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Review

Xanthine oxidoreductase in atherosclerosis pathogenesis: Not only oxidative stress

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ABSTRACT

Endothelial xanthine oxidoreductase (XOR) together with NAD(P)H oxidase and nitric oxide (NO) synthase plays a physiologic role in inflammatory signalling, the regulation of NO production and vascular function. The oxidative stress generated by these enzymes may induce endothelial dysfunction, leading to atherosclerosis, cardiovascular diseases and metabolic syndrome. XOR activity creates both oxidant and anti-oxidant products that are implicated in the development of hypertension, smoking vascular injury, dyslipidemia and diabetes, which are the main risk factors of atherosclerosis. In particular, uric acid may have a protective as well as a detrimental role in vascular alterations, thus justifying the multi-directional effects of XOR inhibition. Moreover, XOR products are associated with cell differentiation, leading to adipogenesis and foam cell formation, as well as to the production of monocyte chemoattractant protein-1 from arterial smooth muscle cells, after proliferation and migration. The role of XOR in adipogenesis is also connected with insulin resistance and obesity, two main features of type 2 diabetes.

Contents

1. Introduction

Xanthine oxidoreductase (XOR) is responsible for purine catabolism, catalysing the oxidation of hypoxanthine to xanthine and of xanthine to uric acid, which *in vivo* possesses both anti-oxidant (primarily in plasma) and pro-oxidant (primarily within the cell) activities [reviewed in Ref. [1]].

Abbreviations: COX-2, cyclooxygenase-2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; XDH, xanthine dehydrogenase; XO, xanthine oxidase; XOR, xanthine oxidoreductase; *XOR*, gene for XOR.

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In mammals, XOR is present in two interconvertible forms [2]: xanthine dehydrogenase (XDH, EC 1.1.1.204), which prefers NAD^+ as an electron acceptor, and xanthine oxidase (XO, EC 1.1.3.22), which transfers the electrons directly to molecular oxygen, with the production of the reactive oxygen species (ROS) superoxide anion and hydrogen peroxide. The production rate of these two ROS depends on O_2 tension, pH and purine concentration, the formation of hydrogen peroxide being favoured when O_2 levels and pH are reduced as under ischaemic and/or hypoxic conditions [reviewed in Ref. [3]].

XOR is a molybdo-flavo homodimer that consists of two identical subunits of approximately 145 kDa, each containing two iron sulphur centres. The conversion from XDH to XO may occur through the oxidation of sulfhydryl residues or limited proteolysis [reviewed in Ref. [4]]. Thus, XOR activity generates both oxidant and anti-oxidant products.

XOR is constitutively expressed in the dehydrogenase form, primarily by epithelial cells in the liver, intestine and mammary gland; XOR serum levels are usually very low in humans. However, XOR levels may become more elevated in some pathological conditions, which cause XOR release from damaged cells into the circulation, where it is converted to the oxidase form. Circulating XOR may bind to endothelial cells, where it is responsible for remote organ injury and has been implicated in the development of endothelial dysfunction. In a recent review, the experimental and clinical pathologies leading to increased serum level of XOR were exhaustively discussed, and the vascular effects of oxidants produced by circulating enzymes and their role in cardiovascular diseases were addressed [reviewed in Ref. [5]]. The present review focus on the role of XOR in the endothelial dysfunction that is central to atherosclerosis.

2. Oxidants-producing endothelial enzymes and atherosclerosis

The investigation of oxidative stress has generated a large body of evidence underlining its implications in cytotoxicity and tissue injury. Today, ROS are receiving growing consideration for their physiologic roles in inflammatory signalling and the regulation of vascular function. In both cases, these oxidants are produced in endothelial cells largely by NAD(P)H oxidase, nitric oxide (NO) synthase, and XOR. The dysregulation of these enzymes is associated with impaired vascular function and cardiovascular diseases [reviewed in Ref. [6]]. The role of these enzymes in the cardioprotection secondary to nitrite administration was investigated in a rat model of myocardial ischemia/reperfusion injury [7]. Infarction was associated with an increase in myocardial XOR activity. The protective effect of nitrite was dependent on the drug dose and was abolished by the inhibition of NAD(P)H oxidase or XOR activities, although it was unaffected by the inhibition of NO synthase. These results indicate that ischaemic conditions abolish NO synthase activity and that the generation of NO from the reduction of nitrite is the result of the activities of NAD(P)H oxidase and XOR. In these conditions, the inhibition of XOR activity with allopurinol may abolish the salutary outcomes due to nitrite treatment [reviewed in Ref. [3]].

Moreover, XOR-derived ROS may activate NAD(P)H oxidase activity and *vice versa*; thus, the roles of these two enzymes in cardiovascular diseases are strictly interrelated [reviewed in Ref. [8]]. Furthermore, XOR activity is inhibited by NO or by reactive nitrogen species that may originate from the reaction between XOR-derived superoxide and NO; however, on the other hand, XOR-derived ROS can inhibit NO synthase activity. Thus, enhanced NO production may occur in response to XOR inhibition *in vivo* [reviewed in Ref. [9]]. The interplay between endothelial NAD(P)H oxidase, NO synthase and XOR activities is presented in Fig. 1.

The pro-inflammatory action of XOR products has been shown to be relevant to pathologies resulting from vascular dysfunction. Oxidants produced by endothelium-bound XOR have been implicated in the pathogenesis of atherosclerosis by a variety of studies [reviewed in Ref. [10]]. In atherosclerotic plaques from patients who underwent carotid endarterectomy, the accumulation of uric acid and XOR, which were more concentrated in endothelium and smooth muscle cells and were co-localised with cholesterol, was identified using an immune-staining based test [11].

Bovine aortic endothelial cells exposed to oscillatory shear stress exhibit enhanced superoxide production that is associated with an elevated ratio of XO to XDH. The addition of oxypurinol or tungsten to endothelial cell cultures inhibited this superoxide production, suggesting that the mechanical stress exerted by blood flowing over the endothelium induces oxidative stress through the conversion of XOR to its ROS-generating form, thus indicating a link between XOR activity and the pathogenesis of atherosclerosis [12].

Recently, the effects of XOR activity inhibition by treatment with allopurinol or febuxostat have been compared to analyse the protection given in heart failure, chronic kidney diseases, and other pathologies [reviewed in Ref. [13]]. In a mouse model, febuxostat provided protection by attenuating systolic overload-induced left ventricular hypertrophy and dysfunction [14]. In rats with fructose-induced metabolic syndrome, febuxostat was able to correct hyperuricemia, hypertension, and hypertriglyceridemia. Moreover, this treatment prevented the increase in fasting plasma insulin [15]. However, XOR inhibition may lead to clinical improvement as well as to opposite results in cardiovascular diseases depending on the level of serum uric acid [reviewed in Ref. [16]].

The main risk factors of atherosclerosis, i.e., hypertension, smoking, dyslipidemia, and diabetes, may trigger the pathogenesis of endothelial dysfunction at least in part through XOR-induced oxidative stress (Fig. 2). As a consequence of this ROS-driven vascular dysfunction, loss of vasodilatation, inflammation and platelet aggregation may be elicited, thus promoting cardiovascular diseases [reviewed in Ref. [17]].

3. Xanthine oxidoreductase activity in hyperuricemia and hypertension

XOR activity in the myocardial tissue of spontaneously hypertensive rats was higher than in controls [18]. An increase in oxy-radicals production has been reported in the microvascular endothelium of spontaneously hypertensive rats, which coincided with the elevated arteriolar tone compared with control animals.

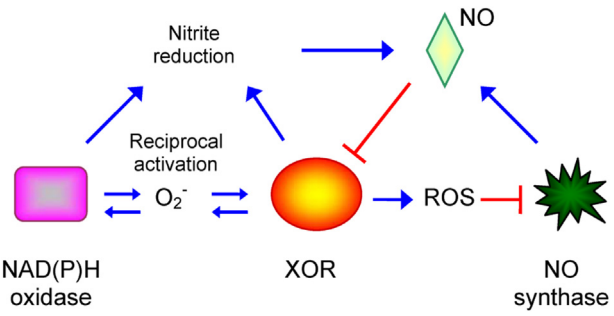

Fig. 1. Regulation of nitrogen oxide (NO) production by endothelial enzymes. NO may be produced by NO synthase activity and also through the reduction of nitrite by NAD(P)H and xanthine oxidoreductase (XOR) activities. NAD(P)H oxidase and XOR may activate each other through the production of superoxide anion, whereas NO synthase and XOR may inhibit each other through their products [reviewed in Ref. [8]].

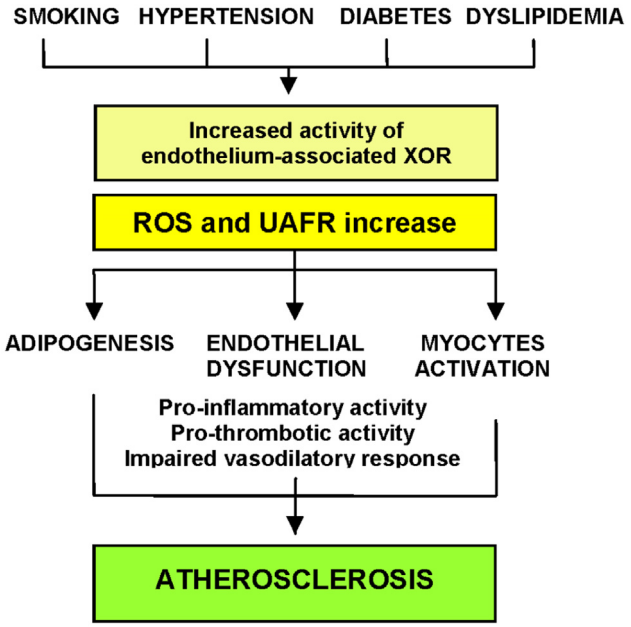

Fig. 2. Role of xanthine oxidoreductase (XOR) in atherosclerosis pathogenesis. Increased endothelium-associated XOR activity contributes to the pathogenesis of vascular oxidative stress and inflammatory responses related to smoke damage, hypertension, diabetes, and hypercholesterolaemia [reviewed in Ref. [10]]. ROS: reactive oxygen species; UAFR: uric acid-derived free radicals.

Pre-treatment with a tungsten diet, which decreases the expression of XOR, or chemical inhibition of XOR activity prevented ROS overproduction and the elevation of blood pressure and arteriolar tone [19].

The involvement of XOR-derived oxidants in the pathogenesis of salt-induced hypertension was indicated by the lowering of blood pressure and the reduction of ROS production in the endothelium of arterioles and venules of salt-sensitive rats after pre-treatment with tungsten [20]. Additionally, renal XOR activity was higher in spontaneously hypertensive rats than in control rats, and blood pressure was increased in both groups by a high-salt diet. Hypertension was correlated to renal XOR activity and to induced left ventricular and renal hypertrophy, which were prevented by allopurinol treatment [20].

The XOR activity was shown to be a regulator of cyclooxygenase-2 (COX-2) expression in studies with *XOR* null mice [21]. Moreover, the expression of COX-2 can be stimulate by uric acid *in vitro* and *in vivo* providing a link between elevated serum uric levels and inflammatory response, suggesting a correlation of hyperuricemia with systemic hypertension. However, the inhibition of XOR by allopurinol or febuxostat did not alter the progression of high blood pressure in deoxycorticosterone acetate-salt treated rats [22]. The results obtained in this animal model of hypertension suggest that a cause–effect relationship could not be established between hyperuricemia and hypertension.

The elevation of systolic blood pressure in ageing rats, which was associated with increased ROS production, appeared to be dependent on the vascular activity of XOR, not on NAD(P)H oxidase activity [23]. On the other hand, XOR inhibition by allopurinol treatment abolished the nitrite-induced dose-dependent blood pressure-lowering effects in spontaneously hypertensive rats, possibly because of the simultaneous inhibition of XOR-dependent nitrite reductase activity localised to the erythrocyte, whereas no effect was observed on the reduction of nitrite at the blood vessel wall [24].

In a comparative study, two groups of patients with similar increased cardiovascular risks but differing in the presence/absence of hyperuricemia were observed. Hyperuricemic patients exhibited impaired flow-mediated vasodilatation that was reversed to the level of controls through XOR inhibition by allopurinol treatment [25]. Allopurinol ameliorated endothelial function and reduced oxidative stress by inhibiting XOR and decreasing myeloperoxidase levels in patients with metabolic syndrome [26]. Also, an improvement of endothelial function and artery flow together with a decrease in circulating uric acid and markers of oxidative stress has been reported in a meta-analysis study investigating the outcomes of XOR activity inhibition in patients with, or at risk of, cardiovascular disease [reviewed in Ref. [27]].

Many experimental data indicate that a high level of serum uric acid is an independent risk factor for cardiovascular disease and is predictive of mortality if associated with diabetes and hypertension in metabolic syndrome. Indeed, this molecule is not inert and gives rise to free radicals by interacting with superoxide anion, NO, peroxynitrite and myeloperoxidase. Uric acid-derived free radicals may cause endothelial dysfunction and vasoconstriction by lowering NO availability and by stimulating the up-regulation of the renin/angiotensin pathway (Fig. 3). Also, these free radicals may induce the migration, proliferation, and production of monocyte chemotactic protein-1 by arteriolar smooth muscle cells [reviewed in Ref. [28]], at least in part mediated by uric acid–induced COX-2 expression [reviewed in Ref. [29]].

However, the epidemiological evidence that supports the role of uric acid as a marker of hypertension is thus far suggestive but inconclusive to confirm the usefulness of uric acid-lowering therapy in cardiovascular diseases and metabolic syndrome [reviewed in Ref. [30]].

4. Xanthine oxidoreductase activity in cardiovascular damage by smoking

XOR activity could be one of the links between cigarette smoking and cardiovascular diseases. The up-regulation of XOR by

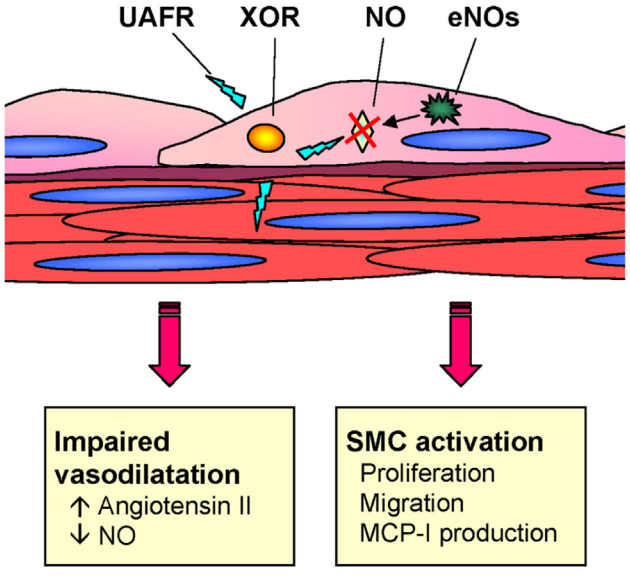

Fig. 3. Arteriolar effects of uric acid-derived free radicals (UAFR). Impaired vasodilatation may be caused by UAFR, which induce the up-regulation of the renin/angiotensin pathway and decrease nitric oxide (NO) availability. Furthermore, UAFR may activate smooth muscle cells (SMC) by inducing the migration, proliferation, and production of monocyte chemotactic protein-1 (MCP-1) [reviewed in Ref. [28]]. XOR, xanthine oxidoreductase; eNOs, endothelial nitric oxide synthase.

tobacco smoke condensate has been reported in cultured rat pulmonary endothelial cells after both acute (1 day) and chronic (7 days) exposures through an increase of XOR mRNA expression and XOR gene promoter activity [31]. Cigarette smoke exposure in hamsters elicits an early rise in XOR activity, followed by leukocyte adhesion to the endothelium of arterioles and post-capillary venules. Both responses are attenuated by ROS scavenging treatment, suggesting the implication of XOR in the pathogenesis of smoke-induced vascular alterations [32].

Increased pulmonary XOR activity and protein levels have been reported in mice exposed to cigarette smoke. Similar increased XOR expression was obtained in cultured pulmonary human lung microvascular endothelial cells after exposure to cigarette smoke extract. XOR activation and the consequent ROS production resulted in DNA double-strand break formation, which mediated the induction of endothelial cell apoptosis. Cell pre-treatment with allopurinol or febuxostat demonstrated that DNA damage was dependent on XOR activity, thus establishing a role for XOR in cigarette smoking-induced endothelial cell injury [33].

Allopurinol reversed the alteration of vessel resistance induced by smoking in subjects with no other risk factors for atherosclerosis, suggesting that XOR contributes to endothelial dysfunction caused by cigarette smoking [34,35].

5. Xanthine oxidoreductase activity in hypercholesterolaemia

Increased superoxide production by endothelial cells has been reported in diet-induced hypercholesterolemic rabbits, possibly due to XOR activity [36]. Accordingly, XOR activity inhibition decreased the superoxide production in aortic rings from hypercholesterolemic [36–38] or diabetic rabbits [39]. The increased XOR concentration in the plasma of hypercholesterolemic rabbits suggested a role for XOR in vascular dysfunction [38].

The vascular responses to infusion of an endothelium-dependent vasodilator and a direct smooth muscle dilator were studied in hypercholesterolemic and essential hypertensive patients. XOR inhibition with oxypurinol improved endothelial vasodilator function in hypercholesterolemic patients but not in hypertensive patients, suggesting that enzyme-generated ROS are partly responsible for the impaired vascular response of hypercholesterolemic patients, whereas ROS do not appear to play a significant role in essential hypertension [40]. Increased NAD(P)H oxidase and XOR activity has been reported in the coronary arteries of patients with coronary artery disease with respect to impaired vasodilatory response [41]. The early increase of vascular XOR activity has been described in asymptomatic young individuals with familial hypercholesterolaemia [42].

The role of XOR in atherosclerosis development was studied together with the effects of the XOR inhibitor allopurinol on this process in an ApoE knockout mouse model and in a murine macrophage cell line [43]. In vivo, the treatment with allopurinol lowered lipid accumulation and calcification in arteries. The inhibition of XOR activity or knockdown of *XOR* suppressed the *in vitro* transformation of macrophage cells into foam cells after treatment with lipoproteins, whereas the over-expression of *XOR* promoted this transformation.

6. Xanthine oxidoreductase activity in obesity and diabetes

The adipocytes of perivascular adipose tissue have been proposed to contribute to the pathogenesis of atherosclerosis through their ability to transduce signals to adjacent blood vessels, in particular modulating inflammation, vasoreactivity, and smooth muscle cell proliferation [reviewed in Ref. [44]]. The regulatory function of adipocytes that is most relevant to this review includes their sensitivity to insulin and the production of cytokines, which influence energy homoeostasis and metabolism in other tissues [45].

In rats, a high-fat diet induces an increase in body weight and in mean arterial blood pressure and elevated serum levels of insulin, cholesterol, and glucose. In obese rats, increased *XOR* expression was detected by immunostaining in the arteriolar endothelium. Moreover, the arterioles exhibited impaired responses to vasomotor stimulus, which was reversed by the inhibition of XOR but not of NAD(P)H oxidase activity. Additionally, increased superoxide production, associated with increased XOR, but not NAD(P)H oxidase activity was observed in rat carotid arteries, suggesting that XOR-derived superoxide production may increase peripheral resistance via altered arteriolar tone in high-fat diet-induced obesity [46].

Treatment with allopurinol decreases haemoglobin glycation, glutathione oxidation, and lipid peroxidation in type 1 diabetic patients, suggesting a role for XOR inhibition in the prevention of vascular oxidative stress and complications of diabetes [39]. Prolonged treatment with allopurinol normalised endothelial dysfunction in type 2 diabetic patients with mild hypertension compared with matched controls. Both groups received intra-arterial infusions of endothelium-dependent and endothelium-independent vasodilators, and forearm blood flow responses were measured after bilateral venous occlusion [47]. Moreover, XOR activity was implicated in the pathogenesis of diabetes consequences because treatment with metformin significantly decreased serum XOR activity and molecular markers of oxidative stress in diabetic patients [48].

Increased plasma XOR activity was significantly correlated to NF-kB activation and high levels of inflammatory markers and insulin resistance in familial combined hyperlipaemia [49]. The hypothesis has been formulated that an imbalance in both oxidative stress and oxidative deficiency could lead to insulin resistance [50]. Also, increased plasma XOR activity has been reported in association with a single exercise session in patients with metabolic syndrome as compared to control subjects, suggesting the involvement of XOR in the pathogenesis of metabolic syndrome [51].

XOR-derived ROS have been proposed amongst the main actors in the interaction between oxidative and nitrosative/nitrative stress, that may promote inflammation and may lead to myocardial dysfunction and diabetic cardiomyopathy [reviewed in Ref. [52]].

7. Role of xanthine oxidoreductase activity in adipogenesis and differentiation

XOR expression significantly increased in fibroblastic-like 3T3-L1 pre-adipocytes at an early time frame during the commitment to differentiate into a mature adipocyte. Shortly after, increased XOR activity levels were detected, primarily in the dehydrogenase form, whereas the level of the oxidase form was unchanged. XOR mRNA levels correlated *in vivo* with adipose mass: obese ob/ob mice exhibited a high enzyme level, which decreased after treatment with leptin, and *XOR* null mice exhibited decreased adiposity [53]. In *XOR* knockout 3T3-L1 cells, reduced lipid accumulation was observed in relation with the extent of the inhibition of adipocyte differentiation. Adipogenesis appeared to be dependent on the activation of the transcription factor PPARγ by the NADH-oxidizing activity of XOR [53].

These results are consistent with previous findings regarding defective fat-droplet secretion in *XOR* knockout mice, suggesting a role for XOR in the production and exportation of milk-fat globules during lactation [54]. Moreover, XOR gene knockout mice exhibited renal tubule injury with an accumulation of triglycerides that was possibly related to an increased expression of adipogenesis genes

[55]. Additionally, *XOR* null mice exhibited increased endothelial–mesenchymal transition associated with interstitial fibrosis [55], supporting the existence of a maturation/differentiating function associated with the XOR gene and with the production of oxygen radicals or hydrogen peroxide [reviewed in Ref. [56]], which is in agreement with the reported low *XOR* expression levels in anaplastic cancer cells [reviewed in Ref. [57]].

Despite the differences between the purine metabolism of humans and mice, these findings suggest a role for XOR in the regulation of adipogenesis. The pharmacologic activation of PPARγ by bezafibrate may improve insulin sensitivity by up-regulating adipogenesis, thus decreasing free fatty acid levels and reversing insulin resistance. For this reason, the therapeutic use of bezafibrate has been suggested in patients with atherogenic dyslipidemia and/or metabolic syndrome to reduce the risk of cardiovascular events and to delay the onset of type 2 diabetes mellitus [reviewed in Ref. [58]].

The physiological and pathological roles of XOR in adipogenesis and differentiation are presented in Fig. 4.

8. Conclusions

This study overviews the role of XOR as a systemic modulator of redox equilibrium by giving rise to both oxidant and anti-oxidant products. This function is performed by endothelial cell-bound XOR together with NAD(P)H oxidase and NO synthase activity. These enzymes play a physiologic role in the inflammatory signalling and regulation of vascular function, to which XOR contributes directly through the generation of ROS and uric acid and also indirectly with uric acid-derived free radicals. Additionally, XOR products are associated with cell differentiation, leading to adipogenesis in different experimental and clinical settings, as well as to the production of monocyte chemoattractant protein-1 from arterial smooth muscle cells after proliferation and migration. The data collected and analysed here indicate that XOR activity is associated with hypertension, cigarette smoking, dyslipidemia and diabetes and could play a role in the pathogenesis of endothelium damage induced by the main risk factors for atherosclerosis. Although, no conclusive results have been obtained thus far, evidence is growing regarding the beneficial effects of lowering the serum level of uric acid in metabolic syndrome. The interconnection between XOR, NAD(P)H oxidase and NO synthase activities may justify the variable results obtained in clinical trials utilizing XOR inhibitors and suggests the opportunity of taking in consideration the cross-talk between these enzymes in designing future experimentations. The knowledge of the intricate interplay of oxidative/nitrosative stress with inflammation is essential to the understanding of atherosclerosis pathogenesis and is crucial to develop novel targeted therapies for metabolic syndrome.

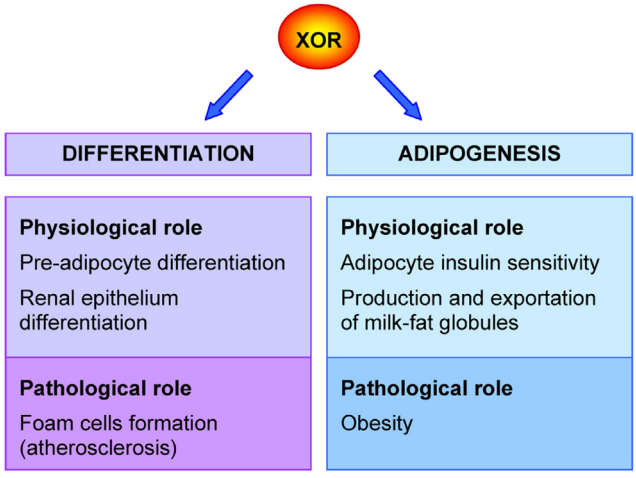

Fig. 4. Roles of xanthine oxidoreductase (XOR) in differentiation and adipogenesis. Experimental studies demonstrate that XOR activity has physiological functions in promoting differentiation and adipogenesis [53–55] as well as pathological effects by inducing the transformation of macrophages to foam cells [43] and fat over-accumulation [53].

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Conflict of interest

There are no conflicts of interest.

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