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REVIEW

Obesidomics: contribution of adipose tissue secretome analysis to obesity research

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Abstract Obesity is presently reaching pandemic proportions and it is becoming a major health concern in developed and developing countries due to its comorbidities like type II diabetes, cardiovascular pathologies, and some cancers. The discovery of the adipose tissue role as an endocrine gland able to secrete adipokines that affects whole-body energy homeostasis has become a key break-through toward a better molecular understanding of obesity. Among the known adipokines involved in the regulation of energy metabolism very few have been clearly seen as central regulators of insulin sensitivity, metabolism, and energy homeostasis. Thus, the discovery and characterization of new adipocyte-derived factors is still in progress. Proteomics technology has emerged as a useful tool to analyze adipose tissue secretion (secretome) dynamics giving a wider picture into the molecular events that control body weight. Besides the identification of new secreted proteins, the advantage of using this approach is the possibility to detect post-translational modifications and protein interactions that generally cannot be predicted by genome studies. In this review, we summarize the recent efforts to identify new bioactive adipokines by proteomics especially in pathological situations such as obesity.

Keywords Secretome · Proteomics · Obesity · Adipose tissue

Introduction

Obesity is currently considered an epidemic in developed and developing countries causing a major health concern that has become a priority in public health policies. This phenomenon is especially troubling since it implies an increased risk of premature death from related diseases such as type II diabetes, cardiovascular disease, and some cancers among others. While obesity may be caused by genetic alterations, it is now well accepted that it is a multifactorial condition that requires a long-term management being strongly influenced by environmental factors such as lifestyle and diet and above all, by the absence of physical activity [1, 2]. Under this context, strategies to prevent, to control and to fight against obesity stress the importance of diet and exercise; nevertheless, little progress has been made in reducing this epidemic. In recent years, the development of basic research for the study of energy homeostasis has strikingly improved our understanding on body weight control at the molecular level in central and peripheral level upbringing new approaches for

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obesity treatment [3]. However, there is still a necessity to improve the knowledge of the molecular events implicated in obesity development.

The recognition that adipose tissue acts as an endocrine gland affecting whole-body energy homeostasis was a major break-through toward a better molecular understanding of obesity and its co-morbidities. Accordingly, many studies have demonstrated that excess of adipose tissue mass or ectopic lipid accumulation is responsible of metabolic disorders that cause type 2 diabetes and dyslipidemia [4, 5]. Adipose tissue communicates with other central and peripheral organs by secreting a variety of molecules known as adipokines, and it is also a source of inflammatory cytokines [6]. The levels of some adipokines correlate with specific metabolic states and have the ability to directly affect the regulation of the metabolic homeostasis in such a way that the deregulation of adipokines has been implicated in obesity, type 2 diabetes, hypertension, cardiovascular disease, and a growing list of pathological situations. There is an ever-increasing number of adipokines described in the literature [7, 8]. Among the known adipokines involved in the regulation of energy metabolism such as leptin, adiponectin, retinol-binding protein-4, visfatin, adipsin, vaspin, apelin, tumor necrosis factor (TNF-1), and interleukin 6 [9–12], very few have been clearly seen as central regulators of insulin sensitivity, metabolism, and energy homeostasis. Under this context, the discovery and characterization of new adipocyte-derived factors is still in progress.

There is no doubt whatsoever that to get a deeper knowledge into the molecular events that control body weight is of great interest to analyze adipose tissue secretion dynamics by proteomics technology. More precisely, the global analysis of a set of proteins released by the adipose tissue at a particular time would constitute this tissue secretome. In addition to the identification of new secreted proteins, the benefit of using this approach is the ability of this technology to detect post-translational modifications (PTMs) and protein interactions [13]. In this review, we summarize the current efforts to identify potential adipokines by proteomics especially in pathological situations such as obesity.

Protein secretion: classical and non-classical secretory pathways

Secreted proteins constitute an important class of molecules expressed by approximately 10% of the human genome [14].Typical secreted proteins are serum proteins (e.g., albumin, transferrin immunoglobulins), extracellular matrix proteins (e.g., collagens, proteoglycans, fibronectin, laminins), digestive enzymes (e.g., trypsin) or milk proteins [15].Others such growth factors, cytokines, hormones or extracellular matrix-processing proteases are in comparison low abundant although highly bioactive [16].

The secretome comprises all the proteins released by a cell, tissue or organism through classical and alternative non-classical secretion mechanisms. In the classical secretion mode, once synthesized, proteins translocate into the lumen of endoplasmic reticulum (ER), transported through Golgi complex and released by exocytosis [17]. These proteins are expressed by messenger RNAs containing a signal sequence that guides its journey from the ribosome to the ER membrane during translation and initiates the transport of the growing polypeptide across the ER membrane into ER lumen. The characteristic ER signal sequence is classically amino terminal and comprises one or more positively charged amino acids followed by a continuous stretch of 6–12 hydrophobic residues. This signal sequence is normally cleaved from the proteins in the ribosome and it cannot be found on the final polypeptide [15].

On the other hand, the non-classical pathway of secretion does not involve the route through the ER and Golgi being the exact mechanism still elusive [18]. Relevant regulators of the immune response, cell growth differentiation, and angiogenesis are secreted without signal peptide such as basic fibroblast growth factor (bFGF), b-galactoside-specific lectins (galectin 1 and 3), and certain members of the interleukin family (e.g., IL-1b). At least four different types of non-classical export routes have been described in the literature: export by import into intracellular vesicles which are probably endosomal subcompartments (e.g., IL-1b and HMGB1), direct translocation across the plasma membrane (e.g., FGF-1 and 2) through transporters, direct translocation through a flip-flop membrane system (Leishmania cell surface protein HASPB), or through exosomes that release their contents into the extracellular space (e.g., galectins) [18 for review]. Precisely, the non-classical secretory pathway was studied in 3T3-L1 differentiated adipocytes by the isolation of secreted microvesicles [19]. This study using high pressure liquid chromatography followed by mass spectrometry (HPLC–MS/MS) allowed the identification of typically integral, cytosolic, and nuclear proteins such as caveolin-1, c-Src kinase, and heat shock protein 70 in the extracellular fraction.

Fortunately, there are currently various bioinformatics tools that allow the prediction of a certain protein secretion and location based on its sequence. Thus, for the prediction of non-classical and classical secreted proteins SignalP and SecretomeP software are widely used [20, 21]. In addition, for the prediction of subcellular location other tools may be used such as Protein Prowler or TargetP [22, 23].

Proteomics technology and secretome analysis

Until very recently, protein analysis tools were expensive, time-consuming, and not sensitive. The development of soft ionization techniques such as MALDI (matrix-assisted laser desorption) and electrospray is currently allowing the study of macromolecules through mass spectrometry (MS). These ionization methods together with different mass analyzers [time of flight (TOF), quadrupole or ion tramp] are now permitting protein identification and sequencing with unprecedented versatility and accuracy [13].

The term Proteome was used for the very first time back in 1995 to describe all the proteins expressed by the Genome. Currently, the most precise definition of proteome refers to the set of proteins that compose a cell, tissue, organism or biologic fluid at a precise moment including all PTMs such as glycosylation, sulfatation, phosphorylation, and acetylation that actively affect protein function and cannot be predicted by Genomics or Transcriptomics [13]. Proteomics include all the technologies that are used for the study of the proteomes and they can be classified in three main groups: (a) Tools for protein separation and global analysis of proteomes [e.g., two-dimensional electrophoresis (2-DE)], (b) Tools for individual analysis of proteome components (MS: MALDI or Electrospray), and (c) Bioinformatics tools to identify peptides from MS spectra in databases.

Clinical proteomics describes the application of proteomics techniques and strategies to medicine. Inasmuch as the cause of the majority of human diseases is related to the functional deregulation of protein interactions, a better knowledge of protein networks role in different illnesses will foreseeable give a broad spectrum of opportunities for clinicians since these networks will be the pharmacologic targets in the next decades. It is believed that in the near future the therapeutic strategies will be designed not only against an isolated protein, but toward the global cellular protein complex. This fact is responsible of proteomics technology development for early stage disease biomarker detection based on the elevation or diminution of proteins as consequence of the disease itself that may be shed to the body fluids [24].

Contrary to other medical disciplines, Proteomics has not been applied to the study of obesity as extensively [25, 26]. However, the publication in 2001 of the first database for 2-DE of mouse tissues (kidney, hepatocyte cell nuclei, muscle, brown and white adipose tissue, and pancreatic islets) has become a helpful tool for the existing studies in human diabetes and obesity (Swiss-2D mouse database: http://www.expasy.org) [27].

When the interest is in cellular communication, it may be interesting to focus the proteomics analysis on the receiving part of the signal, i.e., membrane receptors. However, membrane proteins are known to be very difficult to analyze by proteomics. Otherwise, it is very interesting to analyze the signaling part itself, i.e., the proteins that are secreted by a given cell type to communicate with other cells. Cells and tissues release protein biomarkers into the extracellular fluid through secretion of intact or cleaved peptides. Some of these products can end up in the bloodstream and therefore be affected in pathological situations serving as potential serum biomarkers. Unfortunately, the identification of secreted proteins from body fluids such as serum or plasma is very complex by direct proteome analysis because interest proteins are often masked by high amounts of proteins actually not secreted by the investigated cells or tissues. To overcome the dynamic range of this type of samples, several methods to affinity eliminate the most abundant proteins were designed; nevertheless, these systems have disadvantages such as the lack of specificity and/or the elimination of many other protein species. For this reason, the direct study of cells and tissues secretome either in vitro or in vivo using for example capillary ultrafiltration sampling, it has been referred by proteomics specialists as the best procedure since it reflects a broad variety of pathological conditions and represents a rich source of biomarkers [28, 29].

The main drawback for secretome analysis is related to protein contaminants which may be hiding proteins of interest or result in false discoveries. Thus, cells of tissues may secrete abundant proteins such as albumin, or depending on the approach of election, secretomes may contain unwanted intracellular proteins caused by cell breakage or damage. There are several strategies to solve this issue, although some basal protein contamination has to be assumed. In this sense, the treatment of cells with Brefeldin A may help to identify proteins secreted by the classical secretion route since it blocks ER to Golgi vesicular transport [16, 30]. Other way to discern contaminants is the use of radioactive labeling with 35S methionine to detect newly transcribed proteins and to discriminate for example serum-derived contaminants. More recently, proteomics approaches such as SILAC (stable isotope labeling by amino-acid in cell culture) technology has also revealed a great potential. This method is extremely accurate and fairly easy to apply for the quantification of proteins extracted from cultured cells. It involves cell culture either in regular culture media ("light" protein synthesis) or in media where amino acids have been replaced by their isotopically labeled counterparts ("heavy" protein synthesis). The advantage is that cell situations to be compared can be mixed and treated as a single sample, which avoids the risk of introducing quantification errors during sample preparation. During MS analysis, the relative protein abundance between biological samples can be calculated from the intensities of heavy and light peptides [31] (Fig. 1).

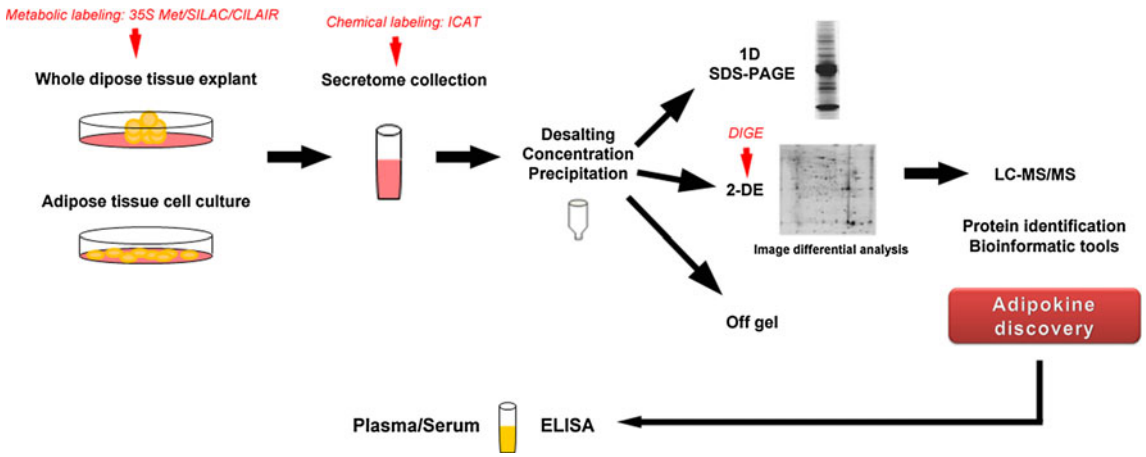

Fig. 1 Schematic representation showing methodological approaches for adipose tissue secretome analysis. Quantitative applications are indicated in *red* (Color figure online)

Adipose tissue secretome

Whole adipose tissue secretome

Adipose tissue is a complex, heterogeneous tissue composed not only by mature adipocytes and fibroblast preadipocytes or stromal/stem cells, but by pericytes, endothelial cells, macrophages, and T-lymphocytes [32]. Under this context, it is reasonable to accept that factors secreted by the stromal-vascular fraction contribute to the adipose tissue secretome and modulate adipokine secretion by adipocytes. Therefore, the characterization of the adipose tissue secretome rather than the secretion of individual cell constituents may exert a more physiological view of adipose tissue secretory function. Although on the other hand, the collection of whole adipose tissue is certainly more difficult to handle since it is more prone to be contaminated with serum proteins and intracellular proteins from damaged cells. Contaminants may dramatically affect the dynamic range of the sample and thus identification of secreted proteins.

The first characterization of whole human adipose tissue secretome by proteomics was recently released [25]. In accordance to the exposed above, in this study visceral adipose tissue secretome collection was standardized by trying five experimental setups to check the final protein concentration and composition. The best protocol for optimal protein quality involved one wash after the first hour in culture followed by two or three additional washes within an 8 h period, starting after overnight culture. Then, adipose tissue was maintained in culture for an additional 48–114 h to obtain the final sample. Besides, to elucidate truly secreted proteins, a labeling experiment was performed by culturing tissue explants in media containing L-[(13)C(6),(15)N(2)] lysine. From 259 identified proteins, 108 proteins contained a secretion signal peptide and 70 of those incorporated the label and were considered secreted by adipose tissue. These proteins were classified into five categories according to function showing a high percentage of proteins components of the extracellular matrix (e.g., perlecan, versican), followed by a second group of proteins involved in cell signaling (e.g., neuropilin-1). As expected, this study also shows that a considerable number of proteins are released not only by adipocytes but by other cell types such as macrophages and endothelial cells (e.g., endoplasmin, gelsolin, protein-disulfide isomerase, protein-disulfide isomerase A3, calreticulin, cathepsin D, and peroxiredoxin 4).

The quantitative assessment of changes in protein secretion of human visceral adipose tissue has been also recently described by comparing isotope-labeled amino acid incorporation rates (CILAIR), a variant of SILAC technology described above [32]. In this study, the authors determined the effect of insulin on the secretome of visceral adipose tissue explants from one patient by comparing incorporation rates of 13C-labeled lysine in the presence and absence of insulin. By analyzing heavy/light ratios in the absence and presence of insulin they show that insulin treatment up-regulate proteins involved in the ER stress response and in extracellular matrix (ECM) remodeling.

Furthermore, the comparative analysis of lean vs. obese rat epididymal fat depots and the effect of thiazolidinedione (TZD) treatment were analyzed by two-dimensional liquid chromatography followed by MS (2D-LC–MS/MS) and quantitative ^{18}O proteolytic labeling proteomics approaches [33]. This study shows that obese adipose tissue releases more inflammatory and ECM molecules, collagens, and proteases than lean adipose tissue. The treatment with TZD inhibited the secretion of these proteins in obese adipose tissue, thus resembling lean tissue.

Mature adipocyte secretome

The first secretion study of mature adipocytes was performed after isolation of rat adipose cells from the epididymal, inguinal, and omental fat pads [34]. In this case, 2D-LC–MS/MS and ^{18}O proteolytic labeling strategies were used to identify and compare levels of secreted proteins in the conditioned medium of the cultured cells with or without insulin stimulation (Fig. 2a). From 84 identified proteins as adipokines, 53 were not previously reported to be secreted by adipose cells and only thirteen were missed when compared to earlier proteomic studies. Upon chronic insulin treatment of primary culture, modulation of several secreted proteins was detected using the quantitative approach, including an increase in adiponectin, and decrease in PAI-1 and osteonectin.

The study of human mature adipocytes culture secretome has become a reality in the present year by using 2-DE and MALDI-TOF. In this study, the in vitro characterization of human visceral adipose tissue secretome has revealed the secretion of PEDF [35] and the identification of dipeptidyl peptidase 4 (DDP4) as a novel adipokine [36].This proteomic approach showed that PEDF is one of the most abundant proteins released by adipocytes. Following the 2-DE and MS analysis, validating experiments have demonstrated that its secretion is inversely regulated by insulin and hypoxia. Moreover, in vitro assays included in this investigation showed that PEDF induces insulin resistance in adipocytes and in human skeletal muscle cells, and leads to inflammatory signaling in human smooth muscle cells. These results parallel previous reports indicating the up-regulation of PEDF in patients with type 2 diabetes and suggest its important role in diabetes and obesity-related disorders. As for the identification of DPP4, further investigation on this new adipokine proved that fully differentiated adipocytes release more DPP4 than preadipocytes or macrophages, and that visceral adipose tissue from obese individuals secretes much higher levels compared to subcutaneous. Moreover, the treatment of fat, skeletal, and smooth muscle cells with DDP4 impeded insulin signaling. Interestingly, DPP4 released from adipose tissue correlated positively with an increasing risk score for the metabolic syndrome.

Stromal-vascular fraction (SVF) secretome

The SVF fraction secretome from human female subcutaneous adipose tissue under un-induced or adipogenic

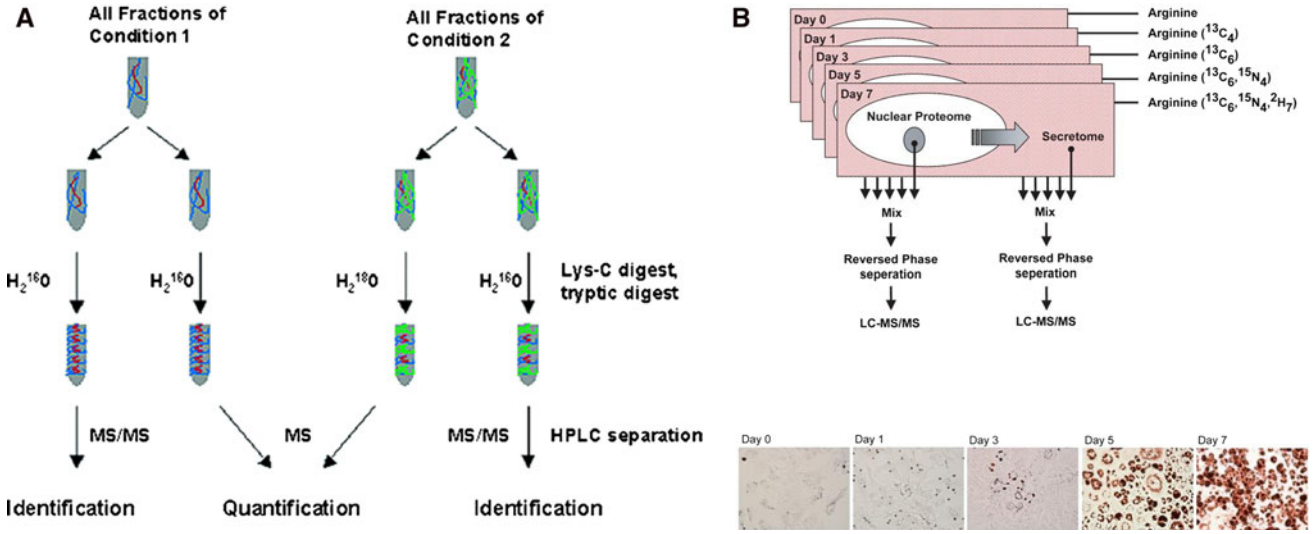

Fig. 2 **a** Labeling strategy for the differential quantification of the secretory proteome of rat adipose cells. Proteins from Condition 1 and 2 were split into two aliquots, and digested with trypsin and $H_2^{16}O$ or $H_2^{18}O$, respectively. The $H_2^{16}O$ digests of Condition 1 and 2 were used for the identification of the proteome of the two conditions. The $H_2^{18}O$-labeled tryptic peptides of the fractions of Condition 2 were mixed 1:1 with the $H_2^{16}O$-labeled tryptic peptides of the fractions of Condition 1 and used for differential quantification. This procedure was repeated with all eight fractions."Reprinted with permission from Quantitative proteomic Analysis of the Secretory Proteins from Rat adipose cells using a 2D liquid chromatography-MS/MS approach by Chen et al. [34]. Copyright 2005 American Chemical Society." **b** 5-plex SILAC strategy and Oil red O staining for adipocyte differentiation. Preadipocytes were grown in different cell culture media containing 5 different isotopic forms of arginine as shown until complete isotopic amino acid incorporation. After the induction of differentiation, cells were harvested on Day 0, Day 1, Day 3, Day 5 and Day 7 of the differentiation process and combined after protein normalization. In parallel, serum free media from five different cell populations were collected at the indicated time points and combined. The nuclear fraction and the secretome were processed and analyzed by LC–MS/MS. Differentiation of adipocytes was confirmed by Oil red O staining. Cells on Day 3, Day 5 and Day 7 show a progressive increase in red staining for fat droplets, indicating the extent of adipocyte differentiation. "Reprinted with permission from Temporal profiling of the adipocyte proteome during differentiation using a five-plex SILAC based strategy by Molina et al. [41]. Copyright 2009 American Chemical Society."

induced conditions was studied by 2-DE and tandem spectrometry [37]. This investigation allowed the identification of proteins without signal peptide classified as cytoplasmatic such as actin and lactate dehydrogenase, and well established adipokines such as adiponectin and plasminogen activator inhibitor 1. Interestingly, multiple serine protease inhibitors (serpins) were identified such as PEDF, placental thrombin inhibitor, pregnancy zone protein, and protease C1 inhibitor.

The SVF secretome from epididymal fat depots of obese Zucker rats and its comparison with adipose cells was also characterized by two-dimensional liquid chromatography followed by MS (2D LC–MS/MS) proteomics approach [30]. The results of this study show that SVF and adipose cells are characterized by differential secretion profiles in the quality and quantity of proteins. The majority of proteins identified in the SVF cell secretome were functionally related to immune response, ECM activity and proteolysis and possibly involved in local activities via a paracrine/autocrine fashion in adipose tissue. On the contrary, adipose cells secreted factors that participate in the regulation of systemic activities.

Under the context of regenerative medicine, human adipose tissue-derived mesenchymal stem cells (hASC) secretomes have been recently studied by LC–MS/MS [38]. To identify hASCs secreted proteins during inflammation, secretomes were analyzed after exposing these cells to tumor necrosis factor-alpha (TNF-alpha). The TNF-alpha-induced secretome comprised a variety of cytokines and chemokines such as interleukin-6 (IL-6), IL-8, chemokine (C-X-C motif) ligand 6, and monocyte chemotactic protein-1 (MCP-1). TNF-alpha also augmented the levels of various proteases including cathepsin L, matrix metalloproteases, and protease inhibitors, and induced the secretion of long pentraxin 3, implicated in innate immunity.

Secretome during adipocyte differentiation

There are currently several reports in the literature analyzing the secretome of differentiating adipose stem cells trying to shed light into the adipogenesis process. Giving support to these in vitro approaches, a gene expression prediction study to elucidate differentiating human preadipocytes secretome is currently available. In summary, this prediction indicates novel candidate secretome genes that are related to components of the coagulation and fibrinolytic systems and reports the matrix gla protein (MGP) and inhibin beta B (INHBB) as new theoretical adipokines [39].

As mentioned above, there are several studies utilizing proteomics technologies for the analysis of the adipose cells secretome during differentiation. This is the case of the characterization of human mesenchymal stem cell secretome at early steps of adipocyte and osteoblast differentiation using mono-dimensional electrophoresis and tandem MS [40]. This approach allowed the identification of a total of 73 proteins among day 0 and day 3 of differentiation. The functional classification of the identified proteins indicated that the majority of proteins belong to extracellular matrix and cytoskeletal components followed by heat shock/folding proteins. Among identified proteins, plasminogen activator inhibitor 1 (PAI-1) was detected at day 0 and day 3 of osteoblast differentiation but never in the adipocyte secretome.

Besides gel based studies, SILAC strategy has been also applied to the temporal profiling of 3T3-L1 mouse cells proteome and secretome during differentiation [41] (Fig. 2b). In addition to previously reported molecules implicated in differentiation such as adiponectin and lipoprotein lipase, this approach permitted the identification of various novel proteins such as THO complex 4 and SNF2 alpha. THO complex, a context-dependent transcriptional activator in the T-cell receptor alpha enhancer complex, was found to have the highest expression at middle stage of adipogenesis, while SNF2 alpha, a chromatin remodeling protein, was found decreased upon initiation of adipogenesis and remained constant during succeeding time points. A similar strategy has been recently published using this time primary human adipocytes and the iTRAQ-based quantitative proteomics technology [42]. The results of this study revealed known differentially secreted adipokines and various proteins whose dynamic expression in this process has not been previously documented such as collagen triple helix repeat containing 1, cytokine receptor-like factor 1, glypican-1, hepatoma-derived growth factor, SPARC related modular calcium binding protein 1, SPOCK 1, and sushi repeat-containing protein.

Finally, it should be highlighted the use of the label-free quantitative approach for the study of human stromal-vascular fraction cells secretomes during adipogenesis by LC–MS/MS [43]. This methodology revealed that the amounts of various extracellular modulators of Wnt and transforming growth factor-β (TGF-β) signaling changed during adipogenesis.

Depot-specific secretomes

There is an increasingly evidence showing numerous biological and genetic differences between adipose tissues depending on its anatomical location [26, 44–46]. In this regard it has been described that upper body/visceral fat distribution in obesity is closely related to metabolic complications in contrast with the accumulation of fat in the lower body [47, 48]. Accordingly, it was shown that visceral fat and resident macrophages produce more inflammatory cytokines and less adiponectin inducing insulin

resistance and atherosclerosis. The anatomical condition of visceral adipocytes located close to the liver, suggest its critical role in this process [49]. Under this context it is of interest to identify biological and functional differences among adipose tissue depots. In this sense a couple of proteomics essays have exhibited tissue-specific proteome characteristics according to the anatomical location [50, 51]. However, there is still very little evidence about protein secretion differences among adipose tissue depots.

By lectin affinity chromatography, Hocking and collaborators have conducted a quantitative analysis of visceral adipose (VAT) vs. subcutaneous tissues (SAT) explant secretomes [52]. In addition, they also compared the secretome of isolated preadipocytes and microvascular endothelial cells (MVECS) from both adipose tissue depots. In this study, they did not find discrete differences in the secretomes of whole VAT and SAT explants, preadipocytes, or MVECS, but they do describe a higher level of protein secretion in VAT samples. This fact is particularly relevant since visceral adipose tissue constitutes only a small fraction of all body fat (on average, 18%) compared to subcutaneous fat stores that comprise approximately 82% [53]. Interestingly, they show that almost 50% of the adipose tissue secretome was composed of factors with a role in angiogenesis suggesting that visceral adipose tissue is a more readily expandable tissue depot.

In this line of research, our group has performed the first secretome mapping and comparative analysis of adipose tissue secretome from different anatomical locations [54]. More precisely, we characterized the secretome of rat visceral, subcutaneous, and gonadal fat specific secretomes and the differentially secreted proteins among the three fat depots by 2-DE and MS (Fig. 3). Paralleling the above study, we also detected a higher level of protein secretion per gram of tissue in visceral adipose depots; however, on the contrary to their study we did find discrete differences among depot secretomes. Hence, reference maps indicating identified proteins for location-specific adipose tissue secretomes are shown and the 45 most significant differences listed. To discern contaminant proteins from cellular breakage, identified proteins were submitted to the sequence-based prediction method SecretomeP. From this scrutiny we detected that approximately 50% of the proteins in the secretome mapping were classified as secreted; a percentage that was significantly increased in the differential analysis reaching a 74%. Among identified proteins we detected known adipose tissue adipokines such as adiponectin, retinol-binding protein 4, angiotensinogen, macrophage migration inhibitory factor, haptoglobin, and serum amyloid P-componentin the secretome mapping. Other proteins were previously described in rat adipose tissue cell culture secretomes like thioredoxin-like protein 1, vimentin, hemopexin, thioredoxin [34], or in human visceral fat secretome analysis such as alpha-1-inhibitor 3, nidogen-2, gelsolin, serotransferrin, 78 kDa glucose-regulated protein, cathepsin D, serum albumin, and cell surface glycoprotein MUC18 [25]. The identification of the former proteins validates our secretion model. Interestingly, many other proteins such as calreticulin, osteocrin, and protein DJ-1 among others had not been associated to fat secretomes previously. From the differential analysis proteins such as enoyl-CoA hydratase, adenosine kinase, carbonic anhydrase 5B, and transgelin were identified exclusively in visceral and gonadal fat secretomes, and an isoform of gelsolin only in visceral adipose tissue. Adipokines or adipose tissue related proteins were found at higher levels in visceral than in subcutaneous fat such as thrombospondin-1, angiotensinogen, fatty acid-binding protein, and galectin-1. On the contrary, vitamin D-binding protein was found to be secreted at more elevated levels by subcutaneous adipose tissue. As conclusion our proteomic approach illustrate that the type of proteins and their role in different biological processes diverge significantly when comparing the secretomes from visceral, subcutaneous and gonadal fat explants. Therefore, this research emphasizes and supports the differential role of adipose tissue in accordance to its anatomical localization.

Final considerations and future perspectives

There is no doubt about the significant contribution of proteomics to the knowledge of adipose tissue biology and physiology, and its application to food related disorders research. The initial technical barriers that impeded the analysis of this delicate tissue seem to be overcome, and the number of publications based on proteomics approaches to study both adipose tissue proteome and secretome has exponentially increased in the last decade.

We believe that future directions should focus on specific adipose tissue depots by analyzing the participation of each cell constituent secretome and the profound role of each depot in reference to its anatomical localization. Taking into account this differential participation, the origin of the sample must be indicated in future studies. In addition, the period of tissue or cell incubation for secretome collection should be indicated and/or agreed for comparison purposes. Other aspects such as site- and sex-related differences, the secretome dynamic changes during development and aging, its affectation in metabolic disorders, or the analysis of brown adipose tissue secretomes will definitively broad our knowledge about energy metabolism regulation. The emerging trends in quantitative proteomics such as DIGE, SILAC, ICAT or label-free methods will definitively improve secretome analysis and the discovery of new bioactive adipokines.

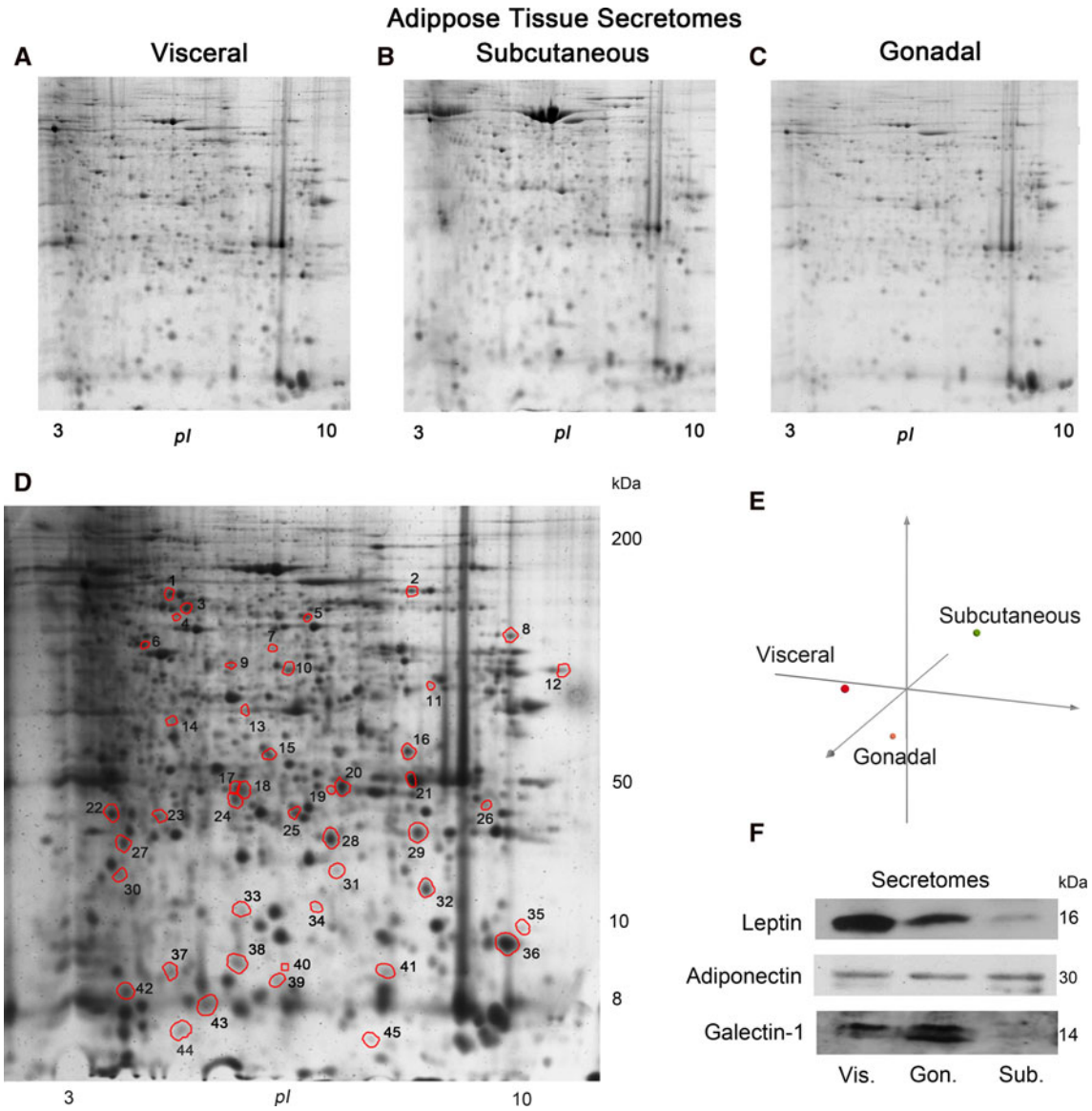

Fig. 3 Depot-specific secretomes. Representative 2-DE (12.5% SDS-PAGE) gel images for visceral (**a**), subcutaneous (**b**) and gonadal (**c**) fat secretomes. Representative gel image showing 45 differences among the three fat depots after applying the most restrictive statistical analysis $P < 0.001$ (**d**); the principal component analysis for all detected spots shows very low variance within each group of secretomes indicating that gels cluster strongly to their secretome group (**e**); and representative images of leptin, adiponectin and galectin-1 western blots for visceral (vis.), subcutaneous (sub.) and gonadal (gon.) fat secretomes (F). "Reprinted from Secretome analysis of rat adipose tissues shows location-specific roles for each depot type by Roca-Rivada et al. [54]. Copyright 2011 with permission from Elsevier"

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