Metabolism, Endocrinology and Differentiation of White Adipose Tissue

Metabolismo, Endocrinologia e Diferenciação do Tecido Adiposo Branco

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Abstract
In the last two decades there has been a growing interest in the scientific community to study the function of adipose tissue (AT), partly due to concerns about obesity and its metabolic consequences and also for recognition that adipocytes integrate a wide range of homeostatic processes. This paper shows the advances in research on the white adipose tissue physiology, with emphasis on its role on endocrine, metabolic and inflammatory process, as well as the adipogenic process. The knowledge of the molecular events that regulate differentiation of pre-adipocytes and mesenchymal stem cells into adipocytes or adipogenesis is critical for understanding the genesis of obesity. This issue will also be approached on this review.

Key-Words: adipogenesis; adipokines; obesity

Resumo
Nas últimas duas décadas tem havido um interesse crescente da comunidade científica no estudo da função do tecido adiposo (TA), em parte devido à preocupação com a obesidade e suas consequências metabólicas e também pelo reconhecimento de que os adipócitos integram uma larga gama de processos homeostáticos. Este trabalho descreve os avanços nas pesquisas no que diz respeito sobre a fisiologia do tecido adiposo branco, com ênfase no seu papel endócrino, metabólico e inflamatório, bem como o processo de adipogênese. O conhecimento dos eventos moleculares que regulam a diferenciação dos pré-adipócitos e células-tronco mesenquimais em adipócitos é fundamental para a compreensão da gênese da obesidade. Esta questão também será abordada nesta revisão.

Palavras-chave: adipogenese; adipocinas; obesidade

Introduction
In the last two decades there has been a growing interest by the scientific community to study the function of adipose tissue, partly due to the discovery of its ability to produce biological substances of remarkable power and desktop them the bloodstream through...
which they can reach other tissues located away from the production site, therefore have the property to act as true hormones. This new capability of fat to produce hormones turned it into an endocrine organ (the largest ever so described) and produces substances that are subject to strict control. Their biological actions, in turn, are re-writing a new chapter in the Endocrine Physiology. Still, the adipose tissue is an abundant and accessible source of mesenchymal stem cells that can be used both in basic research and in clinical practice. This field of study is the subject of intensive research, which is justified by the obvious relationship between adipose tissue and major chronic disease entities that have reached epidemic proportions worldwide, which are: diabetes mellitus (especially type 2 - T2DM), the Hypertension Systemic, Metabolic Syndrome and Obesity.

Adipose tissue as a producer of bioactive molecules

The traditional view of adipose tissue as a lipid storage has been challenged (1) since the discovery of some bioactive molecules produced in this tissue, such as the endocrine factor adipsin (2). The identification of leptin in 1994 (3) definitely established this tissue as an endocrine organ. Nowadays, more than one hundred bioactive peptides (most of them acting as hormones) have been described as mediators released by adipose tissue (4). These proteins share structural properties with cytokines, and most of them are produced and secreted exclusively by adipose tissue being generically called as "adipokines".

Among the various adipokines secreted by adipose tissue leptin, adiponectin, adipsin, resistin, TNF-α, PAI-1, interleukins 1β, 6 and 8, insulin-like growth factor 1 (IGF-1), MCP-1 and visfatin have been extensively studied. Except for adiponectin, production and secretion of most of these factors is intensified with obesity (5,6). Several of them, such as TNF-α, resistin, PAI-1, IL-6 and MCP-1, are directly associated with the induction of insulin resistance, hypercoagulability and atherogenesis. These conditions raise blood pressure, intensify the inflammatory state and increase the risk of cardiovascular and thrombo-embolic events (7,8).

The plasma concentration of IL-6 is considered a marker of T2DM and cardiovascular diseases (9). In addition, obesity decreases the plasma levels of adiponectin in rodents and humans (10). The decrease in plasma adiponectin levels observed in obese subjects (hypoadiponectinemia) is an independent risk factor for T2DM and cardiovascular complications (11,12). In addition to its function as insulin sensitizer, adiponectin can protect against most major diseases related to obesity, including hypertension, atherosclerosis, fatty liver, heart failure, airway inflammation and breast cancer (13,14,15). Leptin also plays an important role in maintaining metabolic homeostasis.
(including insulin sensitivity) in the regulation of energy deposits. Leptin reduces food intake and increases energy expenditure (16) and fertility (17), being considered an antilipotoxic agent. A lipid molecule, the palmitoleic acid (C16:1, ω-7), has also been described to be synthesized and secreted by adipose tissue. This molecule has been named as "lipokine", and presents the ability to associate this tissue to the control of the intermediary metabolism. This fatty acid acts as a hormonal signal that emerges from the adipose tissue and increases the sensitivity of liver and muscle to insulin (18).

**Metabolic Actions of WAT**

Its main metabolic actions can be divided in: lipogenesis (biosynthesis, incorporation and storage of TAG) and lipolysis (release of FFA and glycerol). For the biosynthesis of TAG, the adipocyte needs glycerol-3-phosphate (glycerol-3-P) and esterified with FFA coenzyme A (acilCoA). The first comes from the glycolytic pathway, and the second in the biosynthesis from Acetyl or its catchment lipoproteins (chylomicrons and VLDL).

To produce glycerol-3-P, glucose uptake is necessary, which involves specific transporter proteins, the GLUT (GLUT1 and GLUT4), a process controlled by insulin. Thus, insulin secreted in the postprandial period stimulates translocation of GLUT4 to the cell membrane, increasing the transport glucose. Furthermore, the rate of the metabolism of this hexose is accelerated by insulin, causing an increase in glycerol-3-P. Part of the metabolites of the glycolytic pathway is directed to training pyruvate, which, within the mitochondria is converted in Acetyl CoA by the pyruvate dehydrogenase complex (PDH). This is coupled to oxaloacetate by citrate synthase (CS), generating citrate. Part of the citrate returns to the cytoplasm, where it undergoes the action of ATP-citrate lyase (ATP-CL), reetracing Acetyl. This, under the action of Acetyl carboxylase (ACC) becomes malonilCoA. The latter joins the synthesis fatty acids, catalyzed by fatty acid synthase (FAS), and culminates in the formation of acilCoA, which is esterified with glycerol-3-P to form TAG. This is ultimately incorporated in the cytoplasmic droplet of fat. The FAS needs, for its action, NADPH, provided by pentose via (in parallel to the glycolytic pathway) or by the malic enzyme (ME).

In addition to synthesizing FFA, these are provided in greater amount by lipoproteins. They suffer the action of LPL microcirculation of the AT. Thus, the FFA released from the particles are taken up by adipocytes. The CD36 molecule present in the plasma membrane presents the FFA to another protein, the transporter protein of FFA, which, like CD36, is an integral membrane protein, facilitating its diffusion into the cell. At cytosol the FFA binds to another protein, FABP, that transport this to be esterified with the coenzyme A. This process is performed by another integral membrane
protein, the acilCoA synthase (ACS). Finished this stage, this acilCoA is transported by ACBP (acilCoA binding protein) to local of esterification with glycerol-3-P. The TAG formed is transferred to the lipid droplet.

Another important skill of adipocytes is the lipolysis of the TAG, releasing FFA and glycerol. This process depends on the activation the Hormone-Sensitive Lipase HSL. This is achieved by serine phosphorylation through protein kinase A (PKA). This process is driven primarily by catecholamines, and occurs during fasting or when energy demand is high, as in physical exercise under stress conditions due to the intense sympathetic flow request. Thus, it will generate cyclic AMP (cAMP) intracellular, with consequent activation of PKA, which also phosphorylate perilipin (proteins that surround the fat droplet). The phosphorylated perilipin leave the surface of the droplets are dispersed by the cytosol opening spaces to HSL reach its substrate, the TAG. The FFA released bind to FABP, are brought to the cell membrane and transported to the extracellular milieu by FATP. Glycerol is transported outwards by transporters specific proteins belonging to the family of aquaglyceroporin (or AQP7 aquaporin 7).

**Differentiation of WAT**

Two types of adipose tissues with distinct functional properties are classically described in mammals: WAT (White Adipose Tissue) and BAT (Brown Adipose Tissue). Both are involved in energy balance. BAT is specialized in dissipating energy as heat during the cold-induced thermogenesis and by diet. WAT is the major energy reserve of the body storing lipids in the form of triacylglycerols (TAG). This latter tissue contains adipocytes, preadipocytes (adipocyte precursor cells), endothelial cells, stromal vascular cells, fibroblasts, leukocytes and macrophages. Additionally, mesenchymal stem cells can also be isolated from this tissue. These cells can potentially differentiate into various cell lineages, including myocytes, chondrocytes, osteoblasts and adipocytes (19).

The increase in fat mass, which occurs in obesity, is determined by an augment in adipocyte size (hypertrophy) and/or number (hyperplasia). Changes in size (diameter and volume) occur in response to activation and regulation of its metabolic pathways: lipogenesis and lipolysis. The flux of metabolites through these pathways varies with the need for incorporation or release of lipids. Several conditions affect adipocyte metabolism: the nutritional status, energy expenditure, influence of hormones (catabolic or anabolic), activities of enzymes involved in these processes and the adipose tissue depot (20). Changes in adipocyte number (hyperplasia) depend on the differentiation of preadipocytes into adipocytes, a process called adipogenesis. Thus, the full knowledge of the adipocyte differentiation process may allow
better understanding and/or control of adiposity.

As mentioned, the development of obesity results not only from the hypertrophy, but also from the hyperplasia of fat cells. Increase in the size of adipocytes is not an unlimited process. Eventually, the growth reaches a maximum level beyond which the ability of fat storage is exhausted and new cells are being slowly recruited and emerge from this tissue. Very large adipocytes that get into exhausted their ability to store fat become more lipolytic. This process allows an increase in plasma concentration of free fatty acids that in turn may impair the function of non-adipose organs, a process called lipotoxicity (21). Mature adipocytes are naturally protected from lipotoxicity due to its high capacity for detoxification of fatty acids (22). A proportion of 15-50% of the adipose tissue cells represents a reservoir of mesenchymal stem cells. These latter cells include preadipocytes that can divide and differentiate in response to various extracellular agents as discussed below. These cells, however, have a very limited ability to synthesize and stock neutral lipids (23).

The expansion of TAB in childhood obesity is recognized to result from the combination of both hypertrophy and hyperplasia of adipocytes. In opposition, adults have been thought to present a fixed number of adipocytes and that changes in fat mass were mainly secondary to changes in the volume of fat cell. However, mature adipocytes exhibit remarkably intense and constant renewal (24). The potential for generating new cells persists throughout the life in adipose tissue. Adipocytes derive from multipotent mesenchymal stem cells that reside in the stroma of adipose tissue (25). These multipotent cells become preadipocytes when they lose their ability to differentiate into other mesenchymal lineages and become committed to the adipocyte lineage. This early stage of adipocyte differentiation is known as determination or commitment and it is still poorly characterized. The next phase of adipogenesis is the terminal differentiation. The preadipocytes acquire the characteristics of mature adipocytes, such as accumulation of lipid droplets and the ability to respond to hormones such as insulin. The differentiation involves activation of a cascade of transcriptional events, which lead to the establishment of the differentiated state (25). The understanding of the events involved in adipogenesis has markedly increased in the last two decades with the use of clonal cells and non-clonal precursors of adipocytes from rodents and humans. Glucocorticoids and insulin-like growth factor 1 (IGF-I) were identified as the most efficient adipogenic agents in ex vivo experiments (26).

**Transcriptional regulation of adipogenesis**

Events that occur during adipogenesis have been extensively studied employing ex vivo cellular models. Pre-adipocyte from 3T3-L1 cell line (derived from embryos of Swiss
mice prematurely extracted) have been widely used. Stimulant cocktail containing insulin (Ins), dexamethasone (Dex), methyl-isobutyl-xanthine (MIX) and fetal bovine serum (FBS) triggers adipogenesis in 3T3-L1 cells (27,28). The differentiation of these cells involves precisely controlled stages: cell cycle arrest, clonal expansion and differentiation (initial, intermediate and terminal events) through activation or silencing of hundreds of genes. The exposure of cultured confluent 3T3-L1 pre-adipocytes to the adipogenic cocktail activates glucocorticoid receptors (by dexamethasone), IGF-1 receptor (by insulin) and cAMP signaling pathways (by MIX, a phosphodiesterase inhibitor), which lead to activation of the early events represented by expression of CCAAT/enhancer-binding proteins - C/EBPs: C/EBPβ and C/EBPδ. As a consequence, the cells re-enter the cell cycle, undergo several rounds of regulated cell division (clonal expansion), and then permanently leave the process of cell cycle and enter into terminal differentiation by activation of the peroxisome proliferator-activated receptor gamma (PPARγ) and C/EBPα, the two main regulators of adipogenesis (29,30,31). These two proteins are responsible for regulating expression of genes that dictate much of the mature adipocyte phenotype.

At the confluence, preadipocytes express the very early markers of differentiation [such as lipoprotein lipase (LPL) and collagen type VI] induced by cell-cell contact. After 1 hour of the cocktail addition, a transient expression of proto-oncogenes c-fos, c-jun and myc occurs. They begin the post-confluent mitosis that is important for the occurrence of the unwinding DNA helices allowing access of transcription factors to response elements present in the target genes involved in modulating the phenotype of mature adipocytes. The increased expression of the proto-oncogenes ceases after 2-3 hours of treatment. C/EBP-β and -δ are the first transcription factors being induced following exposure of the cells to the cocktail of differentiation. These transcription factors are directly involved in the process of differentiation (in response to hormonal inducers). In fact, the expression of C/EBPδ stops after 48 hours, whereas the decline in C/EBPβ expression occurs gradually (around the 8th day post-differentiation). C/EBPβ and C/EBPδ activate the expression of PPARγ, which is transcriptionally induced around the two-day period following the induction of differentiation and reaches a maximum in 3-4 days. C/EBPβ and C/EBPδ also induce expression of C/EBPα, that reaches the maximal increase within 4-5 days of differentiation. Once activated, the central regulators of adipogenesis (C/EBPα and PPARγ) reciprocally and positively regulate themselves in order to remain highly expressed, despite the reduced expression of C/EBP-β and -δ. C/EBPα and PPARγ induce transcription of over one hundred previously
silent target genes, including enzymes and proteins involved in the generation and maintenance of adipocyte phenotype, such as those involved in the insulin-sensitive glucose transport, lipogenesis, lipolysis and synthesis and secretion of adipokines. Both factors play a critical role for the later stages of differentiation in a cooperative and synergistic manner, but they are not expressed at high levels in preadipocytes and are not involved in early development.

**Adipogenesis in the living organism**

Until to the middle of last century, the adipose tissue was considered a terminal structure, in terms of differentiation, that is, once the differentiation was complete, the tissue no longer would be renewed. This concept has been reviewed lately. Firstly, there is evidence that it is possible to extract from this tissue cells similar to fibroblasts with high potential for differentiation. Cells similar to fibroblast derived from adipose tissue (32), after appropriated stimulation, can be differentiated into chondrocytes, osteoblasts and adipocytes, which open a field of research on cell differentiation with immense perspectives. Secondly, recovery of the adiposity (or fat mass) was described after lpectomy or denervation of the white adipose tissue (33). The authors attributed the recovery after lpectomy to the effect induced by denervation after surgery. The contribution of sensory information on the size of fat mass to the central nervous system would be compromised, resulting in compensatory neural response. This occurs because in the lipectomized or lipo-denervated region the remaining preadipocytes expand and differentiate rebuilding the tissue.

In a recent study, Spalding and colleagues (24) assessed the development of adipose tissue by determining the content of $^{14}$C resultant from the radioactive contamination of the atmosphere from the post-war period, as a consequence of two nuclear explosions. Part of this radioisotope was accumulated in the adipose tissue and by determining the radioactivity remaining in this tissue and using a complex mathematical analysis, the authors concluded that the number of adipocytes present in the WAT remains unaltered throughout the life of the subject, regardless of the body weight gain or loss. The authors also calculated a possible renewal rate of the tissue in humans and concluded that adipose tissue is completely renewed every 8-9 years. These authors mentioned that the renewal of the tissue is due to a balance between the generation of new adipocytes to replace older apoptotic cells. In a former study, the rate of disappearance of $^{14}$C-thymine from the adipose tissue in rats previously injected with the radiolabel was estimated and the mean lifespan of adipocytes using this methodology was calculated to be 140 days (34).

Little information exists about adipogenesis *in vivo*. Although it is possible to induce adipogenesis in fibroblast like cells
extracted from the WAT of animals or even humans, there is no standardized or established methodology to determine and quantify the phenomenon \textit{in vivo}. This difficulty has led researchers to argue whether adipogenesis actually occurs in living organisms. Contrary to the difficulty of studying adipogenesis \textit{in vivo} and considering that the renewal of adipose tissue involves the replacement of dead adipocytes by new ones, the process of cell death (apoptosis) is better known (35, 36, 37, 38).

Resistance to admit the occurrence of adipogenesis has been gradually overcome and the major challenge is to understand the importance of this process for the physiology of adipose tissue and its association with other important processes as the overall control of metabolism, feeding behavior and body weight. The understanding of the physiology of adipose tissue and the relationship between rates of apoptosis and adipogenesis in this tissue plays a critical role for understanding the events leading to the obesity and the metabolic syndrome. Moreover, the understanding of the dynamics of adipose tissue renewal and the mechanisms governing the balance between adipogenesis and apoptosis will allow a more appropriate intervention in order to control the development of obesity and to prevent or reduce the potential risk of metabolic syndrome, T2DM, atherosclerosis and their evolution to cardiovascular and cerebral strokes.

The balance between the hyperplastic and hypertrophic activities of adipose tissue determines the number of cells in this tissue. In obesity, the prevalence of hypertrophied cells causes an imbalance between the tissue mass increase and the unmatched blood flow, which leads ultimately to hypoxia, inflammation and macrophage infiltration in the tissue (39). Macrophages release factors that affect the adipogenic process in human cells. There is also a decrease in the capacity of lipid buffering by the adipose tissue with subsequent ectopic deposition of fat in other tissues. This fact coupled with the abnormal production of adipokines causes the disorders associated with obesity, mainly insulin resistance. Hypertrophic adipocytes are major producers of proinflammatory cytokines (TNFa, IL6, IL-1, resistin, MCP1) whereas also show limited ability to synthesize and release an important anti-inflammatory adipokine, adiponectin that is the most potent endogenous insulin sensitizer. Stimulation of \textit{in vivo} adipogenesis can replace hypertrophied adipocytes by younger and smaller ones with greater ability to produce adiponectin at the expenses of pro-inflammatory adipokines.

To what extent would be beneficial to promote hyperplasia (or adipogenesis)? The body tends to keep constant the number of cells, that is, as new cells are being recruited, "aged" hypertrophied cells are replaced. The newly differentiated cells become mature, hypertrophied and then assume opposite features. As a result of adipocyte hypertrophy,
obesity arises and the benefits of adipogenesis are lost. It should not be discarded, however, the idea of activating adipogenesis for therapeutic purposes. There are situations in which stimulation of adipogenesis can be extremely beneficial, as are the cases of lipodystrophic syndromes. In other situations, such as aging, that show a strong attenuation of the adipogenic capacity of patients, the pro-adipogenic therapy could also be beneficial to health.

Concluding Remarks

The growth of adipose tissue, which culminates in obesity, results from hypertrophy and hyperplasia of adipocytes, the latter being depends on differentiation of preadipocytes into adipocytes. Obesity is associated with a picture of chronic inflammation which predisposes to insulin resistance and development of type 2 diabetes and increased cardiovascular risk. The main responsible for this scenario is certainly the dysfunction of adipose tissue, whose situation is characterized by high production of cytokines from adipocytes.

The knowledge of the molecular events that regulate the differentiation of pre-adipocytes and mesenchymal stem cells into adipocytes (adipogenesis) is critical for understanding the genesis of obesity. A better understanding of the functioning and development of adipose tissue will have direct impact on the elucidation of issues related to obesity and associated diseases, as well as in developing new therapies that target intracellular pathways located in adipocytes.

References


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