Mitochondrial oxidative stress in obesity: role of the mineralocorticoid receptor

Clara Lefranc¹, Malou Friederich-Persson²*, Roberto Palacios-Ramirez¹* and Aurelie Nguyen Dinh Cat¹

¹INSERM, UMRS 1138, Centre de Recherche des Cordeliers, Pierre et Marie Curie University, Paris Descartes University, Paris, France
²Medical Cell Biology, Uppsala University, Uppsala, Sweden

Correspondence should be addressed to A Nguyen Dinh Cat: cattuong.ndc@gmail.com

*(M Friederich-Persson and R Palacios-Ramirez contributed equally to this work)

Abstract

Obesity is a multifaceted, chronic, low-grade inflammation disease characterized by excess accumulation of dysfunctional adipose tissue. It is often associated with the development of cardiovascular (CV) disorders, insulin resistance and diabetes. Under pathological conditions like in obesity, adipose tissue secretes bioactive molecules called ‘adipokines’, including cytokines, hormones and reactive oxygen species (ROS). There is evidence suggesting that oxidative stress, in particular, the ROS imbalance in adipose tissue, may be the mechanistic link between obesity and its associated CV and metabolic complications. Mitochondria in adipose tissue are an important source of ROS and their dysfunction contributes to the pathogenesis of obesity-related type 2 diabetes. Mitochondrial function is regulated by several factors in order to preserve mitochondria integrity and dynamics. Moreover, the renin–angiotensin–aldosterone system is over-activated in obesity. In this review, we focus on the pathophysiological role of the mineralocorticoid receptor in the adipose tissue and its contribution to obesity-associated metabolic and CV complications. More specifically, we discuss whether dysregulation of the mineralocorticoid system within the adipose tissue may be the upstream mechanism and one of the early events in the development of obesity, via induction of oxidative stress and mitochondrial dysfunction, thus impacting on systemic metabolism and the CV system.

Introduction

Obesity is a chronic inflammatory disease with an ever-increasing prevalence. Excessive and ectopic fat accumulation leads to adverse health effects and reduced life span. Obesity is originally defined by the BMI (BMI = weight/height²). A person is considered obese when their BMI exceeds 30kg/m² (http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). However, this index does not evaluate lean-to-fat mass ratio. Adiposity is more precisely evaluated by taking into account the body fat distribution, using the waist circumference or waist-to-hip ratio.

The high mortality and morbidity rates associated with obesity worldwide are largely attributed to cardiovascular (CV) complications (Pischon et al. 2008). Obesity and obesity-associated metabolic and CV risk factors are grouped under the term ‘cardiometabolic syndrome’ (CMS), including hypertension, dyslipidemia and type 2 diabetes (T2D) (Alberti et al. 2009).
Accumulated adipose tissue, in obesity, stimulates the synthesis of pro-inflammatory cytokines, as well as bioactive substances called ‘adipokines’, which increase generation of reactive oxygen species (ROS) (Fernández-Sánchez et al. 2011). Indeed, inflammation, oxidative stress and mitochondrial dysfunction contribute to the pathogenesis of metabolic disorders, including insulin resistance and T2D (Furukawa et al. 2004, Bournat & Brown 2010, Sivitz & Yorek 2010, Montgomery & Turner 2014).

During the past decade, several studies have reported the importance of aldosterone and the mineralocorticoid receptor (MR) in CMS (Zennaro et al. 2009, Whaley-Connell et al. 2010, Ronconi et al. 2012, Even et al. 2014). Increasing evidence shows a link between obesity, hypertension and hyperaldosteronism, and clinical studies have established a positive correlation between visceral obesity, CMS or BMI and plasma levels of aldosterone (Goodfriend et al. 1999, Fallo et al. 2006, Ingelsson et al. 2007, Rossi et al. 2008). Aldosterone mediates deleterious effects in adipose tissue through genomic and non-genomic pathways, leading not only to alteration of glucose metabolism and insulin sensitivity (Luo et al. 2013, Feliciano Pereira et al. 2014, Luther 2014, Marzolla et al. 2014, Urbanet et al. 2015), but also to inflammation, oxidative stress, endothelial dysfunction and vasoconstriction, as adipose tissue is intimately associated with structural and physiological functions of the vasculature (Guo et al. 2008, Tirosh et al. 2010, Nguyen Dinh Cat et al. 2016).

Little is known of how the mineralocorticoid system regulates obesity-associated oxidative stress and mitochondrial dysfunction. In this review, we focus on factors and pathways in adipose tissue, which link oxidative stress and/or mitochondrial dysfunction to MR activation. Such factors/pathways could represent new, interesting therapeutic targets and give relevance to the use of MR antagonists in the prevention and/or attenuation of metabolic and vascular disorders that occur in CMS.

**Adipose tissue dysfunction in obesity**

**Adipose tissue expansion**

Adipose tissue is a metabolically dynamic organ, playing an essential role in regulating energy balance. This is achieved by storing triglycerides in periods of energy excess and mobilizing energy in the form of fatty acids, available to meet the energy requirements of other tissues, during fasting. White adipose tissue (WAT) is composed of perivascular, subcutaneous (scWAT) and visceral (vWAT) adipose tissue, which, in turn, is divided in epicardial, perirenal, gonadal, retroperitoneal, omental and mesenteric fat. These fat depots display distinct characteristics, as well as clear differences in fatty acid storage and release and inflammatory profile. Thus, it is likely that these depots play specific roles in the regulation of whole body energy homeostasis (Tchkonia et al. 2013).

In obesity, adipose tissue undergoes remodeling, observed as changes in the number (hyperplasia) and/or size (hypertrophy) of the adipocytes. Such remodeling determines the CV and metabolic outcomes of the disease (Spiegelman & Flier 1996). Progenitor cells (pre-adipocytes) are eight-fold more abundant in scWAT than vWAT, which could explain the propensity of visceral adipocytes to become overloaded and thus resistant to insulin and to triglycerides storage (Joe et al. 2009, Kim et al. 2015, Choe et al. 2016). Indeed, vWAT expansion by hypertrophy predisposes to diabetes, insulin resistance and CV diseases, whereas growth of scWAT by hyperplasia may actually be protective, enabling this depot to act as a buffer for lipid excess, and thus protecting other tissues (heart, liver, skeletal muscle) from fatty infiltration (Wajchenberg 2000, McLaughlin et al. 2011). This variability among WAT depots seems to be already determined at the preadipocyte stage, as specific and different expression patterns of developmental genes have been described in stromal vascular fraction from scWAT, compared to the ones from vWAT. These depot-specific differences are maintained during adipogenesis (Gesta et al. 2006).

**Brown adipose tissue activity and WAT browning in obesity**

In humans, brown adipose tissue (BAT) is predominantly found in the neck, supraclavicular and paraspinal regions, and its activity is inversely correlated with BMI (van Marken Lichtenbelt et al. 2009). Activation of the thermogenic BAT has beneficial effects on adiposity and insulin resistance, through activation of mitochondrial uncoupling protein UCP1 that allows the dissipation of energy in the form of heat (Lowell et al. 1993, Poher et al. 2015). Adipocytes from WAT can undergo the process of browning (also known as beiging) in response to stimuli such as cold exposure, exercise, fasting or specific drug treatments (SIRT1 activators and β3-adrenergic receptor (β3-AR) agonists) (Cypress et al. 2015, Stanford et al. 2015, Li et al. 2017, Thayagarajan & Foster 2017). These ‘beige’ adipocytes possess a unique molecular phenotype with expression of brown adipocytes genes (Ucp1, Cidea), as
well as genes that are strictly specific to beige adipocytes, including Tbx1 and Slc27a1 (Wu et al. 2012). Browning of WAT is often associated with increased energy expenditure and decreased lipid metabolism. Thus, promoting WAT browning could represent an alternative therapeutic strategy to combat obesity and its related metabolic disorders (Bartelt & Heeren 2014, Thyagarajan & Foster 2017).

However, the precise mechanism of browning induction is still unclear, as many browning agents have effects on metabolism or can induce heat loss. Thus, this is difficult to determine whether these agents have a direct mechanism of action or if browning is a secondary effect of metabolic modulations (Nedergaard & Cannon 2014). Studies have reported that β3-AR activation may be involved in WAT browning since deletion of β3-AR gene in mice leads to impaired WAT browning (Feldmann et al. 2009). Moreover, UCP1 activity seems to be determining for obesity development, since UCP1-deficient mice display no diet-induced adrenergic thermogenesis and become obese even when fed with chow diet (Jimenez et al. 2003).

Of note, obesity also leads to BAT dysfunction with decreased β-adrenergic signaling, accumulation of enlarged lipid droplets and mitochondrial dysfunction. This process of ‘BAT whitening’ has been associated with vascular rarefaction due to decreased expression of vascular endothelial growth factor A (Vegfa) (Shimizu et al. 2014, Shimizu & Walsh 2015).

Adipose tissue as an endocrine organ

Besides functioning as a storage organ, adipose tissue is a highly active endocrine organ and an important metabolic sensor. Apart from adipocytes, adipose tissue is composed of a wide variety of cells, including pre-adipocytes, fibroblasts, endothelial and vascular smooth muscle cells, nerve cells, as well as immune cells, including B and T cells, macrophages and neutrophils (Elgazar-Carmon et al. 2008, Nishimura et al. 2009, Winer et al. 2011, Cawthorn et al. 2012, Talukdar et al. 2012). Adipocytes and immune cells produce a large number of bioactive substances (hormones, cytokines, ROS), collectively termed adipokines. These molecules influence the response of nearby or remote organs/tissues including adipose tissue itself, heart, blood vessels, kidneys, skeletal muscle and liver, through autocrine, paracrine and/or endocrine mechanisms, with resulting effects on energy metabolism, systemic insulin sensitivity, glucose metabolism, immune response and vascular homeostasis (Trayhurn & Beattie 2001, Kershw & Flier 2004, Hauner 2005, Tilg & Moschen 2006, Eringa et al. 2007, Thalman & Meier 2007, Coelho et al. 2013) (Fig. 1).

Chronic over-nutrition triggers an inflammatory response initiated by WAT, leading to systemic and tissue specific low-grade inflammation and insulin resistance (Malnick & Knobler 2006, Choe et al. 2016). In obese patients, adipose tissue mainly releases pro-inflammatory cytokines, among which are tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and ROS, which induce insulin resistance (Lumeng et al. 2007). Significant upregulation of leptin and downregulation of adiponectin gene expression are seen in mesenteric vWAT compared to scWAT and omental vWAT (Yang et al. 2008).

Figure 1
Endocrine function of adipose tissue. Adipose tissue secretes different factors including adipokines, reactive oxygen species and hormones that will affect a plethora of body physiological functions such as insulin signaling and sensitivity, glucose and lipid metabolism, energy expenditure, inflammation and cardiovascular function. In obesity, this function is altered leading to dysregulation of the production of these adipocytes-secreted factors, resulting in adipose tissue dysfunction and adverse effects on other tissues (cardiovascular, liver, muscle). RAS, renin angiotensin system; ROS, reactive oxygen species.
Renin-angiotensin-aldosterone system in adipose tissue

The renin-angiotensin-aldosterone system (RAAS) is one of the most important biological systems implicated in CV and metabolic diseases. In obesity, RAAS over-activation occurs in different insulin-sensitive tissues, particularly in adipose tissue (Engeli et al. 2000, Goossens et al. 2003). For the past 20 years, we have extended our view of MR-mediated aldosterone effects beyond epithelial tissues, like the kidney and the colon, with the discovery that MR is also expressed in other cell types such as in cardiomyocytes (Pearce & Funder 1987, Barnett & Pritchett 1988), in endothelial and vascular smooth muscle cells (Funder et al. 1989, Lombès et al. 1992) and in adipocytes (Rondinone et al. 1993, Nguyen Dinh Cat & Jaisser 2012). However, for these cell types, target genes are not yet fully identified. Interestingly, adipocytes express all components of the RAAS (Thatcher et al. 2009). In particular, recent work has demonstrated that adipocytes secrete aldosterone and possess functional MRs (Briones et al. 2012). In adipocytes, it has been demonstrated that MR regulates adipokines secretion (Guo et al. 2008) and adipogenesis (Caprio et al. 2011) (Fig. 2). A recent in vivo study by Than and collaborators demonstrated that angiotensin II receptor type 2 (AT2R) activation in mice (treatment with compound C21) promotes WAT browning, raises body temperature and reduces WAT mass. In addition, the authors showed in cultured mouse and human white adipocyte induction of WAT browning through ERK1/2 MAP kinase, AKT and adenosine monophosphate kinase (AMPK) signaling pathways as well as increased UCP1 expression and that Ang II-mediated AT2R activation can enhance brown adipogenesis (Than et al. 2017). In comparison, Armani and coworkers reported that MR antagonism induced WAT browning in mice fed with high-fat diet, as evidenced by the increase in brown adipocytes markers gene expression and upregulation of Ucp1 levels. This may involve a direct regulation of adipocyte autophagy processes by MR (Armani et al. 2014). Hence, one can speculate that AT2R activation and MR blockade are part of a signaling pathway leading to WAT browning. In the studies by Than, it would have been interesting to determine whether there was a reduction in the autophagy process in adipocytes from C21-treated mice. A review on the different roles of the adipose angiotensin receptors in the endocrine, metabolic, immune and vasoactive functions of the adipose tissue has been published and thus will not be detailed here (Kalupahana & Moustaid-Moussa 2012).

An important point is that unlike epithelial tissues that express the 11-beta hydroxysteroid dehydrogenase 2 enzyme (11β-HSD2), which inactivates cortisol and corticosterone and thus confers aldosterone selectivity to the MR, adipose tissue is lacking this enzyme (or expressing it at very low levels) and active glucocorticoids are therefore also capable of activating the MR. Since glucocorticoids circulate at 100- to 1000-fold higher concentrations than those of aldosterone, it is very likely that they are the main endogenous ligand of the MR in adipose tissue. Numerous studies have described the role of glucocorticoids in adipose tissue. This has been summarized by Lee et al. (2014).

The existence of functional MR in adipose tissue has brought it out of the shadow of the glucocorticoid receptor (GR), considered for a long time as the solely player mediating corticosteroid action in adipose tissue and has opened a new area of research.

![Diagram of MR activation in adipose tissue](image_url)

**Figure 2** Effects of MR activation in adipose tissue. A few group of researchers have centered their studies in the implication of MR activation in the pathogenesis of obesity. MR activation in adipose tissue stimulates adipogenesis, insulin sensitivity and glucose uptake, as well as can induce oxidative stress and inflammatory responses. MR, mineralocorticoid receptor.
Different studies discussed the interplay between MR and GR in adipogenesis with contradictory results regarding identification of the main actor in this process. A study by Lee and Fried (2014), using RNA-interference knockdown of either GR or MR on primary cultures of human pre-adipocytes and adipocytes, led to the conclusion that GR mediates cortisol-induced adipogenesis (Lee & Fried 2014). However, other studies showed a major role of the MR in adipogenesis in murine adipocytes cell lines, either 3T3-L1 or 3T3-F442A cells or in cell lines derived from adipocytes of GR- and MR-knockout mice (Caprio et al. 2007, Hoppmann et al. 2010). One of the possible explanation would be an intrinsic difference between species, but this issue is addressed as treatment with the MR antagonist drospirenone inhibited adipogenesis in human pre-adipocytes (Caprio et al. 2011). Taken altogether, these studies suggest that GR and MR could be implicated at different stages of differentiation and play a specific role depending on the fat depot or on the health status of the patient (obese vs lean) (Armani et al. 2014). Further studies are necessary to identify the precise roles of each corticosteroid receptors and to better understand the interactions between MR and GR in the regulation of adipogenesis.

**Aldosterone/MR in obesity and CMS**

Aldosterone has been linked with obesity and associated CMS since (a) it has been reported that patients with visceral obesity have higher levels of aldosterone (Rossi et al. 2008, Nagase & Fujita 2009, Calhoun & Sharma 2010, Vaidya et al. 2013); (b) the Framingham Offspring Study in which aldosterone was found to correlate positively with development of CMS and with a longitudinal change of its components (Ingelsson et al. 2007) and (c) it has been shown that excess of aldosterone exerts detrimental effects on glucose metabolism and insulin sensitivity and secretion. Indeed, aldosterone plasma levels have been reported to be associated with insulin resistance, characterized by a reduced ability of insulin to induce glucose uptake in adipose tissue and skeletal muscle in normotensive healthy subjects independent of traditional risk factors (Garg et al. 2010, Luther et al. 2011, Luther 2014).

Moreover, MR expression is increased in adipose tissue from obese patients as well as from obese db/db mice (Hirata et al. 2012, Urbanet et al. 2015). Our group developed a conditional transgenic mouse model of MR overexpression specifically in adipocytes that displayed various components of the CMS: overweight, insulin resistance, hypercholesterolemia and hypertriglyceridemia (Urbanet et al. 2015), as well as vascular dysfunction (Nguyen Dinh Cat et al. 2016).

Interesting studies in obese mice have demonstrated beneficial effects of chronic treatment with MR antagonist on metabolic parameters with improvement in insulin sensitivity, glucose tolerance and adipose tissue inflammation (Guo et al. 2008, Hirata et al. 2009, Wada et al. 2010). However, the precise mechanisms by which MR activation leads to adipose tissue dysfunction and contribute to obesity and its associated CV complications are yet to be determined.

**Adipose tissue oxidative stress in obesity**

**ROS signaling in the adipose tissue**

ROS are oxygen-derived molecules produced in most cells, either as a result of endogenous (mitochondria aerobic metabolism, cytosolic enzymatic reactions) or exogenous (ionizing radiations, pollutants, drugs) sources (Finkel & Holbrook 2000). Among ROS are the superoxide anion (O$_2^-$), the hydrogen peroxide (H$_2$O$_2$), the hydroxyl anion (OH$^-$) and the peroxynitrite anion (OONO$^-$). Superoxide is generated from molecular oxygen by addition of one unpaired electron. In aqueous solution, it behaves either as an oxidant agent, which is reduced to H$_2$O$_2$ spontaneously or by action of the superoxide dismutase (SOD), or as a reducing agent generating ONOO$^-$ by combination with nitric oxide (NO). H$_2$O$_2$, in turn, can be broken down by action of peroxidases or catalases into H$_2$O and O$_2$ or can be reduced to OH$^-$ in the presence of molecules containing Fe$^{2+}$ (Schiffrin & Touyz 2004) (Fig. 3A).

ROS are highly reactive molecules, which makes them essential second messengers, participating in many cell signaling pathways including proliferation, differentiation, immune response and apoptosis (Simon et al. 1998, Griendling et al. 2000). At physiological levels in adipose tissue, ROS participate in metabolic homeostasis as well as preadipocyte proliferation through an insulin-dependent pathway (Castro et al. 2016, Wagner et al. 2017) and in adipocyte differentiation through H$_2$O$_2$-induced cAMP response element-binding protein beta (C/EBP-beta) DNA-binding activity, along with C/EBP-beta phosphorylation (Lee et al. 2009, Tormos et al. 2011).

Mitochondria are major source of ROS. Complexes I and III of the mitochondrial electron transport chain...
Consequences of ROS imbalance in adipose tissue

Oxidative stress is defined as an imbalance between ROS production and antioxidant capacity of defense systems. WAT is not regarded a mitochondria-rich tissue. Nonetheless, mitochondria and adequate mitochondrial function are essential for ATP synthesis, in order to support the energy requirements of adipocytes for processes like lipid metabolism (tricarboxylic acid cycle, β-oxidation) as well as for adipocyte differentiation and maturation. The relationship between obesity and ROS has been well established in the literature, with the presence of both systemic and organ-specific oxidative stress in adipose tissue, muscle and pancreas being recorded in obese patients (Furukawa et al. 2004). Consequently, long exposure to oxidative stress leads to DNA oxidative damage, triggering mitochondrial dysfunction that ultimately results in a vicious circle causing lipid accumulation and insulin resistance (Bournat & Brown 2010). Imbalanced ROS levels lead to adipocyte dysfunction with impaired adipogenesis and insulin sensitivity, as well as adipocyte hypertrophy, which can lead to dysregulation of adipokines secretion (decreased adiponectin and increased leptin expression and secretion) and adipose tissue inflammation (Lee et al. 2009, Tormos et al. 2011, Yu et al. 2011, Wang et al. 2013, den Hartigh et al. 2017, Wagner et al. 2017) (Fig. 4). Obesity is associated with mitochondrial dysfunction and excessive production of mitochondrial ROS, leading to alteration of cellular components and to premature aging (Harman 1956, Halliwell 2006, Rong et al. 2007, Bjørndal et al. 2011, Kusminski & Scherer 2012, Chattopadhyay et al. 2015, Castro et al. 2016).

Remote effects of ROS in obesity

In obesity, ROS accumulation in adipose tissue has harmful effects on the vasculature causing vascular dysfunction (endothelial dysfunction, vascular hypercontractility, stiffness) and chronic inflammation (Fernández-Sánchez et al. 2011, Manna & Jain 2015). Indeed, perivascular adipose tissue from obese mice (New Zealand obese, high-fat diet and db/db models) promotes endothelial dysfunction, vascular hypercontractility, as well as vascular insulin resistance and inflammation through redox-sensitive dependent mechanisms and pro-inflammatory cytokines induced by adipose-derived ROS (Marchesi et al. 2009, Ketonen et al. 2010, Fernández-Alfonso et al. 2013, Gil-Ortega et al. 2014, Nguyen Dinh Cat et al. 2018).

The renin-angiotensin-aldosterone system and oxidative stress in obesity

MR activation and ROS production in obesity

Among the enzymatic complexes that produce ROS, the family of NADPH oxidases (NOX) is one of the major sources of ROS and counts seven members: NOX1–5 and DUOX1 and 2, which differ in their tissue expression profile as well as in their mode of activation. All of them use NADPH as electron donor to produce O₂⁻, except NOX4 (Dikalov et al. 2008) and DUOXes, which produce H₂O₂ (Bedard & Krause 2007).

The subunit p22phox is required for the formation of a functionally active NOX (except for NOX5). A study showed implication of the MR in the regulation of the...
expression of p22phox in genetically and diet-induced obese mice. NOX subunit p22phox expression was significantly increased in the adipose tissue of obese mice compared to lean control, and treatment with the MR antagonist eplerenone normalized the expression (Hirata et al. 2009).

Oxidative stress does not necessarily result from overproduction of ROS and can be the consequence of a disruption in antioxidant defenses (Fig. 3B). Such antioxidant defenses include the SOD, which converts $O_2^-$ into $H_2O_2$, as well as catalase, thioredoxin reductase, glutathione peroxidases and peroxiredoxins, which are scavenging $H_2O_2$ into $H_2O$. There are three different SODs with specific cellular locations and cofactors: mitochondrial SOD (Mn-SOD or SOD2), cytosolic SOD (Cu/Zn-SOD or SOD1) and extracellular SOD (EC-SOD or SOD3) (Ursini et al. 1995, Faraci & Didion 2004, Johnson & Giulivi 2005, Wassmann et al. 2006). In obese ob/ob and db/db mice, expression of catalase, and Sod1 were reduced compared with lean control mice and administration of eplerenone corrected this decrease (Hirata et al. 2009).

The Nrf2 transcription factor (or NFE2L2) regulates genes involved in antioxidant defenses, including hemoxygenase 1 (HO1), SOD3, glutathione peroxidase (GPX), thioredoxin and peroxiredoxin (Ma 2013). In the kidney, aldosterone has been shown to activate Nrf2 in the context of hypertension-induced oxidative DNA damage (Queisser et al. 2014). Contradicting results from animal studies show beneficial effects either of Nrf2 agonists administration in WT mice under high-fat conditions or of Nrf2 knockout on metabolism and glucose homeostasis (Shin et al. 2009, Pi et al. 2010, Zhang et al. 2012, Choi et al. 2014, Schneider et al. 2016). Interestingly, mice with cell-specific deletion of Nrf2, in adipocytes, hepatocytes (Chartoumpekis et al. 2018) or in myeloid cells (Collins et al. 2012), submitted to a high-fat diet, do not recapitulate the protection against obesity obtained in the global deletion model. This suggests that the Nrf2 pathway may represent a promising target for treatment of obesity and T2D without clear identification of the exact molecular mechanisms.

The renin-angiotensin-aldosterone system and regulators of mitochondrial function

Strong evidence suggests that oxidative stress, in particular mitochondrial ROS, may mediate the effects of RAAS in obesity in multiple organs, contributing to mitochondrial dysfunction and insulin resistance (Ramalingam et al. 2017). In this review, we summarize the potential regulators of mitochondrial function and studies that indicate a putative interaction between RAAS activation and these regulators, although few data are available on adipose tissue (Fig. 5).

Biogenesis, dynamics, mitophagy

Mitochondrial biogenesis, mitophagy and dynamics are essential processes for quality control of mitochondria. In obese and diabetic patients, adipose tissue displays increased autophagy (Kosacka et al. 2015) and several studies have shown that inhibition of autophagy-related pathways (TGF-β, Notch or Atg7) promotes beige adipocyte development and protects mice from diet-induced obesity and insulin resistance (Altschuler-Keylin & Kajimura 2017, Taylor & Gottlieb 2017). In skeletal muscle from Ang II-infused mice, mitochondrial integrity is impaired with reduced mitochondrial content, decreased autophagy, disorganized cristae structure and altered
Mitochondria are highly dynamic organelles organized in a complex network. Fission is the division of the mitochondrial tubular network into fragmented and isolated mitochondria, a process strongly connected to increased oxidative stress and mitophagy (removal of mitochondria). Fusion of mitochondria results in an enhanced tubular network and is associated with a reduction of oxidative stress. Therefore, alterations in the systems regulating mitochondrial dynamics are also major regulators of mitochondrial function, impacting mtDNA copy number. In the kidney from aldosterone-infused mice, mtDNA copy number is diminished and associated with mitochondrial dysfunction (Zhu et al. 2011). Similar results were obtained in aldosterone- and-salt-treated rats as well as in aldosterone-treated human cardiac fibroblasts (Ibarrola et al. 2018).

Sirtuins, AMPK, oxidative stress and MR

AMPK and sirtuins are major regulators of mitochondrial function and can thus influence mitochondrial oxidative stress (Ruderman et al. 2010). In vitro studies reported that inhibition of AMPK activity in 3T3-L1 adipocytes led to increase in both ROS production and lipolysis (Gauthier et al. 2008). Besides, acute oxidative stress induces AMPK activation in HEK-293 cells (Auciello et al. 2014). These elements suggest that reduced AMPK activity and increased oxidative stress could be causal factors for each other (Xu et al. 2012).

Similarly, sirtuin expression can be influenced by oxidative stress: indeed, severe or chronic oxidative stress induces sirtuin degradation by the proteasome while mild oxidative stress causes a compensatory overexpression (Santos et al. 2016). Global knockout of the Sirt1 gene in adult mice prevents resveratrol-induced AMPK activation, indicating that SIRT1 induces AMPK activation (Price et al. 2012).

AMPK pathway

The AMP/ATP cellular ratio is a gauge of the cell energy status. When this ratio increases, AMPK is activated, switching on glucose transport and catabolic pathways as lipolysis to generate ATP and inhibiting fatty acid synthesis and oxidation (Hardie 2003). AMPK has a protective role against obesity and adipose tissue unhealthy expansion. Indeed, high-fat diet-fed AMPKka2-knockout mice exhibit increased fat mass, resulting from adipocyte hypertrophy rather than hyperplasia (Villena et al. 2004). In obese insulin-resistant patients, AMPK activity in adipose tissue is decreased (Xu et al. 2012).

Crosstalk between AMPK and RAAS has been demonstrated in kidney and in cardiomyocytes (Stuck et al. 2008, Hernández et al. 2014, Yang et al. 2016). One can speculate that these interactions might also occur in adipose tissue. Recently, it has been shown that AMPK activation by metformin treatment suppresses aldosterone-induced fibrosis in heart (Mummidi et al. 2016) and adipose tissue remodeling, as well as insulin resistance in obesity (Luo et al. 2016). An interesting study recently provided in vitro and in vivo evidence that Ang II dynamics (Mitsuishi et al. 2009, Silva et al. 2017). Thus, Ang II via stimulation of AT1R can cause mitochondrial morphological and functional abnormalities. This has been suggested to underlie the onset of skeletal muscle insulin resistance (Rogge 2009) as well as in other tissues including fat, liver, heart and vessels (Nisoli et al. 2007, Kim et al. 2008). In other cells, such as cardiomyocytes and vascular smooth muscle cells, mitochondrial ROS participate in the pathways triggered by RAAS activation via mitochondrial ATP-dependent potassium channels opening (Kimura et al. 2005, de Cavanagh et al. 2007, De Giusti et al. 2013). Moreover, RAAS inhibitors protect mitochondria against oxidative stress-induced damage (de Cavanagh et al. 2004).

Mitochondria are highly dynamic organelles organized in a complex network. Fission is the division of the mitochondrial tubular network into fragmented and isolated mitochondria, a process strongly connected to increased oxidative stress and mitophagy (removal of mitochondria). Fusion of mitochondria results in an enhanced tubular network and is associated with a reduction of oxidative stress. Therefore, alterations in the systems regulating mitochondrial dynamics are also major regulators of mitochondrial function, impacting mtDNA copy number. In the kidney from aldosterone-infused mice, mtDNA copy number is diminished and associated with mitochondrial dysfunction (Zhu et al. 2011). Similar results were obtained in aldosterone- and-salt-treated rats as well as in aldosterone-treated human cardiac fibroblasts (Ibarrola et al. 2018).

Sirtuins, AMPK, oxidative stress and MR

AMPK and sirtuins are major regulators of mitochondrial function and can thus influence mitochondrial oxidative stress (Ruderman et al. 2010). In vitro studies reported that inhibition of AMPK activity in 3T3-L1 adipocytes led to increase in both ROS production and lipolysis (Gauthier et al. 2008). Besides, acute oxidative stress induces AMPK activation in HEK-293 cells (Auciello et al. 2014). These elements suggest that reduced AMPK activity and increased oxidative stress could be causal factors for each other (Xu et al. 2012).

Similarly, sirtuin expression can be influenced by oxidative stress: indeed, severe or chronic oxidative stress induces sirtuin degradation by the proteasome while mild oxidative stress causes a compensatory overexpression (Santos et al. 2016). Global knockout of the Sirt1 gene in adult mice prevents resveratrol-induced AMPK activation, indicating that SIRT1 induces AMPK activation (Price et al. 2012).

AMPK pathway

The AMP/ATP cellular ratio is a gauge of the cell energy status. When this ratio increases, AMPK is activated, switching on glucose transport and catabolic pathways as lipolysis to generate ATP and inhibiting fatty acid synthesis and oxidation (Hardie 2003). AMPK has a protective role against obesity and adipose tissue unhealthy expansion. Indeed, high-fat diet-fed AMPKka2-knockout mice exhibit increased fat mass, resulting from adipocyte hypertrophy rather than hyperplasia (Villena et al. 2004). In obese insulin-resistant patients, AMPK activity in adipose tissue is decreased (Xu et al. 2012).

Crosstalk between AMPK and RAAS has been demonstrated in kidney and in cardiomyocytes (Stuck et al. 2008, Hernández et al. 2014, Yang et al. 2016). One can speculate that these interactions might also occur in adipose tissue. Recently, it has been shown that AMPK activation by metformin treatment suppresses aldosterone-induced fibrosis in heart (Mummidi et al. 2016) and adipose tissue remodeling, as well as insulin resistance in obesity (Luo et al. 2016). An interesting study recently provided in vitro and in vivo evidence that Ang II
via AT2R induces browning and brown adipogenesis through, in part, AMPK signaling pathways (Than et al. 2017). Ongoing research investigates the contribution of AMPK in aldosterone secretion by adipocytes and whether AMPK activators drugs would prevent Ang II and aldosterone-induced pro-inflammatory effects in human and mouse adipocytes MR activation (White et al. 2015).

**Sirtuins and RAAS**

Research on caloric restriction has led to the discovery of sirtuins (silent information regulators (SIRT)), redox-sensitive NAD+-dependent deacetylases/deacylases regulating various cellular functions, including glucose and lipid metabolism as well as adipocytes differentiation. There are seven mammalian SIRTs. Their respective roles in energy metabolism have been reviewed by Li & Kazgan (2011). Studies have brought evidence that SIRT1 and SIRT3 can regulate the activity of several mitochondrial enzymes through deacetylation of PGC1-alpha or the forkhead protein FOXO1 or activation of complex I, resulting in control of ATP production (Nogueiras et al. 2012). Recent studies demonstrated beneficial effects of resveratrol (sirtuins activator) on glucose metabolism and insulin sensitivity, closely associated with AMPK activation (Civitarese et al. 2007, Um et al. 2010, Timmers et al. 2011). In rodent studies submitted to high-fat diet, treatment with resveratrol protected the mice against obesity by increasing energy expenditure (Kim et al. 2011, Cho et al. 2012), improving mitochondrial activity of brown adipocytes (Lagouge et al. 2006, Ku & Lee 2016) and inducing browning of WAT (Wang et al. 2015, Arias et al. 2017). RAAS components and sirtuins expression levels in adipose tissue are influenced by diet composition. Indeed a lipid-rich diet induces upregulation of ACE and downregulation of Ace2 and Sirt4 in mice (de Pinho et al. 2013).

Angiotensin-converting enzyme/Ang II/AT1R activation is counterbalanced by ACE2/angiotensin-(1–7)/Mas axis that has been shown to interact with sirtuins in adipose tissue. Indeed, administration of Ang-(1–7) or resveratrol in high-fat diet-induced obese mice improved glucose and lipid metabolic parameters in WAT by modulating Sirt1, Ace1 and Ace2 expressions, respectively (Oliveira Andrade et al. 2014). Other studies, not necessarily in the context of obesity, showed inhibitory effect of resveratrol on AT1R expression through SIRT1 activation in CV tissues (Miyazaki et al. 2008, Kim et al. 2018).

**Clinical perspectives and potential therapeutic applications of selective MR antagonists**

The different options for the management of obesity and its complications include lifestyle changes through progressive increase of physical activity and adapted diets; medical treatments and bariatric surgery in the most severe cases.

Among the different drugs available for obesity complications treatment, biguanides and thiazolidinediones (TZDs) have a mitochondria-targeted mechanism, through inhibition of the ETC complex I. Moreover, it has recently been established that metformin treatment in diabetic mice protected mitochondrial integrity by inhibiting dynamin-related protein 1 (DRP1) activation through an AMPK-dependent mechanism (Li et al. 2016), while TZDs inhibit mitochondrial pyruvate carrier activity (Colca et al. 2014). However, frequent side effects are reported, including kidney and liver complications as well as weight gain. As there seems to be a crosstalk between RAAS and AMPK, MR antagonists could represent an alternative way of raising AMPK levels.

RAAS activation induces oxidative stress and dietary anti-oxidants (zinc, α-lipoic acid, vitamin C and E, lycopene) are a side strategy for management of obesity. With only few studies being available on the exact efficacy of this supplementation, as most studies are observational and there are almost no interventional studies, the causality link is difficult to establish (Abdali et al. 2015). Inhibition of the MR could be a more efficient way of tackling oxidative stress in obesity.

An additional strategy to treat obesity is to increase BAT activity and/or mass to promote energy consumption via non-shivering thermogenesis. In 2015, Liu et al. (2015) showed that transplantation of BAT from control mice in ob/ob mice reduces weight gain, decreases adipose tissue hypertrophy, hepatic steatosis and improves insulin sensitivity and adiponectin plasma levels. Moreover, the activity of the endogenous BAT was increased by the presence of the transplanted BAT. However, transplantation would be difficult in human as the BAT is rather diffused and there are almost no interventional studies, the causality link is difficult to establish (Abdali et al. 2015). Inhibition of the MR could be a more efficient way of tackling oxidative stress in obesity.

An additional strategy to treat obesity is to increase BAT activity and/or mass to promote energy consumption via non-shivering thermogenesis. In 2015, Liu et al. (2015) showed that transplantation of BAT from control mice in ob/ob mice reduces weight gain, decreases adipose tissue hypertrophy, hepatic steatosis and improves insulin sensitivity and adiponectin plasma levels. Moreover, the activity of the endogenous BAT was increased by the presence of the transplanted BAT. However, transplantation would be difficult in human as the BAT is rather diffused and there are almost no interventional studies, the causality link is difficult to establish (Abdali et al. 2015). Inhibition of the MR could be a more efficient way of tackling oxidative stress in obesity.

An additional strategy to treat obesity is to increase BAT activity and/or mass to promote energy consumption via non-shivering thermogenesis. In 2015, Liu et al. (2015) showed that transplantation of BAT from control mice in ob/ob mice reduces weight gain, decreases adipose tissue hypertrophy, hepatic steatosis and improves insulin sensitivity and adiponectin plasma levels. Moreover, the activity of the endogenous BAT was increased by the presence of the transplanted BAT. However, transplantation would be difficult in human as the BAT is rather diffused and would make collection from a donor in a sufficient quantity quite challenging. Luckily, alternative ways of activating the endogenous BAT are available. Among drugs capable of inducing such browning is CL 316243, an agonist of the β3 adrenergic receptor, which not only promotes BAT activity but also protects against WAT...
hyperplasia in the early stages of obesity (Wankhade et al. 2016). The TZD-derived partial PPARγ agonist GQ-16 decreased weight gain in spite of higher energy intake, reduced fat mass and liver TG content and increased morphological and molecular markers of BAT activation in male high-fat diet-fed Swiss mice (Coelho et al. 2016). Another drug, the anti-cancer drug Gleevec improved insulin sensitivity, decreased inflammation in adipose tissues and promoted browning of WAT via PPARγ phosphorylation inhibition at Ser273 (Choi et al. 2016). However, CL 316243 and GQ-16 are not commercialized as drugs and Gleevec causes serious side effects and impacts quality of life. MR antagonists have the advantage of being already used in clinic and with known and manageable side effects.

Conclusion

Far from being a simple reservoir for nutrient storage, adipose tissue is now recognized as one of the central players in the integration and control of metabolic homeostasis and energy balance. Among other regulatory factors, adipose tissue is a target for steroid hormones, since adipocytes express GR, MR, but also androgen and estrogen receptors. For a long time, no one was suspecting a possible role of MR activation in adipose tissue. New and unexpected roles for adipose MRs have been revealed by recent studies demonstrating its direct implication in the regulation of adipocyte differentiation and expansion, pro-inflammatory capacity, insulinsignaling and adipokines secretion. In addition, numerous research studies have confirmed that, in obesity, MR is over-activated and contributes to low-grade inflammation, insulin resistance and CV injury. Moreover, this review has summarized evidence that obesity is often associated with oxidative stress and mitochondrial dysfunction. Thus, further studies are needed to understand the exact mechanisms by which MR over-activation in obesity induces adipose tissue dysfunction. Figure 6 is representing the hypothetical crosstalk between MR activation, oxidative stress and mitochondrial function. Indeed, the close link between the mechanisms controlling oxidative stress and mitochondrial function, allowing to preserve adipose tissue homeostasis, have placed the mineralocorticoid system as an attractive and central candidate in the development of obesity and its associated metabolic complications.

Even if studies in animal models of obesity showed a marked improvement of overall adipose tissue function after treatment with MR blockers, to date, no clinical trials in humans has addressed the impact of MR antagonism on body fat mass and metabolic function as a primary end-point. Nevertheless, indirect evidence have emerged from clinical studies of drospirenone, a potent synthetic anti-mineralocorticoid with progestogenic and moderate anti-androgenic properties, combined with estrogens for contraception and hormone replacement therapy (Palacios et al. 2006). Of particular interest, drospirenone had positive impact on CMS components such as blood pressure and fat mass gain (Tankó & Christiansen 2005, White et al. 2005, 2006, Preston et al. 2007). Based on these observations, Caprio and collaborators demonstrated a potent MR-dependent anti-adipogenic effect of drospirenone on human primary pre-adipocytes from different fat depots, providing some mechanistic insights for the observed favorable effects of drospirenone on human primary pre-adipocytes from different fat depots, providing some mechanistic insights for the observed favorable effects of drospirenone on human primary pre-adipocytes from different fat depots, providing some mechanistic insights for the observed favorable effects of drospirenone on body weight and metabolism (Caprio et al. 2011). Clinical studies using MR antagonists alone or in combination with actual treatments in selected cohorts of obese patients should allow validation of beneficial effects of MR blockade on metabolic and CV parameters.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.
Funding
This work was made possible with funding from CARMA Avenir investment program (ANR-15-RHUS-0003), the Fondation de France (2014-00047968), and the European COST-ADMIRE 1301 network. M P is supported by funding from the Wenner-Gren Foundations, the Magnus Bergvall Foundation and the Åke Wiberg Foundation.

Acknowledgements
The authors would like to thank Dr Maria Dulak-Lis and Dr Sofia Tsiroupolou for critical appraisal and editing of the manuscript.

References
Altschuler-Keylin S & Kajimura S 2017 Mitochondrial homeostasis in adipose tissue remodeling, Science Signaling 10 eaar9248. (https://doi.org/10.1126/scisignal.aar9248)
Bourrat JC & Brown CW 2010 Mitochondrial dysfunction in obesity, Current Opinion in Endocrinology, Diabetes, and Obesity 17 446–452. (https://doi.org/10.1097/MED.0b013e3283c3026)
Bourrat JC & Brown CW 2010 Mitochondrial dysfunction in obesity, Current Opinion in Endocrinology, Diabetes, and Obesity 17 446–452. (https://doi.org/10.1097/MED.0b013e3283c3026)


Goodfellow TL, Kelley DE, Goodpaster BH & Winters SJ 1999 Visceral obesity and insulin resistance are associated with plasma aldosterone levels in women. *Obesity Research* 7 355–362. (https://doi.org/10.1038/35041687)


species and their role in vascular physiology and pathophysiology. Arteriosclerosis, Thrombosis, and Vascular Biology 20 2175–2183. (https://doi.org/10.1161/01.ATV.20.10.2175)


Ku CR & Lee EJ 2016 Response: the effects of high fat diet and resveratrol on mitochondrial activity of brown adipocytes (Endocrinol Metab.2015.03.015)


Lee J-M & Fried SK 2014 The glucocorticoid receptor, not the mineralocorticoid receptor, plays the dominant role in adipogenesis and adipokine production in human adipocytes. International Journal of Obesity 38 1228–1235. (https://doi.org/10.1038/ijo.2014.6)


Kr ¨Ur & Lee EJ 2016 Response: the effects of high fat diet and resveratrol on mitochondrial activity of brown adipocytes (Endocrinol Metab.2016.11.3.482)


Lee M-J & Fried SK 2014 The glucocorticoid receptor, not the mineralocorticoid receptor, plays the dominant role in adipogenesis and adipokine production in human adipocytes. International Journal of Obesity 38 1228–1235. (https://doi.org/10.1038/ijo.2014.6)


tissue of diabetic mice. Molecular and Cellular Endocrinology 434 36–47. (https://doi.org/10.1016/j.mce.2016.06.008)


Marchesi C, Ebrahimiyan T, Angulo O, Paradis P & Schiffrin EL 2009 Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. Hypertension 54 1384–1392. (https://doi.org/10.1161/HYPERTENSIONAHA.109.138305)


Miwa S & Brand MD 2003 Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. Biochemical Society Transactions 31 1300–1301. (https://doi.org/10.1042/)


Palacios S, Foldair JM & Genazzani AR 2006 Advances in hormone replacement therapy with drospirenone, a unique progestogen with


Than A, Xu S, Li R, Leow M-S, Sun I & Chen P 2017 Angiotensin type 2 receptor activation promotes browning of white adipose tissue and...

Wajchenberg BL. 2000 Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocrine Reviews 21 697–738. (https://doi.org/10.1210/edrv.21.6.0415)


Received in final form 16 May 2018
Accepted 6 June 2018
Accepted Preprint published online 6 June 2018