2.94	24.63	tr|Q2HJI1|Q2HJI1_BOVIN	succinate dehydrogenase	1.01	1.05	1.05
478	2.94	2.94	16.40	tr|F1MNN6|F1MNN6_BOVIN	major vault protein	0.87	0.98	1.12
480	2.62	2.62	11.97	tr|A7Z066|A7Z066_BOVIN	calnexin	1.21	1.08	0.89
480	2.93	2.93	14.54	sp|Q08E20|ESTD_BOVIN	esterase d formylglutathione hydrolase	1.00	0.90	0.90
482	2.92	2.93	18.27	tr|Q95M40|Q95M40_BOVIN	thioredoxin domain-containing protein 17	1.01	0.92	0.91

483	2.92	2.92	17.06	sp|Q3T147|DX39B_BOVIN	hla-b associated transcript 1	1.02	1.00	0.98
484	2.92	2.92	17.94	sp|P07514|NB5R3_BOVIN	cytochrome b5 reductase 3	0.98	0.97	0.99
486	2.89	2.89	18.55	tr|A6QPP2|A6QPP2_BOVIN	heparin cofactor 2	1.10	0.90	0.82
490	2.83	2.83	21.62	tr|F1MR86|F1MR86_BOVIN	four and a half lim domains 1	1.26	1.04	0.83
491	2.82	8.91	45.53	tr|F1MPK3|F1MPK3_BOVIN	member ras oncogene family	1.01	0.98	0.98
492	2.82	2.91	7.18	tr|F1N4K8|F1N4K8_BOVIN	fibrillin-1 precursor	1.14	0.90	0.79
493	2.49	2.49	28.62	sp|Q2HJ38|CNN1_BOVIN	calponin smooth muscle	1.21	0.96	0.79
493	2.82	2.84	23.64	tr|F1MZK4|F1MZK4_BOVIN	basic leucine zipper and w2 domain-containing protein 1-like	1.05	1.06	1.01
494	2.82	2.82	16.84	sp|Q3SX44|DDAH2_BOVIN	dimethylarginine dimethylaminohydrolase 2	0.80	0.83	1.03
496	2.81	2.81	24.72	gi|261244968|ref|NP_001159667.1|	prothrombin precursor	0.96	0.88	0.92
496	2.47	2.47	19.16	sp|Q3ZCD7|TECR_BOVIN	synaptic 2	1.13	1.00	0.88
497	2.80	2.84	29.84	tr|F1N3H1|F1N3H1_BOVIN	calumenin	0.84*	0.88*	1.05
500	2.76	3.07	22.22	tr|A7MBI5|A7MBI5_BOVIN	dihydropyrimidinase-related protein 3	0.99	1.06	1.07
501	2.76	2.77	26.71	gi|218783550|ref|NP_001136363.1|	atp synthase subunit mitochondrial-like	1.22	1.34	1.10
502	2.76	2.76	9.56	tr|F1N2W3|F1N2W3_BOVIN	liver carboxylesterase-like isoform 1	0.56	0.74	1.33
503	2.42	2.48	18.11	sp|P04973|CLCA_BOVIN	clathrin light chain a	1.28	1.05	0.83
503	2.75	2.75	17.93	sp|P20072|ANXA7_BOVIN	annexin a7	0.98	1.24	1.26
504	2.74	2.74	23.94	sp|O97764|QOR_BOVIN	quinone oxidoreductase	1.05	0.94	0.89
505	2.74	2.74	9.84	sp|P53620|COPG_BOVIN	coatomer protein subunit gamma	1.01	1.02	1.01
506	2.38	2.38	2.17	sp|Q6URK6|CADH5_BOVIN	cadherin-5 precursor	0.74	0.98	1.32
506	2.72	5.18	25.40	tr|Q2KIE8|Q2KIE8_BOVIN	phosphoenolpyruvate cytosolic	1.03	1.19	1.15
507	2.70	2.70	6.64	gi|168693441|ref|NP_001108236.1|	cd9 antigen	1.08	1.05	0.97
515	2.64	2.64	21.59	sp|Q5E9D3|CHCH3_BOVIN	coiled-coil-helix-coiled-coil-helix domain containing 3	0.95	1.00	1.06
517	2.30	2.30	29.26	tr|A0A027|A0A027_BISBI	mhc class i antigen	1.06	0.98	0.93
517	2.63	2.65	23.04	sp|Q5E9E6|RL10A_BOVIN	ribosomal protein l10a	1.18	1.14	0.97
518	2.63	2.63	12.62	tr|Q58D70|Q58D70_BOVIN	serine threonine-protein phosphatase 2a catalytic subunit alpha isoform	1.00	1.06	1.05
520	2.62	2.62	26.49	tr|F1MYI3|F1MYI3_BOVIN	creatine brain	1.15	1.08	0.93
522	2.60	2.60	44.81	gi|261244964|ref|NP_001159665.1|	eukaryotic translation initiation factor 5a	1.23	1.19	0.97
523	2.59	2.59	12.45	tr|A2VDN8|A2VDN8_BOVIN	actin binding 1c	1.01	1.08	1.08

524	2.59	2.59	22.29	sp|Q862I1|RL24_BOVIN	ribosomal protein l24	0.97	1.16	1.19
525	2.58	2.64	27.38	tr|A2VE06|A2VE06_BOVIN	ribosomal protein s4	0.97	1.02	1.05
527	2.55	6.68	33.21	tr|Q0VC97|Q0VC97_BOVIN	carbonyl reductase 3	0.86	0.90	1.05
529	2.55	2.55	24.75	tr|E1BQ37|E1BQ37_BOVIN	splicing proline- and glutamine-rich	1.19	0.95	0.80
530	2.55	2.55	12.03	tr|F1N5F8|F1N5F8_BOVIN	alcohol dehydrogenase 5 (class iii) chi polypeptide	0.96	0.89	0.93
531	2.54	2.55	16.80	sp|P11181|ODB2_BOVIN	dihydrolipoamide branched chain transacylase e2	1.06	1.11	1.04
532	2.54	2.54	16.74	sp|P08760|KAD3_BOVIN	adenylate kinase 3	1.06	1.03	0.97
533	2.52	2.52	24.96	tr|E1BJA2|E1BJA2_BOVIN	apoptosis-inducing factor mitochondrial	0.97	1.06	1.09
534	2.51	2.52	26.42	sp|Q2HJ97|PHB2_BOVIN	prohibitin 2	0.93	1.00	1.08
535	2.19	2.22	22.41	tr|Q3ZC41|Q3ZC41_BOVIN	acetyl-coenzyme a acyltransferase 1	0.70	1.04	1.48
536	2.51	2.51	38.01	tr|Q862H8|Q862H8_BOVIN	ribosomal protein sa	1.13	1.02	0.90
537	2.50	2.50	20.99	sp|Q2KJI3|FA49B_BOVIN	protein fam49b-like isoform 2	0.88	1.01	1.14
538	2.50	2.50	26.15	tr|F1MNZ2|F1MNZ2_BOVIN	cytochrome c-1	1.28	1.16	0.91
539	2.49	2.49	13.53	tr|F1MPE5|F1MPE5_BOVIN	importin 5	1.08	1.18	1.10
542	2.15	2.15	22.22	sp|Q2KHU0|SERB_BOVIN	phosphoserine phosphatase	0.88	0.80	0.91
545	2.46	2.46	26.56	tr|F1N3R2|F1N3R2_BOVIN	60s ribosomal protein l12-like	1.17	1.15	0.99
548	2.12	2.12	13.73	tr|F1N0I0|F1N0I0_BOVIN	adaptor-related protein complex mu 1 subunit	0.94	1.11	1.19
548	2.44	2.44	36.82	sp|Q5E9K0|PSB2_BOVIN	proteasome (macropain) beta 2	1.00	0.91	0.91
552	2.10	2.12	18.74	tr|Q29RQ2|Q29RQ2_BOVIN	kh domain rna signal transduction associated 1	0.97	1.07	1.10
555	2.42	2.52	15.22	tr|A7MBA2|A7MBA2_BOVIN	26s proteasome non-atpase regulatory subunit 1	1.01	1.07	1.06
555	2.09	2.20	9.53	tr|E1BGJ0|E1BGJ0_BOVIN	prolow-density lipoprotein receptor-related protein 1	0.95	1.01	1.06
557	2.42	2.42	38.98	tr|Q3SZ32|Q3SZ32_BOVIN	transcription elongation factor b polypeptide 2-like	0.98	0.93	0.95
559	2.40	6.90	18.66	tr|F1MYX5|F1MYX5_BOVIN	lymphocyte cytosolic protein 1 (l-plastin)	0.84	0.90	1.08
561	2.39	2.39	66.87	sp|Q8SQ21|HINT2_BOVIN	histidine triad nucleotide binding protein 2	0.98	1.01	1.04
564	2.38	2.38	19.41	sp|Q5E964|PSD13_BOVIN	proteasome (macropain) 26s non-13	1.25	1.11	0.89
565	2.06	2.06	19.58	sp|Q3Y5Z3|ADIPO_BOVIN	adiponectin	0.80	0.94	1.17
568	2.37	2.37	20.80	sp|P00517|KAPCA_BOVIN	protein camp- alpha	0.98	0.96	0.98

569	2.37	2.37	14.94	sp|Q2KJ39|RCN3_BOVIN	reticulocalbin ef-hand calcium binding domain	0.87	1.36	1.57
570	2.36	2.37	34.69	sp|P79342|S10AD_BOVIN	protein s100-a13	0.85	1.01	1.19
574	2.34	2.34	30.18	gi|7687909|gb|AAD42800.2|	glutathione s-transferase a1	1.04	1.07	1.02
576	2.33	2.35	15.82	tr|F1MKG2|F1MKG2_BOVIN	type alpha 2	0.90	0.78	0.86
579	2.32	2.32	14.63	tr|E1B819|E1B819_BOVIN	copine iii	0.78*	0.97	1.25*
582	2.03	2.03	14.26	tr|F1MCU7|F1MCU7_BOVIN	erythrocyte protein band	1.17	1.21	1.04
582	2.30	2.30	16.08	sp|Q5EA79|GALM_BOVIN	aldose 1-epimerase	0.70*	0.72	1.03
584	2.29	2.29	22.40	tr|Q2KIF3|Q2KIF3_BOVIN	cytochrome p-450	1.23	0.89	0.72
585	2.26	2.26	32.65	tr|Q3SX47|Q3SX47_BOVIN	heterogeneous nuclear ribonucleoprotein c (c1 c2)	0.90	1.10	1.22*
586	2.26	2.26	28.21	sp|Q3T0Y5|PSA2_BOVIN	proteasome subunit alpha type-2-like	0.98	1.00	1.02
587	2.25	3.80	27.66	tr|F1N6N3|F1N6N3_BOVIN	eh-domain containing 1	0.86*	0.98	1.13*
588	2.25	2.25	22.19	tr|F2Z4F5|F2Z4F5_BOVIN	dipeptidyl-peptidase 3	0.88	0.91	1.03
589	2.25	2.25	68.67	sp|Q32PB8|RS21_BOVIN	ribosomal protein s21	0.99	1.02	1.03
592	2.21	2.53	15.72	tr|F1MNI4|F1MNI4_BOVIN	ras-related protein rab-5b	1.11	1.02	0.91
595	2.01	2.04	9.97	tr|F1MW44|F1MW44_BOVIN	coagulation factor a1 polypeptide	0.81*	1.15*	1.41*
595	2.18	2.34	13.89	tr|C6K7D3|C6K7D3_BOVIN	sirtuin 2	0.83	0.85	1.03
596	2.01	2.02	17.53	sp|Q32LE5|ASGL1_BOVIN	l-asparaginase	0.94	1.05	1.11*
596	2.17	2.17	55.38	tr|E1BLY1|E1BLY1_BOVIN	ribosomal protein s15a	1.09	1.11	1.02
597	2.01	2.01	30.31	tr|F1MF48|F1MF48_BOVIN	hydroxysteroid dehydrogenase-like protein 2	0.87	0.84*	0.96
597	2.17	2.17	37.01	sp|Q9NZ50|SYUG_BOVIN	synuclein gamma	1.08	0.91	0.84
598	2.15	2.15	19.95	tr|Q148I9|Q148I9_BOVIN	alpha-methylacyl- racemase	1.24	1.04	0.84
599	2.15	2.15	34.03	tr|A1XED5|A1XED5_BOVIN	ribosomal protein s5	1.11	1.23	1.11
600	2.15	2.15	46.48	sp|P63212|GBG2_BOVIN	guanine nucleotide-binding protein g g g subunit gamma-2-like	1.13	0.97	0.86
601	2.14	2.97	10.69	tr|E1BF20|E1BF20_BOVIN	heterogeneous nuclear ribonucleoprotein h	0.93	1.02	1.09
602	2.14	2.19	12.08	sp|A7YWE4|AL4A1_BOVIN	aldehyde dehydrogenase 4 member a1	1.09	1.02	0.94
603	2.14	2.14	40.14	tr|E1BKU3|E1BKU3_BOVIN	glutathione s-transferase omega-1-like	1.13	0.94	0.83
606	2.13	11.48	35.03	tr|E1BLN2|E1BLN2_BOVIN	desmin	0.97	0.92	0.95
607	2.00	9.19	31.71	sp|P68509|1433F_BOVIN	tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation eta polypeptide	1.14	1.04	0.91
608	2.13	2.13	42.72	tr|Q3ZCK2|Q3ZCK2_BOVIN	ras-related protein ral-a-like	1.03	0.98	0.95

609	2.13	2.13	24.91	tr|Q3ZC44|Q3ZC44_BOVIN	heterogeneous nuclear ribonucleoprotein a b	0.95	1.19	1.25
610	2.13	2.13	15.17	tr|Q3SZZ9|Q3SZZ9_BOVIN	fibrinogen gamma chain	1.06	1.24	1.17
611	2.00	2.10	20.46	tr|F1MR38|F1MR38_BOVIN	mitochondrial carrier homolog 2 (elegans)	0.72	1.07	1.49
611	2.13	2.13	42.14	sp|Q3T057|RL23_BOVIN	ribosomal protein l23	1.18	1.18	1.00
615	2.12	2.12	20.26	sp|Q29460|PA1B3_BOVIN	platelet-activating factor acetylhydrolase ib subunit gamma	0.89	1.16	1.31
616	2.11	2.11	28.43	tr|A6QPM9|A6QPM9_BOVIN	parathymosin	0.85	0.82	0.97
618	2.10	2.10	22.88	tr|A5D9H5|A5D9H5_BOVIN	heterogeneous nuclear ribonucleoprotein d	0.82	1.03	1.24*
619	2.10	2.10	35.75	tr|F1MJQ1|F1MJQ1_BOVIN	ras-related protein rab-7a	1.04	0.97	0.93
623	2.08	2.37	15.19	tr|A7YY47|A7YY47_BOVIN	lamin b1	0.87	0.78	0.89
624	2.08	2.08	7.61	tr|Q2KIW1|Q2KIW1_BOVIN	paraoxonase 1	1.07	1.12	1.05
627	2.05	4.66	24.93	gi|12649541|gb|AAB92374.2|	complement component c3d	1.09	1.00	0.92
632	2.04	2.04	73.72	gi|47606778|sp|P80601.3|UK114_CAPHI	heat-responsive protein 12	0.87	1.04	1.19
633	2.04	2.04	11.90	tr|F1MK08|F1MK08_BOVIN	tripeptidyl-peptidase 1 precursor	0.90	0.86	0.96
634	2.04	2.04	22.52	sp|Q3SYX9|ARPC5_BOVIN	actin-related protein 2 3 complex subunit 5	0.97	1.06	1.09
636	2.04	2.04	13.69	sp|P05630|ATPD_BOVIN	atp synthase subunit mitochondrial precursor	1.08	0.97	0.90
637	2.04	2.04	14.63	gi|51702791|sp|Q7M323.1|PLMN_CAPHI	plasminogen precursor	0.86	1.05	1.22
641	2.03	2.03	10.00	tr|A7MB23|A7MB23_BOVIN	phosphatidylinositol-binding clathrin assembly protein	1.02	0.91	0.89
642	2.03	2.03	9.23	sp|Q17QN6|EM55_BOVIN	55 kda erythrocyte membrane protein	0.96	0.98	1.01
648	2.02	2.02	36.00	sp|P81644|APOA2_BOVIN	apolipoprotein a-ii	0.99	0.80	0.81
651	2.01	2.01	21.65	tr|F1MX45|F1MX45_BOVIN	aspartyl aminopeptidase	1.17	1.00	0.85
652	2.01	2.01	8.44	sp|Q9MZ08|BCAM_BOVIN	basal cell adhesion molecule	1.14	0.96	0.84
653	2.01	2.01	18.79	sp|A6QLY4|ISOC1_BOVIN	isochorismatase domain-containing protein 1	0.81	1.02	1.26
655	2.01	2.01	18.16	tr|F1N7M0|F1N7M0_BOVIN	cd5 antigen-like	0.93	1.10	1.17
658	2.01	2.01	10.04	sp|A7YY49|SURF4_BOVIN	surfeit 4	0.92	0.95	1.03
659	2.01	2.01	20.56	gi|242247463|ref|NP_001156018.1|	adp-ribosylation factor 4	0.95	0.90	0.94
664	2.01	2.01	7.86	sp|P68401|PA1B2_BOVIN	platelet-activating factor acetylhydrolase ib subunit beta	0.91	1.08	1.19
667	1.72	1.73	24.42	tr|E1BME9|E1BME9_BOVIN	nadh dehydrogenase	0.85	1.45	1.71
667	2.00	12.72	30.43	tr|Q3ZCI4|Q3ZCI4_BOVIN	phosphogluconate dehydrogenase	0.86	0.92	1.07
668	2.00	12.02	59.56	gi|7274396|gb|AAF44753.1|	mitochondrial malate dehydrogenase nad	1.19	0.62	0.52

669	2.00	10.66	52.38	sp|P28801|GSTP1_BOVIN	glutathione s-transferase pi	1.02	0.94	0.92
670	2.00	9.72	35.06	gi|339522395|gb|AEJ84362.1|	serum deprivation-response protein	0.76	0.91	1.20
671	2.00	9.29	53.88	sp|Q3SZI4|1433T_BOVIN	tyrosine 3-monooxygenase tryptophan 5-monooxygenase activation theta polypeptide	0.99	0.96	0.97
674	2.00	2.99	16.03	sp|Q5E9H7|DHB12_BOVIN	estradiol 17-beta-dehydrogenase 12	0.97	0.94	0.97
680	2.00	2.22	15.63	sp|Q32LA7|H2AV_BOVIN	histone h2a.v	1.09	1.01	0.92
683	2.00	2.02	6.95	tr|B1PK18|B1PK18_BOVIN	1,4-alpha-glucan branching enzyme 1	1.04	0.99	0.96
684	2.00	2.02	20.83	sp|Q148J6|ARPC4_BOVIN	actin-related protein 2 3 complex subunit 4	1.03	1.17	1.13
686	2.00	2.00	23.09	tr|Q08DB4|Q08DB4_BOVIN	copine i	0.79	0.78	0.98
689	1.60	3.31	23.37	sp|P27214-2|ANX11_BOVIN	annexin a11	0.75	0.85	1.13
694	2.00	2.00	9.36	tr|Q3ZC60|Q3ZC60_BOVIN	cd200 molecule	0.98	1.06	1.08
695	2.00	2.00	10.27	tr|Q2NKZ9|Q2NKZ9_BOVIN	serine carboxypeptidase 1	1.01	0.78	0.77
698	2.00	2.00	8.72	sp|Q5E9E2|MYL9_BOVIN	myosin regulatory light polypeptide 9-like	0.97	1.02	1.05
702	2.00	2.00	18.45	tr|Q862H7|Q862H7_BOVIN	s100 calcium binding protein a11	0.95	1.11	1.17
705	2.00	2.00	23.01	tr|F1MW03|F1MW03_BOVIN	thiosulfate sulfurtransferase rhodanese-like domain-containing protein 1-like	1.33	1.06	0.80
710	2.00	2.00	28.99	sp|Q56JX6|RS28_BOVIN	ribosomal protein s28	1.01	1.14	1.12
712	2.00	2.00	8.36	sp|Q3SZD1|PEX19_BOVIN	peroxisomal biogenesis factor 19	0.82	1.04	1.28
718	2.00	2.00	4.37	sp|Q3T0M7|RANG_BOVIN	ran-specific gtpase-activating protein	1.08	1.08	1.00
719	2.00	2.00	11.25	gi|146395421|gb|ABQ28660.1|	enoyl coenzyme a short mitochondrial	0.91	1.00	1.10
721	1.98	1.98	21.88	sp|Q3ZBF7|TEBP_BOVIN	prostaglandin e synthase 3	1.08	1.12	1.04
725	1.91	1.91	31.55	tr|Q862I9|Q862I9_BOVIN	ribosomal p0	1.23	0.97	0.79
727	1.41	1.41	31.15	sp|Q3B7M5|LASP1_BOVIN	lim and sh3 protein 1	0.98	0.90	0.92
727	1.90	1.90	53.01	tr|F1MGG6|F1MGG6_BOVIN	cytochrome c oxidase subunit mitochondrial-like	0.88	0.79	0.90
728	1.89	3.70	17.19	tr|Q3SYT9|Q3SYT9_BOVIN	poly binding protein 2	1.02	1.05	1.03
729	1.89	1.91	17.93	tr|E1BGC1|E1BGC1_BOVIN	methylcrotonoyl- carboxylase subunit mitochondrial	0.93	0.94	1.01
729	1.41	1.41	9.20	sp|Q3ZCK9|PSA4_BOVIN	proteasome subunit alpha type-4-like	0.96	0.97	1.01
730	1.88	1.88	17.30	tr|F1MM14|F1MM14_BOVIN	cd2-associated protein	0.78	0.87	1.12
732	1.39	2.43	12.34	tr|E1BDG9|E1BDG9_BOVIN	aldehyde dehydrogenase family 1 member mitochondrial	0.93	0.99	1.07

733	1.79	1.81	4.84	tr|F1N632|F1N632_BOVIN	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	1.17	0.92	0.79
737	1.77	1.78	29.41	tr|F1MWQ2|F1MWQ2_BOVIN	annexin a3	0.89	0.98	1.11
739	1.77	1.78	17.11	tr|Q2NKR5|Q2NKR5_BOVIN	adipose most abundant gene transcript 2	1.08	0.92	0.85
740	1.76	1.76	70.41	sp|P25417|CYTB_BOVIN	cystatin b	0.89*	0.94	1.07
740	1.35	1.35	11.92	sp|P01044|KNG1_BOVIN	kininogen-1 isoform 1	0.95	0.85	0.89
741	1.75	1.75	12.39	sp|Q3MHP2|RB11B_BOVIN	member ras oncogene family	0.79	1.00	1.26
742	1.74	13.03	52.26	tr|F1MVB3|F1MVB3_BOVIN	neuroblast differentiation-associated protein ahnak-like	0.94	1.19	1.27
743	1.33	1.33	10.77	sp|P51176|TGM2_BOVIN	protein-glutamine gamma-glutamyltransferase 2	1.03	1.17	1.14
749	1.72	2.58	65.00	tr|Q7M371|Q7M371_SHEEP	plasma proteinase inhibitor	1.24	0.95	0.76*
754	1.71	1.71	25.09	sp|Q58DQ3|RL6_BOVIN	ribosomal protein l6	1.22	1.27	1.04
756	1.70	19.75	38.43	sp|Q3MHM5|TBB2C_BOVIN	tubulin beta-2b chain	1.01	1.14	1.12*
766	1.70	1.70	18.35	tr|E1BJX1|E1BJX1_BOVIN	ribosomal protein l30	0.86	1.18	1.38
770	1.68	1.68	17.62	tr|F1MXX0|F1MXX0_BOVIN	inner membrane mitochondrial	1.15	1.07	0.93
771	1.67	30.65	60.66	gi|166157508|ref|NP_001107245.1|	perilipin-1	1.00	1.01	1.00
773	1.63	1.82	19.33	sp|Q0P5L8|KBL_BOVIN	2-amino-3-ketobutyrate coenzyme a mitochondrial	0.83	0.89	1.08
774	1.61	1.62	14.52	tr|Q3SYU8|Q3SYU8_BOVIN	tumor protein d54	1.01	0.97	0.96
778	1.57	1.57	34.42	tr|E1BM28|E1BM28_BOVIN	deoxyribonucleoside 5 -monophosphate n-glycosidase	0.99	1.14	1.15
779	1.56	1.60	15.76	tr|F1MGJ7|F1MGJ7_BOVIN	methylglutaconyl- partial	1.55	0.90	0.58
784	1.54	1.54	17.48	sp|P52556|BLVRB_BOVIN	flavin reductase	0.85	0.88	1.04
788	1.53	1.53	11.29	tr|Q9GJT9|Q9GJT9_BOVIN	hypoxanthine phosphoribosyltransferase 1	1.19	1.00	0.84
789	1.52	1.71	8.97	tr|F1N757|F1N757_BOVIN	uncharacterized protein	0.96	0.96	1.00
799	1.52	1.52	4.40	gi|242247491|ref|NP_001156025.1|	adp-ribosylation factor-like 3	0.95	0.90	0.95
800	1.52	1.52	11.02	REV_tr|Q6Y1E0|Q6Y1E0_BOVIN	myotrophin	1.03	0.86	0.83
801	1.51	1.54	27.93	REV_tr|E1BBS9|E1BBS9_BOVIN	ciliary rootlet coiled- rootletin	0.93	1.05	1.12
805	1.48	1.48	80.72	sp|P0CH28|UBC_BOVIN	ubiquitin-isoform a	1.00	1.04	1.04
808	1.46	1.46	38.96	tr|F1MC72|F1MC72_BOVIN	ubiquitin-conjugating enzyme e2l 3	1.06	1.05	0.99
809	1.44	1.55	29.33	sp|Q5E983|EF1B_BOVIN	eukaryotic translation elongation factor 1 beta	1.01	1.01	1.00

					2			
810	1.44	1.44	38.97	sp|P84227|H32_BOVIN	histone h3.2	1.36	1.20	0.88
813	1.41	7.83	37.79	sp|Q3ZC09|ENOB_BOVIN	beta-enolase-like isoform 1	1.01	0.99	0.98
816	1.41	1.43	9.58	tr|F1MC48|F1MC48_BOVIN	iq motif containing gtpase activating protein 1	0.89	0.86	0.97
817	1.41	1.42	25.51	sp|A2VDZ9|VAPB_BOVIN	vesicle-associated membrane protein-associated protein b	1.03	1.10	1.07
818	1.41	1.41	17.42	sp|A7YW98|SYRC_BOVIN	arginyl-trna cytoplasmic	1.40	0.99	0.71
827	1.39	1.39	17.38	tr|Q3MHP6|Q3MHP6_BOVIN	eukaryotic translation initiation factor 4b	1.15	1.18	1.03
828	1.37	1.37	21.52	sp|Q3T0V4|RS11_BOVIN	ribosomal protein s11	0.97	1.05	1.09
831	1.32	1.56	13.32	tr|E1BF59|E1BF59_BOVIN	plectin isoform 1hij	1.07	1.04	0.98
833	1.32	1.32	14.29	tr|Q32KR9|Q32KR9_BOVIN	chromatin modifying protein 1a	1.16	1.24	1.07
834	1.32	1.32	7.33	sp|Q148C9|HEBP1_BOVIN	heme binding protein 1	0.82	1.09	1.33

* Statistically significant changes (p<0.05)

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