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Redox homeostasis and nitro-oxidative stress in obesity-linked inflammation.

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Abstract

It is now well accepted that most chronic diseases have a common feature which is “low-grade” inflammation. Whether inflammation is causal or rather consequent to these diseases is still a matter of debate. A key factor of inflammation is considered to be “oxidative stress”, which is the result of an alteration of redox homeostasis which is critical for the regulation of physiological cell and organ metabolism and proliferation. The term “oxidative stress” is however often used in an inappropriate manner as the primary target of the initial oxidative radical, superoxide ion, is nitric oxide which, being in large excess, acts as a “buffer”, yielding reactive nitrogen species. It is only once the superoxide fluxes exceed the nitric oxide fluxes that true “oxidative stress” occurs. Nitro-oxidative stress is a more appropriate term which takes into account the evolving generation of reactive nitrogen and oxygen species and their effects on cell and organ

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pathophysiology. The molecular bases of redox homeostasis and nitro-oxidative stress will be presented and discussed using obesity-linked inflammation as a pathophysiological example.

I. Redox homeostasis and nitro-oxidative stress

Introduction and major molecular players

Severe oxidative stress is fortunately a rather extreme situation observed mainly under pathological situations and the reductive mechanisms present in the cell are most often able to prevent or revert the oxidized molecules to their native reduced state [1], with the notable exception of carbonylated proteins [2], certain lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal [3] and DNA [4]. These mechanisms are referred to as “redox homeostasis or regulation” and determine the activity of a series of key enzymes involved in cell metabolism, differentiation and proliferation. A major actor of redox regulation which is often neglected is $NO^{\cdot}$ [5,6]. This low reactivity free radical is the preferential target of the primary oxidative species superoxide ion, $O_2^{\cdot -}$, which oxidizes it ten times more rapidly than any cellular macromolecule [7,8] (Fig. 1). It therefor acts as a buffer for $O_2^{\cdot -}$, especially as under “basal” physiological conditions its concentration exceeds that of superoxide (10^{-12} – 10^{-11}M) by at least two orders of magnitude [9]. This ratio is subject to change, depending on the activation of superoxide generating enzymes including NADPH oxidases (NOX 1-5 and DUOX 1 and 2), xanthine oxidase (XO), mitochondrial respiratory chain complexes and uncoupled endothelial $NO^{\cdot}$ synthase (NOS 3) and reducing systems including thioredoxins, glutaredoxins, peroxiredoxins, catalase, glutathione peroxidase (GR) and the thioredoxin antioxidant (Trx) system [5,10] (Fig. 1).

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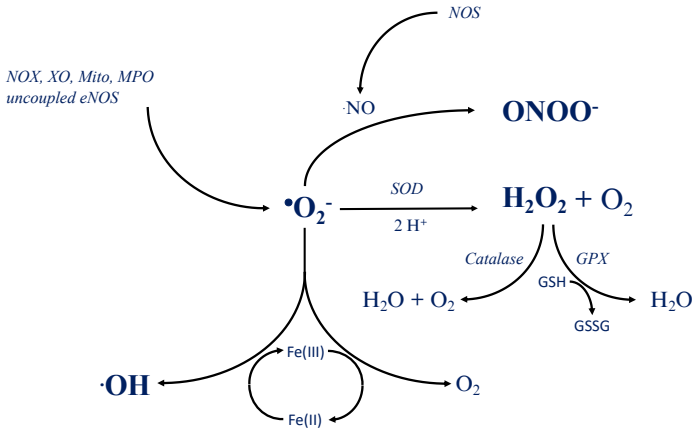

Fig. 1: Schematic representation of the major mechanisms and reactions involved in redox signaling with emphasis on the generation and degradation of reactive oxygen species (ROS)

At low $NO^{\cdot}$ (i.e. ~ 10^{-9} M), and $O_2^{\cdot-}$ (i.e. 10^{-11} - 10^{-10} M) concentrations where $[NO^{\cdot}] >> [O_2^{\cdot-}]$, the latter will essentially regulate $NO^{\cdot}$ bioavailability for the activation of soluble guanylate cyclase (sGC) which produces cyclic GMP (cGMP), responsible for vasodilation. Activation of sGC occurs through reversible binding of $NO^{\cdot}$ with sub-nanomolar affinity, and is called nitrosylation. $O_2^{\cdot-}$ can also downregulate the generation of $NO^{\cdot}$ by endothelial NOS (eNOS) by uncoupling it from tetrahydrobiopterin (BH_4). The major oxidation product of $NO^{\cdot}$ when $O_2^{\cdot-}$ production increases to yield an $[NO^{\cdot}] / [O_2^{\cdot-}] = 2 – 3$ [11] , is the nitrosonium ion NO^+. This unstable ion can S-nitrosate specific Cys residues (R-Cys-SH) of glutathione (GSH) and several proteins to R-Cys-SNO [12,13] (Fig. 2).

The exact mechanism of S-nitrosation is unknown, but may involve the formation of N_2O_3 as the concentration of NO^+ under “cellular” conditions is elusive due to its extremely short half-life. Another hypothesis is that S-nitroso-glutathione (GSNO) may serve as a source for protein S-nitrosation [14] (Fig. 2). This is directly involved in the cell’s redox equilibrium, regulating thioredoxin activity through modification of its Cys^{69} residue [7]. Indeed, thioredoxin together with thioredoxin reductase and glutaredoxin together with glutathione are major reductive systems of the cell, depending on NADPH. Conversely, thioredoxin is inactivated by oxidation of its Cys^{32} and Cys^{35} residues, leading to apoptosis [7]. This emphasizes the importance of the $NO^{\cdot} / O_2^{\cdot-}$ ratio at low fluxes of these species for maintaining cell metabolism

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and life. The half-life of superoxide in aqueous solution at pH 7 is around 5" [15], but much shorter in the cell due to the presence of SOD, and that of NO· ranges between 0.01" and 2" in cells and around 2 x 10^{-3}" in blood [5]. Considering the slow diffusion rate in cytoplasm, it is clear that in order to react, both radicals need to be produced at the same subcellular location.

Thus, S-nitrosation is a very unstable posttranslational modification which is readily reduced under physiological conditions. However, it may regulate the activity of a variety of enzymes, e.g. caspases, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), aldose reductase and transcription factors (e.g. NF-κB), among others [13,16]. Due to its instability, it is very difficult to assess S-nitrosation as the cell's redox status is almost impossible to maintain during sample extraction procedures [17].

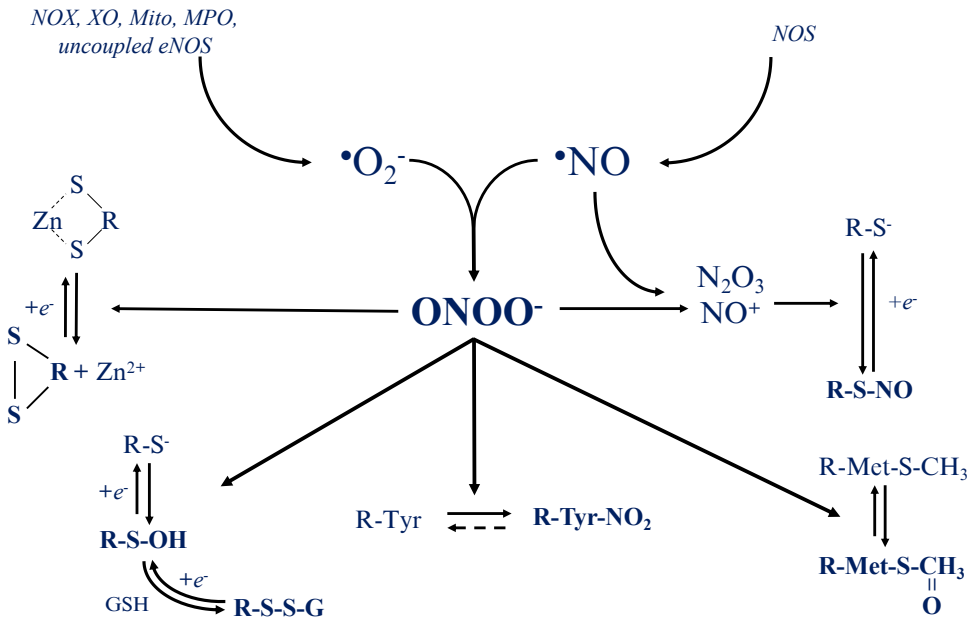

Fig. 2: Schematic representation of the major mechanisms and reactions involved in redox signaling with emphasis on the generation and effects of reactive nitrogen species (RNS)

Peroxynitrite and macromolecular targets

When $O_2^{\cdot-}$ fluxes increase further and its levels become equimolar with or higher than those of NO·, the two radicals react to form peroxynitrite ($ONOO^-$) [5] (Fig. 2). This ion has a half-life

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of less than 1" in the cell and in the extracellular milieu, and its biological effects are thought to occur at concentrations between 10 nM and 5 µM [13]. However, whereas $ONOO^-$ concentrations may reach micromolar concentrations during inflammatory events, the levels at which it exerts its physiological effects is probably rather in the low nanomolar range [18] . The local subcellular or extracellular concentrations of peroxynitrite entirely depend on the expression level and localization of the superoxide- and $NO^{\cdot}$ generating enzymes. Its biological effects are concentration dependent. At concentrations < 500 nM, peroxynitrite specifically nitrates defined tyrosine (Tyr) residues of selected proteins (Fig. 2). Although peroxynitrite has also been reported to nitrate tryptophane (Trp) residues, this seems to be a rare event and its physiological relevance, especially in eukaryotes, is questionable [19].

The list of Tyr-nitrated proteins has been steadily growing over the past 10 years reaching close to 100 [20], which remains however far below that of phosphorylated proteins. Nitroproteins have been reported in virtually all cellular compartments and in the extracellular milieu [21]. Of note, whereas nitration was initially thought to be an irreversible posttranslational modification, it has now been reported to be reversible, with kinetics corresponding to those of enzymatic reversion of other protein modifications such as e.g. phosphorylation [22-24].

At higher concentrations (low micromolar), $ONOO^-$ will also oxidize methionine (Met) residues, resulting in their sulfoxidation [25] and sulfenylate certain cysteine thiols (Cys-SH) [8,26]. These can react with GSH to cause protein glutathiolation as e.g. for eNOS [27]. In zinc fingers, which allow binding of transcription factors to DNA, oxidation of adjacent Cys residues causes the formation of disulfide bridges, releasing Zn-atoms and disrupting their structure, blocking transcription. Peroxynitrite can also oxidize and inactivate protease inhibitors as well as Ca^{++} pumps, causing major cell dysfunction. It will also peroxidize lipids, break DNA strands and nitrate mitochondrial respiratory chain complexes, which are unaffected at lower concentrations [5,8,13] (Fig. 2). Even under these conditions, nitration of mitochondrial proteins appears to be reversible [28]. However, such effects can be very deleterious to the cell, leading to apoptosis.

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nNOS, eNOS, iNOS and cellular location

In addition to the proximity and simultaneous activation of $NO^{\cdot}$ and $O_2^{\cdot -}$ generating systems, substrate availability also is an important factor. Whereas O_2, being the substrate for $O_2^{\cdot -}$ production is generally available at sufficient concentrations (except in mitochondria during metabolic or respiratory stress), this is not always the case for arginine, the only substrate of NOS to generate $NO^{\cdot}$. An example of this is what occurs during macrophage activation. When iNOS (NOS2) is expressed at very high levels and turned on, the arginine pool is rapidly depleted. Under such circumstances, iNOS will change its catalytic activity to oxidation, generating $O_2^{\cdot -}$ instead of $NO^{\cdot}$. In addition to arginine, NOS also requires NADPH, FAD, Zn^{++} and molecular oxygen.

The third parameter is the presence and activity of the reductive systems: mainly the Trx system which includes thioredoxin, thioredoxin reductase and NADPH, the Grx system which comprises glutaredoxin, glutathione reductase, reduced glutathione (GSH) and NADPH, as well as the superoxide dismutases SOD-and 2 together with catalase and glutathione peroxidase (GPX). As indicated, these reductive systems also need common cofactors, essentially NADPH and GSH resulting in a cross-talk between them[2]. Finally, the subcellular location of the generation systems also plays a crucial role. The NOXs are transmembrane proteins located essentially in caveolae, XO is cytosolic and respiratory chain complexes are mitochondrial. Even more complex, NOSs show translocation depending on (patho)physiological situations and their S-nitrosation or oxidation state. It is thus impossible to draw general conclusions regarding the effects of $ONOO^{-}$ on cell functions and biological responses without taking these factors into account.

The three NOS isoforms nNOS (neuronal NOS, NOS1), iNOS (NOS2) and eNOS (NOS3) are active under a homodimeric form and use L-arginine as a substrate and O_2 and NADPH as co-substrates. They all use FAD, FMN and BH_4 as cofactors. When activated, they produce $NO^{\cdot}$, citrulline and limited amounts of $O_2^{\cdot -}$. All three are activated by calmodulin, but whereas binding of this molecule to nNOS and eNOS requires increased cytosolic Ca^{++} concentrations (via intracellular mobilization), it is tightly bound to iNOS even at low Ca^{++} concentrations [29].

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Thus, as opposed to eNOS/nNOS, which are activated by various extracellular mediators, iNOS is constitutively active and its effects seem to be regulated primarily through its expression levels [29].

nNOS essentially generates $NO^{\cdot}$ in the CNS, acting as a “long-term” mediating neurotransmitter. It is also secreted by nitrergic nerves, which innervate smooth muscle, eliciting the generation of cGMP. This mechanism seems essential for regulating vascular tone and thus blood pressure. It is also expressed in the myocardium, where it is mainly localized in the sarcoplasmic reticulum (SR), regulating ryanodine receptor 2 Ca^{++} release channel and phospholamban phosphorylation, important for Ca-influx into the SR. In skeletal muscle, nNOS plays a major role in muscle mass regulation. A splice-variant, nNOSα has been reported to translocate to the nucleus, inducing mitochondriogenesis through $NO^{\cdot}$ mediated activation of the PGC1□ pathway [30]. Another variant, nNOSβ colocalizes with soluble guanylate cyclase (sGC) in the cis-golgi to produce cGMP [31]. A third variant, nNOSμ is localized in the dystrophin glycoprotein complex (DGC), involved in vasodilation and activation of the Akt/PKB pathway through $ONOO^{-}$ [32]. In addition, nNOS can be regulated by mitochondrial ROS [33] and by S-nitrosation in skeletal muscle [34]. These are examples of how the subcellular localization of NOS can determine firstly the generation of $NO^{\cdot}$, secondly the targets and effects of $NO^{\cdot}$ and thirdly, its potential oxidation to nitrosonium and peroxynitrite.

The regulation of eNOS is comparable to that of nNOS in that its activation requires Ca^{++} concentrations of at least 100 nM for calmodulin binding. There are other mechanisms which can modulate its activity, among which the phosphorylation by PKB/Akt and its subcellular translocation [9]. This translocation depends on redox regulation [35]. Superoxide ions can cause glutathiolation of eNOS, inhibiting it through “uncoupling” of the BH_4 cofactor [36]. In this configuration, eNOS generates $O_2^{\cdot-}$ instead of $NO^{\cdot}$. In addition, eNOS has been reported to be subject to S-nitrosation, which inhibits its dimerization required for its enzymatic activity [37]. Regarding its subcellular localization, under basal conditions eNOS appears to be mainly located in caveolae bound to caveolin-1, functioning as an inhibitor [38]. By contrast, caveolin-1

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stimulates eNOS expression and eNOS-produced $NO^{\cdot}$ stimulates endocytosis [31]. Interestingly, eNOS induces S-nitrosation of caveolin-1 which depolymerizes its oligomers [39]. These regulatory mechanisms are remarkable and extremely interesting, as they have been observed in diabetic patients' skeletal muscle vessels [28]. Finally, eNOS appears to also be regulated by Tyr-nitration. When nitrated, calmodulin binds to eNOS in a Ca^{++}-independent fashion. Subsequent activation of eNOS then depends on the nitration site of calmodulin. Whereas nitration of Tyr^{99} inhibits it, Tyr^{138} nitration results in increased $NO^{\cdot}$ production [40].

Whereas n- and eNOS activities are essential for maintaining physiological metabolism at the cellular and at the systemic levels, iNOS is the major actor in inflammation, especially during acute inflammation [41]. This does not mean that the first two isoforms are not involved in inflammation as their activity and location are directly modulated by ROS up to the point that eNOS can switch to an $O_2^{\cdot-}$ generating enzyme when uncoupled. This uncoupling is a typical consequence of low-grade chronic inflammation, observed in hypertension, dyslipidemia, metabolic syndrome (MetS), prediabetes (PD) and type 2 diabetes (T2D), resulting in endothelial damage and vascular remodeling [42,43]. Similar examples exist for nNOS [44,45].

iNOS, being constitutively active, differs in that its biological effects are primarily regulated by its degree of expression, which is modulated by among others by inflammatory mediators and cytokines. Once its expression is induced it can generate $NO^{\cdot}$ for several days at micromolar fluxes [46]. Besides its regulation at the transcriptional and translational levels, its activity is also tightly controlled both by proteolytic degradation and, as stated earlier, by arginine bioavailability [47]. iNOS is found in a variety of cell types, but its major expression site is leucocytes. In these cells, cytokines and bacterial lipopolysaccharides (LPS) mimicking infection, can induce a strong expression. Other factors such as protein-protein interactions, e.g. with p53 and thrombospondin, also regulate iNOS activity [48]. An interesting role of iNOS is the dominant $NO^{\cdot}$ concentration gradient it induces in tissues. Whereas local $NO^{\cdot}$ levels adjacent to cytokine-activated macrophages are micromolar and thus cytotoxic, a few cell layers further its concentration is much lower, exerting anti-apoptotic and proliferative effects, protecting tissue and stimulate healing following damage by invading pathogens [48].

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In summary, generation of micromolar fluxes of $NO^{\cdot}$ and formation of RNS/ ROS cannot be considered as deleterious or detrimental per se. Since most posttranslational modifications caused by RNS are less toxic than those due to ROS and are reversible, $NO^{\cdot}$ can be considered as a buffer for $O_2^{\cdot -}$ and thus as a complement to the reductive systems, allowing restoration of the redox equilibrium and normal cellular homeostasis. Cytotoxic effects will only occur when RNS concentrations exceed the cells' reducing capacity, causing lipid peroxidation and DNA strand breaks, leading to apoptosis. This happens when the $O_2^{\cdot -}$ concentration exceeds that of $NO^{\cdot}$, producing highly reactive oxidants ($OH^{\cdot}$ and $NO_2^{\cdot}$ radicals) which can cause irreversible protein carbonylation and DNA strand breaks [5,8,13] (Fig. 2). Oxidative stress is a major risk factor for non-communicable diseases, including cardiometabolic, neurodegenerative, osteoarticular, kidney and oncologic pathologies, which are leading causes of disability and early death [49-53]. High oxidative stress levels have been associated with low-grade chronic inflammation. In fact and more precisely, oxidative stress and inflammation have been shown to exacerbate one another [54] in MUO obesity which is a well-known chronic pro-inflammatory pathology.

II. Obesity and inflammation

Obesity is associated with severe comorbidities, including metabolic and cardiovascular diseases such as type 2 diabetes mellitus (T2DM), dyslipidemia, nonalcoholic steatosis (NASH) and hypertension [50,56]. This is a major concern since the prevalence of obesity is increasing worldwide. However, the heterogeneity of phenotypes with regard to metabolic features raises questions. Two major subphenotypes can be distinguished: the metabolically healthy obesity (MHO) and the metabolically unhealthy obesity (MUO) [55,56]. MUO refers to excess weight associated with at least one cardiometabolic abnormality, including inflammation, oxidative stress, hyperglycaemia, insulin resistance, dyslipidaemia and/or hypertension, as well as an increased risk of developing cardiometabolic comorbidities [56-59]. MUO is the most frequent phenotype, and represents approximately 70% of all obesities [58]. Conversely, metabolically

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healthy obesity (MHO), is characterised by the absence of the aforementioned cardiometabolic diseases [58,60,61]. Nevertheless, there is no accepted standard definition for MHO and MUO which explains the limitation of comparisons between studies. The transition from MHO to MUO has been reported to be linked to the limited capacity of adipose tissue (AT) to expand [62]. Adipose tissue expansion depends on both genetic predisposition and the environment. Familial Partial Lipodystrophy type 2 (FPLD2) could be an adequate model to better understand the consequences of the AT incapacity to expand (5). In familial partial lipodystrophy Dunnigan type 2 (FPLD2), subcutaneous adipose tissue atrophy leads to the inability of affected subjects to regulate lipid overflow [63,64]. Ectopic lipid deposits of free fatty acid (FFA) are responsible for early severe insulin resistance [65]. Observational studies in Dunnigan populations demonstrated a severe burden of diabetes, dyslipidaemia and NASH [65-67] due to AT dysfunction secondary to the impact of the responsible mutation (cellular senescence, mitochondrial dysfunctions, oxidative stress and low-grade inflammation) [68-70]. Systemic low-grade inflammation is considered to be one of the primary markers of adipose tissue (AT) dysfunction in obesity and one of the most important factors of the metabolically unhealthy evolution. In response to calorie overflow, AT modulation is driven by hyperplasia (cell number increase) and/or hypertrophy (cell size increase) of adipocytes. In lean adipose tissue, the adaptative response favors hyperplasia with numerous and relatively smaller adipocytes. However, inability of storage to expand through hyperplasia leads to pathogenic hypertrophic remodelling which promotes inflammation [71-72] (Figure 3). Many processes are incriminated in the development of inflammation in AT and are described below. Poor vascular perfusion with regard to adipocyte expansion promotes hypoxia with the release of the transcription factor hypoxia-inducible factor 1 (HIF-1), which regulates the expression of several genes involved in inflammation [73]. Dysregulation of adipogenesis and of FFA homeostatis in hypertrophic AT activate the inflammatory NF-kB and JNK pathways [74,75]. Furthermore, pro-inflammatory cytokines and chemokines expression by preadipocytes and lipolytic environment trigger the infiltration of AT by immune cells which scavenge the necrotic adipocytes [73-77]. MCP-1/CCR2 pathways contribute to exacerbate inflammation by macrophages and T

lymphocytes recruitment [76,77] and MIP-2α is responsible for the recruitment of neutrophils in AT [78]. Consistent with this, it has been shown that macrophage infiltration is more frequent in visceral (VAT) than in subcutanous adipose tissue (SAT) and correlates with adiposity.

In white adipose tissue, 90% of the macrophages are localized close to apoptotic cells [79]. Adipocytes interact with immune cells through the expression of a large panel of inflammation mediators and biomarkers (PAI-1, C-reactive protein (CRP), IL-1B, IL-6, TNFα and more) [74,75,77,80,81]. Therefor, in hypertrophic AT, the switch to MUO emphasizes the shift towards a pro-inflammatory profile with an excess of immune cells and a dysregulation of the immune-modulatory system [82-84]. Initial hypotheses have opposed two macrophage phenotypes: the pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages [83-85] with a shift of M2-like to M1-like macrophages in MUO. However, the classical M1–M2 paradigm seems to be more complex [86]. Indeed, in rodent and human model, macrophages in VAT polarize into a specific metabolically activated macrophage, with markers from both the M1 and M2 phenotypes [87]. In addition to CD11c+ expression which contributes to inflammation, some M2-like markers such as CD36, ABCA1 (ATP-binding cassette transporter A1) and Plin2 (Perileptin 2) are also expressed, supporting a mixed AT macrophage phenotype [87]. In conclusion, the adipocyte dysfunction in MUO is responsible for profound changes in the adipose tissue environment towards a self-maintaining pro-inflammatory state [77,80-84]. Macrophage infiltration and AT inflammation are tightly linked to the degree of insulin resistance and cardiometabolic outcome [50,55-59,88-91] (Figure 3).

Many inflammatory adipokines have been associated with increased adiposity [92-94]. Some of the biomarkers are correlated with total body fat (resistin, leptin), or with VAT mass (IL-6 and C-reactive protein (CRP)) [95-97]. About one third of the circulating IL-6 comes from adipose tissue. IL-6 triggers hepatic expression of CRP [96]. Similar to the differences in number and types of immune cells in AT between unhealthy and healthy state, adipocytokines expression also differs between MHO and MUO. The expression of anti-inflammatory and/or insulin sensitizing adipocytokines such as adiponectin, IL-4 and IL-10, is decreased in hypertrophic

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VAT. Conversely adipocytokines with pro-inflammatory and/or insulin desensitizing proprieties are increased (leptin, TNF-α, IL-6, visfatin, resistin) [83]. Given the link between inflammation and insulin pathways, inflammation in MUO is associated with insulin resistance: TNF-α and IL-1β activate the IKK/NF-κB or JNKs and MAPKs pathways [98] and consequently, insulin signalling is impaired through inhibition of serine–threonine phosphorylation of insulin receptor substrate 1 (IRS-1), leading to decreased PI3K/PKB signalling [99]. JAK1/JAK2/STAT1 and STAT3 also promote the degradation of IRS proteins via their downstream effectors Socs1 and Socs3, [100]. Toll-like receptors 2 and 4 (TLR2, TLR4) mediate the crosstalk between adipocytes and immune cells induced by FFA such as palmitic acid, resulting in the activation of Ikkb/NFκB and JNK/AP-1 pathways which in turn interact with insulin pathways [101]. In contrast, the anti-inflammatory system is blunted in MUO as evidenced by the decrease of anti-inflammatory markers. Adiponectin which is mainly secreted by adipose tissue, has anti-inflammatory effects by inhibiting TNF-α expression [102]. It also enhances insulin sensitivity via IRS-2 [103] and has beneficial vascular effects by enhancing $NO^{\cdot}$ production [104]. Furthermore, adiponectin promotes the M2-like phenotype and the secretion of the essential anti-inflammatory cytokine IL-10 [105, 106]. Adiponectin levels are low in obesity and are negatively correlated with insulin resistance [107]. In obese insulin-resistant KKAy mice, replenishment of adiponectin significantly improves insulin sensitivity and hypertriglyceridemia [108] suggesting that adiponectin could be a potential treatment for obesity. Elevated CRP and IL-6 levels have been identified in some studies as independent predictors of both initial T2DM and cardiovascular disease [109-112]. However, direct and independent association between many inflammatory markers and cardiometabolic outcomes remains unclear. Indead, studies describing the impact of anti TNF-α / anti IL-6 administration on metabolic parameters in patients with rheumatic diseases have shown little or no effect [113-115].

Hypertrophic adipose expansion in MUO impairs tissue vascularisation and thereby AT oxygenation, leading to hypoxic conditions [46]. This hypoxic state is particularly marked in the visceral adipocytes as compared to the subcutaneous adipocytes [116,117]. As described pre-

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viously, the lack of vascularisation and hypoxic signals lead to the upregulation of the inflammatory genes in the expanded adipocytes [116] and to insulin resistance [118-119] and is therefor a critical factor for the occurrence of long-term complications in MUO. In addition, the development of a hypoxic status favours ROS and RNS generation due to altered mitochondrial function [120-122].

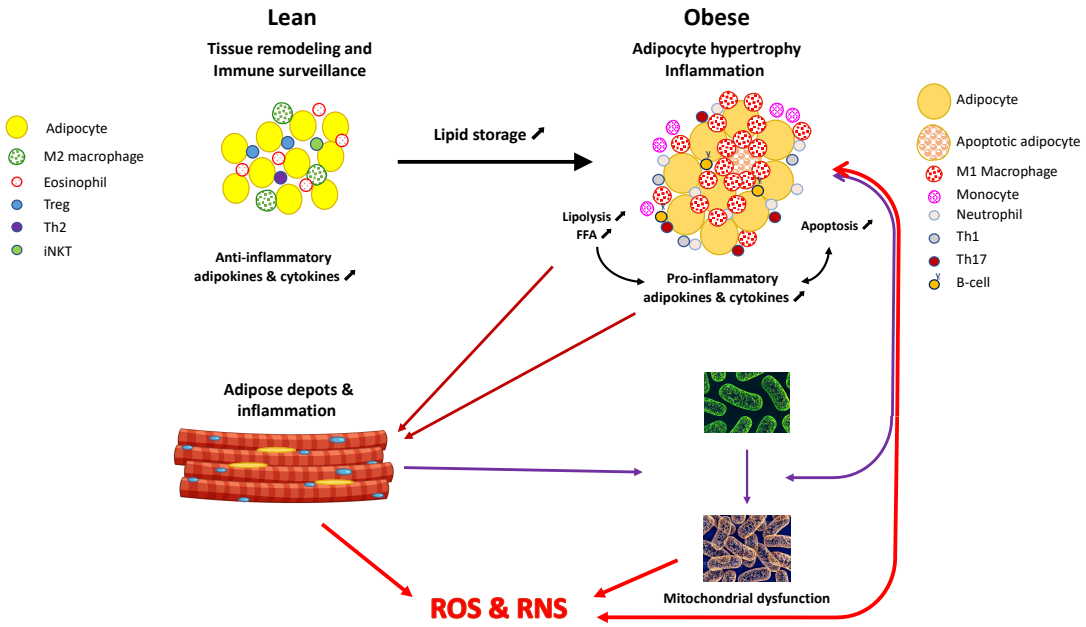

Fig. 3: Impact of obesity on the metabolism and immune status of visceral adipose tissue and its effects on reactive species generation. Consequences on skeletal muscle and mitochondrial dysfunction are highlighted.

III. Obesity and nitro-oxidative stress

As indicated above, in MUO VAT becomes a site of chronic inflammation [123,124]. In addition to the immune cells, especially the M1 macrophages which generate large amounts of ROS and hence also of RNS, the hypertrophic adipocytes also display increased ROS production due to altered mitochondrial function [125]. Not only is the biogenesis and thus the density of mitochondria decreased in these adipocytes, but so are also their metabolic functions including fatty acid and branched-chain aminoacid (BCAA) oxidation, oxidative phosphorylation, beta oxidation and Krebs or tricarboxylic acid (TCA) cycle. Slowdown of the TCA cycle which is

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further aggravated by reduced glucose uptake and oxidation, results in additional reduction of mitochondrial ATP synthesis and respiration. This decreased respiration due to substrate deficit is the major cause for increased ROS and RNS generation by the hypertrophic adipocytes' mitochondria (Fig. 3).

Recently a new paradigm called mitochondrial transfer has emerged. This mechanism, reported in several cell types, consists in the extrusion of mitochondria which are subsequently taken up by "acceptor" cells [126]. In obesity, the hypertrophic adipocytes extrude dysfunctional mitochondria which are taken up either locally by macrophages [127], fibroblasts and progenitors or by more distant targets where they may contribute to affect cell differentiation and metabolism [128,129].

Interestingly, similar observations have been made in skeletal muscle [130-132] in obesity as well. Indeed, skeletal muscle becomes infiltrated by adipose tissue which exhibits the same inflammatory phenotype as that found in visceral fat in MUO [133]. The same causes having the same effects, muscle also displays an inflammatory profile in this situation [134] (Fig. 3).

Skeletal muscle accounts for the main tissue and mitochondrial mass of the body. Mitochondrial dysfunction therefor not only has major metabolic effects including insulin-resistance, but also contributes in a very significant manner to increased ROS and RNS generation [135]. In the case of obesity, skeletal muscle together with VAT therefor becomes the major site of nitro-oxidative stress. Obviously, the complex regulation of inflammation and mitochondrial dysfunction in other organs as a consequence of obesity goes beyond the scope of this review but has become the subject of intensive research [136-139].

Whereas the question of whether nitro-oxidative stress precedes or follows inflammation and mitochondrial dysfunction is still a matter of speculation and debate, but their close entanglement is a clear fact which leads to a vicious circle [140-141]. Understanding these complex pathophysiological mechanisms paves the way for novel therapeutic strategies.

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Figure Legends

Fig. 1: Schematic representation of the major mechanisms and reactions involved in redox signaling with emphasis on the generation and degradation of reactive oxygen species (ROS)

Fig. 2: Schematic representation of the major mechanisms and reactions involved in redox signaling with emphasis on the generation and effects of reactive nitrogen species (RNS)

Fig. 3: Impact of obesity on the metabolism and immune status of visceral adipose tissue and its effects on reactive species generation. Consequences on skeletal muscle and mitochondrial dysfunction are highlighted.