Corticosteroid receptors, their chaperones and co-chaperones: How do they modulate adipogenesis?

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Abstract

It is well known that glucocorticoids and mineralocorticoids are part of the list of hormones that control adipogenesis as well as different aspects of the physiology of the adipose tissue. Their actions are mediated through their binding to the glucocorticoid and the mineralocorticoid receptors (GR and MR, respectively), in complex with heat shock proteins (Hsps) and high molecular weight immunophilins (IMMs). Albeit many aspects of the molecular mechanism of the corticosteroid receptors are not fully elucidated yet, it was not until recently that the first evidences of the functional importance of Hsps and IMMs in the process of adipocyte differentiation have been described. Hsp90 and the high molecular weight IMM FKBP51 modulate GR and MR activity at multiple levels i.e. hormone binding affinity, their subcellular distribution and the transcriptional status, among other aspects of the NR function. Interestingly, it has recently been described that Hsp90 and FKBP51 also participate the control of PPARγ, a key transcription factor in the control of adipogenesis and the maintenance of the adipocyte phenotype. In addition, novel roles have been uncover for FKBP51 in the organization of the nuclear architecture through its participation in the reorganization of the nuclear lamina and the control of the subnuclear distribution of GR. Thus, the aim of this review is to integrate and discuss the actual understanding of the role of corticosteroid receptors, their chaperones and co-chaperones in the process of adipocyte differentiation.

Introduction

The pandemic of obesity has brought the attention on adipose tissue and the development of fat cells. Undoubtedly, adipose tissue plays a central role in the control of energy balance and lipid homeostasis; however during the last two decades it has been demonstrated that adipocytes release a variety of factors, including cytokines, chemokines, and many other biologically active molecules, generically called adipokines that made adipose tissue to be regarded as an active endocrine organ [1]. Adipokines signal to organs of metabolic importance including brain, liver, skeletal muscle, and the immune system [2, 3]. These functions appear to be modulated by the location of the adipose tissue (visceral vs. subcutaneous vs. bone marrow adipose) [4; 5], by the size of the average adipocyte in the tissue [6], by the cross-talk between adipocytes and other
cell-types present in this tissue like macrophages [7, 8], as well as by adipocyte metabolism of glucose [9] and corticosteroids [10]. In this way, adipose tissue has a central role modulating lipid and glucose metabolism homeostasis, blood pressure, and inflammation, in other words, it is a master regulator of the metabolic homeostasis of the organism. In obese individuals, the secretion of adipokines is deregulated [1] and adipose tissue is generally hypertrophic, and infiltrated by a higher number of macrophages compared to normal tissue [7], events that correlate with measures of adiposity and insulin resistance [11, 12]. This leads to the establishment of a state of chronic inflammation leading to metabolic disease [13]. However, a very recent report shows that up to certain level proinflammatory signaling is necessary in the adipocyte for the adequate remodeling and expansion of the adipose tissue [14]. Failure of the normal proinflammatory response leads to increased ectopic lipid accumulation due to deficient adipogenesis, accompanied by glucose intolerance and systemic inflammation, demonstrating that adequate control of adipose tissue inflammatory signaling facilitates the appropriate storage of excess nutrients maintaining the metabolic equilibrium [14].

Conversely, lipodystrophy a disorder characterized by selective total or partial loss of body fat, is also accompanied by similar metabolic consequences as obesity, including insulin resistance, dyslipidemia, hepatic and myocellular steatosis and increase risk for diabetes and atherosclerosis [15, 16], reinforcing the notion of the key role of adipose tissue in the control of the homeostasis of body metabolism. Taken in consideration what has been mentioned, it is relevant to uncover the factors that control not only adipogenesis but also those that exert control in the function of the adipose cell itself. It is well known that corticosteroids are key regulators not only of fat distribution, but also of adipocyte differentiation as demonstrated both in vitro and in vivo [17-21], are required for the induction of lipogenic genes and lipolysis in adipocytes [22, 23] as well as to restrain adipose tissue inflammation in obesity [24].

Corticosteroids exert their action through the binding to their cognate receptors, the glucocorticoid- and mineralocorticoid receptor (GR and MR, respectively) that are present in the cytoplasm. For proper steroid hormone action, GR and MR need to be part of an heterocomplex with the 90-kDa and 70-kDa heat shock proteins, Hsp90 and Hsp70, respectively, the acidic protein p23, and a protein that belongs to the conserved and a large family known as immunophilins (IMMs) [25, 26]. Among the members of the IMMs family, FK506 binding
protein (FKBP)52, FKBP51, Cyclophilin (CyP) 40, and three IMM-like proteins, protein phosphatase 5 (PP5), hepatitis virus B X-associated protein 2 /AhR-associated protein 9 (XAP2/ARA9), and WAF-1/CIP1 stabilizing protein (WISp) 39 have been recovered to date in steroid receptor•Hsp90 complexes [25, 27]. Detailed revisions have been made on the roles of glucocorticoids and mineralocorticoids actions in adipose tissue biology [28, 29], thus our aim will be to summarize the molecular mechanism of action of gluco- and mineralocorticoids, and then discuss recent findings of how chaperones and co-chaperones not only regulate GR and MR at different levels but also exert new roles in the process of adipogenesis.

**Glucocorticoids actions in the adipose tissue**

Glucocorticoids are required for proper adipocyte differentiation [17, 30, 31] and have a wide spectrum of actions on adipose tissue biology, for a recent review refer to [28]. As already mentioned, they exert their action through their binding to the GR that is present in the cytoplasm as part of a heterocomplex with Hsp90, Hsp70, p23 and the high molecular weight immunophilin FKBP51 or FKBP52. GR mRNA [32] and protein [33] exhibit a particular pattern of expression, with higher levels during the first several hours post-induction of the adipogenic program of 3T3-L1 preadipocytes, follow by a period of lower level of expression that gradually increases back to preadipocytes level after several days. There is still some controversy about GR requirement for proper adipogenesis to occur. It has been reported that knock-down of GR by specific siRNA blocks the differentiation of 3T3-L1 preadipocytes [34] but other report provides some evidence that this is not the case [21]. However the role of GR appears to be more complex, base on the fact that it has been shown that brown adipose cell lines generated from newborn homozygous GR-knockout mice, showed mildly impaired adipocyte differentiation with a decrease of lipid accumulation mainly at early time points compared to wild-type adipocytes [35]. Thus, differences in cell lines and/or cell sources may account for different results, and more studies are needed to shed light on this complex issue.

There is no doubt that glucocorticoids have a pro-adipogenic effect that is evident in the development of central obesity in patients with high levels of circulating glucocorticoids, as observed in Cushing’s syndrome or in patients that required prolonged administration of this steroid hormone therapeutically [28, 36]. Further, adipose tissue-dependent amplification of glucocorticoid production in transgenic mice results in a full metabolic syndrome, including
central obesity, insulin resistance and hypertension [10]. In contrast, glucocorticoid inactivation is associated with resistance to metabolic dysfunction [37, 38].

**Mineralocorticoids actions in the adipose tissue**

Aldosterone and its cognate receptor MR besides controlling water and salt homeostasis, are also involved in the complex biology of the adipocytes [29]. Many reports have shown that aldosterone is required for proper adipogenesis [20, 21]. 3T3-L1 preadipocyte differentiation is blocked in the presence of aldosterone antagonists such as ZK91587 [20], spironolactone [21] or drospirenone [39]. Further, knock-down of MR by siRNAs markedly inhibited 3T3-L1 cells differentiation [21]. Taken together these studies demonstrate the importance of aldosterone/MR in the adipogenic process. Significantly higher MR mRNA levels are present in white adipose tissue (WAT) of obese ob/ob and db/db mice than in lean control mice [40]. Treatment of ob/ob and db/db obese mice with eplerenone, a selective MR antagonist, does not result in significant changes in body weight; however this treatment reduces the number of hypertrophic adipocytes, decreases the infiltration of adipose tissue by macrophages, and corrects glucose tolerance test [40]. Therefore, MR may play a key role in the development of the metabolic syndrome and its blockade could be beneficial for obese patients who suffer insulin resistance and type2 diabetes.

Interestingly, several reports, some of them even published when no role was known for aldosterone in adipogenesis, indicated an association between high serum aldosterone levels and obesity [41-46]. In this regard, secretory products isolated from human adipocytes strongly stimulate steroidogenesis in adrenocortical cells with a predominant effect on mineralocorticoid secretion [47]; the identity of these products still remains elusive. Further, visceral adipocytes isolated from SHR/NDmcr-cp, a rat model of metabolic syndrome, secrete factors that stimulate aldosterone production in adrenocortical cells, activity that was not detected in adipocytes from non-obese SHR [48]. The stimulatory effect of adipocyte-conditioned medium on aldosterone secretion is independent of adipose angiotensin II since the effect is not block in the presence of antagonists of angiotensin type1 receptor [47, 48].

Importantly, the adipocyte itself is another source of both aldosterone synthesis and secretion [49, 50]. Preadipocytes as well as adipocytes express CYP11B2 (aldosterone synthase) gene and have a functionally active aldosterone synthase, making these cells aldosterone-
producing cells [50]. Specific inhibition of CYP11B2 interferes in the normal process of adipogenesis [50]. It remains to be investigated whether local synthesis of aldosterone may depend on fat depot and whether changes in the local aldosterone production in the hypertrophic adipocyte may also contribute to the pathophysiology of obesity.

Healthy adipose tissue around small arteries secretes factors that influence vasodilation by adiponectin-dependent increase in nitric oxide bioavailability [51]. In perivascular fat from obese subjects with metabolic syndrome this dilator effect is lost possibly due to defects of hypertrophic adipocytes to properly secrete adiponectin [51]. However, adipose local production of aldosterone may also exert a paracrine action modulating the microvascular tone. Small arteries from db/db mice exhibit endothelial dysfunction [52], and acetylcholine-induced relaxation of mesenteric arteries is improved by pretreatment of db/db mice with eplerenone [50]. Thus, local aldosterone secreted by perivascular adipocytes may contribute to endothelial cells dysfunction, explaining the beneficial effect of the selective MR antagonist eplerenone on the microvasculature [50].

Do glucocorticoids and mineralocorticoids exert coordinated actions in adipose tissue?

Glucocorticoids and mineralocorticoids have their own cognate receptors, however the way they exert their biological actions is more complex. Circulating levels of aldosterone are 100 to 1000 times lower than cortisol which can bind to MR with 10 fold higher affinity than to GR [53]. In order to control GR/MR activation by glucocorticoids, 11-beta hydroxysteroid dehydrogenases (11β-HSDs) type 1 and 2 determine the availability of intracellular concentrations of active glucocorticoids [54, 55]. 11β-HSD1 is expressed primarily in glucocorticoid- target tissues, such as liver, the central nervous system, and the adipose tissue, and acts as a predominant 11β-reductase NADP(H) dependent that amplifies glucocorticoid action. On the other hand, 11β-HSD2 is a high affinity NAD-dependent dehydrogenase expressed primarily in mineralocorticoid target tissues, such as kidney, and metabolizes glucocorticoids to their 11-dehydro derivatives i.e. cortisone, 11-dehydrocorticosterone, that have weak or no affinity for MR [56, 57]. Thus, the presence of 11β-HSD2 “protects” MR from its activation through the binding of glucocorticoids. In adipose tissue, 11β-HSD2, which reduces active levels of glucocorticoids, is expressed at much lower levels than 11β-HSD1 [58-60]. In Flier’s lab transgenic mice that expressed 11β-HSD2 driven by the adipocyte fatty acid binding protein 2
promoter were generated. These mice have the highest levels of 11β-HSD2 in adipose tissue, and when they are exposed to high-fat diet they are resistant to weight gain, event that is associated with decreased food intake, increase energy expenditure, and improved insulin sensitivity [38]. Further, 11β-HSD-1 null mice showed reduced visceral fat accumulation upon high-fat feeding, and expressed higher PPARγ, adiponectin and lower resistin and tumor necrosis factor-α, pattern of gene expression that indicates high insulin sensitivity [37]. Therefore, it is possible that high concentration of cortisol within the adipose tissue that cannot be inactivated due to low levels of 11β-HSD2, may exert their action not only upon GR activation but also through MR. In obese patients, serum cortisol levels are not increased and can be even lower than serum aldosterone levels [61, 62]; therefore increased local activation of cortisol from cortisone observed in obesity have detrimental consequences in adipose tissue physiology.

The requirement of an adequate level of gluco- and mineralocorticoids stimulus is highlighted by the fact that the activation of GR and MR has some opposite outcomes in adipocytes. For example, MR activation increases the expression of interleukin-6 and plasminogen activator inhibitor (PAI) 1 while GR activation decreases them [35]. It has also been reported that the synthetic glucocorticoid dexamethasone (DEXA) decreases the expression of proinflammatory cytokines [24, 63-65] as well as macrophage infiltration in adipose tissue [24]. Further, treatment of obese diabetic db/db mice with eplerenone, a selective MR antagonist, decreases the expression of tumor necrosis factor –α, monocyte chemoattractant protein-1, and macrophage protein CD69 while increases the expression of adiponectin and peroxisome proliferator-activated receptor (PPAR)γ in adipocytes from the retroperitoneal fat depot [66]. Eplerenone treatment also decreases macrophage infiltration and the generation of reactive oxygen species in adipose tissue [40]. Since the pattern of expressed genes is different dependent on the participation of GR or MR, the strict control of their activation is required in the adipose cell to achieve the proper biological response. We need to keep in mind that in the genome there are approximately 2-5 x10^6 potential recognition elements for binding of this nuclear receptors (NRs), however a few thousand binding sites are occupied [67, 68]. Therefore, how are GR and MR specificities achieved? Several coordinately layers of GR/MR control allow them to gain such specificity, among them – regulation of their subcellular distribution, posttranslational modifications that may modulate their interaction with factors that control their transcriptional
capacity, and mechanisms that mediate the recognition of the response elements in the architecture of the genome contribute to it.

**GR and MR expression during adipogenesis.**

In order to gain insight in a better understanding of the NRs function during adipogenesis, Fu *et al* analyzed the gene expression profiles of NRs during differentiation of murine 3T3-L1 preadipocytes. Of the forty-nine NRs analyzed, thirty are expressed at some point during 3T3-L1 adipogenesis and seventeen of them are expressed in a temporal-specific manner [32]. GR and MR belong to a group of NRs that are expressed in a biphasic manner, with higher levels of expression during the first several hours post-induction of the adipogenic program, follow by a period of lower level of expression that gradually increases back to preadipocytes levels after several days [32]. GR and MR expression is found substantially higher in mature primary adipocytes relative to preadipocytes [32]. In agreement with mRNA, GR protein expression levels follow a similar pattern [33]. It was proposed that this temporal pattern of GR and MR expression possibly marked distinct, transcriptionally regulated boundaries at early, middle and late stages of adipocyte differentiation [32]. In addition, GR mRNA levels exhibit differences that depend on the fat depots. GR mRNA is 2 to 4-fold higher in omental than subcutaneous adipose tissue [22, 69-71]. It will be relevant to determine whether MR expression is similar or not in different fat depots.

Besides differences in GR and MR levels of expression, it has to be taken in consideration the existence of GR and MR isoforms. GRα and -β are GR isoforms generated by alternative splicing of exon 9 [72, 73]. The resulting proteins are identical except that GRα contains 5 additional amino acids and GRβ additional non-homologous 15 amino acids. Both are widely expressed in all tissues with GRα being present at relatively higher levels in the majority of the tissues examined in humans and mice [74, 75]. GRβ functions as a dominant negative inhibitor of GRα, therefore the GRα/GRβ ratio may also mediate differential glucocorticoid responses. Insulin increases GRβ mRNA without affecting GRα mRNA in mouse embryonic fibroblasts [75]. Importantly, GRα/GRβ ratio changes in the liver when fasted mice are re-fedded indicating that the GRs ratio changes in response to insulin [75]. To add more complexity, different isoforms from the GRα transcript can be generated by alternative translation initiation mechanisms [76]. No significant differences have been observed in their affinity for
glucocorticoids or their capacity to interact with glucocorticoid response elements (GREs) present in GR target genes, but their subcellular distribution shows some differences [76]. Importantly, it has been shown that translationally generated GRα isoforms regulate both common and distinct sets of genes in the same cell. Lu et al showed that in U-2 OS cells, a human osteoblastic sarcoma cell line that lacks endogenous GR, expression of different GR isoforms have distinct capabilities to activate the cell death program [76, 77]. Since they have identical DNA- and ligand binding domains, it has been proposed that this functional difference may relay on their differential ability to recruit co-regulators and/or the recognition of chromatin modifications on target genes [77].

MR has also isoforms and protein variants that create diversity around one single gene [78-81]. MR is expressed as two different protein variants, MRA and MRB, resulting from Kozak sequences that initiate alternative translation [82]. Thus, future studies are require to address the contribution of GR and MR isoforms in the process of adipogenesis as well as their expression in the different adipose depots where they may possibly contribute to the differential response to corticosteroid hormones.

GR and MR undergo different posttranslational modifications that constitute another layer of control to achieve specificity in the corticosteroids response. GR can be substrate for phosphorylation [83-87], sumoylation [88-92], ubiquitination [93, 94] and acetylation [95]. These post-translational modifications regulate the final biological outcome dependent on GR by modulating GR subcellular distribution, protein stability, chromatin binding, transcriptional capacity, and its interaction with co-regulatory complexes. In the case of MR, it has been reported that it can undergo phosphorylation [96-99], acetylation [100], and sumoylation [101], having consensus sites for possible ubiquitination. It still remains to be elucidated in detail how posttranslational modifications may control hormonal responses as well as the crosstalk of steroid hormones with other signaling molecules in adipose tissue.

**GR and MR transformation and nuclear translocation**

For proper steroid hormone action, it is well established that GR and MR need to be part of a heterocomplex with the 90-kDa and 70-kDa heat shock proteins, Hsp90 and Hsp70, respectively [25, 26]. In the absence of ligand, GR and MR reside primarily in the cytoplasm, and
it has been accepted that upon hormone binding, they dissociate from the Hsp90 heterocomplex for their translocation to the nucleus by free diffusion. However, during the last decade several reports provided evidence that movement of GR and MR is more complex than simple diffusion. It was demonstrated that the intermediate chain of the motor protein dynein co-immunoprecipitates with the Hsp90•FKBP52 complex of the GR [102-104] and MR [105, 106] suggesting that this motor protein can facilitate the retrograde movement of the steroid receptor (Fig. 1). Blockage of the Hsp90•FKBP52 or FKBP52•dynein interactions by over-expressing the tetratricopeptide repeat motif (TPR) domain or the peptidyl-prolyl isomerase domain of the IMM, respectively slows the rate of receptor translocation to the nucleus in a similar manner as inhibition of the Hsp90 function by geldanamycin treatment [107]. In all these cases, the nuclear localization of the receptor was not fully abrogated, but it was delayed. Therefore, GR and MR possess two mechanism of transport, a rapid Hsp90•FKBP52•dynein complex-dependent mechanism and a slow heterocomplex-independent mechanism by simple diffusion.

The classic model of GR/MR receptor activation establishes that Hsp90 “anchors” NR in the cytoplasmic compartment and, upon hormone binding, the NR has to undergo a process known as “transformation”, that corresponds to its dissociation from Hsp90, for then translocate to the nucleus. Since Hsp90•FKBP52 complex facilitates GR and MR movement towards the nucleus, consequently their transformation should not take place immediately upon ligand binding (Fig. 1). Moreover, it has been shown that the whole MR•Hsp90-based heterocomplex can be transiently recovered in the nucleoplasmic fraction of the nucleus shortly after steroid hormone incubation [106, 108]. Thus, the steroid-receptor transformation could possibly take place in the nucleus [109], raising the possibility that the entire oligomeric structure could pass intact through the nuclear pore for transformation to be a nuclear rather than a cytoplasmic event (Fig. 1). In this regard, GR and its associated chaperones bind with proteins of the nuclear pore such as nucleoporins (Nups) and importin (Imp)-β, and it has been shown that the entire Hsp90 heterocomplex cross-linked to GR translocates intact through the nuclear pore in digitonin-permeabilized cells [110]. There is evidence that Imp-α is co-internalized with the GR [111], whereas Imp-β is not. Nonetheless, the knock-down of Imp-β significantly delayed GR nuclear import [110]. It has been reported that many importins including Imp-β effectively suppress the aggregation of cargoes [112]. It can be envisioned that the presence of chaperones and co-
chaperones associated to importin, Nups and the cargo itself may prevent the aggregation of cargoes when a relatively hydrophobic domains are exposed [109]. When these complexes are disrupted by Hsp90 inhibitors such as radicicol or geldanamycin, the nuclear translocation rate of GR [102, 104] and MR [105, 113] undergoes a substantial delay, in line with the idea that importin-Nup-chaperone complexes may facilitate their nuclear pore translocation step.

**The chaperone Hsp90: does it play a role in adipogenesis?**

During the process of differentiation of 3T3-L1 preadipocytes no change is observed in the protein levels of the chaperones and co-chaperones Hsp90, Hsp70, and p23 [33] Hsp90 accounts for 1-2% of the total soluble proteins in resting cells, ~6-7% in cancer cells and up to 10% in stressed cells [114-116]. Hsp90 is a molecular chaperone that associates with numerous substrate proteins called clients modulating their folding and function, among them protein kinases and transcription factors [117-119]. In this manner, Hsp90 controls metastable proteins that are regulatory hubs in biological networks. The master regulator for acquisition and maintenance of the adipocyte phenotype PPARγ has been recently incorporated to the large list of Hsp90 client proteins. Inhibition of Hsp90 by treatment of 3T3-L1 cells with geldanamycin or its analogs at early time points of the adipogenic process prevented the cells to differentiate properly [120-122]. It has been proposed that the anti-adipogenic effect of geldanamycin may result from the destabilization of PPARγ that is targeted to degradation by the proteosome [120], to decrease PDK1-Akt activities leading to the blockade of the mitotic clonal expansion at the onset of adipogenesis [121], and the decrease of GR and MR activities [122]. Hsp90 is essential for wide spectrum of cellular processes such as protein folding, protein degradation, and signal transduction cascades [117, 123], having been recently shown that Hsp90 also participates in the maintenance of RNA polymerase II pausing, function required for the adequate gene expression when cells have to respond to environmental stimuli [124]. Therefore the blockade of the adipogenic program upon Hsp90 inhibition could be the resultant of a more widely disruption of signaling pathways as well as nuclear events dependent on Hsp90 surveillance.

**High molecular weight immunophillin: regulatory functions beyond NR control**

IMMs comprise a family of proteins classified by their ability to bind immunosuppressant drugs in which cyclophilins bind cyclosporine A whereas FKBPs bind FK506. The high
molecular weight IMMs FKBP51 and FKBP52 do not play a role in immunosuppression, and rather have been related to steroid receptor regulation [125]. The FKBP5s are modular proteins that possess FKBP12-like peptidyl-prolyl isomerase (PPIase) domains 1 and 2 (FK1 and FK2) and a tetratricopeptide repeat motif (TPR). The FK1 domain is required for the binding of the immunosuppressive drug FK506, it confers PPIase activity, and it is also the primary domain required for steroid hormone receptor regulation [125-127]. The TPR domain contains sequences of 34 amino acids repeated in tandem through which FKBP5s interact with Hsp90. FKBP51 and FKBP52 share 60% identity and 70% similarity, however the former has been so far mainly reported to be a negative regulator of steroid hormone receptors while the latter is a positive one [103-105, 125, 127]. When differentiation of 3T3-L1 preadipocytes is induced, it was reported that FKBP51 has a transient expression at very early time points (day 1 up to day 4 of differentiation) and then its expression decreases to undetectable protein levels [128]. More recent studies demonstrate that FKBP51 and FKBP52 exhibit opposite changes in their level of expression during the process of adipocyte differentiation. FKBP51 expression progressively increases whereas FKBP52 decreases as adipogenesis progresses [33, 129]. The differences observed between early and recent studies may possibly depend on the development of highly sensitive and specific antibodies now available for the study of these IMMs. Further, the changes in FKBP51 and FKBP52 observed during 3T3-L1 preadipocytes differentiation are in agreement with their expression level in adipose tissue that is high for FKBP51 and non detectable for FKBP52 [130].

To uncover the functional importance of these IMM, knocked-out mice were generated [125]. Fkbp51-deficient mice were initially observed to display no overt phenotype, but these mice are less vulnerable to the detrimental effects of stress [131-133]. Interestingly, Fkbp51 knockout mice showed reduced body weight compared to wild type littermates; however, upon exposure to chronic stress, these animals exhibited a significant increase in body weight [133], results that suggest that the process of adipogenesis may not be impaired in the absence of FKBP51. It has been recently reported by Balsevich et al a differential spatial pattern of Fkbp51 gene induction in different areas of the brain dependent on either diet or stress conditions. In mice exposed to high-fat diet, Fkbp51 is induced in the ventromedial hypothalamic nuclei, in accordance with the hypothalamus being involved in the control of energy balance [134].
contrast under conditions of chronic stress, the expression of this IMM increases in the hippocampus, area of the brain involved in the response to stress [134]. Inasmuch as environmental stress is another risk factor for the development of obesity [135], future studies will uncover the role of FKBP51 in different areas of the brain, whether this IMM plays a role in the control of appetite, energy balance and whether FKBP51 is implicated in the relationship between control of energy, metabolic homeostasis and stress response. On the other hand, Fkbp52-deficient male mice display phenotypes related to partial androgen insensitivity syndrome [136, 137]. Heterozygous Fkbp52-deficient mice show increased susceptibility to high fat-diet-induced hyperglycemia and hyperinsulinemia that correlates with reduced insulin clearance, hepatic steatosis and glucocorticoid resistance [130]. However Fkbp51-Fkbp52 doubled knock out results in embryonic lethality [125], indicating that these IMMs have some physiologic functional redundancies that need to be uncover by tissue-specific conditional double knockout.

FKBP51 is present in mitochondria [138], and upon oxidative stress the mitochondrial fraction of this IMM rapidly translocates to the nucleus protecting cells from apoptosis [138]. When 3T3-L1 preadipocytes are induced to differentiate, FKBP51 also rapidly and transiently translocates to the nucleus [33]. Adipogenesis is controlled by many signaling pathways that coordinately modulate the sequential activation of transcription factors required for cells to differentiate [139]. We found that IBMX (3-isobutyl-1-methylxanthine), a phosphodiesterase inhibitor that increases intracellular cAMP, and to a lesser extent DEXA are responsible for the rapid relocalization of mitochondrial FKBP51 to the nucleus [33]. Several reports have shown that the second messenger cAMP is associated with immediate events of adipogenesis by the classic PKA signaling pathway, as well as by the non-classic pathway, the exchange proteins activated by cAMP (EPAC) that function as guanine nucleotide exchange factor for the Ras-like small GTPases Rap1 and Rap2 [140-143]. FKBP51 nuclear translocation depends on PKA but not on EPAC pathway activation, demonstrating another differential role of PKA and EPAC/Rap during adipogenesis [33]. In the case of DEXA, it has been reported that corticosterone possibly facilitates arachidonic acid (AA) release and increased synthesis of prostacyclin, which in an autocrine manner leads to cAMP generation required for the differentiation of Ob1771 preadipocytes [18, 144]. The effect of corticosterone on AA metabolism in Obl711 cells could be
contradictory considering the antiinflammatory properties of glucocorticoids upon inhibition of phospholipase A2; however there are studies that questioned this effect [145, 146]. Further, based on the pro-adipogenic action of glucocorticoids, AA and prostacyclin signaling in adipose tissue development [147], it is tempting to speculate that, in adipose tissue, glucocorticoids may possibly mediate a different biological response in AA pathway, possibility that needs to be investigated. DEXA treatment also results in increased levels of intracellular cAMP in other cell systems. For example, glucocorticoid treatment of 3B4.15 T cells causes activation of adenylate cyclase and a decrease in phosphodiesterase activity [148], and in human airway epithelial cells DEXA increases the beta2-adrenergic receptor-adenylate cyclase system [149] leading to the increase in cAMP. It will be relevant to investigate these possibilities in adipose cells, to better understand corticosteroids actions both in adipogenesis and the adipose tissue biology.

FKBP51 interacts with PKA-cα as shown by immunoprecipitation assays, and when PKA signaling is blocked dramatic changes in the electrophoretic pattern of migration of FKBP51 are observed supporting the notion that FKBP51 is a PKA substrate [33]. By using NetPhosk 1.0, we found that Serine 312 of FKBP51 is a candidate PKA phospho acceptor site. Serine 312 is in the TPR domain that confers to the IMM the ability to bind Hsp90 through the EEVD motif present in the extreme C terminus of the chaperone. FKBP51 localization in mitochondria depends on TPR integrity, since FKBP51 TPR deficient mutants are constitutively nuclear [138]. Therefore, changes in phosphorylation in Serine 312 present in the TPR domain of FKBP51 may possibly regulate its interaction with Hsp90 and consequently its subcellular localization, possibility that is under current investigation. Interestingly, when the interaction of FKBP51 with Hsp90 is disrupted by Hsp90 inhibitors such as radicicol, FKBP51 is no longer in mitochondria and concentrates in the nucleus [138]. As mentioned already, geldanamycin and radicicol inhibit 3T3-L1 preadipocytes differentiation [120-122]. Therefore, it is also possible that the Hsp90 inhibitors not only affect PPARγ, GR and MR function but may also alter the dynamic mitochondrial-nuclear shuttling of FKBP51 at the onset of the differentiation process required for adipogenesis to proceed.

However, during the last years several reports demonstrate that FKBP51 functions are not circumscribed only to the control of NRs. FKBP51 participates in the control of the protein kinase Akt activity. The IMM is a scaffold protein for the interaction between Akt and the PH
domain leucine-rich repeat protein phosphatase (PHLPP) that dephosphorylates Serine 473 in Akt inhibiting the kinase activity [150]. It has been recently demonstrated that FKBP51 interacts with over-expressed PPARγ in COS7 cells, and reporter gene assays shows that FKBP51 is a positive regulator of this NR [87]. PPARγ like other NRs can be regulated by changes in its phosphorylation status. MAPK ERK1/2, and JNK are able to phosphorylate PPARγ at serine 112 reducing its transcriptional capacity [151-153]. Further, inhibition of p38MAPK increases PPARγ expression and its transcriptional activity [154]. GR is also a substrate of p38MAPK, post-translational modification that increases GR transcriptional capacity [155]. Taken together, MAPK-dependent phosphorylation of PPARγ and GR has opposite effects on the transcriptional capacities of these NRs, PPARγ transactivation decreases while GR transactivation increases. Stechschulte et al showed that in mouse embryonic fibroblasts (MEFs) null for FKBP51 elevated Akt activity causes an increased activation of p38MAPK leading to the phosphorylation of GR and PPARγ, post-translational modification that induces the transcriptional activation of the former and inhibition of the latter [87]. Further, they show that knock down of FKBP51 in 3T3-L1 preadipocytes makes cells resistant to differentiation and MEFs from mice 51KO have impaired differentiation [129]. The authors proposed a model, in which FKBP51 restrains Akt activation by scaffolding PHLPP [87], favoring the inactive state of p38MAPK that prevents PPARγ phosphorylation and keeps this NR in a transcriptionally active state to induce the expression of the adipogenic genes [129]. While the role of p38MAPK in adipogenesis is rather controversial [154, 156-158], several lines of evidences demonstrate that Akt is required for proper adipogenesis. Akt is a key component of insulin signaling and is required for PPARγ expression [139, 159]. Expression of constitutive active Akt induces spontaneous differentiation of 3T3-L1 preadipocytes [160], and mice null for Akt1 and Akt2 have impaired adipogenesis [159]. Akt is responsible for phosphorylation and nuclear exclusion of anti-adipogenic factors such as the forkhead proteins FOXO-1 [161] and FOXA-2 [162], and the transcription factor GATA2 [163]. Therefore, proper activation of Akt is required for normal adipogenesis and it can be speculated that Akt inhibition by the FKBP51-PHLPP could have a negative effect on this process. In line with this possibility, Toneatto et al showed that knock down of FKBP51 facilitates the process of adipogenesis and its over-expression blocks 3T3-L1 preadipocyte differentiation, based on the fact that this IMM also restrains the adipogenic potential of GR [33], as well as MR. It is possible that the discrepancies between these two studies could result, in part,
from differences in the protocol of adipogenesis used in each case. More studies are required to precise the role of FKBP51 during adipogenesis and, in this way, shed light on this conundrum.

**FKBP51 and the organization of the nuclear architecture during adipogenesis**

In a very simplistic description, as depicted in figure 2, the following compartments can be distinguished in the interphase nucleus: 1-the nuclear lamina that lies below the nuclear envelope; 2-the nuclear matrix or nucleoskeleton; 3-the chromosome territories, that comprise the volume of the nucleus in interphase occupied by each chromosome; 4-the interchromatin domain; and 5-nuclear bodies, including the nucleolus, spliceosomes or nuclear speckles, paraspeckles, the Cajal bodies, the promyelocytic bodies, and transcription factories, among others [164-168]. The importance of the organization of the nuclear architecture is highlighted by the evolutionary conservation of chromosome territories [169], by the observation that chromosome positions are heritable through the cell cycle in mammalian cells [170] and interphase chromosomes, as well as chromatin organization, undergo changes during terminal differentiation [171-174]. Great body of evidence shows that the genome has a dynamic 3D organization that impinges in the transcriptional status of the cell. Chromatin is not static and genes can be repositioned from repressive to transcriptionally favorable nuclear compartments and *vice versa* for their proper expression or repression [175-182]. It is relevant to understand how the architecture of the nucleus is delineated to uncover how the cell acquires and sustains the pattern of gene expression require for the acquisition and maintenance of the final phenotype.

The nuclear lamina (NL) is a filamentous protein mesh-work that lines the nucleoplasmic surface of the nuclear envelope (NE) interacting with inner nuclear membrane proteins and the nuclear pores (Fig. 2) [183, 184], and reviewed in [185, 186]. It consists of a polymeric assembly of lamins, members of the type V intermediate filament proteins family [187] that are the A-type (LA and LC) and the B-type lamins (LB1 and LB2), respectively. LA and LC are derived from a single gene by alternative splicing and are expressed only in differentiated cells. On the other hand, LB1 and LB2 are expressed in all cells throughout development [188]. The NL is thought to provide a structural framework for the NE contributing to the size, shape and mechanical stability of the nucleus. It also provides anchoring site for interphase chromosomes at the nuclear periphery, and plays important roles in DNA replication and repair, RNA polymerase II
transcription, and the epigenetic control of chromatin remodeling [186, 189]. The functional importance of NL is demonstrated by the fact that mutations in the lamin A/C or lamin-associated proteins genes are responsible of a group of genetic diseases known as laminopathies [186, 190] to which belong Dunnigan-type familial partial lipodystrophy (FPLD) [191-194], partial lipodystrophy with madibuloacral dysplasia (MAD) [195], and lipoatropy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules [196], diseases that affect the adipose tissue. In addition, laminopathies such as Hutchinson-Gilford progeria and atypical Wermer’s syndrome show generalized lipodystrophy, often combined with insulin resistance [197-200].

In a recent study, analysis of a mouse and human model systems for adipogenesis showed fragmentation of the nuclear lamina and subsequent loss of lamins A, C, B1, and emerin at the nuclear rim, which coincides with reorganization of nesprin-3/plectrin/vimentin complex [201]. We have also observed fragmentation of nuclear lamina at the early stages of 3T3-L1 preadipocytes differentiation upon detection of lamin B. Interestingly, fragmented lamin B not only colocalizes but interacts with FKBP51 as well as PKA-cα [33]. Several phosphorylation sites are important in the NL disassembly, including those for the cyclin B1-(CCNB1)-CDC2 complex, PKC and PKA [202, 203]. Therefore, it is possible that enrichment of FKBP51 and PKA-cα in the NL may facilitate its reorganization by phosphorylation of lamins during adipocyte differentiation.

During the last few years, several studies revealed a dramatic and dynamic modulation of the chromatin landscape during the first hours of adipocyte differentiation [67, 68, 204-206]. These changes coincide with cooperative binding of early adipogenic transcription factors, including GR, to enhancers and promoters of many genes and high level of chromatin relaxation [68, 205]. However, genes such as PPARγ are not transcriptionally activated until later time points of the adipogenic program, and it has been proposed that the activation of additional factors and/or signals is required for their later activation [68]. It can be speculated that, in spite of chromatin relaxation and the increased binding of transcription factors at the early stages of adipogenesis, gene expression is kept controlled by factors that restrain the transcriptional capacity of complexes already bound to those sites. When adipogenesis is triggered FKBP51 translocates to the nucleus, its interaction with GR progressively increases rendering a GR less
transcriptionally active [33]. When cells are treated with DEXA in the presence of IBMX GR has an increased presence in the nuclear rim, area that corresponds to the NL (Fig. 2, panel C vs. B), a domain that is also transiently enriched in PKA-α [33]. Therefore, we hypothesize that FKBP51 “sequesters” GR in the nuclear rim to control its nuclear bioavailability as shown for the control of AP-1 transcriptional activity upon the sequestration of c-fos in the NL in an ERK1/2 dependent manner [207, 208]. Interestingly, it has been shown that GR interacts in vitro and in vivo with the catalytic subunit of PKA in a ligand dependent manner, and that GR transcription depends on PKA signaling [209]. In this way PKA may play a dual role in the regulation of GR, on one hand, modulating positively GR transcriptional capacity [209] and, on the other hand, restraining it by increasing the nuclear availability of FKBP51, a known GR negative regulator [104] that possibly modulates its nuclear distribution in different compartments. It is possible that the presence of FKBP51 in the nucleus may be critical for the control not only of GR but also for MR at the onset of adipogenesis. Future studies will possibly demonstrate the existence of other transcription factors that do not belong to the NR family, that need to be repressed or even activated by nuclear FKBP51 to keep their transcription under control, at a step of the adipogenic program in which high level of chromatin remodeling takes place.

**Final remarks**

Undoubtedly during the last decade, great progress has been accomplished in the understanding of the complex biology of the adipose tissue, the pathophysiology of obesity and its participation in the metabolic syndrome. However, many aspects of the physiology of the adipocyte including how corticosteroids modulate its biological responses need to be explored further in depth. Uncovering at the molecular level how different fat depots respond to the gluco- and mineralocorticoid stimuli, how corticosteroid receptors, their chaperones and co-chaperones control adipocyte differentiation will not only enriched our basic knowledge but also will be key for the design of new therapeutic strategies for the treatment of obesity, lipodystrophies and metabolic problems associated to this pathologies.

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References


**Figure legends**

**Figure 1 - Model of GR and MR action** – The adipogenic media contains DEXA, IBMX, insulin and is supplemented with fetal bovine serum. Upon corticosteroid hormone binding to its receptor (GR or MR) FKBP51 is exchanged for FKBP52 that facilitates the retrograde movement of the NR towards the nucleus. IBMX increases cAMP leading to PKA activation that triggers the translocation of FKBP51 from mitochondria to the nucleus, possibly upon changes in its phosphorylation status. In the nucleus GR binds to its target genes, localizes in the NL (nuclear lamina) and possibly in the NM (nuclear matrix). FKBP51 is retained in the nucleus by its interaction with the NL, the NM and chromatin, regulating GR-target genes, and possibly other genes.

**Figure 2 - A)** Schematic representation of the compartments of the nucleus in interphase NE: nuclear envelope, NPC: nuclear pore complex, NL: nuclear lamina, NM: nuclear matrix, CTs: chromosome territories; MARs: matrix attachment regions that correspond to DNA sequences that allow the interaction of chromatin to NM, LADs: lamin attachment domains, corresponds to DNA sequences that allow the interaction of chromatin to the nuclear lamina. **B-C)** 3T3-L1 preadipocytes grown on coveslips were incubated with DEXA in the absence (B) or presence (C) of IBMX and subjected to indirect immunofluorescence with anti-GR, anti-FKBP51, and secondary antibodies labeled with Alexa 488 or Alexa 546, respectively, and images were analyzed by confocal microscopy. GR is detected in red, FKBP51 in green and chromatin is stained by DAPI in blue. Inserts in each panel were magnified. Observed in panel C that DEXA plus IBMX treatment caused enrichment of GR (red signal) in the nuclear area limited by the white dotted lines that correspond to NL.
Figure 1