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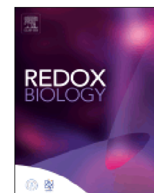
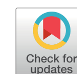


Xanthine oxidase-lactoperoxidase system and innate immunity: Biochemical actions and physiological roles



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ABSTRACT

The innate immune system in mammals is the first-line defense that plays an important protective role against a wide spectrum of pathogens, especially during early life before the adaptive immune system develops. The enzymes xanthine oxidase (XO) and lactoperoxidase (LPO) are widely distributed in mammalian tissues and secretions, and have a variety of biological functions including in innate immunity, provoking much interest for both *in vitro* and *in vivo* applications. The enzymes are characterized by their generation of reactive oxygen and nitrogen species, including hydrogen peroxide, hypothiocyanite, nitric oxide, and peroxynitrite. XO is a major generator of hydrogen peroxide and superoxide that subsequently trigger a cascade of oxidative radical pathways, including those produced by LPO, which have bactericidal and bacteriostatic effects against pathogens including opportunistic bacteria. In addition to their role in host microbial defense, reactive oxygen and nitrogen species play important physiological roles as second messenger cell signaling molecules, including cellular proliferation, differentiation and gene expression. There are several indications that the reactive species generated by peroxide have positive effects on human health, particularly in neonates; however, some important *in vivo* aspects of this system remain obscure. The primary dependence of the system on hydrogen peroxide has led us to propose it is particularly relevant to neonate mammals during milk feeding.

1. Introduction

The mammalian immune system comprises two parts: adaptive (or acquired) immunity and innate immunity. Adaptive immunity comprises specific antigen-antibody binding and long-lived memory T-cells and B-cells. The memory feature of the adaptive immune system allows it to react rapidly to a previously recognized pathogen, however, its response to any new antigen exposure may take days or weeks [1]. The innate immune system, as the first line of host defense against pathogens, does not require prior exposure to specific pathogens or antigens and is rapid but nonspecific. Innate immunity provides barriers to infection for the skin and the linings of the gastrointestinal, respiratory, and genitourinary tracts, and consists of antimicrobial peptides and enzymes (e.g. lactoferrin, lysozyme, and oxidative enzymes), as well as cellular components (e.g. neutrophils and dendritic cells) [2].

Oxidative enzymes play important roles as part of host innate immunity [3–5]. These enzymes are able to generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) through oxidoreductive mechanisms. Reactive free radicals are produced as byproducts of metabolism and turnover in cells and secretions. These are tightly regulated, as excess levels of these molecules result in peroxidation of proteins, lipids, and nucleic acids, thereby leading to cell death [6,7]. However, when at physiological concentrations they also have important roles in cell signaling [8,9] in addition to destroying invading pathogens [10,11]. For example, NADPH oxidase mediates the release of an oxidative burst of ROS in response to infection, a mechanism utilized for the elimination of pathogens particularly by neutrophils [12].

The enzyme xanthine oxidase (XO) acts together with lactoperoxidase (LPO) to generate ROS and RNS [4,13]. These two enzymes comprise what can be referred to as the 'XO-LPO system' [14]. Hydrogen peroxide (H_2O_2) and superoxide are generated by XO, and these are antimicrobial agents alone, or H_2O_2 activates secreted LPO to oxidize the pseudo-halide substrate thiocyanate (SCN^-) or other physiological halides, to produce potent antimicrobial agents such as hypothiocyanite ($OSCN^-$). Table 1 shows the reported concentrations of XO-LPO system components in some human body fluids and in bovine milk, while the major XO-LPO pathway and reactions are summarized

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Table 1
Concentrations of XO-LPO components in some human body fluids and bovine milk.

Components	[a]Concentration	Sample type	Number of subjects	Ref
Breastmilk				
XO (mU/mL)	0.52	Mature milk	7	[98]
	8	Colostrum	24	[40]
	0.91	Mature milk	14	[39]
H_2O_2 (μM)	25	Mature milk	60	[51]
	27	Mature milk	22	[40]
SCN (μM)	76	Mature milk	10	[99]
	80	Mature milk	45	[47]
	52	Mature milk	75	[100]
LPO (mU/mL)	3.28	Colostrum	45	[47]
	0.89	Mature milk (whey milk)	13	[101]
	0–12.6	Colostrum	4	[102]
Bovine milk				
XO (mU/mL)	20	Mature milk	8	[13]
	49	Mature milk	33	[103]
	80	Fresh mature milk	3	[39]
H_2O_2 (μM)	280	Fresh mature milk	7	[13]
SCN (μM)	86	Mature milk	7	[104]
	60	Mature milk	7	[13]
	36	Mature milk	2059	[105]
LPO (mU/mL)	5478	Mature milk	9	[106]
	4000	Mature milk	8	[13]
	2300	Mature milk	7	[104]
Neonates & Infants saliva				
XO (mU/mL)	[b] No activity			
H_2O_2 (μM)	[b]Not detected			
SCN (μM)	285	Unstimulated	75	[47]
	380	Unstimulated	31 (infants)	[54]
	420	Unstimulated	60 (neonates)	[40]
LPO (mU/mL)	0–34	Unstimulated	63 (neonates)	[47]
	474	Unstimulated	31 (infants)	[54]
	7	Unstimulated	12 (neonates)	[40]
Adult saliva				
XO (mU/mL)	No activity			
H_2O_2 (μM)	8–13	Calculated	Calculated	[65]
SCN (μM)	672	Unstimulated/ nonsmokers	20	[107]
	990	Unstimulated/ smokers	39	[108]
	643	Unstimulated/ nonsmokers	10	[109]
LPO (mU/mL)	573	Unstimulated/ nonsmokers	16	[110]
	711	Unstimulated/ nonsmokers	39	[108]
	620	Unstimulated/ nonsmokers	12	[40]

[a] All concentrations are expressed as the mean values unless otherwise specified.
[b] Sourced through breastfeeding.

in Fig. 1.

Thus, XO and LPO are classes of enzymes that generate ROS and RNS [4,13], acting together as a primal immunity function. The components of the system are: XO substrates, XO enzyme, H_2O_2 generated by XO, secreted LPO, and its pseudo-halide substrate SCN^- (or other halides). The system is active only in the presence of all of these components.

The aim of this review is to highlight the key biochemical actions, physiological roles, and health implications of the XO-LPO system as part of the natural host defense innate immunity, describing the enzymes' structures, reactions and mode of innate immunity action in some important biological fluids and secretions. In addition, it points out further investigations needed in the field of mammalian innate immunity.

2. Xanthine oxidase structure and reaction

Xanthine oxidase (XO; E.C. 1.17.3.2) has been studied for more than 100 years [15]; it has attracted attention in research partly because of its ability to generate ROS and RNS, and it is a major source of H_2O_2. Previously, research into the generation of reactive free radicals in the body has focused on the pathogenesis of ischemia-reperfusion injury [16]. However, free radical species produced by XO have been found also to fulfil important physiological roles in innate immunity. XO is widely distributed in mammalian tissues, where the highest enzyme activity in humans is found in the small intestine and liver, with very low levels of mRNA expression reported in major organs such as the heart, brain, lung and kidneys [17]. Interestingly, XO is highly expressed in the milk, with bovine milk having the highest activity of XO among the mammalian species examined [18].

XOR is a homodimer with a molecular mass of approximately 300 kDa. Each subunit contains a single peptide chain of approximately 1330 amino acids, which binds one molybdopterin cofactor (Mo-co), two nonidentical Fe_2S_2 centers, and one FAD cofactor. Proteolytic cleavage of XOR has demonstrated that the different cofactors are located in the C-terminal 85 kDa, N-terminal 20 kDa and intermediate 40 kDa domains, respectively (for a review of XOR structure, see Ref. [19]).

XO and xanthine dehydrogenase (XDH) are two forms of the xanthine oxidoreductase (XOR) enzyme expressed by the same gene [17]. The enzyme is synthesized as XDH, which uses NAD^+ as the electron acceptor, but can be readily converted reversibly to XO by oxidation of sulfhydryl residues, or irreversibly by proteolysis [20]. XDH exists mainly in intracellular spaces, while in the extracellular secretions and circulatory system XO is the predominant form [21].

Intracellularly, XDH plays a major role in the final stages of catabolism of purine nucleotides by catalyzing the oxidation of the bases hypoxanthine and xanthine to uric acid, the end-product of purine catabolism in humans (Fig. 1) [22]. The reaction occurs at the Mo-co site in which electrons are transferred via the two Fe_2S_2 domains to the FAD site, where NAD^+ is reduced by XDH to form NADH [23]. Furthermore, it has been shown that in the presence of NADH and low oxygen concentrations (< 5%), XOR can also reduce organic and inorganic nitrate (NO_3^-) and nitrite (NO_2^-) to generate nitric oxide (·NO) [24], although xanthine is a more effective reducing substrate than NADH [25]. Nitrite reduction takes place at the molybdenum site of the enzyme, unlike the reduction of NAD^+ (and molecular oxygen) which has been long known to occur at the FAD cofactor site [26]. Extracellularly, oxygen is reduced by XO to yield H_2O_2, which can then be used by LPO.

3. Lactoperoxidase structure and reaction

Lactoperoxidase (LPO; E.C. 1.11.1.7) is a member of the heme-containing peroxidase enzyme family [27]. All mammalian peroxidases consists of 576–738 amino acids, with a covalently attached heme moiety in the center [27,28]. The primary structure of LPO has been described as a globular single-chain monomer of approximately 80 kDa for human milk [29], and 78 kDa for bovine milk [30]. Some studies portray LPO as a scavenger enzyme, which acts to decrease the concentration of H_2O_2 in cells and secretions and consequently to prevent cellular oxidative stress and membrane peroxidation [31]. Other studies have described LPO as an essential part of the mammalian natural host defense system because of its ability to generate a highly reactive pseudohalide, $OSCN^-$, which exhibits antimicrobial effects against bacteria, fungi and viruses [3]. LPO is mainly found in secretions

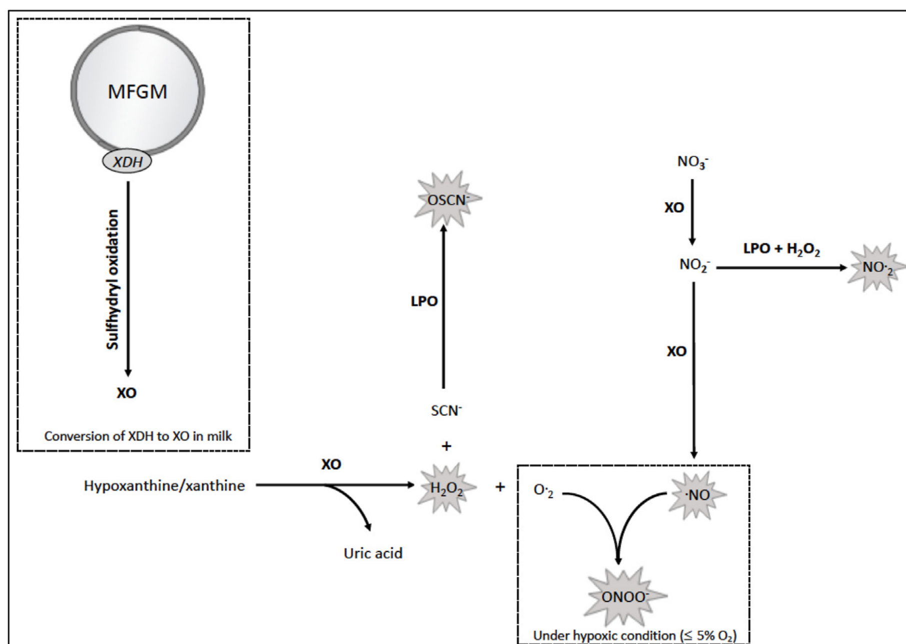
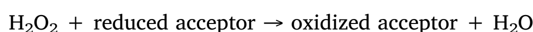


Fig. 1. The major XO-LPO system pathway and metabolism. XDH is synthesized but then converted to XO during secretion, in the milk fat globule membrane (MFGM). The system is associated with a wide range of antimicrobial (✹) ROS and RNS. The system is triggered by its main substrates, which include the purines hypoxanthine/xanthine, thiocyanate, nitrates, and nitrites.

including tears, saliva, airway mucus, and milk [32].

The LPO heme moiety comprises a heterocyclic protoporphyrin ring holding a ferric ion in the center [27]. All peroxidases catalyze a similar reaction, as shown in the general formula below:



$$H_2O_2 + \text{reduced acceptor} \rightarrow \text{oxidized acceptor} + H_2O$$

The reduced acceptor in this scheme is typically a halide or pseudohalide, which is converted to an oxidized hypohalide or hypothiocynous acid [5]. The enzymatic mechanisms of LPO are complex. Briefly, the reaction is initiated when H_2O_2 oxidizes the ferric (Fe^{3+}) moiety of the porphyrin from its resting state to the ground state, Fe = O. This 'compound I' state may then enter either a 'halogenation' or a 'peroxidation' cycle. In the halogenation cycle, physiological concentrations of reduced acceptor (halides such as $Cl^-/Br^-/I^-/NO_2^-$ or the pseudo-halide SCN^-) react with compound I to form Compound II (Fig. 1). The iron-bound oxygen is then transferred to form the oxidized acceptor (hypothiocyanite or a hypohalide ion), and LPO continuously returns at a low rate to its resting enzymatic state. In the presence of excess, non-physiological H_2O_2 concentrations, Compound II can also react to form Compound III, producing complete irreversible inactivation of LPO. It has been shown, based on the LPO kinetics and reaction with its substrates, that SCN^- is the preferred substrate, followed by halides I^-, Br^-, and Cl^- (for a review of the LPO reaction see Ref. [3]). SCN^- is distributed in mammalian extracellular fluids including plasma, saliva, tears, milk, gastric juice and trachea secretions [33].

4. Innate immunity role of the XO-LPO system

4.1. XO-LPO system in milk

XO and LPO are both expressed in milk, to varying degrees in different mammalian species, and are capable of generating free radical species. XO is an abundant enzyme in human milk and is present in all mammalian milks investigated, with bovine milk containing the highest activity relative to human milk (Table 1) [34]. XO is secreted in association with milk fat globule membrane (MFGM) and is generally a major protein component of MFGM in mammals [35]. In bovine milk, the enzyme is located in two major sites: the inner MFGM contains a mixture of XO and predominantly XDH, while a second pool of XO is attached to the outer MFGM and in the milk serum [36]. This suggests that XDH is converted into XO by oxidation of a sulfhydryl residue after the milk fat droplets are excreted into the mammary gland lumen [37,38]. It has been demonstrated that approximately 90% of human milk XOR is found in the demolybdo state and this could lower H_2O_2 production from the oxidation of purines. It may also produce H_2O_2 via oxidation of NADH to NAD^+ at the FAD but the yield will be both considerably less and dependent upon the steady-state of NADH. However, the activity of XO in human milk was assayed *in vitro* using the purine bases xanthine and hypoxanthine, and the enzyme was able to produce H_2O_2 particularly in colostrum compared to that in subsequent milk [39–41]. It has been found that XOR mRNA transcripts are increased in mice mammary alveolar epithelial cells during the second trimester of pregnancy and are significantly amplified at the beginning of lactation, and then decline with induced involution [42], suggesting that the secretion of the enzyme in milk is regulated at a genetic level. Interestingly, lactation and the administration of oxytocin were found to enhance both XDH and XO levels, and were associated with increased H_2O_2 generation in the milk of lactating rats, with the level of XO being two-to three-fold higher compared to those in non-lactating rats [43].

LPO is also secreted from the mammary glands, into milk serum. This enzyme is the second most abundant enzyme in bovine milk after XO [44] and is commonly present in ruminant [45] and human [46] milk throughout lactation. Bovine milk contains approximately 1–19 U/ml of LPO, which is much higher than in human milk (Table 1). Similar to XO, human milk LPO has the highest activity in colostrum then declines gradually throughout the lactation period [47].

Researchers have demonstrated the presence of H_2O_2 in freshly-expressed bovine and human milk in a pattern consistent with the expression of XO in mammary glands. It has been shown that the substrates of XO, hypoxanthine and xanthine, are secreted in bovine milk [48]. In one study, a fresh milk sample was obtained, and then after emptying the udder, cows were injected with a dose of oxytocin to stimulate additional fresh milk secretion. These fresh milk samples were immediately analyzed and found to contain approximately 40 μM of both xanthine and hypoxanthine. These XOR substrates were then consumed within 30 min, and this effect was suppressed by the addition of allopurinol, an XO inhibitor. These findings demonstrate that xanthine and hypoxanthine are continuously secreted by mammary glands and subsequently oxidized by XO to generate H_2O_2. Simultaneously, the XO product uric acid is significantly increased in milk serum. Thus it may be presumed that peroxide provides the mammary glands with

continuous innate immunity protection against invading pathogens and mastitis [13].

In addition to the role of XO as a generator of the antibacterial H_2O_2, the enzyme has been suggested to be a physiological source of peroxide substrate for LPO in milk. It has been suggested that XO can generate as much as 360 μM/h H_2O_2 in bovine milk [48], making XO a major physiological source of this oxidant in the mammary glands and milk. The H_2O_2 is then metabolized by LPO to oxidize SCN^- into the potent bactericidal $OSCN^-$ [32].

Studies have shown that the XO-LPO system also extends its microbiocidal effect in milk through the synthesis of reactive nitrogen species (RNS). Enzymatically, XO can convert nitrate (NO_3^-) to nitrite (NO_2^-), and the latter is an important substrate for formation of the nitric oxide free radical (·NO) by the action of XO and under hypoxic condition [49]. Simultaneously, LPO can convert NO_2^- into the potent microbiocidal radical, nitrogen dioxide ($NO_2^{\cdot}$); this reaction step requires the presence of H_2O_2, supplied by XO [50]. It has been shown that with regard to the generation of RNS by the XO-LPO system, ·NO and $NO_2^{\cdot}$ have an inhibitory effect against the major invasive mammary gland pathogens such as *Escherichia coli* and *Staphylococcus aureus* in bovine milk [13]. Thus, the interactions between the XO and LPO enzymes play a crucial role in innate immunity and exert a more powerful microbiocidal function when they are present together.

This suggests that breastmilk is a source of significant factors essential for the natural host defense mechanism for the mammary glands and for the suckling infant. Surprisingly, the importance of the milk XO-LPO system for mammary gland resistance to infection, and its positive effect on neonatal and infant health, has only been partially investigated *in vivo* [40,43,47]. In humans, freshly-expressed breastmilk has been found to contain H_2O_2 of 25–27 μM [40,51], about 10 times lower than bovine milk [48]. The highest concentrations of H_2O_2 in breastmilk have been found in colostrum, with the concentration declining until it reached a mean of about 9 μM in the fourth week of lactation [51]. It is worth noting that although the XO-LPO activity of human milk is lower than that of bovine milk, the presence of peroxide in fresh breastmilk suggests it uses the same mechanism as bovine milk, i.e. the secretion of substrates xanthine and hypoxanthine by the mammary gland, with subsequent reaction with XO to produce H_2O_2. *In vitro* addition of serial concentrations of exogenous hypoxanthine to fresh breastmilk showed a significant dose dependent inhibitory effect against *E. coli* and *S. aureus* [41]. It has been demonstrated that the concentrations of human milk NO_3^- and NO_2^- are relatively higher during early days of lactation, suggesting that elevated RNS concentrations are also important for innate immune defense in mammary glands, and in the stomachs of breastfed neonates [52].

Thus, the XO-LPO system is a key innate immunity component of milk against invading pathogens and common infectious diseases such as mastitis [40]. It has an equally essential antibacterial protective role for neonates and infants in the upper gastrointestinal tract, as discussed below.

4.2. XO-LPO system in saliva

Human whole saliva contains a variety of antimicrobial factors that work together to regulate the colonization of oral microorganisms and fight against pathogens [53]. In human neonates, salivary LPO activity was identified a few hours after delivery, albeit with high inter- and intra-individual variability. LPO activity was shown to be raised throughout the first week after birth, followed by a gradual decrease with increasing age of infants [47]. While the LPO substrate SCN^- has been found in infants' saliva, with higher levels found in neonatal saliva, presumably deriving from the mother's circulation [40,54], there was doubt about the availability of H_2O_2 as an essential substrate for LPO. Studies have demonstrated that in adults, H_2O_2 can be provided for LPO by oral bacteria [55,56]. Production of H_2O_2 in adult saliva mainly occurs through the action of the metabolic processes of the oral microbiota. Streptococcal species such as *S. mutans, S. mitis* and *S. sanguinis* have been found to be important in H_2O_2 generation [55,57]. It has been demonstrated that H_2O_2 produced by *S. mitior* is able to activate LPO using synthetic saliva [58]. In contrast, bacterial colonization and formation of biofilms may not be well established in the neonatal mouth. Therefore the presence of milk XO as a generator of H_2O_2 may be of critical importance as a selective pressure for mammals within the early neonatal period.

Recently, the XOR substrates hypoxanthine and xanthine were found to be at higher concentrations in saliva of neonates than adults [40,59]. It was shown that human milk, which contains XO, generated H_2O_2 when mixed *in vitro* with neonatal saliva, and this mixture exhibited an inhibitory effect against opportunistic bacteria such as *S. aureus* and *E. coli*. This bacterial inhibitory effect was cancelled by oxypurinol, an inhibitor of XO. It is probable that the observed microbiocidal activity was not only caused by H_2O_2 generation, but also by the activation of LPO present in both saliva and milk [40]. Nevertheless, several studies have reported that oral streptococci are highly susceptible to H_2O_2 by itself, with complete inhibition or killing at concentrations as low as 100 μM [55,57,60].

Thus, in addition to H_2O_2 present in freshly-expressed breastmilk, the H_2O_2 generated by breastmilk-saliva interaction may further activate LPO in the neonatal mouth during suckling, so providing a host defense mechanism that could have crucial implications for infant health [40]. The presence of XO in breastmilk therefore has a function beyond antibacterial protection of mammary glands. While it has been demonstrated that the microbiocidal activity of breastmilk-saliva is selective against opportunistic pathogens, commensal bacteria were found to be insensitive to this inhibitory effect [40,60]. Since the interaction of breastmilk with neonatal saliva has a host defense role, it has been suggested that this mechanism could then affect the colonization of the gut by oral bacteria during early infancy, creating distinct microbiota patterns between breastfed and formula-fed infants [61,62]. Of course, the variations in infant oral and gut microbiota cannot be attributed solely to the XO-LPO system, as saliva and breastmilk contain other antibacterial and growth-enhancing factors, including amino acids, organic acids, nucleotides, proteins, enzymes, and immunoglobulins. There is also the contribution of skin bacteria from the breast to the breastfed oral bacteria, as compared with bottle-fed babies [61].

Another source of H_2O_2 may exist within salivary glands. Saliva collected directly from these glands has been shown to contain $OSCN^-$, indicating that H_2O_2 in the cells of the glands can oxidize SCN^- via LPO prior to excretion into the oral cavity [63,64]. Although H_2O_2 has not been directly measured in saliva, because of the presence of active scavenging systems, the concentration of H_2O_2 in adult unstimulated saliva prior to secretion has been estimated to be 8–13 μM, increasing to 18 μM in stimulated saliva [65]. It has been found that mRNA of Duox2, an isoform of dual oxidase that produces H_2O_2, is highly expressed in human and rat salivary glands, including the excretory ducts that showed high oxygen demand for generation of ROS [66]. These findings suggest that H_2O_2 is continuously synthesized in salivary glands and secreted (increasing with stimulation) into the oral cavity to activate salivary peroxidase.

The concentration of SCN^- in adult saliva is higher than in neonates and infants and shows less inter- and intra-individual variation [40]. The highest and most stable level of salivary LPO was observed in a group of subjects aged 10–24, and then the activity decreased with age [67]. In the context of adult oral health, LPO provides one of the main mechanisms of anti-caries defense, regulating the composition of microbiota and preventing the growth of pathogens present in periodontitis. The biochemical roles of LPO and its physiological products have been tested in several clinical trials aiming to produce an enriched preparation as an oral hygiene treatment. Bacterial biofilm formation on the teeth is one of the most common causes of caries formation, as bacteria ferment dietary carbohydrates into organic acids. It has been

shown that LPO is effective in the prevention of dental cariogenic biofilm formation. LPO, together with lysozyme, lactoferrin, and immunoglobulins, is able to inhibit the formation of *S. mutans* biofilms, the most common cause of dental caries. In cases of multispecies biofilms, the application of toothpaste containing LPO has been shown to produce significant decreases in microbial viability, but no decrease in biofilm integrity. A similar finding was observed when using enzymatic toothpastes with children, with the bioactivity of *S. mutans* being significantly decreased [68].

Thus, it can be concluded that there are four major sources of H_2O_2 in the human oral environment that contribute to the activation of LPO. First, secretions collected directly from the adult salivary glands contain $OSCN^-$ [63] indicating that H_2O_2 is generated in the glands prior to excretion into the oral cavity, as evidenced by Geiszt, M. et al. [66]. Second, when whole adult saliva is incubated *in vitro*, the $OSCN^-$ concentration increases because of H_2O_2 production by oral bacteria, primarily streptococci [55,56]. Third, freshly-secreted milk provides neonates with H_2O_2 produced within mammary glands by the action of XO [40,51]. Finally, the XO substrates xanthine and hypoxanthine are secreted into neonatal saliva, and react with breastmilk XO during suckling to generate H_2O_2 [40].

4.3. XO-LPO system in the gastrointestinal tract

H_2O_2 generated by milk XO, as well as milk/salivary LPO and its oxidative products, may contribute to innate immunity within the gastrointestinal tract (GIT), particularly in neonates [40,41]. Cieslak M. et al. reported the presence of H_2O_2 in rat fresh milk curds, suggesting that its gastric biological activity is continued [43]. Studies on mice demonstrated that orally-administered LPO was effective in preventing infection with influenza virus [69] and reducing colitis [70]. Furthermore, Millar TM et al. showed that XO can catalyze the reduction of NO_3^- and NO_2^- to ·NO under hypoxic conditions, such as the gut [24]. This has lead to the proposal that the neonatal stomach provides a suitable environment for peroxidative reactions to take place [41], noting that neonatal stomach pH ranges between 4 and 6, with a hypoxic oxygen tension equal to or below 5% [71].

In the presence of superoxide from milk XO, ·NO generates the powerful microbiocidal peroxynitrite ($ONOO^-$). Generation of ·NO was demonstrated *in vitro* with samples of breastmilk collected from a full-term mother between days 2 and 112 postdelivery, under conditions similar to that in the neonatal stomach, i.e. oxygen less than 5%, pH 5.5, 37 °C. The highest levels of ·NO were measured in milk from the first 3 weeks postpartum, while no activity was found in any milk formulae [41]. ·NO derives from nitrite (NO_2^-), which is normally present in the gut from the metabolism of enteric bacteria [72,73]. Thus, the XO-LPO system may contribute to neonatal GIT innate immunity by playing an important role in the prevention of opportunistic infections and selection in favor of commensal microbiota.

XO has long been known to be present in high activity in the small intestine, being highly expressed in both basal and apical layers of the intestinal villi. Rat digestive tract studies also indicated that the enzyme is highly active in intestinal goblet cells, enterocytes, and duodenum mucus secretions. The presence of XO in the upper small intestine, particularly on the surface of epithelial cells, suggests an innate immunity role, in addition to its traditionally assigned role of catabolizing dietary purine bases to uric acid. The immunity hypothesis was supported by Van Den Munckhof et al., who found that bacteria surrounded by the enzyme showed signs of membrane destruction and cell toxicity death [74].

XOR has also been shown to be present in the epithelial cells of the bile duct, where its highest activity is detected in the luminal surface. The enzyme has been shown to be secreted into human and rat bile, where it may act as an innate immunity agent to protect the biliary system and upper gut from infection [75]. These studies did not clearly confirm if XOR is present in the form of XO or XDH when secreted, however Van Den Munckhof et al. [74] demonstrated that ROS generation on the intestinal apical surface layers and in the secretions was complete inhibited by allopurinol. It has been found that the form of the enzyme in organs other than liver of the rat can be reversibly converted between XO and XDH by sulfhydryl oxidation, except for the intestine where the luminal proteolytic enzymes rapidly and irreversibly convert XDH to XO [76].

4.4. XO-LPO system in cellular signaling

In addition to the direct role of XO-LPO enzymes as an antimicrobial system, the generation of a wide range of ROS and RNS under various conditions has important roles in cellular signaling and growth when present at physiological concentrations.

In eukaryotes, H_2O_2 is able to activate signaling transduction, to stimulate cellular proliferation [77], migration [78], regulation of gene expression [79,80], differentiation [81], apoptosis [82], and T-cell activation [83]. Pan et al. showed that low H_2O_2 concentrations stimulated cell proliferation and enhanced adhesion, migration, and wound healing in cornea cells [84]. Fajardo et al. found that H_2O_2, at a concentration similar to that in human milk, enhanced gastric motility and emptying in rat neonates, a finding that directly implicates XOR in neonatal health and wellbeing [85].

In addition, ·NO has a well-known regulatory role in the control of smooth muscle relaxation, platelet aggregation, and neurotransmission (for reviews see Ref. [86–88]). This suggests that both ROS and RNS produced by the XO-LPO system may also provide these active metabolites in appropriate concentrations for rapid cell signaling and growth. These metabolites may come from breastmilk, saliva or from the upper GIT, to be utilized by gastrointestinal cells for proliferation and growth, and hence improve neonatal immunity and gut motility at a time when other mechanisms are not fully developed. This mechanism of cell growth and signaling related to the XO-LPO system has not been fully investigated, and research into this field may have important implications for the development of normal neonatal immunity and health.

4.5. Exploitation of the XO-LPO system for preservation of bovine milk

In the dairy industry, the microbial quality of raw milk is usually strictly controlled during collection, storage and transportation, through the use of cooling systems. However, refrigerated facilities may not be available in rural areas of developing countries, for technical, economical, or practical reasons. As an alternative, the shelf life of raw milk can be significantly prolonged by the LPO reaction [89–91]. The UN Food and Agriculture Organization (FAO), in collaboration with the World Health Organization (WHO), has developed a protocol based on the reactivation of the endogenous milk LPO enzyme by the addition of sodium thiocyanate and hydrogen peroxide to maintain the hygienic quality of the milk without refrigeration [92]. However, preservation of raw dairy milk by activation of the entire XO-LPO system, for example by adding XO substrate (hypoxanthine), may provide a physiological alternative approach to dairy milk product preservation.

4.6. XO-LPO system and infant formulae

Preterm infants who are provided with formula feeding have greater susceptibility to GIT infections compared to those that are given human milk [93]. If preterm infants are fed pasteurized breastmilk, the milk will have low XO-LPO activity, no H_2O_2 [43], and markedly reduced levels of other milk defense proteins [94,95].

Since the activity of the XO-LPO system is absent from formula preparations as well as from pasteurized breastmilk [40,41], the absence of this antimicrobial defense mechanism in the neonatal mouth, and thus the GIT, may partially explain the greater susceptibility of bottle-fed infants to GIT infection. An additional factor is that saliva is

normally aspirated from the mouths of preterm babies, due to the absence of a mature swallowing reflex: this removes the salivary hypoxanthine and xanthine that activates the XO-LPO system in breastfeeding babies. It is also possible that the XO-LPO mechanism provides an additional protection against the risk of middle ear infection for babies if fed in a prone position [40]. Because the XO-LPO system generates a wide range of ROS/RNS that are important mediators and second messengers, as discussed earlier, the absence of these free radical species may also delay or affect the rapid growth of the gut cells in infants relying on formula feeding.

It has been shown that feeding bovine calves with preparations containing LPO enzymes is effective in curing diarrhea [96], while calves fed XO-supplemented formula showed greater increases in weight and a marked decreased incidence of infective scours compared to controls [97]. Thus, this review raises questions of whether supplementation of infant formula preparations with the XO-LPO system, for therapeutic purposes, could be a potentially important factor in clinical practice. Further studies and clinical trials are important to evaluate the benefits and risks of supplementation of infant formula with the XO-LPO system.

5. Conclusion

Apart from the roles of XO and LPO in normal catabolism, their physiological roles extend to participating in the innate immunity of mammals, particularly in the neonatal period. This arises from the capacity of the XO-LPO system to generate physiologically active concentrations of ROS and RNS, which have important roles as antimicrobial agents, and in cellular signaling. Although the studies reviewed in this article demonstrate that the microbiocidal activity of oxidative radicals produced at physiological concentrations by the XO-LPO system, and their role in cellular second messengers, most studies have been conducted *in vitro*, with only a few addressing these functions *in vivo*. Yet such research is important, especially with regard to their relevant physiological roles of oxidative radicals for the health of full-term breastfed babies, *versus* preterm neonates who receive pasteurized or formula milk that has an inactivated XO-LPO system. Several studies have now provided strong evidence for therapeutic use of the XO-LPO system, in particular by ensuring that the system is active in infant formula preparations. This will need additional studies and clinical trials for justification, but may have important positive outcomes for neonatal and infant health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101524.

References

[1] M. Riera Romo, D. Pérez-Martínez, C. Castillo Ferrer, Innate immunity in vertebrates: an overview, Immunology 148 (2) (2016) 125–139.
[2] M. Hamosh, Protective function of proteins and lipids in human milk, Neonatology 74 (2) (1998) 163–176.
[3] F. Bafort, O. Parisi, J.-P. Perraudin, M. Jijakli, Mode of action of lactoperoxidase as related to its antimicrobial activity: a review, Enzym. Res. 2014 (2014).
[4] C. Vorbach, R. Harrison, M.R. Capecchi, Xanthine oxidoreductase is central to the evolution and function of the innate immune system, Trends Immunol. 24 (9) (2003) 512–517.
[5] D. Sarr, E. Tóth, A. Gingerich, B. Rada, Antimicrobial actions of dual oxidases and lactoperoxidase, J. Microbiol. (Seoul, Korea) 56 (6) (2018) 373–386.
[6] K. Samardzic, K.J. Rodgers, Oxidised protein metabolism: recent insights, Biol. Chem. 398 (11) (2017) 1165–1175.
[7] J. Cadet, K.J. Davies, Oxidative DNA damage & repair: an introduction, Free Radic. Biol. Med. 107 (2017) 2–12.
[8] R. Breton-Romero, S. Lamas, Hydrogen peroxide signaling in vascular endothelial cells, Redox biol. 2 (2014) 529–534.
[9] A. Weidinger, A. Kozlov, Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction, Biomolecules 5 (2) (2015) 472–484.
[10] C. Kohchi, H. Inagawa, T. Nishizawa, G.-I. Soma, ROS and innate immunity, Anticancer Res. 29 (3) (2009) 817–821.
[11] F.C. Fang, Antimicrobial reactive oxygen and nitrogen species: concepts and controversies, Nat. Rev. Microbiol. 2 (10) (2004) 820.
[12] A.M. Franchini, D. Hunt, J.A. Melendez, J.R. Drake, FcγR-driven release of IL-6 by macrophages requires NOX2-dependent production of reactive oxygen species, J. Biol. Chem. 288 (35) (2013) 25098–25108.
[13] N. Silanikove, F. Shapiro, A. Shamay, G. Leitner, Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates, Free Radic. Biol. Med. 38 (9) (2005) 1139–1151.
[14] D. Al'perovich, E. Kesel'man, A. Shepelev, L. Chernavskaia, Free-radical mechanism of antimicrobial action of xanthine oxidase and lactoperoxidase, Biulleten Eksp. Biol. Meditsiny 114 (9) (1992) 272–274.
[15] V. Massey, C. Harris, Milk xanthine dehydrogenase: the first one hundred years, Biochem. Soc. Trans. 25 (1997) 750–755.
[16] D.N. Granger, P.R. Kvietys, Reperfusion injury and reactive oxygen species: the evolution of a concept, Redox biol. 6 (2015) 524–551.
[17] C.A. Pritsos, Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system, Chem. Biol. Interact. 129 (1–2) (2000) 195–208.
[18] R. Harrison, Milk xanthine oxidase: properties and physiological roles, Int. Dairy J. 16 (6) (2006) 546–554.
[19] R. Harrison, Structure and function of xanthine oxidoreductase: where are we now? Free Radic. Biol. Med. 33 (6) (2002) 774–797.
[20] E. Della Corte, F. Stirpe, The regulation of rat liver xanthine oxidase. Involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme, Biochem. J. 126 (3) (1972) 739–745.
[21] R. Hille, Structure and function of xanthine oxidoreductase, Eur. J. Inorg. Chem. 2006 (10) (2006) 1913–1926.
[22] R. Huber, P. Hof, R.O. Duarte, J.J. Moura, I. Moura, M.Y. Liu, J. LeGall, R. Hille, M. Archer, M.J. Romao, A structure-based catalytic mechanism for the xanthine oxidase family of molybdenum enzymes, Proc. Natl. Acad. Sci. U. S. A. 93 (17) (1996) 8846–8851.
[23] E.E. Kelley, N.K. Khoo, N.J. Hundley, U.Z. Malik, B.A. Freeman, M.M. Tarpey, Hydrogen peroxide is the major oxidant product of xanthine oxidase, Free Radic. Biol. Med. 48 (4) (2010) 493–498.
[24] T.M. Millar, C.R. Stevens, N. Benjamin, R. Eisenthal, R. Harrison, D.R. Blake, Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions, FEBS Lett. 427 (2) (1998) 225–228.
[25] B.L. Godber, J.J. Doel, G.P. Sapkota, D.R. Blake, C.R. Stevens, R. Eisenthal, R. Harrison, Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase, J. Biol. Chem. 275 (11) (2000) 7757–7763.
[26] H. Li, A. Samouilov, X. Liu, J.L. Zweier, Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrite reduction evaluation of its role in nitric oxide generation in anoxic tissues, J. Biol. Chem. 276 (27) (2001) 24482–24489.
[27] S. Sharma, A.K. Singh, S. Kaushik, M. Sinha, R.P. Singh, P. Sharma, H. Sirohi, P. Kaur, T.P. Singh, Lactoperoxidase: structural insights into the function, ligand binding and inhibition, Int. J. Biochem. Mol. Biol. 4 (3) (2013) 108.
[28] P.J. O'Brien, Peroxidases, Chem. Biol. Interact. 129 (1–2) (2000) 113–139.
[29] K. Shin, H. Hayasawa, B. Lonnerdal, Purification and quantification of lactoperoxidase in human milk with use of immunoadsorbents with antibodies against recombinant human lactoperoxidase, Am. J. Clin. Nutr. 73 (5) (2001) 984–989.
[30] F. Borzouee, M.R. Mofid, J. Varshosaz, S.Z.A.S. Shariat, Purification of lactoperoxidase from bovine whey and investigation of kinetic parameters, Adv. Biomed. Res. 5 (1) (2016) 189.
[31] S. El-Chemaly, M. Salathe, S. Baier, G.E. Conner, R. Forteza, Hydrogen peroxide-scavenging properties of normal human airway secretions, Am. J. Respir. Crit. Care Med. 167 (3) (2003) 425–430.
[32] K.D. Kussendrager, A. Van Hooijdonk, Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications, Br. J. Nutr. 84 (S1) (2000) 19–25.
[33] J.D. Chandler, B.J. Day, Thiocyanate: a potentially useful therapeutic agent with host defense and antioxidant properties, Biochem. Pharmacol. 84 (11) (2012) 1381–1387.
[34] N. Silanikove, U. Merin, G. Leitner, Physiological role of indigenous milk enzymes: an overview of an evolving picture, Int. Dairy J. 16 (6) (2006) 533–545.
[35] I. Mather, Proteins of the milk-fat-globule membrane as markers of mammary epithelial cells and apical plasma membrane, The mammary gland (1987) 217–267.
[36] N. Silanikove, F. Shapiro, Distribution of xanthine oxidase and xanthine dehydrogenase activity in bovine milk: physiological and technological implications, Int. Dairy J. 17 (10) (2007) 1188–1194.
[37] M.G. Battelli, E. Lorenzoni, F. Stirpe, Milk xanthine oxidase type D (dehydrogenase) and type O (oxidase). Purification, interconversion and some properties, Biochem. J. 131 (2) (1973) 191–198.
[38] M. Nakamura, I. Yamazaki, Preparation of bovine milk xanthine oxidase as a

dehydrogenase form, J. Biochem. 92 (4) (1982) 1279–1286.
[39] A.M. Brown, M. Benboubetra, M. Ellison, D. Powell, J.D. Reckless, R. Harrison, Molecular activation-deactivation of xanthine oxidase in human milk, Biochim. Biophys. Acta 1245 (2) (1995) 248–254.
[40] S.S. Al-Shehri, C.L. Knox, H.G. Liley, D.M. Cowley, J.R. Wright, M.G. Henman, A.K. Hewavitharana, B.G. Charles, P.N. Shaw, E.L. Sweeney, et al., Breastmilk-saliva interactions boost innate immunity by regulating the oral microbiome in early infancy, PloS One 10 (9) (2015) e0135047.
[41] C.R. Stevens, T.M. Millar, J.G. Clinch, J.M. Kanczler, T. Bodamyali, D.R. Blake, Antibacterial properties of xanthine oxidase in human milk, Lancet 356 (9232) (2000) 829–830.
[42] J.L. McManaman, C.A. Palmer, R.M. Wright, M.C. Neville, Functional regulation of xanthine oxidoreductase expression and localization in the mouse mammary gland: evidence of a role in lipid secretion, J. Physiol. 545 (Pt 2) (2002) 567–579.
[43] M. Cieslak, C.H. Ferreira, Y. Shifrin, J. Pan, J. Belik, Human milk H_2O_2 content: does it benefit preterm infants? Pediatr. Res. 83 (3) (2018) 687.
[44] J. De Wit, A. Van Hooydonk, Structure, functions and applications of lactoperoxidase in natural antimicrobial systems, Neth. Milk Dairy J. 50 (1996) 227–244.
[45] X. Ye, S. Yoshida, Lactoperoxidase and lactoferrin: changes in post partum milk during bovine lactational disorders, Milchwissenschaft 50 (2) (1995) 67–71.
[46] K. Shin, H. Hayasawa, B. Lönnerdal, Purification and quantification of lactoperoxidase in human milk with use of immunoadsorbents with antibodies against recombinant human lactoperoxidase, Am. J. Clin. Nutr. 73 (5) (2001) 984–989.
[47] L. Gothefors, S. Marklund, Lactoperoxidase activity in human milk and in saliva of newborn infants, Infect. Immun. 11 (6) (1975) 1210–1215.
[48] N. Silanikove, F. Shapiro, G. Leitner, Posttranslational ruling of xanthine oxidase activity in bovine milk by its substrates, Biochem. Biophys. Res. Commun. 363 (3) (2007) 561–565.
[49] H. Li, A. Samouilov, X. Liu, J.L. Zweier, Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrate reduction: evaluation of its role in nitrite and nitric oxide generation in anoxic tissues, Biochemistry (Mosc.) 42 (4) (2003) 1150–1159.
[50] A. Van Der Vliet, J.P. Eiserich, B. Halliwell, C.E. Cross, Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite a potential additional mechanism of nitric oxide-dependent toxicity, J. Biol. Chem. 272 (12) (1997) 7617–7625.
[51] E.A. Al-Kerwi, A.H. Al-Hashimi, A.M. Salman, Mother's milk and hydrogen peroxide, Asia Pac. J. Clin. Nutr. 14 (4) (2005) 428–431.
[52] T. Iizuka, M. Sasaki, K. Oishi, S. Uemura, M. Koike, M. Shinozaki, Non-enzymatic nitric oxide generation in the stomachs of breastfed neonates, Acta Paediatr. 88 (10) (1999) 1053–1055.
[53] W. van't Hof, E.C. Veerman, A.V.N. Amerongen, A.J. Ligtenberg, Antimicrobial defense systems in saliva, Saliva: Secretion and Functions, vol. 24, Karger Publishers, 2014, pp. 40–51.
[54] J. Tenovuo, O.P. Lehtonen, A.S. Aaltonen, P. Vilja, P. Tuohimaa, Antimicrobial factors in whole saliva of human infants, Infect. Immun. 51 (1) (1986) 49–53.
[55] J. Carlsson, Y. Iwami, T. Yamada, Hydrogen peroxide excretion by oral streptococci and effect of lactoperoxidase-thiocyanate-hydrogen peroxide, Infect. Immun. 40 (1) (1983) 70–80.
[56] L. Zhu, J. Kreth, The role of hydrogen peroxide in environmental adaptation of oral microbial communities, Oxid. Med. Cell Longev 2012 (2012).
[57] E.L. Thomas, T.W. Milligan, R.E. Joyner, M.M. Jefferson, Antibacterial activity of hydrogen peroxide and the lactoperoxidase-hydrogen peroxide-thiocyanate system against oral streptococci, Infect. Immun. 62 (2) (1994) 529–535.
[58] H. Donoghue, D. Hudson, C. Perrons, Effect of the lactoperoxidase system on streptococcal acid production and growth, J. Dent. Res. 66 (2) (1987) 616–618.
[59] S. Al-Shehri, M. Henman, B.G. Charles, D. Cowley, P.N. Shaw, H. Liley, A. Tomarchio, C. Punyadeera, J.A. Duley, Collection and determination of nucleotide metabolites in neonatal and adult saliva by high performance liquid chromatography with tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 931C (2013) 140–147.
[60] E. Sweeney, S. Al-Shehri, D. Cowley, H. Liley, N. Bansal, B. Charles, P. Shaw, J. Duley, C. Knox, The effect of breastmilk and saliva combinations on the in vitro growth of oral pathogenic and commensal microorganisms, Sci. Rep. 8 (1) (2018) 15112.
[61] S.S. Al-Shehri, E.L. Sweeney, D.M. Cowley, H.G. Liley, P.D. Ranasinghe, B.G. Charles, P.N. Shaw, D. Vagenas, J.A. Duley, C.L. Knox, Deep sequencing of the 16S ribosomal RNA of the neonatal oral microbiome: a comparison of breast-fed and formula-fed infants, Sci. Rep. 6 (2016) 38309.
[62] P.L. Holgerson, N.R. Vestman, R. Claesson, C. Öhman, M. Domellöf, A.C. Tanner, O. Hernell, I. Johansson, Oral microbial profile discriminates breastfed from formula-fed infants, J. Pediatr. Gastroenterol. Nutr. 56 (2) (2013) 127.
[63] K. Pruitt, B. Mansson-Rahemtulla, J. Tenovuo, Detection of the hypothiocyanite (OSCN−) ion in human parotid saliva and the effect of pH on OSCN− generation in the salivary peroxidase antimicrobial system, Arch. Oral Biol. 28 (6) (1983) 517–525.
[64] B. Lamberts, K. Pruitt, E. Pederson, M. Golding, Comparison of salivary peroxidase system components in caries-free and caries-active naval recruits, Caries Res. 18 (6) (1984) 488–494.
[65] K.M. Pruitt, J. Tenovuo, B. Mansson-Rahemtulla, P. Harrington, D.C. Baldone, Is thiocyanate peroxidation at equilibrium in vivo? Biochim. Biophys. Acta Protein Struct. Mol. Enzymol. 870 (3) (1986) 385–391.
[66] M. Geiszt, J. Witta, J. Baffi, K. Lekstrom, T.L. Leto, Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense, Faseb. J. 17 (11) (2003) 1502–1504.
[67] E. Salvolini, D. Martarelli, R. Di Giorgio, L. Mazzanti, M. Procaccini, G. Curatola, Age-related modifications in human unstimulated whole saliva: a biochemical study, Aging Clin. Exp. Res. 12 (6) (2000) 445–448.
[68] M. Magacz, K. Kędziora, J. Sapa, W. Krzyściak, The significance of lactoperoxidase system in oral health: application and efficacy in oral hygiene products, Int. J. Mol. Sci. 20 (6) (2019) 1443.
[69] K. Shin, H. Wakabayashi, K. Yamauchi, S. Teraguchi, Y. Tamura, M. Kurokawa, K. Shiraki, Effects of orally administered bovine lactoferrin and lactoperoxidase on influenza virus infection in mice, J. Med. Microbiol. 54 (8) (2005) 717–723.
[70] K. Shin, A. Horigome, K. Yamauchi, M. Takase, T. Yaeshima, K. Iwatsuki, Effects of orally administered bovine lactoperoxidase on dextran sulfate sodium-induced colitis in mice, Biosci. Biotechnol. Biochem. 72 (7) (2008) 1932–1935.
[71] A.M. Widström, K. Christensson, A.B. Ransjö-Arvidson, A.S. MATTHIESEN, J. Winberg, K. Uvnäs-Moberg, Gastric aspirates of newborn infants: pH, volume and levels of gastrin-and somatostatin-like immunoreactivity, Acta Paediatr. 77 (4) (1988) 502–508.
[72] M. Tiso, A.N. Schechter, Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions, PloS One 10 (3) (2015) e0119712.
[73] T. Sobko, C. Reinders, E. Jansson, E. Norin, T. Midtvedt, J. Lundberg, Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite, Nitric Oxide 13 (4) (2005) 272–278.
[74] D.M.R.J. Van, H. Vreeling-Sindelarova, J.P. Schellens, N.C.J. Van, W.M. Frederiks, Ultrastructural localization of xanthine oxidase activity in the digestive tract of the rat, Histochem. J. 27 (11) (1995) 897–905.
[75] H.M. Martin, K.P. Moore, E. Bosmans, S. Davies, A.K. Burroughs, A.P. Dhillon, D. Tosh, R. Harrison, Xanthine oxidoreductase is present in bile ducts of normal and cirrhotic liver, Free Radic. Biol. Med. 37 (8) (2004) 1214–1223.
[76] M. Battelli, E.D. Corte, F. Stirpe, Xanthine oxidase type D (dehydrogenase) in the intestine and other organs of the rat, Biochem. J. 126 (3) (1972) 747.
[77] J. Foreman, V. Demidchik, J.H. Bothwell, P. Mylona, H. Miedema, M.A. Torres, P. Linstead, S. Costa, C. Brownlee, J.D. Jones, Reactive oxygen species produced by NADPH oxidase regulate plant cell growth, Nature 422 (6930) (2003) 442.
[78] M.M. Reddy, M.S. Fernandes, R. Salgia, R.L. Levine, J.D. Griffin, M. Sattler, NADPH oxidases regulate cell growth and migration in myeloid cells transformed by oncogenic tyrosine kinases, Leukemia 25 (2) (2011) 281.
[79] L. Li, S-h Cheung, E.L. Evans, P.E. Shaw, Modulation of gene expression and tumor cell growth by redox modification of STAT3, Canc. Res. 70 (20) (2010) 8222–8232.
[80] A.N. Lyle, E.W. Remus, A.E. Fan, B. Lassègue, G.A. Walter, A. Kiyosue, K.K. Griendling, W.R. Taylor, Hydrogen peroxide regulates osteopontin expression through activation of transcriptional and translational pathways, J. Biol. Chem. 289 (1) (2014) 275–285.
[81] L. Diebold, N.S. Chandel, Mitochondrial ROS regulation of proliferating cells, Free Radic. Biol. Med. 100 (2016) 86–93.
[82] T.S. Gechev, J. Hille, Hydrogen peroxide as a signal controlling plant programmed cell death, J. Cell Biol. 168 (1) (2005) 17–20.
[83] L.A. Sena, S. Li, A. Jairaman, M. Prakriya, T. Ezponda, D.A. Hildeman, C.-R. Wang, P.T. Schumacker, J.D. Licht, H. Perlman, Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling, Immunity 38 (2) (2013) 225–236.
[84] Q. Pan, W.Y. Qiu, Y.N. Huo, Y.F. Yao, M.F. Lou, Low levels of hydrogen peroxide stimulate corneal epithelial cell adhesion, migration, and wound healing, Invest. Ophthalmol. Vis. Sci. 52 (3) (2011) 1723–1734.
[85] A.F. Fajardo, C. Sobchak, Y. Shifrin, J. Pan, T. Gonska, J. Belik, Hydrogen peroxide promotes gastric motility in the newborn rat, Pediatr. Res. 84 (5) (2018) 751.
[86] S. Moncada, A. Higgs, The L-arginine-nitric oxide pathway, N. Engl. J. Med. 329 (27) (1993) 2002–2012.
[87] L. Adams, M.C. Franco, A.G. Estevez, Reactive nitrogen species in cellular signaling, Exp. Biol. Med. 240 (6) (2015) 711–717.
[88] E.A. Veal, A.M. Day, B.A. Morgan, Hydrogen peroxide sensing and signaling, Mol. Cell. 26 (1) (2007) 1–14.
[89] E. Seifu, E.M. Buys, E. Donkin, Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review, Trends Food Sci. Technol. 16 (4) (2005) 137–154.
[90] N.E. BARRETT, A.S. GRANDISON, M.J. LEWIS, Contribution of the lactoperoxidase system to the keeping quality of pasteurized milk, J. Dairy Res. 66 (1) (1999) 73–80.
[91] M. Haddadin, S.A. Ibrahim, R. Robinson, Preservation of raw milk by activation of the natural lactoperoxidase systems, Food Contr. 7 (3) (1996) 149–152.
[92] Food, Organization A: Benefits and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation: Report of an FAO-WHO Technical Meeting; FAO Headquaters, Rome; 28 November-2 December 2005, FAO, 2006.
[93] J. Miller, E. Tonkin, R.A. Damarell, A.J. McPhee, M. Suganuma, H. Suganuma, P.F. Middleton, M. Makrides, C.T. Collins, A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants, Nutrients 10 (6) (2018) 707.
[94] H. Akinbi, J. Meinzen-Derr, C. Auer, Y. Ma, D. Pullum, R. Kusano, K.J. Reszka, K. Zimmerly, Alterations in the host defense properties of human milk following prolonged storage or pasteurization, J. Pediatr. Gastroenterol. Nutr. 51 (3) (2010) 347–352.
[95] P.W. Lin, B.J. Stoll, Necrotising enterocolitis, Lancet 368 (9543) (2006) 1271–1283.
[96] B. Reiter, R.J. Fulford, V.M. Marshall, N. Yarrow, M.J. Ducker, M. Knutsson, An evaluation of the growth promoting effect of the lactoperoxidase system in newborn calves, Anim. Sci. 32 (3) (1981) 297–306.
[97] T.M. Millar, J.M. Kanczler, T. Bodamyali, D.R. Blake, C.R. Stevens, Xanthine oxidase is a peroxynitrite synthase: newly identified roles for a very old enzyme,

Redox Rep. 7 (2) (2002) 65–70.
[98] S. Abadeh, J. Killacky, M. Benboubetra, R. Harrison, Purification and partial characterization of xanthine oxidase from human milk, Biochim. Biophys. Acta 1117 (1) (1992) 25–32.
[99] A.B. Kirk, J.V. Dyke, C.F. Martin, P.K. Dasgupta, Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk, Environ. Health Perspect. 115 (2) (2006) 182–186.
[100] S. Narkowicz, E. Jaszczak, Polkowska Ż, B. Kiełbratowska, A. Kotłowska, J. Namieśnik, Determination of thiocyanate as a biomarker of tobacco smoke constituents in selected biological materials of human origin, Biomed. Chromatogr. 32 (3) (2018) e4111.
[101] K. Shin, M. Tomita, B. Lönnerdal, Identification of lactoperoxidase in mature human milk, J. Nutr. Biochem. 11 (2) (2000) 94–102.
[102] B. Langbakk, T. Flatmark, Demonstration and partial purification of lactoperoxidase from human colostrum, FEBS Lett. 174 (2) (1984) 300–303.
[103] K. Granelli, L. Björck, L.Å. Appelqvist, The variation of superoxide dismutase (SOD) and xanthine oxidase (XO) activities in milk using an improved method to quantitate SOD activity, J. Sci. Food Agric. 67 (1) (1995) 85–91.
[104] F.A. Fonteh, A.S. Grandison, M.J. Lewis, Variations of lactoperoxidase activity and thiocyanate content in cows' and goats' milk throughout lactation, J. Dairy Res. 69 (3) (2002) 401–409.
[105] L. Yong, Y. Wang, D. Yang, Z. Liu, G. Abernethy, J. Li, Investigation of concentration of thiocyanate ion in raw cow's milk from China, New Zealand and The Netherlands, Food Chem. 215 (2017) 61–66.
[106] X. Lan, J. Wang, D. Bu, J. Shen, N. Zheng, P. Sun, Effects of heating temperatures and addition of reconstituted milk on the heat indicators in milk, J. Food Sci. 75 (8) (2010) C653–C658.
[107] Minarowski Ł, D. Sands, A. Minarowska, A. Karwowska, A. Sulewska, M. Gacko, E. Chyczewska, Thiocyanate concentration in saliva of cystic fibrosis patients, Folia Histochem. Cytobiol. 46 (2) (2008) 245–246.
[108] N. Goi, Y. Hirai, H. Harada, A. Ikari, T. Ono, N. Kinae, M. Hiramatsu, K. Nakamura, K. Takagi, Comparison of peroxidase response to mental arithmetic stress in saliva of smokers and non-smokers, J. Toxicol. Sci. 32 (2) (2007) 121–127.
[109] B.D. Paul, M.L. Smith, Cyanide and thiocyanate in human saliva by gas chromatography-mass spectrometry, J. Anal. Toxicol. 30 (8) (2006) 511–515.
[110] A.Z. Reznick, I. Klein, J.P. Eiserich, C.E. Cross, R.M. Nagler, Inhibition of oral peroxidase activity by cigarette smoke: in vivo and in vitro studies, Free Radic. Biol. Med. 34 (3) (2003) 377–384.