



Is oxidative stress of adipocytes a cause or a consequence of the metabolic syndrome?



Leonid N. Maslov^{a,*}, Natalia V. Naryzhnaya^a, Alla A. Boshchenko^a, Sergey V. Popov^a, Vladimir V. Ivanov^b, Peter R. Oeltgen^c

^a Cardiology Research Institute, Tomsk National Research Medical Centre, Russian Academy of Sciences, Tomsk, Russia

^b Siberian State Medical University, Tomsk, Russia

^c Department of Pathology, University of Kentucky College of Medicine, Lexington, KY, USA

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ABSTRACT

Metabolic syndrome is accompanied by oxidative stress in animals and humans. The main source of ROS in experimental metabolic syndrome is NADPH oxidase and possibly adipocyte mitochondria. It is now documented that oxidative stress induces insulin resistance of adipocytes and increases secretion of leptin, MCP-1, IL-6, and TNF- α by adipocytes. It was established that oxidative stress induces a decrease in adiponectin production by adipocytes. It has also been shown that obesity itself can induce oxidative stress. Oxidative stress can cause an alteration of intracellular signaling in adipocytes that apparently leads to the formation of insulin resistance of adipocytes. Chronic stress, glucocorticoids, mineralocorticoids, angiotensin-II, TNF- α also play an important role in the pathogenesis of oxidative stress of adipocytes. Oxidative stress is not only a consequence of metabolic syndrome, but also a reason and a foundational link in the pathogenesis of the metabolic syndrome.

Introduction

Metabolic syndrome (MS) is a pathological condition that, according to the International Diabetes Federation (IDF) criteria [1], is characterized by obesity, dyslipidemia, hyperglycemia, high blood pressure. Metabolic syndrome is widespread in both developed and developing countries. For example, in the USA this syndrome occurs in 33% of the adult population [2]. In Germany, metabolic syndrome occurs in 19.4% of women and 30.2% of men [3]. In Russia, the incidence of MS in men is 23%, and in women 32.4% [4]. It has also been shown that patients with MS had a fourfold increase in mortality from cardiovascular diseases [5].

The pathogenesis of MS has been intensively studied for almost 30 years. However, the mechanism(s) of this metabolic disturbance remains a mystery in many ways. The intent of this review is to draw the reader's attention to the role of reactive oxygen species (ROS) in the pathogenesis of disturbance of adipocyte metabolic state and, as a consequence, in the mechanism of MS occurrence (see Fig. 1).

Oxidative stress in metabolic syndrome, experimental data

In 2003, Tailor et al. published the results of their experiments on

adipocytes isolated from adipose tissue from mice fed on a diet enriched with fat [6]. Such a diet led to the formation of a state similar to MS in humans. Isolated adipocytes were characterized by insulin resistance and a twofold increase in the ROS production. In KKAY strain mice with obesity and type 2 diabetes mellitus, an increase in the malondialdehyde (MDA) level in plasma and white adipose tissue was observed in comparison to C57BL/6 mice (without MS), which had an increase in the plasma H₂O₂ concentration [7]. The inhibitor of NADPH oxidase (Nox) apocynin reduced the MDA level in white adipose tissue in KKAY mice and did not affect the MDA content in white fat of C57BL/6 mice [7].

Later, Kurata et al. also found [8] that in KKAY mice compared with C57BL/6 mice, an increase in the MDA level in the subcutaneous adipose tissue and H₂O₂ was observed in the blood plasma. Even more significant were experiments in mice with congenital obesity and insulin resistance (ob/ob strain and db/db strain) [9]. It turned out that the MDA level in the plasma and in the white fat tissue of these mice was 2 times higher than in the control C57BL/6J mice. In experiments on rats fed a diet with a high fructose content, an increase in the activity of Nox in epididymal adipose tissue was noted [10]. These investigators hypothesized that a high fructose diet contributes to MS. Nox generated a superoxide radical (O₂[•]) [10]. Farina et al. [11] in experiments with

* Corresponding author.

E-mail address: maslov@cardio-tomsk.ru (L.N. Maslov).

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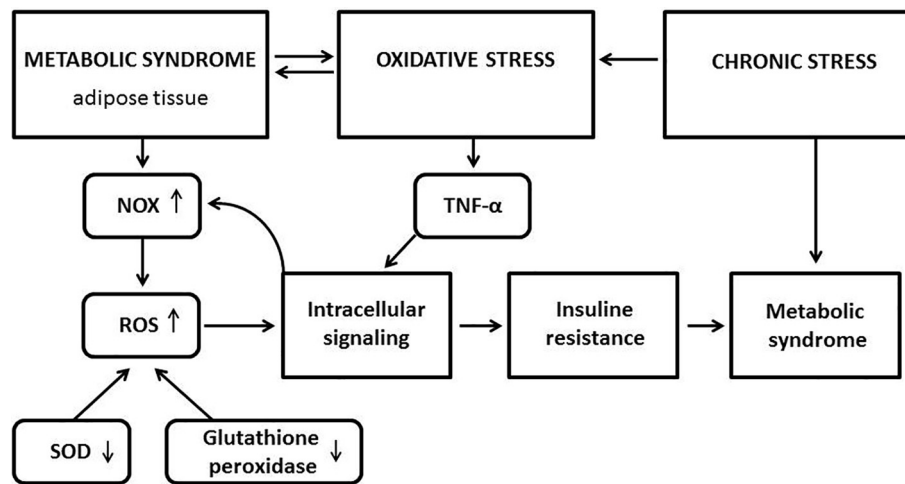


Fig. 1. The relationship of metabolic syndrome and oxidative stress. ROS, reactive oxygen species; SOD, superoxide dismutase; TNF- α , tumor necrosis factor- α .

rats on a diet with a high fructose content, found Nox activation in adipose tissue and an increase in the level of MDA in blood plasma. It has also been shown that not only Nox, but also mitochondria of adipocytes can be a source of ROS [12]. The state which is similar to MS is developed in rats on diet with a sucrose-rich diet for 4 months [13]. These rats have high levels of MDA, protein carbonyl groups and TNF- α in blood plasma. In female rats, MS was caused by ovariectomy and drinking water with sucrose (30%) [14]. Such an effect after 6 months resulted in insulin resistance and obesity. The MDA level in the intra-abdominal fat of these rats was increased by almost 5-fold in comparison with the control group.

Recent data indicate that the source of ROS during experimental MS is Nox and, possibly, mitochondria. However, the reason for increasing lipid peroxidation can be not only enhanced ROS production, but also a decrease in antioxidant protection of the adipocyte. Thus, in KKAY mice with obesity and type 2 diabetes mellitus, compared with C57BL/6 mice, a decrease in the activity of superoxide dismutase (SOD) and glutathione peroxidase in white fat was also noted [7]. Similar changes in the activity of enzymes in the liver and skeletal muscles could not be detected by the authors. It has also been demonstrated that glutathione peroxidase activity was decreased in epididymal fat ob/ob mice [15]. A decrease in the activity of SOD, glutathione peroxidase, and catalase has been observed in the intra-abdominal fat of female rats with MS [14].

The aforementioned studies indicate that experimental induced metabolic syndrome promotes oxidative stress in adipose tissue, due to activation of the ROS production and reduction of antioxidant protection of the adipocyte.

Oxidative stress in metabolic syndrome, clinical data

There are few clinical studies on oxidative stress in patients with MS. Furukawa's research referring to patients with oxidative stress is frequently cited [7]. However, there was no data on patients with MS in the article since only healthy volunteers and people with obesity without diabetes mellitus and cardiovascular diseases were included in the study. Since one of the symptoms of MS is arterial hypertension [1], the exclusion of cardiovascular disease suggests that patients with MS may have been excluded from the study. However, Furukawa et al. did present interesting findings which should be mentioned [7]. These authors found that there was a direct correlation between the level of MDA in blood plasma and the body mass index. In addition, an inverse correlation was found between the MDA level in the blood and the concentration of adiponectin in the plasma [7]. A study in Iran included 37 MS patients and 30 healthy volunteers [16]. The authors were unable to identify differences between groups in regard to MDA and

antioxidant activity in serum. However, they were able to detect an increase in the total oxidant status of serum in patients with MS [16].

Our study included patients with MS and volunteers without MS (control group) [17]. The diagnosis of MS was made in accordance with the recommendations of the International Diabetes Federation [1]. The material of the study was visceral adipose tissue obtained during surgical interventions. The level of ROS in adipocytes was determined using the dichlorofluorescein diacetate dye by flow laser cytometry on the day of isolation [18]. The results were expressed in relative units (R.U.). We found [17] that the production of ROS in adipocytes of visceral fat in patients with MS increased four-fold from 0.074 ± 0.07 ($M \pm SD$, $n = 29$), in the control group (healthy volunteers) to 0.298 ± 0.09 ($n = 6$) R.U. ($p < 0.05$) in the MS group. The production of ROS in mesenchymal stromal cells of visceral fat in patients with MS was 0.498 ± 0.08 , and was 0.314 ± 0.04 R.U. ($p < 0.05$) in the control group.

The aforementioned studies indicate that the metabolic syndrome promotes oxidative stress in adipose tissue, mainly due to the activation of the products of ROS by adipocytes.

Oxidative stress and obesity

A study performed in China showed that the MDA level in adolescents with obesity is 20% higher ($P < 0.01$) than in adolescents without obesity (control) [19]. In 2012, Karbownik-Lewinska et al. [20] reported that patients which were overweight or obese as compared to volunteers with normal weight that the lipid peroxidation (LPO) level in the serum (MDA, 4-hydroxyalkenali) was increased. The level of LPO products correlated with body weight and body mass index. Similarly, it was demonstrated that in children and adolescents with insulin resistance and obesity without diabetes mellitus that the serum MDA level was increased 3.6-fold [21]. In patients with obesity and type 2 diabetes, the level of leptin (one of the main adipokines) in serum is doubled, and the MDA concentration is increased by 32% compared to the control group (without obesity and diabetes) [22]. In a study performed by Becer and Çırakoğlu [23] included 150 obese patients and 120 volunteers with normal weight. The body mass index in obese patients was 35, the level of total cholesterol was increased, HOMA-IR (homeostatic model assessment-insulin resistance) was 2 fold higher than in the control group (patients without obesity). The concentration of leptin in the blood plasma of obese patients was 3 times higher than in the control group [23]. The MDA level in the serum of obese patients was almost 2 fold higher than in the control group. It should be noted that patients with obesity included in this study, according to IDF criteria, closely matched MS patients, however they did not have arterial hypertension.

Thus, obesity itself can cause oxidative stress. However, it should be noted that in these studies there was no attempt to evaluate the production of ROS by fatty tissue or to determine the source of MDA in the blood of obese patients.

Oxidative stress, as a cause of impaired secretion of adiponectin, leptin, MCP-1, IL-6, TNF- α by fat tissue

It has been shown that the level of leptin was increased in the blood of MS patients in comparison with volunteers without MS [24,25,26] while the concentration of adiponectin was decreased [24,26,27]. Many researchers [25,28,29,30] feel this is directly related to the pathogenesis of MS and its associated complications. Both hormones are synthesized by adipocytes [28]. In patients with MS, the level of proinflammatory cytokines was increased: MCP-1 (monocyte chemoattractant protein 1), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) [24,31,32,33]. It is believed that these cytokines are synthesized by macrophages of adipose tissue [28] and are involved in the pathogenesis of MS [30].

In experiments on murine pre-adipocyte 3T3-L1 culture, oxidative stress has been shown to decrease the secretion of adiponectin [34]. In 2006, Chen et al. [35], performing experiments with of pre-adipocyte 3T3-L1 culture, found that ROS reduced the expression of adiponectin mRNA. In another study, 3T3-L1 pre-adipocytes were exposed to oxidative stress by adding H₂O₂ or glucose oxidase to the incubation medium [36]. This causes a decrease in the adiponectin level in adipocytes and increases the production of TNF- α and IL-6. Reduction in the expression of adiponectin mRNA in the pre-adipocyte 3T3-L1 culture after oxidative stress was observed by other investigators [37]. In 2015, Pan et al. [38] found that H₂O₂ reduced adiponectin production by 3T3-L1 adipocytes by a factor of 2 and contributed to a 3-fold increase in the synthesis of TNF- α and IL-6. Oxidative stress caused by the addition of H₂O₂ to the incubation medium of 3T3-L1 pre-adipocytes caused an increase in the mRNA levels of leptin, IL-6 and MCP-1 (monocyte chemoattractant protein 1), and an increase in the secretion of these proteins by adipocytes. Especially noticeable was an almost 3-fold increase in secretion of IL-6 [39]. The ability of H₂O₂ to induce an increase in the mRNA of levels of TNF- α , IL-6, MCP-1 in 3T3-L1-adipocytes while reducing the amount of adiponectin mRNA and decreasing the concentration of this adipokine in the incubation medium was noted by other authors [40].

Thus, it has now been shown that oxidative stress promotes a reduction of adiponectin production and an increase in the synthesis and secretion of leptin, MCP-1, IL-6 and TNF- α by adipocytes.

Oxidative stress and intracellular signaling

In 2003, Talior et al. [6] found that high-fat feed promotes the activation of protein kinase C δ (PKC- δ), the same effect was exerted by H₂O₂. In experiments with 3T3-L1 adipocytes, it was shown that H₂O₂ promotes the activation of a number of kinases: Akt (anti-apoptotic kinase), JAKs (Janus kinases), ERK1/2 (extracellular signal-regulated kinase) [41]. Simultaneously, the transcription factor STAT (signal transduced and activator of transcription) is activated. The authors obtained data that the decrease in adiponectin secretion under oxidative stress is associated with activation of Akt, JAK/STAT, and an increase in IL-6 production is a result of an increase in the activity of Akt, JAK/STAT and ERK1/2 [41]. It was found that ROS can not only activate but inhibit signaling [40,42]. Thus, oxidative stress has been shown to reduce insulin-induced activation of protein kinase B (Akt kinase) and PI3 kinase (phosphatidylinositol 3-kinase) in 3T3-L1 adipocyte culture, which, according to the authors, contributes to the disruption of translocation of GLUT4 (Glucose transporter) into cellular membrane [42]. In this connection, it should be noted that PI3K plays an important role in insulin-induced signaling [43]. In experiments with 3T3-L1-adipocytes, it was shown that H₂O₂ inhibits insulin-

induced phosphorylation of the insulin receptor and phosphorylation (activation) of Akt [40]. The phosphorylation of JNK, on the contrary, enhanced.

In experiments with young mice deletion of the gene encoding PKC- δ was deleted specifically in muscle using Cre-lox recombination. It was shown that the deletion of the gene encoding PKC- δ promotes the formation of insulin resistance [44]. In 2011, Bezy et al. [45] received more revealing data. They found that expression of PKC- δ mRNA and PKC- ϵ was increased in the liver of obese patients compared to people with normal weight. In mice with a liver-specific disturbance of PKC- δ expression, an increase in glucose tolerance, an increase in insulin sensitivity, and an increase in insulin signaling in hepatocytes was observed [45]. Specific liver overexpression of PKC- δ , on the contrary, led to the formation of a state similar to metabolic stress.

The aforementioned data indicate that oxidative stress caused a disturbance of insulin-dependent stimulation of PI3K, while the activity of JNK and PKC- δ in adipocytes increased. Reduced PI3K activity, which is a key enzyme in the insulin-dependent signaling, undoubtedly plays an important role in the formation of ROS-induced insulin resistance. An increase in the activity of PKC- δ in oxidative stress, apparently, also has a direct bearing on the formation of MS. The role of other kinases in regulating the sensitivity of cells to insulin has yet to be completely delineated.

Oxidative stress, as a cause of insulin resistance of adipocytes

Thus, oxidative stress can cause the formation of insulin resistance of adipocytes. In 1997, in experiments with the culture of 3T3-L1-adipocytes, it was shown that oxidative stress leads to a decrease in insulin-dependent glucose transport into the cell [46]. In experiments with 3T3-L1 adipocytes, it was shown that insulin causes translocation into the GLUT4 cell membrane [47], which leads to an increase in glucose transport to the cell. Oxidative stress led to disruption of this process. In 2003, Tailor et al. [6] have shown that oxidative stress *in vivo* also contributes to the formation of insulin resistance of adipocytes. The authors found that mice fed for 4 months on a high fat content diet became obese and developed an insulin resistant state similar to MS. Adipocytes were isolated from epididymal fat. It turned out that in the presence of glucose the ROS production by adipocytes of animals with MS increased almost 2-fold compared to the adipocytes of healthy mice [6]. It was also shown that H₂O₂ contributes to the disturbance of glucose uptake of 3T3-L1-adipocytes [36,48]. If ROS is really involved in the pathogenesis of MS, then antioxidants should have a positive effect on the course of MS.

In 2009, research results were published that were performed on high-fat diet-induced insulin resistance rats [49]. It turned out that the antioxidant SS31 contribute to decline the glucose and insulin levels in the blood after oral intake of glucose. In a study that was performed by Gao et al. [50], it was shown that flavonoids, which are natural antioxidants, decreased serum MDA levels, improved insulin resistance, ameliorated lipid disorders in high fat diet fed rats as obesity model and KK-ay mice as diabetic model. In 2009, the results of a study were published that included 5220 adult volunteers without MS who received natural antioxidants (vitamins C and E, β -carotene, zinc, and selenium) for 7.5 years [51]. The risk of MS was assessed. It has been established there are no benefit or adverse effect of multiple antioxidant supplementation on MS incidence. It has been found that adipocyte-specific deficiency of Nox-4 delays the onset of insulin resistance in mice with a high fat/high sucrose diet [52]. Recently, in experiments with mice with a high fat/high sucrose diet, it has been shown that overexpression of genes encoding catalase and SOD improved insulin sensitivity of adipocytes in comparison with adipocytes of wild type of mice [53]. In contrast, ROS-augmented mice, in which glutathione was depleted specifically in adipocytes, exhibited deteriorated insulin sensitivity of adipocytes.

Thus, oxidative stress can cause the formation of insulin resistance

of adipocytes. It should be noted that some authors propose that not only oxidative stress but also a decrease in ROS levels resulting from over supplementation with antioxidants can contribute to adipocyte dysfunction and subsequently insulin resistance [54].

Pathogenesis of oxidative stress and insulin resistance of adipocytes

The pathogenesis of the oxidative stress of adipocytes in MS is still a mystery. In 2003, Taylor et al. [6] have shown that the oxidative stress of isolated adipocytes of mice with MS is noted only in the presence of glucose and the resulting hyperglycemia may be the cause of oxidative stress. It is believed that the cause of oxidative stress in MS may be an increase in blood and tissue free fatty acid levels in patients with metabolic syndrome [55]. It has also been documented that TNF- α , whose level is increased in the blood of MS patients, can cause oxidative stress of adipocytes [56,57]. However, there is no conclusive evidence that hyperglycaemia, dyslipidemia or elevated TNF- α levels are the primary cause of oxidative stress and subsequent insulin resistance in MS formation. We noted above that oxidative stress induces activation of PKC- δ , which in turn activates Nox, which synthesizes $O_2^{\cdot -}$ [58].

Thus, a positive feedback loop can be formed. Oxidative stress increases the TNF- α production, which, in turn, ensures the enhancement of ROS production. Oxidative stress stimulates PKC- δ , it also activates Nox, which in turn causes an increase in $O_2^{\cdot -}$ production. However, it is still unclear if this process occurs in vivo.

We concur with Eriksson's hypothesis [59] that the primary cause of insulin resistance, dyslipidemia in MS is chronic stress. This hypothesis is confirmed by the data of clinical observations [60]. Indeed, clinical data suggest a positive effect of losartan, an angiotensin II receptor antagonist, on MS course [38]. It has been established that direct renin inhibition with aliskiren improves metabolic syndrome, and reduces angiotensin II levels and oxidative stress in visceral fat tissues in fructose-fed rats [61]. It has also been documented that the mineralocorticoid receptor antagonist eplerenone reduces insulin resistance and adipocyte dysfunction in ob/ob and db/db mice with a dysmetabolic state similar to MS [9]. In experiments on 3T3-L1-adipocytes, it has been shown that dexamethasone can induce insulin resistance [62]. The glucocorticoid receptor antagonist RU486 has been shown to reduce dysfunction of adipose tissue in DahlS.Z-Lepr (fa)/Lepr (fa) rats with MS [63].

Conclusion

The presented data indicate that oxidative stress causes insulin resistance of adipocytes, and contributes to increased secretion of leptin, IL-6, TNF- α by adipocytes. The impact of the resulting ROS leads to decreased adipocyte secretion of adiponectin. It is now documented that obesity itself can cause oxidative stress. The aforementioned studies indicate that a positive feedback loop can be formed: oxidative stress increases the production of TNF- α , which, in turn, ensures the enhancement of AFK production. Oxidative stress also stimulates PKC- δ , and activates Nox, which causes an increase in $O_2^{\cdot -}$ production. However, it is still unclear whether these processes occur in vivo. Oxidative stress can cause the formation of insulin resistance of adipocytes. Oxidative stress can also cause impaired insulin-dependent stimulation of PI3K, while the activity of JNK and PKC- δ in adipocytes is increased. Reduction of PI3K activity and stimulation of PKC- δ of adipocytes in oxidative stress seems to have a direct bearing on the formation of the metabolic syndrome. We hypothesize that chronic stress, glucocorticoids, mineralocorticoids, angiotensin II, TNF- α play an important role in the pathogenesis of oxidative stress of adipocytes. The presented data suggest that oxidative stress is not only a consequence of MS, but also a link in the pathogenesis of MS. Our knowledge of the pathogenesis of metabolic syndrome could be greatly expanded by the in vitro studies of adipocytes derived from patients

with MS. These studies are necessary to find the source of ROS in patients with MS, but also to determine which pharmacological agents are capable of reducing ROS production by adipocytes in patients with MS. This knowledge would be a great benefit in developing new approaches to MS therapy.

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Conflict of interest

There is no conflict of interest.

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