Chapter 11

Dietary and Hormonal Factors Involved in Healthy or Unhealthy Visceral Adipose Tissue Expansion

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Abstract

White adipose tissue (WAT) expansion is related to the development of metabolic disorders found in obesity. WAT expansion is the result of generation of new adipose cells by activation of adipogenesis and/or the increase in adipose cell size (hypertrophy). The balance between these two processes determines whether WAT expansion occurs predominantly by hyperplasia, which means the increase in the number of adipocytes, or hypertrophy. Hypertrophic adipocytes are characterized by changes in adipokine secretion pattern, insulin resistance and altered lipid metabolism, which is the reason why WAT-hypertrophic expansion is considered unhealthy. Conversely, the generation of new mature adipocytes by adipogenesis contributes to reduction of the development of hypertrophic adipocytes and therefore maintain normal WAT functions, leading to healthy hyperplastic expansion. The adipogenic capacity of adipose tissue depends on the adipogenic potential and the number of adipocyte precursor cells. Different factors are known to regulate adipogenic process and adipose tissue function, contributing to a healthy or unhealthy expansion that occurs under positive energy balance. This chapter discusses the role of fructose intake and glucocorticoids and testosterone as regulators of adipose tissue function and expansion.

Keywords: fructose-rich diet, glucocorticoid, testosterone, adipogenesis

1. Introduction

Obesity has been defined by the World Health Organization as the excess of adipose tissue (AT) mass that can be harmful or not for the health. The incidence of this disorder has reached epidemic levels during the last few decades. It is associated with a high risk of developing different pathologies such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, dyslipidaemias

and cancer, among others. White AT (WAT) dysfunction plays an important role in the development of metabolic disorders associated to obesity. Anatomically, WAT presents a discontinuous distribution in the organism and it is divided into two major depots, visceral and subcutaneous (VAT and SAT, respectively). VAT is mainly located in the abdominal cavity (peri-renal, retroperitoneal (RPAT), mesenteric, omental and gonadal) and SAT is distributed below the dermis (femoral, gluteal, abdominal and gonadal). Studies in humans and animal models indicate that VAT expansion is associated with an increase in metabolic risk and mortality, whereas accumulation of SAT improves insulin sensitivity and reduces the risk of developing T2DM.

WAT increase is the consequence of two processes: the increase in the size (hypertrophy) and/or in the number of adipocytes (hyperplasia). The form in which WAT expands correlates with the presence or absence of WAT-functional alterations. It is well known that hypertrophy of adipose cells is associated with the change in the pattern of adipokine secretion, increasing the release of leptin [1] and pro-inflammatory cytokines [2], and decreasing the release of adiponectin [2]. This profile of adipokine secretion contributes to the development of insulin resistance (IR) observed in hypertrophic adipocytes [3]. Moreover, the increase in cell size is associated with changes in lipid metabolism [4]. Conversely, adipogenesis activation leads to an increase in the number of adipose cells, and therefore prevents the development of hypertrophy thus contributing to normal WAT function.

WAT has been recently reported to express brown AT (BAT) markers, and its exposure to cold or beta-adrenergic receptors stimulation to increase the presence of brown-like-multilocular cells [5]; this process is called WAT 'browning'. Since these cells are different from brown and white adipocytes, they have been called beige or brite adipocytes (from the combination of brown and white). It is known that brown and beige adipocytes originate from different lineages; while brown adipocytes are generated from $MYF5^+$ precursors, beige ones derive from precursors that express platelet-derived growth factor receptor ($PDGFR\text{-}\alpha^+$) and stem cells antigen 1 ($Sca1^+$) or from smooth muscle-like precursors that express myosin heavy-chain 11 ($MYH11^+$) [6, 7]. Previous reports have suggested that beige adipocytes could arise through a less-studied mechanism of transdifferentiation of pre-existent white adipocytes. The most important functional difference between beige and white adipocytes is the expression of the mitochondrial-uncoupling protein 1 (UCP-1), which allows the production of heat via the respiratory-uncoupling reaction. UCP-1 is activated by an increase of free fatty acids (FFAs), product of cold-induced lipolysis [8]. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) is a transcription co-activator which promotes the expression of thermogenic genes during cold-induced browning of WAT. Thereby, enhancing the number of beige adipocytes results quite relevant because of the consequent increase in energy expenditure that would avoid or reduce unhealthy hypertrophic WAT expansion. This could protect organisms against metabolic disorders associated to obesity.

Adipogenesis is a process that can be divided into two sequential steps: (a) commitment of mesenchymal stem cells into adipocyte precursor cells (APCs), acquiring the adipogenic potential and restricting them to the adipocyte lineage; and (b) the terminal adipocyte differentiation wherein APCs under specific adipogenic stimuli differentiate into mature adipocytes. In the first step, APCs begin to express CD34, a cell surface antigen that distinguishes between adipogenic and non-adipogenic cell subpopulations [9]. It is important to highlight that APCs are not a homogeneous cell population, presenting different ability to differentiate

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into mature adipocytes. This cell ability has been called APCs competency and is mainly determined by the expression of two transcription factors, zinc finger protein 423 (Zfp423) and peroxisome proliferator-activated receptor (PPAR)-γ2, that are considered competency markers. Initially, APCs express Zfp423 [10], which in turn activates the basal expression of PPAR-γ2, a key pro-adipogenic signal that assures APCs conversion into adipocytes [11]. APCs differentiation capacity is inversely correlated with the expression of anti-adipogenic factors, such as preadipocyte factor 1 (Pref-1) and wingless-type MMTV 10b (mouse mammary tumour virus) (Wnt-10b). Pref-1 is produced by APCs and exerts the most potent inhibitory signal of the adipogenic process, by suppressing CCAAT/enhancer-binding proteins (C/EBPs) gene expression [12]. Pref-1 expression decreases progressively in cells undergoing differentiation, becoming undetectable in mature adipocytes. Also, the increase of Wnt-10b levels inhibits APCs differentiation *in vitro* and *in vivo* [13].

Terminal adipocyte differentiation is under the control of different transcription factors and hormones. PPAR-γ2 is one of the most important factors that induce adipocyte differentiation, and its expression is absolutely required for adipocyte differentiation. C/EBP-α, -β and -δ have been the first transcription factors described as involved in the differentiation of AT cells. The expression of C/EBP-β and -δ increases immediately after induction of adipogenesis and stimulates the expression of C/EBP-α and PPARγ-2. The increase in C/EBP-α occurs at the end of adipogenesis and is more abundant in mature adipocytes, being crucial for the normal sensitivity to insulin in adipocytes.

Several factors influence the biology and adipogenic potential of WAT, which contribute to a healthy or unhealthy expansion that occurs under positive-energy balance. The purpose of this chapter is to discuss the role of three of these factors, such as fructose intake, glucocorticoids (GCs) and testosterone, affecting WAT function and expansion (**Figure 1**).

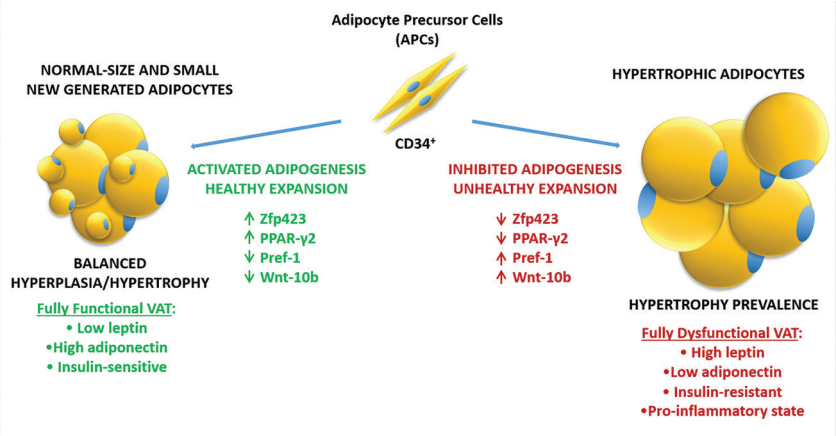

Figure 1. Adipocyte precursor cells (APCs) are committed mesenchymal stem cells that have acquired the adipogenic potential, and are restricted to adipocyte lineage. During healthy adipose tissue (AT) expansion, adipogenesis is active and AT is composed by normal sized and small new generated adipocytes, maintaining normal functions. When AT undergoes an unhealthy expansion, VAT expands mainly by hypertrophy, and becomes dysfunctional, characterized by insulin-resistant adipocytes, with abnormal endocrine function and local pro-inflammatory state.

2. Deleterious effects of high-fructose diet

2.1. Fructose-rich diet intake and fructose metabolism

Fructose is a natural sugar found in fruits and vegetables, also known as levulose and fruit sugar. Although the intake of natural sources of fructose has been relatively stable for the last 40 years, the introduction of high-fructose corn syrups has led to an exponential increase of free fructose in food supply [14].

Fructose is metabolized primarily by the liver, although both the small intestinal mucosa and kidney also contain the enzymes necessary for fructose catabolism [15]. Fructose is transported into cells by two membrane proteins: GLUT5, a specific fructose transporter highly expressed along the small intestine, and GLUT2, a transporter of both glucose and fructose which is expressed in the liver, small intestine and pancreas. Most of ingested fructose passes via the portal circulation to the liver where it is rapidly cleared. Fructose liver metabolism bypasses the main glycolysis metabolic control. Therefore, fructose metabolism will generate pyruvate and acetyl-CoA even during positive-energy balance, and both of them will end in fatty acid synthesis. This phenomenon explains why high-fructose consumption, more than other sugar intake, increases *de novo* lipogenesis, dyslipidaemia and visceral fat deposition, all of them components of the metabolic syndrome (MS).

2.1.1. Fructose impacts on adipose tissue function and adipogenesis

Fructose effects were initially described by observational studies showing the association between cardiometabolic diseases and consumption of fructose-containing sugars, but not with lactose [16]. These considerations were also confirmed by several pre-clinical and clinical studies globally showing that dietary fructose can induce several metabolic alterations closely similar to the MS phenotype.

There is considerable evidence suggesting that the intake of added sugars or sugar-sweetened beverages is associated with increased body weight, presence of unfavourable lipid levels, IR, fatty liver, cardiovascular disease and MS [17], while these alterations are not found using artificial sweeteners [18]. Specifically, fructose-sweetened beverages have been reported to cause body-weight increase and intra-abdominal fat deposition, which would be related to high circulating levels of triglycerides found after fructose-rich diet (FRD) [19]. Indeed, dietary fructose activates de novo lipogenesis in liver and therefore increases AT fatty acid uptake. This powerful lipogenic effect has not been observed after the ingestion of other carbohydrates, since fructose metabolism is driven almost completely to fatty acid synthesis, as mentioned above. Another effect related to fructose consumption is the advanced glycation end products (AGEs) formation. Fructose produces 10 times more AGEs than glucose does and these AGEs indirectly contribute to the inflammation and oxidative stress that characterize the MS phenotype [20].

As in men, fructose ingestion by rats induces body-weight gain, morphological and functional VAT modifications and MS-like phenotype, while this does not occur when glucose or starch is administered to rats [21–23]. Previous studies from our group showed that FRD

http://dx.doi.org/10.5772/65927

given to adult rats during 3 and 8 weeks, induced an increase in circulating levels of key adipokines secreted by RPAT, such as leptin, adiponectin and PAI-1. Interestingly, this altered secretion pattern was accompanied by enlarged adipocytes and decreased expression of insulin receptor substrates (IRS-1 and IRS-2) in adipocytes [24–28], suggesting an IR state. On the other hand, Pektas et al. showed that FRD increases gene expression of insulin-signalling pathway components and pro- and anti-inflammatory markers in WAT from male and female rats. Gender-dependent differences in fructose feeding were not significant, suggesting that females were not protected from harmful effects of fructose [29]. In summary, FRD ingestion during short or long periods of time induces deep changes in VAT functionality, predominantly by favouring hypertrophic VAT mass expansion.

The deleterious effect of high-fructose intake on hypertrophic AT mass expansion has been extensively studied; however, the study of fructose effects on adipogenesis is now emerging. It has been observed that rats fed with FRD during short and long periods showed an increase in the adipogenic potential of APCs from RPAT, displaying high levels of competency markers, PPAR-γ2 and Zfp423 [26, 28]. These changes in the cell expression pattern of both competency factors are related to an increased APCs ability to differentiate into mature adipocytes. Indeed, APCs from FRD-fed rats differentiated into adipocytes showed high intracellular lipid content and high expression levels of adipogenic genes indicating that FRD intake generates APCs with a greater ability to become mature adipocytes [26, 28]. Another factor that could influence cell adipogenic potential in AT depots is APCs number. High-fat diet intake increases APCs number in different AT depots [30, 31]. Interestingly, we previously reported that a 3-week FRD intake did not induce any change in RPAT APCs number [26]. However, prolonged FRD intake (8 weeks) increased RPAT APCs number, indicating that this effect is dependent on the time period of fructose consumption; thus, prolonged periods of fructose intake increase RPAT APCs number.

Though literature about the effects of fructose on WAT browning is rather scarce, some authors have shown that dietary factors are related with this phenomenon. It has been observed that high-fat ingestion is associated with an increase in UCP-1 expression in BAT and WAT, in mice [32, 33] and rats [34]. Conversely, other studies show a decrease or no differences in UCP-1 WAT expression [35]. Moreover, changes in the micronutrient composition induce WAT browning in rodents [36]. Nevertheless, dietary effects on beige adipocytes generation and precursors remain to be further studied.

2.1.2. Direct effects of fructose on adipocyte precursor cells

As mentioned above, fructose is extensively extracted and metabolized (50–75%) primarily by the liver; however, a percentage of fructose enters into the systemic circulation and is metabolized by extra-hepatic tissues, such as the AT [37]. In fact, fructose concentration in the portal circulation around VAT can easily reach 5–10 mM [38, 39]. So, it suggests that some of the effects of FRD intake on AT mass expansion could be the result from a direct fructose effect on APCs.

In this regard, there are some available reports describing direct fructose effects on adipogenesis, and most of them have been focused on the terminal differentiation stage of 3T3-L1

preadipocytes [40]. The addition of fructose into the culture medium of 3T3-L1 preadipocytes stimulates the terminal stage of adipogenesis, a mechanism that is GLUT5-dependent [40, 41]. Both 3T3-L1 preadipocytes and adipocytes express GLUT5 [40, 42]. It has been described that GLUT5 gene expression is higher in undifferentiated than in differentiated 3T3-L1 cells, in which it is almost undetectable [40, 43]. This indicates that adipocyte precursors are a better target for fructose action than mature adipocytes. Several effects have been described using fructose in the culture medium. In differentiated 3T3-L1 cells, fructose increased lipolysis and the activity of 11-β hydroxysteroid dehydrogenase-1 [41]. Also, the presence of fructose (55–5500 μM) during adipocyte differentiation induced an increase in several pro-adipogenic factors, and either GLUT5 knockdown or its over-expression reduced or increased this effect, respectively [40].

Previous reports have described direct effects of fructose on cultured RPAT APCs from adult normal male rats when they were cultured in the presence of fructose (5500 μM) in the culture medium. Under this condition, APCs expressed high levels of competency factors, showing greater APCs potential to become adipocytes. Similar results were found when cells were cultured with a comparable concentration of glucose [28]. It has been proposed that the balance between mineralocorticoid (MR)/glucocorticoid (GR) receptors plays a key role in the pro-adipogenic effect of GCs. Fructose decreases GR expression in adipocytes [44], in agreement with these data APCs grown in the presence of fructose, but not in the presence of glucose, displayed higher MR and lower GR mRNA levels. Interestingly, when fructose-exposed APCs were induced to differentiate, they accumulated high lipid content, indicating that the imprinting of fructose in APCs is reflected in the mature adipocytes differentiation capacity [28].

As mentioned before, there are two possible mechanisms by which adipogenic potential of AT can be increased, that is, by enhancing APCs competency and increasing APCs number. Fructose effects on APCs competency have already been presented. Regarding the direct effect of fructose on APCs number, our previous studies showed that fructose directly increased the $CD34^+$ adipogenic cell subpopulation in the WAT stromal vascular fraction (SVF), indicating an increase on APCs [28]. Interestingly, these results were not observed when glucose was used, which confirms a fructose-specific effect. Taken together, these results are similar to those observed in cells from FRD-fed rats, thus it is plausible that some effects observed in RPAT mass expansion from FRD-fed rats can be a consequence of direct fructose effects on APCs.

2.1.3. Fructose-rich diet intake and metabolic imprinting

The concept of 'developmental origins of adult disease' states [45] that environmental factors, including maternal nutrition, experienced *in utero* and during early postnatal life, can elicit permanent metabolic and physiological modifications in individuals, leading to enhanced susceptibility to develop diseases later in life. Limited data are available on the long-term effects of high fructose exposure during gestation, lactation and infancy. Emerging research suggests that fructose consumption by mothers and/or their offspring during early life can lead to persistent neuroendocrine and metabolic dysfunctions.

Our group has studied the effect of fructose exposure during gestation or lactation, on adult male pups. Maternal consumption of FRD during gestation alters offspring development causing impaired insulin sensitivity and RPAT dysfunction, evidenced by hypertrophic adipocytes that secrete larger amounts of leptin *in vitro*, though with decreased AT mass. A paradoxical situation could at least partially be the result of a reduced RPAT APCs number [46]. Adult male offspring born to FRD-fed dams throughout gestation were reported to develop IR, dyslipidaemia, with a distorted pattern of peripheral adipokines and enhanced oxidative stress [47]. Interestingly, later in life, the offspring normalized their metabolic profile [48]. Conversely, another study showed that FRD intake by gestating mothers resulted in pronounced maternal dysfunctions without major undesirable metabolic effects on the offspring, even after following their progress up to 6 months of age [49].

When FRD was administered to lactating dams, the offspring showed increased body weight, hypothalamic leptin resistance, increased food intake, IR and increased VAT mass (due to both fat mass and adipocyte size) [50].

It was reported that offspring born from mothers consuming FRD during pregnancy and lactation displayed decreased body weight, hyperinsulinaemia and hypoglycaemia at weaning [51]. Moreover, rat pups consuming high-carbohydrate milk during lactation did develop obesity at adulthood [52], characterized by increased body weight, hyperinsulinaemia and augmented skeletal muscle fatty acid transport at adult life [53]. Excessive insulin secretion in turn promotes enhanced lipogenesis [54] and adipogenesis [55]. Regarding WAT browning, FRD effects have not been yet studied. Nevertheless, maternal perinatal undernutrition has been reported to increase the appearance of beige adipocytes in gonadal WAT of rats at weaning [56]. There is still much to investigate about this mechanism.

In summary, FRD administration during an individual´s development (gestation or lactation periods) induces a permanent alteration in AT development, increasing its susceptibility to have an unhealthy RPAT expansion, and leading to unfavourable metabolic consequences seen at adult age.

2.2. The role of glucocorticoids in adipose tissue biology

GCs have numerous effects on AT biology and functionality. Among others, they regulate AT endocrine function, AT inflammation in obesity and lipogenic-lipolysis balance [57]. High GC levels in blood or in AT depots would be expected to increase the breakdown of lipids; however, the GC effects on AT metabolism are controversial. Many reports agree that GCs increase lipolysis in mature adipocytes [58, 59], while others state that GCs have an inhibitory effect on lipolysis [59, 60]. Regarding lipogenesis, dexamethasone (DXM), a synthetic GC, has been shown to potentiate the stimulatory effect of insulin on *de novo* lipogenesis in adipocytes [61]. GCs also may cause an increase in VAT lipoprotein lipase (LPL) activity, particularly in men [62]. Consequently, a greater amount of fatty acids would be available for uptake in the VAT, and could help to explain central AT accumulation seen in individuals with high GC levels. The global balance of GC effects on AT lipid metabolism seems to indicate that GCs may favour lipid accumulation in adipocytes, contributing to cell hypertrophy.

Another important effect of GCs on AT is the regulation of fat distribution, promoting VAT deposition [63]. One clear example is Cushing's syndrome (CS) phenotype, mainly characterized by high-serum GC levels and increased VAT rather than SAT mass [64]. CS has several features in common with MS phenotype, such as the presence of VAT-hypertrophic adipocytes, altered lipid metabolism and impaired adipokine secretion [65]. Chronic treatment with GCs induces obesity and MS, impairing AT metabolism. These alterations suggest that GCs have a pivotal role in the pathogenesis of central obesity and the associated alterations seen in the CS phenotype. Interestingly, the restoration of normal peripheral levels of GCs in a rat model of CS reverses most of dysfunctions [66].

Although plasmatic levels of GC seem not to be increased in human obesity, increased local production of cortisol within the AT is associated with this disorder [67]. Local cortisol levels are regulated by 11β-hydroxysteroid dehydrogenase type-1 and -2 (HSD1 and HSD2, respectively). Both enzymes are expressed in AT, where they act regulating the interconversion from the inactive to the active forms of GCs and vice versa, respectively. HSD1 is expressed at higher levels than HSD2 in AT, generating higher concentrations of the active form, which may play an important role in GCs-driven AT mass expansion. In animal models, overexpression of HSD1 in mature adipocytes has generated a model for VAT accumulation [68], whereas HSD1 knockout mice are resistant to central obesity [69].

GCs are required for the differentiation of APCs [70] and for the maintenance of adipogenic gene expression in cultured adipocytes and AT [71]. In fact, DXM has been widely used *in vitro* as a component of traditional differentiation cocktails, due to its potent adipogenic stimulus. The main effect of GCs during early stages of adipogenesis results from the inhibition of anti-adipogenic and the activation of pro-adipogenic transcriptional factors, as well as the increase of APCs competency factors. GCs decrease Pref-1 and Wnt-10b expression [72, 73], both factors are highly expressed in preadipocytes, absent in mature adipocytes and responsible for the undifferentiated phenotype maintenance [74, 75]. Experiments in 3T3-L1 preadipocytes showed that Pref-1 is an early target for DXM action and that its expression decreases with high DXM concentrations, at the same time that adipocyte differentiation increases [72]. Similarly, methylprednisolone (another synthetic GCs) or DXM inhibit Wnt/b-catenin-signalling pathway, promoting adipocyte differentiation [73, 76]. In cultured APCs from hypercorticosteronaemic rats, mRNA levels of Pref-1 and Wnt-10b decreased at the time that all differentiation parameters increased, for example, lipid content, expression levels of mature adipocytes genes (Ob, adiponectin, C/EBP-α, PPAR-γ2) and leptin secretion [77]. On the other hand, GCs increased mRNA levels of the pro-adipogenic factors C/EBP-δ and C/EBP-β [78], which subsequently regulated the expression of mature adipocytes genes. Additionally, it has been previously shown that GCs can activate APCs by enhancing the expression of the competency factors, PPAR-γ2 and Zfp423 [77], and consequently increasing their adipogenic potential.

The actions of GC on AT cells can be exerted through their binding to MR and GR [79, 80], although the contribution of MR and GR in mediating GC effects on AT cells has not been fully understood. In 3T3-L1 and in mouse and human preadipocytes, MR rather than GR has been reported to be important in mediating the pro-adipogenic effects of GCs [79, 81, 82].

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However, another study by Lee et al. found that human preadipocytes express lower levels of MR than those of GR. Moreover, the blockade of GR but not of MR inhibited adipogenesis activation caused by GCs [80]. Nevertheless, the participation of MR or GR in the biological actions of GCs upon the AT is still a matter for debate, while there is currently no consensus about this.

Most studies on the stimulatory role of GCs on preadipocyte differentiation have been largely limited to the 3T3-L1 cell line [83, 84]. However, the role of GCs *in vivo* should not be assumed on the basis of those studies. Taking into account the potent pro-adipogenic action of GCs, it is difficult to explain the presence of hypertrophic adipocytes under conditions of high GC levels, when they are supposed to favour the generation of new cells and therefore small adipocytes through a continuously activated adipogenesis. In this regard, previous reports show evidence for a dual behaviour of the adipogenesis in a model of high GC levels, characterized by initial activation and a subsequent inhibition of the adipogenic process [66, 77]. This dual behaviour could be in part because of differential expression levels of MR: while MR expression does not change in early stages of hypercorticosteronaemia, it decreases under chronic high GC levels condition. This fact could suggest that MR is involved in the development of a GCs-resistant state in AT and it could explain, at least in part, the inhibition of adipogenesis in a powerful pro-adipogenic environment. However, the contribution of GR to the lack of GCs effect cannot be disregarded, even in the presence of similar GR expression levels.

The recruitment of immune cells, such as macrophages, lymphocytes and natural killer (NK) cells, occurs in VAT during obesity and contributes to the development of a chronic inflammatory state [85]. Furthermore, there is a macrophage polarization towards the M1 pro-inflammatory type in detriment to the M2 anti-inflammatory type [85]. The role of GCs mediating the inflammatory response in AT depends on MR or GR activation, which will determine a pro- or anti-inflammatory response, respectively. While GR activation induces a decrease of pro-inflammatory cytokine secretion, MR activation generates the opposite effect [86]. Nevertheless, in CS patients the establishment of an AT inflammatory state is debated [87, 88]. In our animal model of high GC levels, the RPAT expression of macrophages infiltration markers (TNF-α, IL-6, MCP-1 and F4/80) does not increase [66]. This suggests an anti-inflammatory effect of GCs exerted through GR activation or a lower pro-inflammatory effect due to low MR expression [66].

It has been described that GCs suppress thermogenesis in rodents BAT, by decreasing the expression of UCP-1 [89]. Also, GCs treatment decreases BAT-specific genes expression in a brown adipose cell line [90]. However, available information about GCs action on beige adipocytes generation is contradictory and inconclusive. MR antagonist in high-fat-fed mice has been observed to promote WAT browning, inducing the expression of UCP-1 in VAT and SAT (inguinal) and the generation of brown-like adipocytes [91]. On the other hand, human and mouse adipocytes expressed lower levels of UCP-1 when cultured in the presence of DXM [92]. Additionally, DXM-treated mice showed decreased UCP-1 mRNA levels in both SAT and WAT depots, at the same time that developed glucose intolerance and hypertrophic adipocytes [92]. Further studies are needed to clarify the role of GCs on WAT browning and the potential effects on metabolic disorders.

The molecular basis and a more complete understanding of GCs effects on *in vivo* AT mass expansion remain to be defined. Adipogenesis activation or inhibition, depending on the competency and number of APCs, could be one factor modulating the way in which an AT depot expands. This fact is crucial because the metabolic dysfunctions associated with obesity are dependent on the development of adipocyte hypertrophy. Thus, the possibility to increase APCs adipogenic potential could result in the activation of adipogenesis (hyperplastic AT mass expansion), and probably the compensation of adipocyte hypertrophy, with consequent benefits for AT functionality. The fact that CS patients as well as GCs-treated rodents show enlarged adipocytes [93, 94] suggests that GCs must also stimulate hypertrophy, through either increased lipogenesis or decreased lipolysis, in addition to hyperplasia, through adipogenesis activation, probably in balance where hypertrophy exceeds hyperplasia.

2.3. Testosterone modulates fat store deposition and function

It is well known that AT mass and distribution pattern display a clear dimorphism between genders, which has been observed in humans, non-human primates and laboratory animals. Women have greater percentage of AT and proportionately lower lean mass than men. Furthermore, there is a differential distribution of AT among individuals, while men have greater predisposition to accumulate VAT (android distribution), women accumulate gluteal-femoral AT (gynoid distribution). Since VAT expansion is associated to high risk of T2DM and cardiovascular disease development, the greater predisposition to VAT accumulation in men is one of the reasons for higher male incidence in metabolic disorders development. In physiological range, plasma testosterone levels are inversely correlated with VAT mass and therefore associated with a favourable metabolic profile. The same relation has been shown with plasmatic sex hormone-binding globulin (SHBG) concentration and VAT mass. Dehydroepiandrosterone (DHEA) is an adrenal precursor of the peripheral steroid synthesis and it is considered a weak androgen. Some studies have found an inverse correlation between the DHEA levels and central obesity. However, this relationship is not clear with supra- or sub-physiological testosterone levels. Treatments with testosterone in transsexual individuals are accompanied by an increase in AT mass [95], while in patients with hypogonadism a decrease of AT has been observed [96]. In both examples, the risk of developing cardiovascular diseases was enhanced [95–97].

The relationship between blood androgen levels and AT function in women is more complex. It is accepted that androgen excess is associated with central obesity, although there are studies that are not consistent with this assumption. Women with polycystic ovary syndrome (PCOS) often have hyperandrogenaemia associated with IR and accumulate VAT mass. It has been seen that in humans, testosterone induces IR in adipocytes, in part by decreasing glucose uptake by these cells. Neonatal androgenization, an experimental model of PCOS, clearly showed that transiently testosterone excess altered AT function, increasing VAT mass [98], adipocyte size and plasmatic leptin, PAI-1 and FFA levels. These alterations shifted towards those favouring IR and inflammation in adult life [27]. On the other hand, neonatal treatment with a non-steroidal antagonist of the androgen receptor (AR), flutamide, induced a decrease

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in the levels of leptin and greater LPS-induced TNF-α secretion [99]. In general, the treatment with testosterone in the first days of life increases the susceptibility to the development of MS [76] and, on the contrary, the treatment with flutamide improves this condition, showing that testosterone effects are specific receptor-dependent (AR) [100].

The effects of androgens on lipid metabolism, insulin sensitivity and adipogenic process are well known. Androgens exert their biological actions through its specific receptor, which is part of the nuclear receptors family that includes GR, MR and PPAR-γ, among others. AR is expressed in both adipose cells and APCs [101]. However, the levels of its expression differ among AT depots. VAT has higher AR expression levels than SAT [101–103], which would explain in part the differential actions of testosterone on these different AT depots.

It is accepted that androgens have stimulating effects on lipolysis. In rats, castration inhibits catecholamines- or cAMP-induced lipolysis [104], while testosterone treatment increases forskolin- and adrenaline-induced lipolysis [105]. Also, DHEA has positive effects on the lipolytic process. It has been found that rats treated with DHEA show an increase in plasma glycerol and FFA, and in the epididymal AT pad it increases the expression levels of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [106]. In humans, androgen effects on lipolysis are dependent of the AT depot being studied. Treatment with testosterone in transsexual individuals has been reported to increase lipolysis in VAT, but not in the SAT. *In vivo* studies also show that testosterone increases catecholamine-induced lipolysis in abdominal AT, but not in the femoral fat pad. There is a correlation between plasma testosterone levels and the degree of post-stimulation lipolysis in omental AT [107].

In vitro studies with preadipocyte cell line 3T3-L1 and multipotent cell lines C3H10T1/2, have shown that androgens (e.g. testosterone, dihydrotestosterone (DHT) and DHEA) inhibit cell proliferation and differentiation into mature adipocytes [108–110]. The same inhibitory effect was observed in human APCs from different AT depots (mesenteric, omental and abdominal subcutaneous). In rats, castration produces a dual effect according to the AT depot studied; while increasing adipogenic potential of APCs from peri-renal AT, a decreased effect was observed in epididymal AT [111]. Part of the anti-adipogenic action of androgens would be exerted through the inhibition of PPAR-γ2 and C/EBP-α. On the other hand, it has been observed that testosterone and DHT also inhibit the commitment of mesenchymal cells into APCs obtained from lean and obese women [112, 113]. The induction of the APCs to differentiate into mature adipocytes increases the expression of the AR [103], although Dieudonne et al. showed that this protein level decreases [101]. It is important to emphasize that most of the literature related to androgen effects on adipogenesis is focused on the terminal phase of this process, but very little is known about the actions on APCs number and competency.

Androgens effects on WAT browning have not been yet explored. However, the thermogenic capacity of BAT is associated with sexual dimorphism, evidenced by differential UCP-1 expression levels between males and females [114, 115]. It has been observed that testosterone inhibited the expression of UCP-1 [116] and PGC1-α in cultured precursors of brown

adipocytes [117]. These results coincide with the lowest mitochondrial activity observed in male compared to female rats [118]. These observations show that androgens inhibit thermogenic capacity in brown adipocytes and therefore the same effect could be expected on beige adipocytes.

In obesity, plasmatic testosterone levels are diminished, favouring the increase in VAT mass. At the same time, low levels of testosterone induce inhibition of lipolytic metabolism and stimulate LPL expression favouring lipogenesis [119]. This altered lipolysis/lipogenesis balance contributes to an increase lipid storage in adipose cells and therefore to the development of unhealthy VAT mass expansion. Adipocyte hypertrophy is associated with higher leptin secretion into circulation. Leptin impacts on reproductive axis function by inhibiting testis testosterone production [120]. These feedback effects contribute to generate a vicious cycle between AT dysfunction and androgen.

The development of a pro-inflammatory state is one of the features of unhealthy VAT mass expansion. Androgens have been described as anti-inflammatory factors. In hypogonadism, pro-inflammatory cytokines levels increase, while androgen replacement therapy decreases them [121]. Therefore, the decrease of testosterone levels associated to obesity contributes to the pro-inflammatory state observed. On the other hand, low testosterone levels in circulation would be one of the factors that activate adipogenesis, contributing to the increase of VAT adipocyte number, as observed in obese individuals. However, the effect of low androgen levels on cell hyperplasia is not strong enough to prevent adipocyte hypertrophy development, and therefore VAT depot dysfunction, main characteristics of the hypertrophic obese phenotype.

3. Conclusion

Endocrine-metabolic alterations associated to obesity are related to WAT dysfunction, mainly VAT. However, the increase of VAT mass per se is not an unequivocal indication of VAT dysfunction, whereas the adipocyte size actually is. Therefore, the balance between hypertrophy and hyperplasia will determine the appearance of enlarged adipocytes and, consequently, the development of VAT dysfunction. There are many factors that regulate this balance in WAT expansion. In this chapter, we have addressed three of them: fructose intake, GCs and testosterone. Both, fructose intake and GCs, stimulate adipogenesis by modulating APCs competency and number, and thus terminal differentiation. However, in both cases the chronic exposure to these factors led to hypertrophic adipocytes, and therefore to an unhealthy WAT expansion. Chronic exposure to high GC levels seems to induce a resistance state in APCs that would limit their adipogenic potential, partially by a lower response to GCs stimuli due to MR expression decrease. Conversely, testosterone is an anti-adipogenic factor that favours unhealthy expansion. Obesity is associated with reduced testosterone levels, which would promote adipogenesis; however, much extensive research is needed to determine the role of androgens in APCs adipogenic potential in obesity. Finally, factors inducing adipogenesis could become a therapeutic target against

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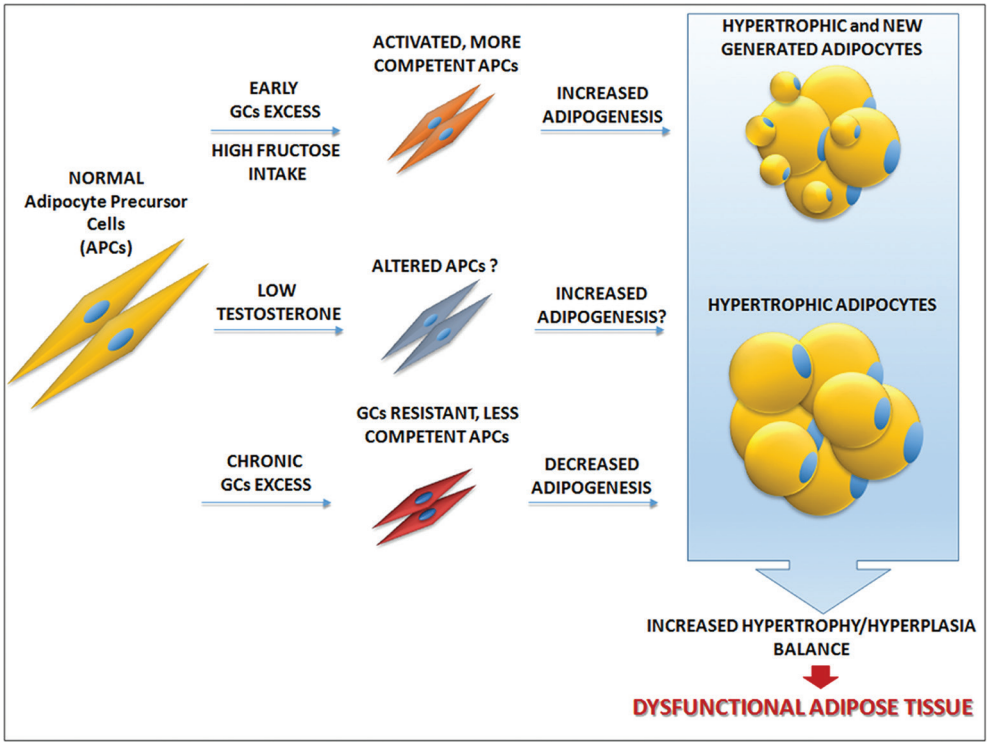

Figure 2. The analysis of three different factors regulating adipogenesis and VAT expansion shows differential effects, depending on the factor analysed. Early GC excess and FRD intake make APCs more competent, this means favouring their ability to differentiate into mature adipocytes, consequently increasing adipogenesis. Nevertheless, this increased adipogenesis occurs in parallel with hypertrophic VAT expansion. GCs chronic excess causes APCs competency to decrease, adipogenesis to fall and VAT expansion mainly by the hypertrophy of pre-existent adipocytes. Effects of low testosterone levels associated to obesity need to be further studied. Nonetheless, it is already known that testosterone is an anti-adipogenic factor involved in unhealthy VAT expansion, favouring adipocyte hypertrophy. In summary, all three factors are involved in increased hypertrophy/hyperplasia balance, generating a dysfunctional VAT.

endocrine-metabolic disorders, favouring WAT healthy expansion and thus mitigating obesity-associated pathologies (**Figure 2**).

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