



## Obesity and diabetes: the link between adipose tissue dysfunction and glucose homeostasis

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### Abstract

Obesity and type 2 diabetes mellitus (T2DM) epidemics, which have already spread, imply the possibility of both conditions being closely related. Thus, the goal of the present review was to draw a parallel between obesity, adipose tissue (AT) changes, and T2DM development. To this end, a search was conducted in PubMed, MEDLINE and SciELO databases, using the following key words and their combinations: obesity; diabetes; insulin resistance; diet; weight loss; adipocin; inflammation markers; and interleukins. Based on a literature review, AT dysfunction observed in obesity is characterised by adipocyte hypertrophy, macrophage infiltration, impaired insulin signalling and insulin resistance. In addition, there is release of inflammatory adipokines and an excessive amount of NEFA promoting ectopic fat deposition and lipotoxicity in muscle, liver and pancreas. Recent evidence supports the hypothesis that the conception of AT as a passive energy storage organ should be replaced by a dynamic endocrine organ, which regulates metabolism through a complex adipocyte communication with the surrounding microenvironment. The present review demonstrates how glucose homeostasis is changed by AT dysfunction. A better understanding of this relationship enables performing nutritional intervention strategies with the goal of preventing T2DM.

**Key words:** Inflammation: Type 2 diabetes mellitus: Fat deposition: Obesity

### Introduction

Obesity is the chronic and excessive accumulation of body fat and, due to its high prevalence in various locations in the world, is considered a pandemic<sup>(1)</sup>. It predisposes increased risk of premature morbidity and mortality, since there is a relationship between the increase in BMI and related diseases, such as hypertension, type 2 diabetes mellitus (T2DM) and CVD<sup>(2)</sup>.

Currently, obesity is characterised by a metabolic process associated with low-intensity chronic inflammation, which is evidenced by an increased concentration of circulating inflammatory mediators in an asymptomatic form<sup>(3–5)</sup>. In addition to the functions of mechanical protection of organs, body temperature control and energy storage, adipose tissue (AT) is also considered an active endocrine organ. It is able to send and respond to signals in order to modulate appetite, energy intake, insulin sensitivity, the endocrine and reproductive system, bone metabolism and inflammation, among other functions<sup>(4,6–9)</sup>.

More than fifty inflammatory proteins have been linked to obesity and its co-morbidities, and the mechanisms by which

these proteins influence the manifestation of these diseases seem to involve the attenuation of insulin activity, fat mobilisation, endothelial dysfunction and oxidative stress<sup>(5)</sup>. On the other hand, it has also been suggested that reduction in weight and body fat percentage can decrease the concentration of circulating pro-inflammatory molecules and increase anti-inflammatory molecules<sup>(4,6)</sup>.

Based on the above-mentioned considerations, the goals of the present review were drawing a parallel between changes in AT, the genesis of obesity and the development of T2DM, and discussing whether the reduction in risk of co-morbidities resulting from body-weight reduction may be due to a decrease in pro-inflammatory factors.

A search was conducted using PubMed, MEDLINE and SciELO scientific databases with the following key words and their combinations: obesity; diabetes; insulin resistance; diet; weight loss; adipocin; inflammation markers; and interleukins. Reviews were consulted in order to clarify the mechanisms involved in the pathogenesis of obesity and insulin resistance.

**Abbreviations:** AT, adipose tissue; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; T2DM, type 2 diabetes mellitus; TZD, thiazolidinediones.

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## Adipose tissue: a lipid storage organ

Positive energy balance leads to adipocyte hypertrophy and hyperplasia. In the context of obesity, even though AT may have a strategic protector effect, it can also be considered dangerous when in excess<sup>(2)</sup>. Despite the clinical importance of obesity, knowledge about its origin, development and functions related to AT is still limited.

## Origin of adipose tissue

AT is the primary site for the storage of energy and it also functions as an endocrine organ that regulates energy homeostasis via secretion of adipokines, such as adiponectin, leptin and resistin<sup>(10,11)</sup>. Although the origin and development of adipocytes are not completely elucidated, the functions of AT under normal conditions and adverse effects of excessive adiposity linked to weight gain can be related to these biological markers<sup>(10)</sup>.

Only white AT was taken into consideration in the present review, because the adipocytes that compose it are especially involved in obesity. The development of AT begins during pregnancy in higher mammals, and shortly after birth in rodents. Dense regions of mesenchymal cells linked to vascular structures form the sites where AT is developed. Depending on the epigenetic event, multipotent mesenchymal stem cells turn into unipotent adipoblast cells, and the latter turn into pre-adipocytes<sup>(12,13)</sup>. According to the stimulus, pre-adipocytes suffer adipogenic conversion, i.e. they accumulate lipids in a single lipid droplet and become sensitive to insulin, thus being called functional or mature adipocytes<sup>(12)</sup>.

In addition to the adipocytes and their precursors, AT contains a matrix formed by collagenous and reticular fibres, nerve cells, blood vessels, lymph nodes, immune cells (leucocytes, macrophages) and fibroblasts<sup>(14)</sup>. A study conducted by Dudakovic *et al.*<sup>(15)</sup> revealed that mesenchymal stem cells from AT spontaneously express fibroblastic, osteogenic, chondrogenic and adipogenic biomarkers when kept in confluent cell cultures, which demonstrates their versatility.

The stimuli for adipogenesis in AT-derived stem cells result in increased activity of glycerol-3-phosphate dehydrogenase, which is a lipogenic enzyme involved in the synthesis of TAG. In addition, so that adipocytes become mature, AT-derived stem cells increase the expression of other proteins involved in the biosynthesis and storage of lipids, such as: lipoprotein lipase

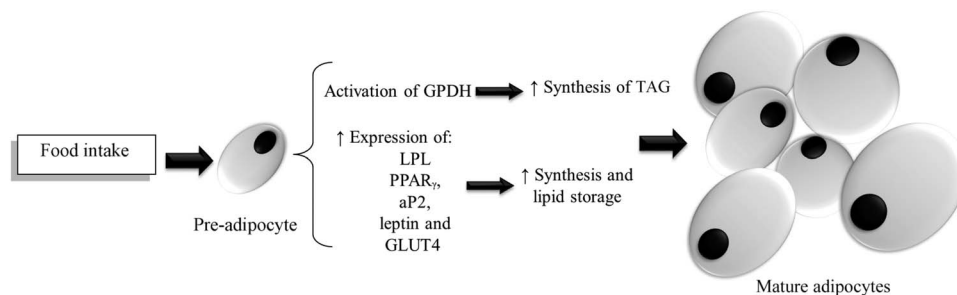
(LPL), which is an exchange enzyme; PPAR $\gamma$ , a transcription factor that causes pre-adipocyte differentiation; adipocyte fatty acid binding protein (aP2); glucose transporter GLUT; and leptin<sup>(16)</sup>.

AT can expand throughout life in response to increased food intake resulting in adipocyte hypertrophy due to the accumulation of TAG. Still, there may be hyperplasia due to resident pre-adipocyte differentiation and mesenchymal progenitor cells in new adipocytes (Fig. 1). AT mass also increases in response to treatment with thiazolidinediones (TZD) used clinically as anti-diabetogenic agents. These agents stimulate the emergence of new adipocytes by activating the nuclear receptor PPAR $\gamma$ . Weight gain and treatment with TZD are associated with changes in circulating concentration of cytokines and chemotactic factors (CCR2, CXCL10 and RANTES) that can regulate progenitor cell mobilisation, trafficking and recruitment<sup>(10)</sup>.

Even though there is consensus on the origin of adipocytes from mesenchymal progenitor cells residing in AT, some studies have shown that mesenchymal stem cells and multipotent progenitor cells can be isolated from other tissues and induced to adipocyte differentiation *in vitro* and *in vivo*<sup>(11,17–19)</sup>. Thus, distinct populations of adipocytes may be identified in AT from different regions of the body.

Functional variations between white fatty tissues can result from specific differences between the progenitors of these fat deposits. Crossno *et al.*<sup>(10)</sup> assessed whether bone marrow progenitor cells could contribute to the development of new adipocytes in AT through bone marrow transplantation from transgenic mice expressing green fluorescent protein (GFP) to C57BL6 wild-type mice subjected to treatment with a hyperlipidic diet or rosiglitazone for 8 weeks. The analyses revealed the presence and the increase in GFP<sup>+</sup> multilocular adipocytes in both treatments. In turn, these adipocytes expressed adiponectin, perilipin, fatty acid-binding protein, leptin and PPAR $\gamma$ , but not uncoupling protein-1, a marker of the CD45 haematopoietic line. The authors concluded that bone marrow-derived progenitor cells can traffic to AT and differentiate into adipocytes.

Tang *et al.*<sup>(20)</sup> found a subpopulation of stromal-vascular cells expressing PPAR $\gamma$ , i.e. a marker of newly formed adipocytes. In addition, the authors assessed the adipogenic potential of these cells when subjected to spontaneous adipogenesis or stimulation by insulin. This procedure revealed that blood vessels of AT seem to function as an adipocyte progenitor niche and may provide signs for their development.



**Fig. 1.** Influence of positive energy balance on adipogenesis. An excess of energy nutrients makes pre-adipocytes increase the expression of mediators associated with the accumulation of intracellular TAG transforming them into mature adipocytes. GPDH, glycerol-3-phosphate dehydrogenase; LPL, lipoprotein lipase; aP2, adipocyte protein 2 – carrier protein for fatty acids.

Changes in adiposity with modification of body fat distribution, AT metabolism and inflammation profile are also associated with age, weight gain and sex. These changes especially with respect to visceral fat may be linked to obesity-related co-morbidities, particularly to T2DM. According to a study conducted by Majka *et al.*<sup>(11)</sup>, bone marrow-derived adipocytes accumulate in a specific way and according to sex in certain regions of the body. The researchers affirm that this differential accumulation emphasises gene expression patterns in adipocytes derived from bone marrow precursors, which exhibited reduced expression of mitochondrial and peroxisomal genes related to the biogenesis of these organelles and lipid oxidation, and increased expression of genes encoding pro-inflammatory cytokines.

These results challenge the paradigm of a resident mesenchymal origin for white AT adipocytes and point out that adipocytes may be originated from bone marrow-derived progenitor cells. Moreover, they reveal that the different sub-populations of adipocytes may perform specific roles according to sex and the physiological changes that occur in the stages of life. Thus, adipocyte precursor cells are indeed heterogeneous cell populations, and this is the origin potentially associated with the various functions that AT can exert and the sites in which it can expand.

#### *Development of adipose tissue via PPAR $\gamma$ : molecular considerations*

It is known that the functions of AT are altered in obese individuals with insulin resistance. In addition, morphological changes, associated with hyperglycaemia and the beginning of T2DM, are also observed in adipocytes<sup>(21)</sup>.

The great interest of researchers in studying PPAR $\gamma$  is due to its high expression in AT, its relevant role in adipogenesis, and the possibility of its induction serving as a model system to study transcriptional and epigenetic regulation of genes expressed in specific cell types<sup>(22)</sup>. In pre-adipose cell lines, the expression of PPAR $\gamma$  during cellular differentiation has been associated with lipid accumulation and the expression of genes involved in adipogenesis, such as aP2, LPL and adipsin. PPAR $\gamma$  also induced the differentiation of pre-adipocytes in mature cells from human subcutaneous AT concomitantly with the activation of genes involved in TAG synthesis and storage<sup>(21)</sup>.

PPAR $\gamma$  can also play an important role in the regulation of lipogenesis; however, this mechanism has not yet been elucidated. Kubota *et al.*<sup>(23)</sup> tried to understand this process by studying PPAR $\gamma^{+/-}$ -deficient mice fed on a hyperlipidic diet. The authors found that these animals had gained less weight and had less AT when compared with those of the control group. Genes under transcriptional control of PPAR $\gamma$  in AT include genes encoding enzymes involved in the metabolism of fatty acids, such as LPL, acyl-CoA synthase and FAT/CD36 (cluster of differentiation 36 – known as fatty acid translocase), suggesting that PPAR $\gamma$  plays an important role in lipid uptake by adipocytes<sup>(24)</sup>. Way *et al.*<sup>(25)</sup> studied the effects of GW 1929 (PPAR $\gamma$  agonist) on glucose and TAG concentrations and the expression of genes in diabetic and obese Zucker rats. The treatment led to reductions in NEFA, glucose and TAG

concentrations. In addition, the authors confirmed an increased expression of genes involved in lipogenesis, such as acetyl-CoA carboxylase, fatty acid synthase and ATP-citrate lyase.

In addition to its role in lipogenesis, PPAR $\gamma$  can also contribute to the inhibition of genes such as the one that encodes leptin through lipolysis activation and fatty acid oxidation<sup>(26,27)</sup>. Kadowaki *et al.*<sup>(28)</sup> assessed the expression of PPAR $\gamma$  and leptin concentration in mice with and without this receptor deficiency when fed on a fat-rich diet. It was observed that the PPAR $\gamma$ -deficient group exhibited an increase in leptin concentration, in addition to the adipocytes being smaller. These results indicate that PPAR $\gamma$  can assist in the regulation of obesity and insulin resistance, since its low expression stimulates lipolysis and hinders glucose uptake.

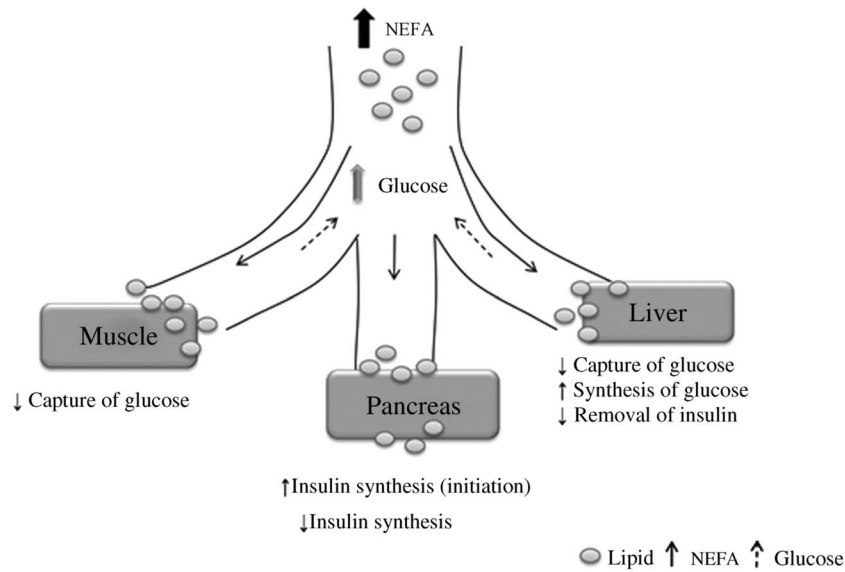
#### *Lipid accumulation and development of type 2 diabetes mellitus*

TAG deposited in AT serve as high-density energy fuel and corresponds to 85 % of adipocyte weight<sup>(29)</sup>. AT is crucial for reducing the influx of dietary fat that reaches the circulation daily, since it suppresses the release of NEFA into the blood and increases TAG clearance<sup>(1)</sup>.

In obesity, lipid storage capacity is reduced, especially in the postprandial period. This process probably occurs because the volume of adipocytes in obese individuals has already reached its limit and cannot accumulate TAG efficiently. Added to this, there is an increase in the rate of lipolysis during the fasting period<sup>(9)</sup>. As a result, other tissues are exposed to excessive NEFA influx<sup>(1)</sup>, thus compromising their functions. The processes derived from lipid accumulation in the bloodstream are illustrated in Fig. 2.

The excess of lipids in the bloodstream and their accumulation in organs, such as the pancreas, liver and muscle, seem to contribute to the development of insulin resistance and reduced secretion of insulin in overweight individuals. Ferrannini *et al.*<sup>(30)</sup> assessed the acute effect of increased NEFA concentration on glucose uptake and found that NEFA infusion in hyperinsulinaemic and normoglycaemic individuals had caused a reduction in glucose uptake. A similar result was observed in subjects with hyperglycaemia and hyperinsulinaemia. On the other hand, in individuals with hyperglycaemia, hypoinsulinaemia and hyperglucagonaemia (simulation of the diabetic condition), the infusion did not affect glucose uptake, but stimulated its production. The authors concluded that in appropriate insulin concentrations, increased concentration of NEFA in the blood competed with glucose during uptake by peripheral tissues, regardless of the presence of hyperglycaemia and, when insulin is deficient (diabetes), the excess of lipids in the bloodstream can contribute to hyperglycaemia, given the increase in glucose synthesis.

Taking into consideration that NEFA may contribute to inhibiting the function of pancreatic  $\beta$  cells, a study<sup>(31)</sup> demonstrated that exposure of rat islets to palmitate raised the TAG content to 70 % in these structures after 6 h cell culturing, and 200 % after 48 h. This increase was associated with the inhibition of insulin secretion. A similar result was found in a study on human pancreatic islets, whose cell cultures exposed



**Fig. 2.** Accumulation of NEFA in the blood and relationship with type 2 diabetes mellitus (T2DM) development. The reduction in lipid storage capacity in adipose tissue (AT) observed in obesity leads to an increase in NEFA in the bloodstream and their influx into muscle, pancreas and liver. This process leads to insulin resistance, reduced glucose uptake, increased glucose synthesis in the liver, reduction in the synthesis and insulin release in the pancreas, and consequent hyperglycaemia, characteristic of T2DM.

to palmitate or oleate had inhibited insulin secretion and proinsulin synthesis<sup>(32)</sup>. A possible explanation is that the addition of palmitate directs the metabolism of  $\beta$  cells to lipid synthesis by increasing the expression of enzymes involved in this process (glycerol-3-phosphate acyltransferase, diacylglycerol acyltransferase and hormone-sensitive lipase)<sup>(33)</sup>.

NEFA represent an important energy source for the skeletal muscle and this organ has insulin resistance due to the increased lipid content in muscle fibres. One of the hypotheses for muscle insulin resistance was proposed by Randle *et al.* (1963) cited by Tabidi<sup>(34)</sup>. They postulated that increased NEFA oxidation causes elevation of acetyl-CoA:CoA and NADH:NAD<sup>+</sup> ratios in the mitochondria, providing the inactivation of pyruvate dehydrogenase. As a result of the increase in acetyl-CoA, the citrate concentration increases, resulting in inhibition of phosphofructokinase and accumulation of glucose-6-phosphate, which, in turn, inhibits hexokinase II reducing glucose uptake. An alternative hypothesis was suggested by Roden *et al.* (1996) cited by Kovacs & Stumvoll<sup>(35)</sup>, who described the increase in NEFA in plasma inducing insulin resistance through inhibition of glucose transport and phosphorylation, given the reduction in phosphatidylinositol 3-kinase activity associated with insulin receptor substrate-1. It is suggested that insulin resistance associated with NEFA involves blocking intracellular signalling by insulin due to fatty acid by-products.

As TAG content correlates with insulin resistance in skeletal muscle, Goodpaster *et al.*<sup>(36)</sup> assessed whether worsening of insulin resistance could result from the increase in TAG, and whether these lipids would be increased in muscle fibres of obese individuals. The study revealed that lipid content in muscle fibres was greater in obese individuals with T2DM than in eutrophic individuals; however, it was not different in

eutrophic individuals with T2DM. The authors also found that weight reduction contributed to the decrease in lipid content in muscle cells.

About 80 % of endogenous glucose production occurs in the liver and it is responsible for increased blood glucose concentration during the fasting period. The endogenous production of glucose occurs in T2DM in spite of hyperinsulinaemia. One of the consequences of the increase in NEFA flow for the liver in T2DM is the inhibition of insulin suppression of glucose production<sup>(35)</sup>. D'Adamo *et al.*<sup>(37)</sup> assessed the role of hepatic lipid accumulation in adolescents and found that the group of individuals with an elevated liver fat percentage (>5.5 %) had exhibited lower insulin secretion and sensitivity, and reduced suppression of endogenous glucose production. A similar result was found by Koska *et al.*<sup>(38)</sup> in a study conducted with adults, whose liver lipid content was a predictor of reduced suppression of liver glucose synthesis mediated by insulin.

Thus, an ectopic deposition of NEFA influences the metabolic balance of nutrients. Possibly the accumulation of fat in other organs is favoured by the density increasing of the extracellular matrix that composes the AT. This characteristic reduces the adipocytes' ability to store the excess of nutrients. Collagen VI is a protein that appears to be involved in the organisation of the matrix architecture. The reduction in gene expression that encodes it leads to the development of larger adipocytes, i.e. it enhances the fat storage capacity, reduces fibrosis and inflammation, and improves macronutrient homeostasis. A comparison between this characteristic and metabolically healthy obese individuals can be defined, once such individuals have mechanisms that lead to lower visceral fat deposition, fibrosis and subclinical inflammation reduction, and reductions in glucose and lipid concentrations in the blood. However,

metabolically healthy obese generally present lower adipocytes when compared with unhealthy obese. Thus, the relationship between hypertrophy of fat cells and improvement of the metabolic profile is difficult to draw. A mechanism that can be thought of is the possible relationship between adipocyte hyperplasia, which results in many small fat cells, and the maintenance of glucose uptake and the secretion of adipokines at normal levels<sup>(39)</sup>.

Gastaldelli *et al.*<sup>(40)</sup> assessed the relationship between hepatic and visceral fat accumulation, and insulin resistance and hepatic gluconeogenesis. The authors found that excess visceral AT increased gluconeogenesis. Both visceral AT and hepatic fat accumulation were associated with insulin resistance. On the other hand, medications such as pioglitazone and metformin can improve insulin sensitivity by reducing intracellular TAG content in the liver and muscle, although the mechanism is still unknown<sup>(41)</sup>. It is known that treatment with thiazolidinedione improves the metabolic profile of obese patients and one possible explanation for this result is the increase in the number of adipocytes and body adiposity<sup>(39)</sup>. In addition to increasing hepatic glucose production, NEFA increase the production of VLDL and reduce the removal of insulin by the liver, causing hyperlipidaemia and hyperinsulinaemia<sup>(1)</sup>. All these results explain the intrinsic relationship between obesity and T2DM development.

### Importance of vascularisation in adipose tissue

Metabolically, the dense network of blood vessels present in AT serves for the systematic transport of lipids towards the adipocytes. On the other hand, these vessels also carry adipokines and adipocyte nutrients to meet the metabolic and physiological demands<sup>(42)</sup>.

Changes in AT blood flow can affect lipid storage in this organ, contributing to the increase in the amount of this nutrient in other tissues. AT blood flow controls, for example, the supply of TAG-rich lipoproteins to LPL, which is responsible for hydrolysing these lipids to NEFA and glycerol. It has been demonstrated that NEFA blood flow is reduced during the fasting period<sup>(1)</sup>. A study conducted by Potts *et al.*<sup>(43)</sup> showed that the release of AT lipids is reduced in obese individuals when compared with eutrophic individuals, both in the fasting and postprandial periods. This result reveals that impaired postprandial blood flow is associated with insulin resistance, which is partially explained by the decrease in TAG uptake in this period. These lipids remain longer in the circulation, which, according to Nellesmann *et al.*<sup>(44)</sup>, implies continuous NEFA availability to other organs. These authors found an association between plasma insulin and the greatest deposition of TAG from VLDL into abdominal subcutaneous AT of obese women. The relevance of the blood flow to lipid metabolism was also assessed in a study based on adrenaline infusion used in healthy individuals. It was found that adrenaline caused significant increase in AT blood flow and NEFA efflux from this tissue<sup>(45)</sup>.

Metabolic AT flexibility represents protection against lipotoxicity in other organs caused by excess energy intake<sup>(8)</sup>, mainly after meals<sup>(46)</sup>. In order to guarantee this protection, the expansion and the reduction in fat mass also depend on the

circulation present in the AT. Local hypoxia occurs when blood vessels are insufficient. Thus, microcirculation of AT is required for the expansion of adipose mass, not only due to its ability to prevent hypoxia, but as a potential entry point of adipocyte progenitors into white AT. The network of blood capillaries also contributes to immune response. An example is that the macrophages heading into AT have multiple functions: they remove necrotic adipocytes, which lead to the formation of foam cells; release pro-inflammatory mediators; and contribute to angiogenesis<sup>(42)</sup>.

The adequate regulation of AT blood flow ensures the communication with other systems and the occurrence of reactions to meet the energy needs of the organism. It is worth noting that these flow modifications involve the initial stages of obesity and take part in the aggravation of disorders associated with this disease.

### Relationship between adipocyte size and insulin resistance

The relationship between adipocyte size and insulin resistance has been studied for more than 40 years. Salans *et al.*<sup>(47)</sup> compared insulin sensitivity of AT in obese and non-obese individuals with respect to cell size. It was found that responsiveness to insulin was higher in smaller-sized cells. These authors reported that insulin sensitivity had been restored after weight loss and consequent decrease in adipocyte size. In this way, the increase in body fat correlates with adipocyte hypertrophy and reduced insulin sensitivity. It should be noted that the increase in adipocyte size, especially in the abdominal area, has been observed in individuals with T2DM<sup>(48)</sup>.

Adipocyte hypertrophy may be due to the impairment of new cell differentiation<sup>(1)</sup>. In this sense, one factor that leads to the development of T2DM seems to be the failure of the cell differentiation mechanism. This fact occurs because existing cells accumulate more TAG and increase their volume in the absence of new adipocytes, gradually becoming insensitive to insulin. Corroborating this hypothesis, Varlamov *et al.*<sup>(49)</sup> observed that small adipocytes of non-diabetic rhesus monkeys responded to insulin by increasing lipid uptake, whereas adipocytes of diameter larger than 80–100  $\mu\text{m}$  were resistant to insulin. It seems that when cell size reaches a critical threshold, adipocytes decrease the transport of fatty acids stimulated by insulin to AT. This negative feedback can protect the adipocytes against excessive lipid accumulation and restrict the expansion of AT, which leads to obesity and associated metabolic complications. In this way, a potential association between adipocyte size and the development and progression of insulin resistance may be the reduction of NEFA uptake in AT.

As a consequence of lower fatty acid uptake, influx into the liver and the accumulation in this organ can lead to the production of endogenous glucose, thus exacerbating insulin resistance. Koska *et al.*<sup>(38)</sup> found that the increase in lipid content in the liver may be associated with hypertrophic obesity, and visceral adiposity increase with peripheral and liver insulin resistance.

This way, what could the reduced NEFA transport due to the lack of stimulus generated by insulin in hypertrophic adipocytes

cause? In contact with its receptor, insulin promotes a cascade of intracellular reactions that direct the vesicles containing GLUT4 glucose transporters to the cell membrane. A study conducted by Franck *et al.*<sup>(50)</sup> found no increase in the amount of GLUT4 in the plasma membrane of human hypertrophic adipocytes after stimulation with insulin; however, a twofold increase was observed in the amount of GLUT4 in the cells of smaller diameter in the same individual. This result suggests that the number of receptors exposed on the cell surface is limited and, considering the cell proportions, lipolysis can be enabled in hypertrophic cells to the detriment of lipogenesis due to glucose uptake stabilisation, even in the presence of insulin.

The relationship between adipocyte hypertrophy and insulin resistance is also evidenced by the restoration of GLUT4 expression and insulin sensitivity increase, as demonstrated in a study conducted by Koenen *et al.*<sup>(51)</sup>. They found that treatment with pioglitazone (45 mg/d), a type of TZD, improved insulin sensitivity (placebo: 0.35 (SD 0.16)  $\mu\text{mol/kg} \times \text{min}$  per milliunit per litre; pioglitazone: 0.53 (SD 0.16)  $\mu\text{mol/kg} \times \text{min}$  per milliunit per litre,  $P < 0.001$ ), which was accompanied by an increase in the surface area of subcutaneous adipocytes (placebo: 2323 (SD 725)  $\mu\text{m}^2$ ; pioglitazone: 2821 (SD 885)  $\mu\text{m}^2$ ,  $P = 0.03$ ), and GLUT4 expression in AT. However, it is worth mentioning that there is a need to assess the trafficking of this receptor to the plasma membrane of fat cells, in order to confirm whether the improvement in sensitivity is due to the increase in the number or the action of these receptors. Still, TZD seems to contribute to the emergence of new pre-adipocytes originating from bone marrow precursors and stimulate their differentiation into mature adipocytes<sup>(1)</sup>.

Although the relationship between increased prevalence of chronic diseases, such as T2DM and non-alcoholic hepatic steatosis with increased weight, there is a subpopulation of obese individuals that seems to be protected or more resistant to the development of obesity-related metabolic changes<sup>(52)</sup>. It is estimated that up to 30 % of obese individuals are metabolically healthy and may be protected against the morbidity and mortality associated with excess weight; however, the factors responsible for this phenomenon are unknown. A study conducted by O'Connell *et al.*<sup>(53)</sup> revealed that the size of omental adipocytes in obese metabolically healthy individuals is smaller than the size of adipocytes in unhealthy individuals. Still, the size of these adipocytes positively correlated with the degree of insulin resistance ( $r$  0.73;  $P < 0.0005$ ), concentration of TAG ( $r$  0.65;  $P < 0.0005$ ), TAG:HDL ratio ( $r$  0.67;  $P < 0.0005$ ) and glycated Hb (HbA1c) ( $r$  0.50;  $P < 0.005$ ). The size of omental adipocytes was a predictor of the progression of hepatic steatosis to fibrosis, a process that has not been observed in metabolically healthy obese individuals.

### Role of hypoxia in the development of insulin resistance

Insulin resistance is often associated with sleep apnoea and respiratory overload triggered by obesity. This process occurs because apnoea and overload cause intermittent cycles of hypoxia, resulting from periodic collapses of the airways during sleep<sup>(1)</sup>. Additionally, studies on adipocyte biology have allowed inferring that hypoxia negatively affects tissue

function<sup>(54,55)</sup>. In this way, which is the relationship between AT hypoxia and insulin resistance?

The increase in adipocyte size (up to 140–180  $\mu\text{m}$ ) observed in obesity causes hypoxia. The reason is the inadequate diffusion of oxygen, which would occur efficiently at a distance of approximately 100  $\mu\text{m}$  from the blood vessel<sup>(1)</sup>. It is suggested that this situation may occur due to reduced AT blood flow, which has already been observed in mice by Hosogai *et al.*<sup>(56)</sup>.

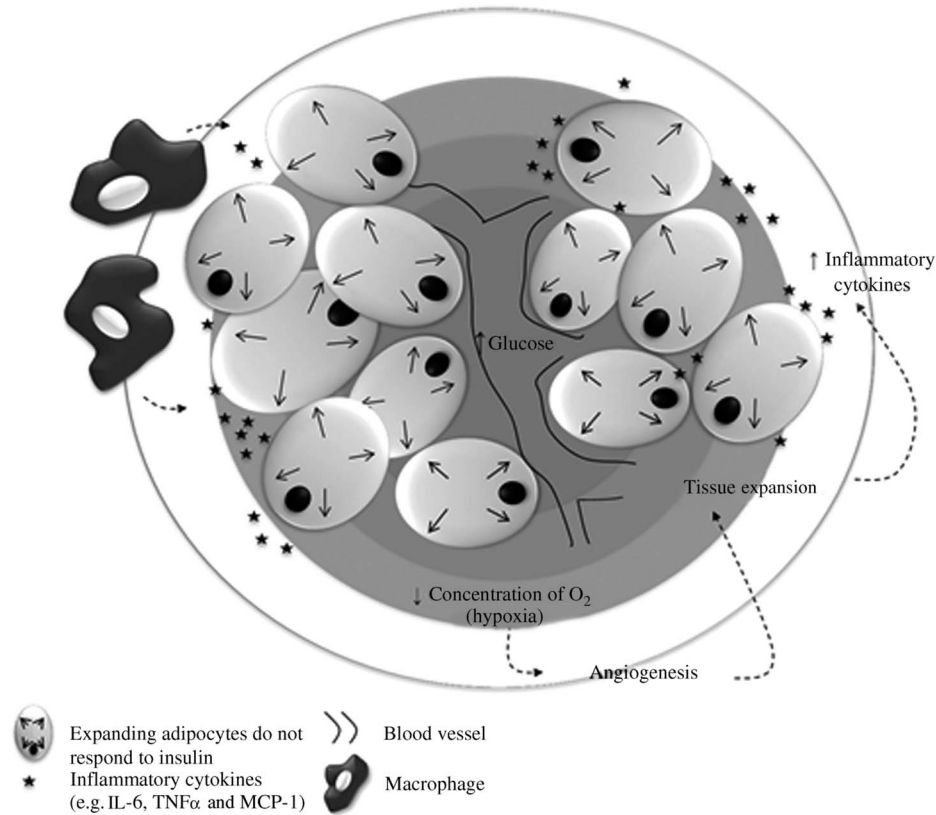
Hypoxia induces angiogenesis, which in turn stimulates more tissue expansion; however, the formation of new vessels is not enough to maintain AT growth<sup>(42,57)</sup>. Thus, hypoxia has become indicative of metabolic disorder, since the increase in AT results in elevation of the concentration of the  $\alpha$ -subunit of the transcription by hypoxia-inducible factor 1 (HIF-1). This factor, in turn, stimulates the expression of adipokines and factors involved in inflammation, which have been implicated in the development of insulin resistance. It is worth mentioning that hypoxia also leads to an increased expression of genes involved in glucose metabolism and tissue fibrosis blocks pre-adipocyte differentiation<sup>(42,57)</sup> (Fig. 3).

Cells that suffer hypoxia also feature adaptive responses which are independent of hypoxia inducible factor 1 $\alpha$  subunit (HIF-1 $\alpha$ ). It has been reported that the response of badly folded proteins is activated in the presence of hypoxia and contributes to cellular adaptation to this stress. Newly synthesised proteins of the secretory pathway and proteins of the membrane are folded correctly and organised by the chaperones of the granular endoplasmic reticulum. However, hypoxia causes the accumulation of badly folded proteins in the endoplasmic reticulum, resulting in the reticulum stress<sup>(56)</sup>.

Chen *et al.*<sup>(58)</sup> tested Sprague–Dawley rats to confirm the hypothesis that GLUT4 changes in intra-abdominal AT caused by intermittent hypoxia could facilitate the emergence of peripheral insulin resistance. These researchers found that the group submitted to intermittent hypoxia exhibited increased plasma insulin concentration in the fasting period (245.07 (SD 53.89) pg/ml *v.* 168.63 (SD 38.70) pg/ml,  $P = 0.038$ ) and a reduced amount of GLUT4 mRNA, as well as GLUT4 protein expression (0.002 (SD 0.002) *v.* 0.039 (SD 0.009),  $P < 0.001$ ; 0.642 (SD 0.073) *v.* 1.000 (SD 0.103),  $P = 0.035$ ) when compared with the rats of the control group. Quantification of GLUT4 is of relevant interest in order to understand the mechanisms by which hypoxia leads to insulin resistance.

Regazzetti *et al.*<sup>(59)</sup> assessed whether the phenomenon that leads to insulin resistance may be due to failures in the intracellular signalling pathway of this hormone. The signalling triggered by hypoxia was modulated in human and murine adipocytes by means of incubation in low oxygen concentration (1 % O<sub>2</sub>) or HIF-1 expression. Insulin signalling was monitored through the phosphorylation state of several important proteins in this pathway and glucose transport. It was found that hypoxia inhibited insulin signalling, which was demonstrated by the reduction in phosphorylation of the insulin receptor and the reversal of this process under normal oxygen conditions.

In addition to apnoea, there are other arguments in favour of the hypothesis of hypoxia, such as: cardiac output and blood flow to AT do not increase in obese individuals, despite the expansion of fat mass; and obese individuals do not exhibit



**Fig. 3.** Hypoxia and insulin resistance. The increase in adipocyte size observed in obesity leads to tissue hypoxia. Hypoxia induces angiogenesis and increased tissue expansion; however, the formation of new vessels is not enough to maintain adipose tissue (AT) growth. The increase in AT promotes the elevation of inflammatory cytokine expression, which has been related to the development of insulin resistance. Hypoxia has also been related to the inhibition of insulin signalling due to the reduction in phosphorylation of its receptor, thus causing hyperglycaemia. MCP-1, chemokine (C-C motif) ligand 2 (CCL2), also referred to as monocyte chemoattractant protein-1.

increased postprandial blood flow, as occurs in eutrophic individuals<sup>(60)</sup>.

The set of information obtained about hypoxia suggests that adipogenesis inhibition and TAG synthesis may be a mechanism for the increase in NEFA in the circulation of obese individuals, which contributes to the triggering of insulin resistance. This information can help understand the beneficial effects of energy restriction and performance of periodic physical exercise. Evidence shows that weight reduction improves tissue oxygenation and reduces inflammation<sup>(61)</sup>. This way, increased AT oxygenation may be a new strategy in the treatment of inflammation and insulin resistance associated with obesity.

### Adipose tissue as an endocrine organ

The ability of AT to secrete a variety of adipokines which can perform as autocrine, paracrine and systemic mechanisms has been discovered in the last 15 years. These chemical mediators revolutionised the concepts about the biological function of AT. There is a consensus that this tissue does not only supply and store energy, but it is also a dynamic and central organ that regulates appetite, food intake, glucose availability and energy expenditure<sup>(1,14,62)</sup>.

Due to the diversity of adipokines and the variety of functions already identified, it can be said that they comprise proteins

related to the immune system (for example, TNF- $\alpha$  and IL-6), growth factors (for example, transforming growth factor- $\beta$  (TGF- $\beta$ )) and proteins of the alternative complement pathway (adipsin). Still, there are also adipokines involved in blood pressure regulation (angiotensinogen), blood clotting (plasminogen activator inhibitor-1; PAI-1), glucose homeostasis (adiponectin, resistin, visfatin and leptin) and angiogenesis (vascular endothelial growth factor; VEGF), among several other actions<sup>(14)</sup>. Besides the fact that AT performs its own metabolic activities, the adipocytes release signals to other tissues and regulate their metabolism. On the other hand, AT expresses numerous receptors allowing the response to afferent signals, such as hormones and signals from the central nervous system<sup>(1)</sup>.

IL-6, TNF- $\alpha$ , and the complement factors B, C3 and D (adipsin) are adipokines with immune function. They are produced by adipocytes in response to infectious or inflammatory stimuli. Although some of these molecules have mainly autocrine and paracrine actions, some of them contribute significantly to systemic inflammation<sup>(63)</sup>.

IL-6 is a cytokine with pro-inflammatory and endocrine actions. It is present in physiological situations of visceral AT, being secreted by many cells, including adipocytes and vascular fraction cells. Like TNF- $\alpha$ , IL-6 inhibits LPL expression, stimulating lipolysis and hepatic lipid secretion, promoting hypertriglycerolaemia. It is also involved in the suppression



of adiponectin expression<sup>(6,7)</sup>. On the other hand, it stimulates the production of acute-phase proteins in the liver (C-reactive protein, fibrinogen, haptoglobin, complement factor 3 and serum amyloid-A protein)<sup>(7,64)</sup>.

The secretion of these substances is increased in adipocytes of obese individuals and has an important role, both as circulating hormones and local regulators of insulin action<sup>(62,64,65)</sup>. Ye *et al.*<sup>(54)</sup> studied ob/ob mice and found that hypoxia was associated with an increased expression of inflammatory genes (TNF- $\alpha$ , IL-1, IL-6 and TGF- $\beta$ ), chemokines (macrophage migration inhibitory factor; MIF), extracellular enzyme (matrix metalloproteinase-9; MMP-9) and macrophage markers proteins (CD11 and F4/80), suggesting an increase in inflammation and macrophage infiltration in obesity. In that study, the authors found an association between inflammation and hypoxia in mice whose obesity was induced by feeding. These results suggest that chronic inflammation is associated with hypoxia in AT of obese mice.

IL-18 is a potent pro-inflammatory mediator, with chemotactic properties in mononuclear cells. This cytokine is produced and secreted by human adipocytes, as well as by skeletal muscle. Its primary production occurs in non-fatty AT cells<sup>(64,66)</sup>. It is considered a key mediator in subclinical inflammation associated with abdominal obesity and its complications<sup>(64)</sup>.

Complement molecules were the first adipokines identified (adipsin or factor D) in AT. There is also production of factors B, C3 and D. The presence of the factors B and D is required for the synthesis of factor C3a. This protein is involved in TAG synthesis and storage and its deficiency in rats is associated with decreased body fat and increased insulin sensitivity. It should be noted that adipsin levels increase due to food intake<sup>(63)</sup>.

PAI-1 stands out in the group of molecules with cardiovascular function. It is a protein that promotes thrombus formation and rupture of unstable atherogenic plaques<sup>(14)</sup>. It is primarily produced in the liver, but also in AT, and this tissue is its main source in obesity. PAI-1 production is stimulated by insulin and corticoids and its expression is regulated by PPAR. PAI-1 levels correlate with visceral fat and it is involved in the development of CVD<sup>(63)</sup>.

Adiponectin stands out among the molecules with metabolic function that participate in energy homeostasis. It is a protein produced specifically by adipocytes and its mRNA is decreased in obesity and insulin resistance<sup>(67)</sup>. This adipocin enhances the action of insulin in the liver and reduces hepatic glucose production. It also induces the oxidation of fats, reducing TAG levels in liver and muscle. Its anti-inflammatory action is a result of decreased synthesis and action of TNF- $\alpha$  and IL-6, and induction of IL-10 production. TZD increase adiponectin, which is another mechanism responsible for increased insulin sensitivity associated with the use of these drugs<sup>(61)</sup>.

Resistin is a mediator that induces insulin resistance. Currently, it is known that its expression in human adipocytes is reduced, but it is high in monocytes and macrophages. In animal models, high concentrations of resistin are associated with insulin resistance and inhibition of pre-adipocyte differentiation. On the other hand, visfatin is mainly produced by visceral AT and has insulin-mimetic action, since by binding to the insulin receptor it promotes its activation<sup>(63)</sup>.

Leptin is a molecule known as the paradigm of the endocrine function in AT and it is vital in regulating energy deposits.

Leptin is a peptide whose synthesis predominates in AT. Its production in adipocytes is regulated by insulin and its concentration correlates positively with AT mass. The binding of leptin to hypothalamic receptors triggers the transmission of information concerning AT mass and existing energy deposits. As a consequence, the efferent response triggered by the sympathetic nervous system determines the reduction of anabolism and increased energy expenditure<sup>(68)</sup>. In addition, one of the peripheral actions of leptin is the reduction in insulin synthesis and secretion, thus establishing an adipo-insular axis.

A study conducted by Blažetić *et al.*<sup>(69)</sup> using an animal model revealed increased distribution of leptin receptors (Ob-R) in the lateral hypothalamic nucleus, but without effect on the arcuate nucleus resulting from a fat-rich diet. This way, in obesity, despite the high concentration of circulating leptin and possible increase in the number of receptors in the brain, leptin does not induce the expected response of decreased intake and increased energy expenditure. As the outcome, there is resistance to the action of leptin, which can lead to interruption of the adipo-insular axis and maintenance of insulin secretion.

Whether the increase in adipocyte volume leads to the expression of inflammatory cytokines has not yet been elucidated, because adipocyte size is associated with this feature. In order to discuss this issue, Ye *et al.*<sup>(54)</sup> assessed the mechanical strain on the cell surface (using compressed air or vacuum condition) and found that adipocytes exhibited changes in endocrine function under vacuum conditions. Since vacuum conditions are associated with low oxygen concentration, the authors also confirmed the hypothesis that hypoxia influences AT.

### Inflammation, macrophage infiltration and insulin resistance: a continuous cycle

AT is a heterogeneous organ that contains different cellular types including mature adipocytes, pre-adipocytes, endothelial cells, vascular smooth muscle cells, leucocytes, monocytes and macrophages<sup>(1)</sup>. Thus, what is the origin of adipokines expressed in AT? Each adipocyte and other TA cells produce a small amount of substances, including cytokines and acute-phase proteins that directly or indirectly increase the production and circulation of factors related to inflammation. However, due to the fact that AT is considered one of the largest organs in the body, the pool of these factors secreted into the circulation can have a great impact on bodily functions<sup>(7)</sup>.

Two mechanisms are suggested in the literature to explain the low-intensity chronic inflammatory process, also known as 'metaflammation' (metabolic inflammation), which is coordinated by cells in response to excess nutrients and energy<sup>(70)</sup>. It is worth pointing out that when it comes to obesity, not only the amount of AT should be taken into consideration, but also the type of adiposity. Adipocytes of subcutaneous AT are highly functional due to their greater ability to store energy, which makes them different from adipocytes found in visceral AT, which are less functional and whose tissue has a greater amount of macrophages<sup>(2)</sup>.

The first suggested mechanism is AT hypoxia and the second is macrophage infiltration into AT. In AT expansion, the increase in cell volume and consequent remoteness of



adipocytes from the proximity of blood vessels can turn them hypoxic. Some authors suggest that the onset of hypoxia stimulates the release of inflammatory cytokines, chemokines and angiogenic factors (VEGF) as a way to increase the blood flow and stimulate vascularisation<sup>(6)</sup>. Cytokines and chemokines, especially chemokine (C-C motif) ligand 2 also referred to as monocyte chemoattractant protein-1 (MCP-1), lead to an increased number of circulating monocytes and infiltration into AT, due to increased expression of adhesion molecules in the endothelium of blood vessels. It is worth mentioning that MCP-1 is produced by resident macrophages, endothelial cells and adipocytes. It has been reported that circulating concentrations of chemokines are elevated in obese and diabetic individuals; however, they seem to diminish as a consequence of weight loss<sup>(1)</sup>.

The basic mechanisms of low-intensity inflammatory processes observed in obesity are not fully understood, but it is known that macrophage infiltration can be an important source of pro-inflammatory adipokines synthesised locally<sup>(71)</sup>. This synthesis is mainly triggered by stress of the endoplasmic reticulum. This deregulation in the synthesis and secretion of adipokines may have local and systemic effects. It has been shown that TNF- $\alpha$  inhibits the differentiation of adipocyte precursors and, similarly, IL-6 also inhibits adipocyte differentiation. Additionally, it has been observed that TNF- $\alpha$  induces apoptosis in pre-adipocytes and mature cells. These effects may result in decreased body fat storage capacity, and increased glucose and NEFA concentration, leading to insulin resistance<sup>(1)</sup> and T2DM. This process occurs due to the inhibition of insulin receptor signalling in peripheral tissues by these fatty acids, concomitant increase of reactive oxygen species, and production of inflammatory cytokines in pancreatic  $\beta$  cells, promoting the attraction of immune cells to the organ<sup>(72)</sup> and reduction in insulin synthesis. At the same time, NEFA induce increased expression of MCP-1 and IL-6 in pre-adipocytes, in a greater amount than in mature adipocytes<sup>(73)</sup>.

The relationship between macrophage infiltration and insulin resistance in obesity occurs in an intriguing way. MCP-1 expression and secretion by pre-adipocytes can be stimulated by TNF- $\alpha$ , insulin, IL-6 and growth hormone, whereas its secretion by mature adipocytes is reduced by stimuli that increase insulin sensitivity, such as adiponectin and treatment with TZD. This way, changes in the secretion of adipokines can modulate MCP-1 synthesis and this, in turn, can influence macrophage infiltration into AT. Studies<sup>(74,75)</sup> showed that CCR2 (receptor for MCP-1) and MCP-1 knockout mice subjected to treatment with a hyperlipidic diet exhibited reduced macrophage infiltration into AT, reduction in pro-inflammatory gene expression, hepatic TAG content and improvement in insulin sensitivity when compared with the mice of the control group. In this way, it is possible to infer that MCP-1 plays an important role in macrophage infiltration into AT and may contribute to the development of insulin resistance. Corroborating these results, the study conducted by Kanda *et al.*<sup>(74)</sup> showed that MCP-1 overexpression in AT of GM mice increased macrophage infiltration and contributed to the development of insulin resistance.

In the inflammatory context, macrophages present in AT suffer changes in number, tissue distribution and function, thus

enlarging the subclinical inflammation. These resident cells are proportionately increased in number and size in AT according to BMI and may represent about 60 % of this tissue components. Given the activity of macrophages in metabolic inflammation, Ye *et al.*<sup>(54)</sup> found that inflammatory gene expression in macrophages can be induced in response to inflammation. Peritoneal macrophages extracted from C57BL/6 mice with hypoxia treated *in vitro* had inflammatory genes significantly expressed. Three inflammatory genes (IL-6, MIF and MMP-9) were also induced by hypoxia in 3T3-L1 adipocytes. Thus, when macrophages are activated, they release biologically active cytokines, such as NO, TNF $\alpha$ , IL-6 and IL-1 that increase the production of acute-phase proteins (PAI-1, CRP and haptoglobin) which, in turn, contribute to systemic inflammation and insulin resistance. However, the activation of macrophages in areas of inflammation is typically temporary, giving way to repair processes that attempt to re-establish the local function of the tissue. In this way, macrophage infiltration into AT observed in obesity is due to increased infiltration of circulating monocytes<sup>(6,66,76)</sup>.

The hypoxic state can also lead to adipocyte death. In obese subjects, the adipocytes surrounded by macrophages exhibit rupture of the plasma membrane, dilated endoplasmic reticulum, cellular debris in the extracellular space, and small lipid droplets in the cytoplasm suggesting necrosis. It is thought that adipocyte death contributes not only to the recruitment of monocytes to AT via MCP-1, but also to its functional change, with formation of multinucleated giant cells<sup>(6,77)</sup>.

In this sense, the infiltration process may be a consequence and, at the same time, the cause of the chronic inflammatory state associated with obesity. The consequence is characterised by the induction of pro-inflammatory cytokine expression by adipocytes, which promotes macrophage infiltration into AT. The cause is characterised by the environment with low oxygen concentration, which makes the macrophages more active producing more cytokines, such as TNF- $\alpha$  and IL-1<sup>(61)</sup>. Even though the initial stimulus for the recruitment of macrophages into AT arises from resident cells in this tissue, they can perpetuate an uninterrupted cycle of macrophage recruitment and production of pro-inflammatory adipokines along with adipocytes and other cell types, thus leading to a progressive loss of adipocyte function and development of insulin resistance related to obesity.

In response to the environment around them, the macrophages have different functional states. Macrophages present in AT of obese individuals can be found in the M1 state, i.e. pro-inflammatory expressing a wide variety of genes important for adhesion, migration and inflammation. However, after weight loss and consequent decrease in fat mass, there seems to be a phenotypic transition of macrophages to an anti-inflammatory state (M2), which is proven by the increased IL-10 expression<sup>(78)</sup>. The adaptation is complex and depends on changes in IL-1, TNF- $\alpha$  and MCP-1 concentrations, and M1 macrophages. This phenotypic change is accompanied by changes in the number and distribution of macrophages in AT<sup>(6,79)</sup>.

Various mechanisms are involved in the pathogenesis of insulin resistance linked to disorders caused by obesity. However, weight loss is the key factor to reverse the situation to

a healthy state before the installation of T2DM. Weight reduction seems to influence not only adipokine concentrations, especially the increase in adiponectin and its receptors, whose effects are anti-diabetogenic but also macrophage infiltration revealed by the repolarisation of these cells in AT<sup>(78,80)</sup>.

## Conclusions

When AT performs the function of 'lipid storage organ', it has great metabolic flexibility, which makes it possible to protect other organs against lipotoxicity. However, AT dysfunction observed in obesity is characterised by adipocyte hypertrophy, increased NEFA concentration, synthesis and inflammatory adipokine secretion, and macrophage infiltration, leading to insulin resistance due to the impairment of this hormone signalling in the liver, muscle and AT. As a result, the number of glucose receptors exposed in the plasma membrane is reduced, which compromises homeostasis and favours hyperglycaemia, which is a characteristic of type 2 diabetes. Thus, a broader understanding of obesity and its relationship with diabetes may be established. In this context, some points need to be further clarified:

- (1) How and what adipocyte maturation pathways are activated by excessive energy intake?
- (2) What are the mediators involved in cell differentiation in preadipocytes and what is the trigger for differentiation to happen?
- (3) How do the molecular mediators orchestrate adipocyte physiology in the disease and how can their action mechanisms be changed in order to restore health?
- (4) What are the changes that occur in AT as a result of excessive consumption of food and what do these changes imply in the dysfunction of other organs and metabolic changes?
- (5) How do adipokines act in the microenvironment of AT and in other tissues that constitute the body, and what are the answers developed by them with glucose homeostasis implications?

Although it may be complex, the network connecting obesity to diabetes emergence may allow different strategies to be created in order to prevent and treat these diseases. An example of this is that the effects of AT dysfunction on glucose homeostasis can be softened or prematurely reversed through the adoption of healthy habits that promote weight and body fat loss. It is worth mentioning that there is no consensus among studies that compare the effects of different amounts and quality of macronutrients, as well as the amount of body weight loss needed to change beneficially the inflammatory markers. Therefore, there is a need of further studies in this area.

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