

Regulation of inflammation-related genes in human adipose tissue

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Abstract. Clement K, Langin D (Inserm, U755 Nutriomique, Paris; University Pierre and Marie Curie-Paris 6, Paris; Hôtel-Dieu Hospital, Paris; Inserm, U586, Unité de Recherches sur les Obésités, Toulouse; Université Paul Sabatier, Toulouse; Institut Fédératif de Biologie de Purpan, Toulouse, France; and Franco-Czech Laboratory for Clinical Research on Obesity, Prague, Czech Republic). Regulation of inflammation-related genes in human adipose tissue (Review). *J Intern Med* 2007; **262**: 422–430.

The identification of a moderate increase in circulating inflammatory factors in obese subjects, the description of changes in inflammatory gene expression in adipose tissue (AT) and the discovery that macrophage cells infiltrate AT are observations contributing to the concept that human obesity is a chronic inflammatory illness. This concept has led to some revision

of the physiopathology of obesity and of its related metabolic and cardiovascular co-morbidities. Low-grade inflammation in the AT and the subsequent production of specific biomarkers could actually link expanded fat mass to obesity complications. This review aims at providing an overview of the current knowledge brought up by human gene expression studies, notably those performed on a large scale in AT depots. The regulation of specific biomarkers related to inflammation and putative new candidates (i.e. cathepsins and serum amyloid A) is discussed in the context of weight loss programmes based on calorie restriction and physical exercise. The foreseen clinical and technological challenges are also summarized.

Keywords: adipose tissue, gene expression, inflammation, macrophages, obesity.

Introduction

Mounting evidence indicates that obesity is associated with a chronic systemic low-grade inflammatory state and that inflammation is one of the potential mechanisms of obesity-related morbidity [1, 2]. Chronic low-grade inflammation, as defined by increased plasma levels of C-reactive protein, is a strong risk factor for cardiovascular and metabolic diseases [1, 3–5]. Additional markers of inflammation such as interleukin-6 (IL-6) and tumour necrosis factor- α (TNF- α) are also associated with increased risk for several chronic diseases including insulin resistance,

coronary artery disease and type 2 diabetes mellitus [6, 7].

Adipose tissue (AT) is an important endocrine tissue that secretes many biologically active proteins, such as adiponectin and leptin, and numerous cytokines and chemokines [8]. Plasma levels of several factors are increased during obesity [9–11]. They are postulated to play a critical role in the pathogenesis of insulin resistance and atherosclerosis but definite proof and the respective importance of each factor are still unknown. The identification of the molecular factors underlying the metabolic disturbances observed in

obesity is a key step in developing better therapeutic strategies. Each stage in the development of obesity, i.e. weight gain, weight maintenance and variable response to weight loss treatments based on nutrition, physical exercise or drugs are probably associated with different molecular mechanisms. At present, there are no validated biological or molecular markers of passing from one stage to the other [12]. Techniques allowing systematic analysis of AT gene expression are useful to identify master genes involved in human obesity and related disorders. In this review, we focus on how transcriptomics has allowed progress on the knowledge of the molecular determinants of inflammation in human AT. Following an overview of the techniques at hand, dysregulation of inflammatory genes in the state of increased fat mass will be surveyed with a focus on subcutaneous and visceral AT. A series of pangenomic studies based on low calorie diets provide interesting information on the dynamics of AT inflammation in combination with data on the cellular origin of the inflammatory markers. The regulation of specific biomarkers related to inflammation will be developed in the context of weight loss programmes based on calorie restriction or physical exercise. In the concluding section, the foreseen clinical and technological challenges are discussed.

The genes involved in AT inflammation have been studied intensely in human and rodent models. For clinical reasons this review will focus on human studies.

The tools of transcriptomics for the study of human adipose tissue

Determination of mRNA levels in human AT is based on two complementary techniques: reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and DNA microarrays. RT-qPCR is the method of choice for a quantitative analysis of candidate gene mRNA levels [13]. Improvement in the efficiency and reproducibility of reverse transcription of mRNA into cDNA and the development of real-time PCR has allowed a considerable change in the analysis of mRNA levels from minute amounts of tissues sampled through biopsy in clinical protocols. This is especially true for human AT which, because of the

high lipid content (more than 90% in wet weight) of the samples, shows a very poor yield during total RNA preparation. RT-qPCR applications are numerous. It is now possible to analyse more than a hundred genes in parallel through the use of low density arrays based on microfluidic cards that enables to perform 384 simultaneous real-time PCR reactions [14].

DNA microarrays, also called DNA chips, are now widely used as a standard tool for transcriptome analysis in the field of obesity research due to its profitability and capacity of simultaneously quantifying thousands of genes in a single experiment [15]. Microarray technology is based on the reverse concept of dot blot and Northern blot analysis. The DNA is attached to the solid phase (probe) whereas labelled cDNA or RNA is in solution (target). One can fix large numbers of cDNA sequences or synthetic DNA oligomers onto a glass slide (or other supports) in known locations on a grid. Arrays with thousands of spotted DNA have been developed by academic consortium or companies. Different kinds of microarrays are available, allowing the study of constitutive genes of a whole genome of an organism or a specific metabolic pathway or cellular process. Each RNA sample is transcribed into cDNA and labelled with different fluorophores (typically Cy3 and Cy5). The measured amount of target bound to each probe reflects the level of expression of the gene. This measurement is frequently performed by simultaneous competitive hybridization of two labelled cDNA. Whilst initially used for simple organisms, this approach now indexes thousands of known and newly discovered genes into various large groups defined by expression similarities in terms of physiological pathways, for example respiration, cell division, or immune and defence response. For this purpose, several bioinformatic tools have been developed. Starting from a list of several hundreds of genes, it is possible to identify a biological process that can be affected by a dietary intervention, a drug treatment or a pathological state. This kind of screening is now applied for the understanding of complex human diseases including cancer, ageing and more recently metabolic diseases, including diabetes and obesity. For example, it was observed that oxidative phosphorylation genes regulated by the

transcriptional coactivator peroxisome proliferation-activated receptor-gamma coactivator-1 (PGC-1 α) are globally down-regulated in skeletal muscle of type II diabetic patients [16, 17]. The key objective is to dissect and characterize the regulatory pathways and networks involved in energy balance and to define the resulting signalling patterns in gene expression. Amongst the goals of future projects that could be achieved is the identification of clusters of genes that are recruited or modified by given nutritional conditions, their links in terms of biological function, their co-regulation in different tissues, the gene markers specific for some nutrients, differences/similarities in different models of obesity, and eventually the patterns of tissue expression in individuals with different genetic polymorphisms located in these genes. Studies of the gene expression profiling in AT could help to understand what happens during weight changes and which mechanisms support the improvement of risk factors for obesity complications. As subcutaneous AT can be obtained by simple needle-biopsy aspiration, gene expression measurements in this tissue may provide a suitable tool to a better individual characterization of obese patients.

Studies of gene expression in subcutaneous and visceral adipose tissues

The anatomical distribution of AT is a key indicator of metabolic alterations and cardiovascular diseases. The excess of fat mass in the upper part of the body constitutes a classical risk factor for diabetes and cardiovascular diseases [18, 19]. There are marked differences between subcutaneous and visceral ATs in the expression and secretion of key adipose genes such as leptin [20] and adiponectin [21] as well as proinflammatory factors like plasminogen activator inhibitor 1, which has been related to the pathogenic effects of visceral fat [22]. Leptin is preferentially secreted by the subcutaneous AT, whilst the expression of adiponectin plasminogen activator inhibitor-1 (PAI-1), IL-8, and IL-1 β is more important in the visceral adipose depot [23, 24]. There have been discrepancies in results for the secretion and modulation of IL-6, as both visceral and subcutaneous AT of obese and nonobese subjects release this cytokine. The large-scale screening of

genes differentially expressed in human subcutaneous and visceral adipose depots allowed the identification of biomarkers of visceral obesity that may represent the mediators of metabolic alterations. Differential expression of genes involved in immune response such as immunoglobulins and proteins of the complement systems were found to be strongly expressed in visceral AT [25, 26]. In other studies, carboxypeptidase-E and thrombospondin-1 were found overexpressed in visceral AT [27]. Higher expression levels of several cytokines in omental compared to subcutaneous fat may mediate part of the relationship between visceral obesity and the associated pro-inflammatory, pro-thrombotic state [28]. The new AT-secreted protein visfatin alters glucose homeostasis and is highly expressed in visceral fat [29] whilst its expression is also found in human subcutaneous AT [30]. Macrophages from visceral AT are a major source of chemokines, visfatin and resistin [31], and the accumulation of macrophages in visceral human AT has been associated with the fibroinflammation state in the liver of morbidly obese subjects [32]. These observations strengthen the idea that the production of proinflammatory factors by visceral AT is mainly of macrophage origin [24] but the biomarkers promoting specific obesity complications are to be identified.

Inflammatory genes associated with weight changes

Gene profiling could help in characterizing the molecular response of obese patients to a nutritional intervention or a treatment. It is well known that weight loss, even if modest, improves numerous complications of obesity. There are numerous data indicating that decreasing the energy intake and increasing the physical activity may be effective for reducing overall systemic inflammation. Ziccardi *et al.*, for example, have shown an improvement in the endothelial stress factors during weight-loss combining diet and exercise counselling [33].

Are these systemic changes associated with modification of gene expression in the AT? We studied the effects of moderate weight loss induced by a very low calorie diet (VLCD) on the inflammatory factor gene expression in subcutaneous AT of obese women

[34]. About 100 genes related to inflammatory processes were modulated after 4 weeks of VLCD (41% increase and 59% decrease). These genes belong to 12 functional families including cytokines, interleukins, complement cascade factors, acute phase proteins and some molecules involved in cell–cell contacts or extracellular matrix (ECM) remodelling. The classes of inflammatory genes were essentially down-regulated after weight loss [35]. After 4 weeks of VLCD leading to an average of 5–6 kg weight loss, the pro-inflammatory gene expression profile of the subcutaneous AT in obese subjects clusters with that of nonobese ones, despite the persistent differences in the clinical and biological phenotypes between the two groups. Most genes (75%) whose expression changed after weight loss were expressed in the stroma-vascular fraction containing macrophages. Indeed the AT is composed of several cellular types: mature adipocytes and various other small cells (i.e. preadipocytes, fibroblasts, endothelial cells, histiocytes and macrophages), usually grouped and indicated as the stroma-vascular fraction. Due to this heterogeneity, the cellular origin of different secreted inflammatory factors by whole AT is debated, but large-scale gene expression profile of isolated adipocytes clearly shows the following; whilst the enriched functions that best characterized the genes over-expressed in adipocytes represent the well-known metabolic and secretory properties, the most significant functions characterizing the genes over-expressed in the stroma-vascular fraction were related to ‘inflammatory’ or ‘immune’ processes. The functional terms that grouped stroma-vascular fraction genes were related to ‘defence response’, ‘chemotaxis’, ‘humoral immune response’, ‘antigen processing’ and ‘inflammatory response’ [36]. Globally the importance and the contribution of the stroma-vascular fraction cells in the secretion of inflammatory factors are now well recognized, especially in pathological conditions such as obesity.

The marked improvement of the inflammatory pattern in the AT was further confirmed when analysing large-scale gene expression change after 3 months drastic weight loss induced by bypass surgery [36]. A significant decrease in AT macrophage infiltration was

associated with this improvement in the inflammatory profile (Fig. 1) following weight loss. Crown-like structures completely surrounding adipocytes found in obese subjects disappeared after fat mass loss [36]. This improvement of the inflammatory profile in subcutaneous AT was associated with an increased gene and protein expression of anti-inflammatory factors such as IL-10 or IL-1Ra; an observation that suggests a switch in macrophage inflammatory phenotype state (Fig. 1). The phenotype of these very plastic cells nevertheless needs to be characterized in human AT. The modification in the inflammatory profile of the subcutaneous AT could provide a biological support to the observation of an improvement of metabolic parameters during weight loss even when the loss of weight is modest. The molecular origin of the macrophage changes in AT during weight variation is under investigation, but the analysis of gene clusters that change after weight loss revealed the suppression of several pro-inflammatory and chemoattractant genes that can provide candidate mechanisms. Concomitantly with reduced macrophage number, we observed that the expression levels of chemoattractant molecules such as monocyte chemoattractant protein-1, colony stimulating factor 3 and urokinase plasminogen activator receptor (UPAR or CD87) for example clustered together and were strongly increased in the

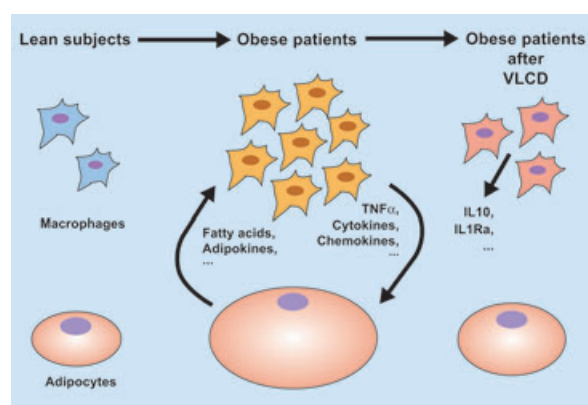


Fig. 1 Schematic representation of the interplay between adipocytes and macrophages during the development of obesity and the weight loss induced by a very low calorie diet (VLCD). Changes in adipocyte size and macrophage phenotype (represented by different shading) influence adipokine and inflammation-related gene expression and secretion during the phases of weight gain and weight loss.

AT of morbidly obese subjects with a high degree of macrophage infiltration [36]. MCP-1 mRNA levels correlate with circulating MCP-1 ($P < 0.05$) and with the level of corpulence ($P < 0.05$) [37]. MCP-1 – also known as CCL2 – is a powerful chemoattractant cytokine, and its action is mediated via its receptor CCR2. Interestingly knock out mice for the CCR2 gene present a reduction in AT macrophage infiltration [38]. Although MCP-1 is a plausible candidate, others remain to be explored. For example, local adipose hypoxia is a plausible mechanism that could facilitate the attraction and retention of macrophages in AT. Local tissue hypoxia is indeed a well-known inducer of macrophage attraction and maintenance in some solid tumours and in atheroma plaques. We observed that hypoxia inducible factor-1 α (HIF-1 α) – a transcription factor strongly induced by hypoxia – is over-expressed in obese AT and its expression decreases during weight loss. These observations raise the hypothesis that the AT of obese patients may present hypoxic areas with an increased local expression of hypoxia stimulated chemoattractant factors. Interestingly, leptin itself is a well known HIF-1 α -inducible gene [39] and was shown to display chemo-attractant properties [40].

Regular exercise also offers protection against mortality primarily by protection against cardiovascular disease and type 2 diabetes mellitus. Longitudinal studies show that regular training induces a reduction in plasma levels of C-reactive protein, suggesting that physical activity may suppress systemic low-grade inflammation [41]. Little is known however of the adaptations of AT inflammatory gene profile to physical exercise. In moderately obese women, a 3 month endurance training programme resulted in an improvement in insulin sensitivity with moderate weight loss [42]. No change in plasma levels and AT mRNA expression of adiponectin, TNF- α and IL-6 was observed. A different type of physical exercise programme, a 3 month dynamic strength training in obese men, produced similar results. An improvement in insulin sensitivity without change in body fat mass was associated with no variations of plasma levels and AT mRNA expression of adiponectin, IL-1 β , IL-6 or TNF- α [43]. These data suggest that the effect of

physical exercise on the inflammatory status of AT may be different from the effect of weight loss induced by calorie restriction. However, a definite answer on the inflammatory status of AT awaits pangenomic gene expression analysis during training programmes.

Candidate biomarkers of inflammatory status in adipose tissue: relevance for obesity complications

Obesity is characterized by a deregulation of many inflammatory biomarkers in the AT. Some of them might link obesity to its downstream complications. Adiponectin was one of the most extensively studied. Adiponectin is secreted at high levels by adipocytes and shows a characteristic NH₂-terminal collagen-like region and a COOH-terminal complement factor C1q-like globular domain. Adiponectin has been shown to exert an anti-inflammatory effect notably in atherosclerosis plaques [44]. This property is explained by suppression of TNF- α and pro-inflammatory cytokines such as IL-6 and interferon- γ and induction of anti-inflammatory factors such as IL-10 and IL-1 receptor antagonist [45]. Adiponectin levels are low in several situations of insulin resistance. Together with its metabolic and anti-inflammatory effect, it has been proposed that adiponectin contributes to the beneficial effect of body weight loss on reducing insulin resistance. The numerous studies conducted so far give a blurry picture. Following bariatric surgery in morbidly obese patients and long-term improvement of insulin sensitivity, an increase in plasma levels and AT gene expression of adiponectin has been observed [46]. However, during low-calorie diets in nonmorbidly obese women, the lack of variation of adiponectin mRNA, AT secretion rate and plasma levels suggests that adiponectin does not play a major role in the improvement of insulin sensitivity observed during moderate weight loss [47, 48]. A further confounding factor is the variability in individual response. Some morbidly obese subjects respond to a short term severe calorie restriction by a decrease in AT adiponectin mRNA levels whereas others do not [49]. Whether or not the polymorphisms in the adiponectin gene associated with the circulating level of adiponectin explain the

differential response to caloric restriction is still to be determined [50].

We and others have observed the decreased expression of a gene encoding the acute phase reactant serum amyloid A (SAA) after weight loss [34, 51–53]. The SAA are apolipoprotein A, usually known to be synthesized by the liver and to be involved in cholesterol transport and in early response to injury. We have shown that SAA gene expression is increased in AT of obese subjects and significantly correlates with adipocyte size and inflammatory biomarkers. This finding was confirmed by immunohistochemistry analysis of AT showing mainly staining in adipocytes in contrast to many inflammatory-related proteins [51]. The production of SAA by human AT has been confirmed by several independent teams [52, 54, 55]. SAA could play a local role in the AT, notably in enhancing the synthesis of inflammatory protein by the macrophage and the free fatty acid release from adipose cells [52]. Although no obvious evidence of a relationship between insulin sensitivity surrogates and SAA was observed in morbidly obese subjects [51], improvement of insulin sensitivity using thiazolidinedione (TZD) showed a decrease of SAA in AT and in the serum of less severely obese subjects [52]. It was then suggested that this inflammatory adipokine could link obesity with its metabolic and vascular complication complications. In agreement with this hypothesis, studies in obese subjects revealed significant associations between AT and plasma levels of SAA and surrogates of sleep apnoea, a condition frequently linked with cardiovascular diseases [56].

The second biomarker identified in human AT by large-scale analysis was cathepsin S (CTSS) [57]. Cathepsin S is a cysteine protease (CTSS), known to degrade several components of the ECM, notably in atherosclerotic plaques, and to be involved in immunity processes, notably antigen presentation. Several studies showed that various models of atherosclerosis-prone mice had higher CTSS levels in their atherosclerosis lesions compared with their lean counterparts [58]. Human investigations showed the abnormal presence of CTSS in atherosclerotic lesions, whereas no expression of CTSS was detected in normal

arteries [59]. Finally, the direct role of CTSS in atherogenesis has been established in transgenic mice model, atheroma-prone LDLR^{-/-} mice crossed with CTSS^{-/-} mice, subjected to high cholesterol diet, that showed significant reductions in atheroma lesions in the absence of CTSS [60]. A recent study performed on a large cohort of patients not selected on body mass index showed that serum CTSS levels were increased in patients with atherosclerotic stenosis [61]. Specialists in the cardiovascular field suggest that elevated levels of CTSS in vascular wall promote atherosclerosis. Using pangenomic arrays and real-time PCR combined with bioinformatics treatment of the data, we have shown in independent groups of individuals that CCTSS is produced by human adipose cells, increased in obesity and decreased with weight loss both in AT and in serum [62]. Its secretion in AT explants is enhanced by inflammatory markers [57]. As ECM remodelling is a key process associated with adipogenesis and cathepsin S, a potent elastolytic protein, this prompted us to assess the potential role of CTSS in promoting preadipocyte differentiation. A set of experiments analysing fibronectin cleavage using both a specific inhibitor and a recombinant protein showed that CTSS facilitates adipogenesis at least in part by degrading fibronectin in the early steps of differentiation [63]. Other cathepsins such as cathepsin K may also be involved in the facilitation of adipose differentiation [64]. Taken together, these results indicate that CTSS and eventually other members of the cathepsin family, released locally by preadipocytes, promote adipogenesis, suggesting a possible contribution of this protease to fat mass expansion in obesity. In addition, given the potential deleterious effect of CTSS on the arterial wall, this protease represents a plausible molecular link between enlarged fat mass and developing atherosclerosis (Fig. 2).

Conclusion

As the physiopathology of obesity is complex, it becomes apparent that a multidisciplinary research effort, involving the combination of various fields (e.g. the clinical, biochemical and genetic fields) is necessary with the aim of increasing our knowledge

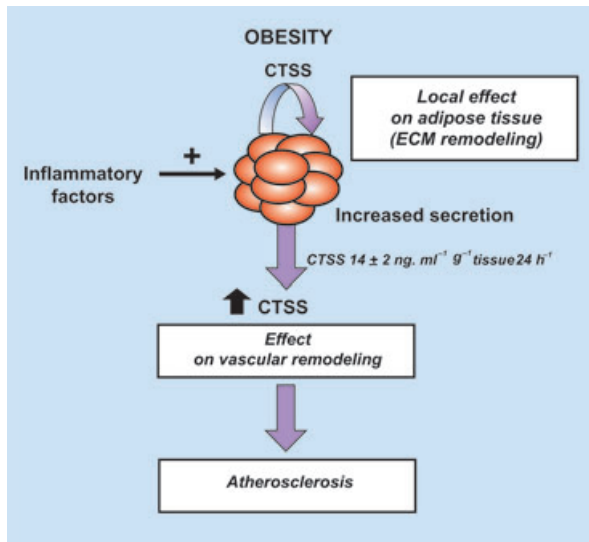


Fig. 2 Working hypothesis regarding cathepsin S (CTSS) a novel biomarker of adipose tissue potentially linking enlarged adipose tissue to its vascular complications.

of the complexity of biological traits and processes of the disease. The use of other 'omics' technologies such as proteomics and metabolomics which assess the protein and metabolites products offers additional and complementary opportunities [65, 66]. In the complex picture of the physiopathology of obesity at its different stages of evolution (weight gain, weight maintenance, resistance to weight loss), studies of genes, proteins and metabolites may unravel the role of certain signals and then could provide better understanding of the mechanisms of fat mass enlargement and related adverse effects. Several European networks aim at completing this task. Optimization of AT biopsies and use of the samples for transcriptomics and peptidomics applications are performed under the MolPAGE program (<http://www.molpage.org>). The Diogenes program (<http://www.diogenes-eu.org>) will allow a concomitant analysis of genetic background, gene expression, peptide and protein analyses during different weight loss and weight maintenance programmes in obese subjects from several European countries. Finally, one of the goals of the HEPADIP project (<http://www.hepadip.org>) is to investigate adipose and hepatic metabolic and inflammatory gene expression in relation to the metabolic syndrome.

Gene expression profiling studies associated with the study of AT cell types have revealed the role of a vast panel of inflammatory biomarkers that could be involved not only in the pathogenesis of obesity but also in molecular mechanisms linking expanded fat mass to obesity co-morbidities. What is lacking now is information on the specific roles of these factors on the occurrence of obesity-related disorders.

Conflict of interest statement

No conflict of interest was declared.

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