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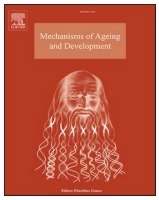

Sex differences in markers of oxidation and inflammation. Implications for ageing

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ABSTRACT

Sexual dimorphism is a key factor to consider in the ageing process given the impact that it has on life expectancy. The oxidative-inflammatory theory of ageing states that the ageing process is the result of the establishment of oxidative stress which, due to the interplay of the immune system, translates into inflammatory stress, and that both processes are responsible for the damage and loss of function of an organism. We show that there are relevant gender differences in a number of oxidative and inflammatory markers and propose that they may account for the differential lifespan between sexes, given that males display, in general, higher oxidation and basal inflammation. In addition, we explain the significant role of circulating cell-free DNA as a marker of oxidative damage and an inductor of inflammation, connecting both processes and having the potential to become a useful ageing marker. Finally, we discuss how oxidative and inflammatory changes take place differentially with ageing in each sex, which could also have an impact on the sex-differential lifespan. Further research including sex as an essential variable is needed to understand the grounds of sex differences in ageing and to better comprehend ageing itself.

1. Introduction

Ageing is a complex biological process characterized by the progressive deterioration of an individual's function and capacity to recover from different inner and external disturbances, resulting in increased morbidity and mortality. The oxidative-inflammatory theory of ageing (De la Fuente and Miquel, 2009) proposes that the general decline in function experienced with age is the result of the establishment of both chronic oxidative and inflammatory stresses in the body. This theory combines two very interlinked and important ageing characteristics: the age-related establishment of an oxidative stress state with the age-related establishment of a low-grade chronic inflammation by the interplay of the immune system. Thus, according to the free radical theory of ageing (Harman, 1956), ageing is the consequence of the accumulation of damage by deleterious oxidation in biomolecules caused by the reactivity of free radicals of oxygen when chronic oxidative stress occurs as a consequence of the higher amount of oxidant compounds than antioxidant defenses. In addition, the term inflamm-ageing denotes an upregulation of the inflammatory response that occurs with age, resulting in a low-grade chronic systemic proinflammatory state (Franceschi, 2000). The oxidative-inflammatory theory of ageing proposes that reactive oxygen species (ROS) leakage from mitochondria and ROS produced by oxidant enzymes over time, when exceeding antioxidant defense mechanisms, would lead to the establishment of an oxidative stress situation, which maintained over time will damage mitochondrial and nuclear DNA, proteins, and lipids in all cells of the organism. In addition, this chronic oxidative stress translates into a chronic inflammatory condition by the interplay of the immune system. Accordingly, oxidatively damaged dysfunctional cells can eventually become senescent and acquire the senescence-associated

Abbreviations: ROS, reactive oxygen species; XO, xanthine oxidase; NOX, NADPH-oxidase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2-deoxy Guanosine; PAMP, Pathogen-Associated Molecular Patterns; DAMP, Damage-Associated Molecular Patterns; PRR, Pattern Recognition Receptors; TLR, Toll-like receptors (TLRs); NLR, NOD-like Receptor; ccfDNA, circulating cell free DNA.

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secretory phenotype (SASP) and produce inflammatory mediators to signal immune cells to clear up the damage. This is a homeostatic adaptive response that, under normal conditions, facilitates their removal from tissues by immune cells (Ovadya and Krizhanovsky, 2014). Nevertheless, due to the age-related decline of the immune system (so-called immunosenescence), immune cells are functionally defective and unable to resolve the situation. However, immune cells will keep producing ROS and pro-inflammatory cytokines in response to the age-related increase in senescent cells, whereas senescent cells will keep producing pro-inflammatory mediators, creating a positive feedback loop of oxidation and inflammation (Martínez de Toda et al., 2021).

In fact, we consider that ROS could be responsible for or, at least, contribute largely to the so-called "hallmarks of ageing" such as genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis (López-Otín et al., 2023). For instance, we believe that the oxidative-inflammatory theory of ageing aligns to the epigenetic theory of ageing or epigenetic clock, as on the one hand, ROS could contribute to the age-related epigenetic changes (Robert and Wagner, 2020) and at the same time, epigenetic changes could be responsible for the uncontrolled production of ROS and inflammatory compounds by immune cells.

The acceptance of ROS as the primary cause of ageing has been doubted by experimental data showing that in certain conditions increased oxidative stress leads to increased longevity (Honda and Honda, 2002; Ristow and Schmeisser, 2011). These results, however, far from dismantling the free radical theory of ageing, can be explained based on hormesis. Thus, a short-term increase in ROS production can cause an adaptive response by increasing antioxidant expression (Schulz et al., 2007; Mesquita et al., 2010; Yang and Hekimi, 2010), whereas chronic ROS levels beyond a certain threshold are still damaging for cellular components. Other claims against the free radical theory of ageing are based on studies in which the use of antioxidants did not increase longevity (Sadowska-Bartosz and Bartosz, 2014). Nevertheless, the confusion between maximum and average longevity is one of the reasons for this criticism. Species with higher longevity have fewer antioxidants because they do not need them since they produce a lower amount of ROS (Barja, 2000). Another argument against the theory is the fact that overexpression of antioxidant enzymes does not extend the lifespan of invertebrates (*Drosophila melanogaster*) and mammals (*Mus musculus*) (Mockett et al., 2010; Jang et al., 2009). However, care must be taken when interpreting the causes of ageing using genetically manipulated animals, as they can develop other adaptive mechanisms to counteract a specific mutation. Interestingly, a 20% increase in lifespan was observed when the upregulation of catalase expression was targeted to the mitochondria specifically (Schriner et al., 2005).

In contrast to the controversy over what is the primary cause of ageing, all researchers agree that the ageing process is very heterogeneous among individuals with identical chronological age. There is substantial individual variability in the ageing process, with certain human individuals living independently in their 90 s while others need help in daily routines earlier in life. Similarly, isogenic populations, such as a certain mouse strain in a laboratory, still display significant variability in lifespan (Yuan et al., 2009). These different ageing rates within a population led to the need to identify accurate markers for biological age quantification to try to determine the precise biological ageing rate of a subject. According to the oxidative-inflammatory theory of ageing, different mathematical models or biological clocks have been proposed to calculate the biological age of an individual, including redox parameters (Martínez de Toda et al. 2020) and immune markers (Martínez de Toda et al. 2016).

One of the most striking facts regarding ageing variability is sexual dimorphism. In general, women live longer than men, consistent with the observed lower biological ages assessed by different molecular biomarkers (Jylhävä et al., 2017). Moreover, the increased longevity of women is also seen across other species (Austad 2006). A demographic study of 134 mammal populations, encompassing 101 species, showed that the female's median lifespan is on average 18.6% longer than that of conspecific males (Lemaître et al., 2020).

At present, there is limited information on whether biological ageing unfolds differently in men and women. This is because, in the past decades, women were excluded from clinical trials to avoid potential harm in fertile women and due to their hormonal fluctuations (Hägg and Jylhävä, 2021) In experimental animals, male models are more commonly chosen because of the assumed increased variability of females in the results (Beery and Zucker, 2011). Nevertheless, a recent study investigated more than two hundred traits in thousands of male and female mice and concluded that neither males nor females are more variable overall (Zajitschek et al., 2020).

Therefore, in order to shed more light on differences in ageing and life expectancy between sexes, this review aims to describe the sex-related differences in several markers of ageing, with a focus on the oxidative and inflammatory processes, in both human and murine models.

2. Sex differences in redox parameters

ROS are generated as natural by-products of normal oxygen metabolism and have important roles in cell signaling and homeostasis in normal conditions (D'Autréaux and Toledano, 2007). However, with ageing, ROS exceed the buffering capacity of antioxidants, disrupting cell integrity and causing tissue injury.

In general, males are believed to have more oxidative stress than females (Ide et al., 2002). This assumption has been observed in several species, such as in flies (Niveditha et al., 2017), mice (Yuan et al., 2009), rats (Barp et al., 2002), and humans (Kander et al., 2017). However, controversial results have emerged depending on the cell type or tissue investigated. In *Drosophila melanogaster* it has been described that longer-lived females show lower ROS levels and higher antioxidant enzymes than males as a function of age, which could account for their observed longer lifespan (Niveditha et al., 2017).

The rate of ROS generation and detoxification is of foremost importance to maintain ROS between certain levels that allow the normal functioning of cells. This balance is tightly regulated by several enzymes and cycles in which sex-biased differences have been suggested and will be discussed. Main sex differences in oxidative stress parameters and oxidative damage markers have been summarized in Table 1 and Table 2, respectively, and are shown in Fig. 1.

2.1. Production of ROS

Regarding ROS production, mitochondria and more precisely, the mitochondrial electron transport chain are the main source of free radicals. It has been described that mitochondria from females produce fewer superoxide radicals than those of the males in the liver of rats (Borrás et al., 2003), and in the brain and heart of mice (Khalifa et al., 2017). However, sex differences have also been reported depending on the substrate used, with female liver mitochondria producing less H_2O_2 for the substrates pyruvate and α-ketoglutarate but more than males when using succinate as a substrate (Mallay et al., 2019). The general lower production of ROS in females, according to the "Mitochondrial theory of ageing" proposed by Miquel in 1980 (Miquel et al., 1980), would agree with the longer lifespan that females show, as the rate of oxidant production by mitochondria from long-lived species is much lower than that of short-lived ones (Sohal et al., 1990; Barja et al., 1994; Barja, 2013).

In addition to mitochondria, there are specific enzymes that are implicated in free radical production, such as the xanthine oxidase (XO) or NADPH-oxidase (NOX) enzymes. XO is an isoform of the xanthine oxidoreductase, an enzyme system that catalyzes the oxidation of

Table 1
Sex differences in oxidative stress parameters. PDH: pyruvate; KDGH: α-ketoglutarate; PBMCs: peripheral blood mononuclear cells.

	Marker	Species	Cell type/Tissue	Sex-differences	References
ROS production	**H_2O_2 mitochondria**	Mice	Brain	♂> ♀	Khalifa et al. 2017
		Mice	Heart	♂> ♀	Khalifa et al. 2017
		Rats	Liver	♂> ♀	Borrás et al. (2003)
		Mice	Liver	♂> ♀ (PDH and KGDH)	Mallay et al. (2019)
		Mice	Liver	♂< ♀ (Succinate)	Mallay et al. (2019)
	Xanthine Oxidase (XO)	Humans	Plasma	♂> ♀	Watanabe et al. (2021)
	NADPH oxidase	Rats	Cerebral arteries	♂> ♀	Miller et al. (2007)
		Rats	Mesenteric arteries	♂> ♀	Zhang et al. (2012)
Antioxidant defences	**Superoxide Dismutase (SOD)**	Mice	Brain, Lung	♂< ♀	Chen et al. (2011)
		Mice	Kidney, Heart	♂= ♀	Chen et al. (2011)
		Rats	Heart	♂< ♀	Barp et al. (2002)
		Humans	Erythrocytes	♂< ♀	Semenova et al. (2022)
		Humans	Blood cells	♂> ♀	Martínez de Toda et al. (2019)
		Humans	Plasma	♂< ♀	Mendoza-Núñez et al. (2010)
	Catalase (CAT)	Rats	Heart	♂= ♀	Barp et al. 2002
		Human	Plasma	♂= ♀	Ide et al. (2002)
		Rats	Pancreas	♂= ♀	Gómez-Pérez et al. 2011
		Mice	Brain, Lung, Heart	♂= ♀	Chen et al. (2011)
		Mice	Kidney	♂< ♀	Chen et al. (2011)
		Humans	Muscle	♂< ♀ (mRNA), ♂= ♀ (protein)	Maher et al. (2009)
		Humans	Erythrocytes	♂= ♀	Guemouri et al. (1991)
		Humans	Blood cells	♂< ♀	Martínez de Toda et al. (2019)
	Glutathione Peroxidase (GPx)	Rats	Heart	♂> ♀	Barp et al. 2002
		Mice	Kidney, Brain	♂< ♀	Chen et al. (2011)
		Mice	Heart	♂> ♀	Chen et al. (2011)
		Mice	Brain	♂< ♀	Sobocanec et al. 2003
		Rats	Liver	♂< ♀	Yamamoto et al. (2002)
		Mice	Liver	♂< ♀	Liang et al. (2011)
		Rats	Liver (mitochondria)	♂< ♀	Viña et al. (2005)
		Rats	Liver	♂< ♀	Erden Inal et al. (2003)
		Humans	Erythrocytes	♂< ♀	Alkazemi et al. (2021)
		Humans	Blood cells	♂< ♀	Kowalska and Milnerowicz, 2016
		Humans	Plasma	♂< ♀	Mendoza-Núñez et al. 2010
		Humans	Erythrocytes	♂< ♀	Massafra et al. 2002
		Humans	Blood cells	♂= ♀	Martínez de Toda et al. (2019)
	Glutathione Reductase (GR)	Rats	Liver	♂< ♀	Yamamoto et al. (2002)
		Rats	Liver	♂< ♀	Erden Inal et al. (2003)
		Rats	Brain	♂= ♀	Dukhande et al. (2009)
		Humans	Blood cells	♂< ♀	Kowalska and Milnerowicz, 2016
		Humans	Blood cells	♂> ♀	Martínez de Toda et al. (2019)
	Reduced glutathione (GSH)	Rats	Liver (mitochondria)	♂< ♀	Borrás et al. (2003)
		Humans	Erythrocytes	♂< ♀	Alkazemi et al. (2021)
		Humans	PBMCs	♂= ♀	Ferri et al. 2020
Oxidant markers	**Oxidized glutathione (GSSG)**	Humans	PBMCs	♂> ♀	Ferri et al. 2020
	GSSG/GSH ratio	Humans	PBMCs	♂> ♀	Ferri et al. 2020

hypoxanthine to xanthine and xanthine to uric acid in purine metabolism generating free radicals. Its activity has been shown to increase with ageing (Vida et al., 2011). Regarding the sex-related differences in this enzyme, it has been described that plasma XO activity is lower in women than in men (Watanabe et al., 2021).

NADPH-oxidase enzymes are interesting since they are the only ones designed to and whose sole purpose is to produce ROS, unlike other oxidant enzymes that generate ROS as byproducts of a different reaction. Thus, they may play a more decisive role in the different oxidative stress state between sexes. Several studies have observed that NADPH-oxidase activity and function are lower in females (Miller et al., 2007; Kander et al., 2017). This is due to two mechanisms: first, females have lower p47, which is required for the assembly of NADPH-oxidase enzyme, leading to lower levels of superoxide production independently of estrogens, and second, estrogens can directly induce lower NADPH-oxidase activity. The result of these gender differences is lower levels of superoxide in females with lower levels of oxidative stress (Kander et al., 2017).

In addition to their activity, NOX subunits have also been found to exhibit gender discrepancies. The expression of NOX1 and NOX4 was higher in males than in females, suggesting that gender differences in superoxide production can be the result of the differential expression of these two subunits (Miller et al., 2007). This is consistent with another study in which NOX4 levels were significantly lower in the mesenteric arteries of female rats compared to the males (Zhang et al., 2012). The two previous studies also agree in showing that NOX2 levels did not differ between males and females (Miller et al., 2007; Zhang et al., 2012). It could be possible that the higher expression of NOX subunits in males partly explains why they exhibit higher levels of oxidative stress than females.

2.2. *Antioxidant defense mechanisms*

In addition to the gender differences in ROS production, clinical and experimental data have also suggested a greater antioxidant potential in females than males (Kander et al., 2017). It has been proposed that women have an estrogen-linked antioxidant advantage and that this advantage is lost in the postmenopausal stage (Mendoza-Núñez et al., 2010).

In order to allow only transient increases in ROS and thus prevent damage to biomolecules, several antioxidant enzymes that work to keep ROS levels within a certain range. Regarding the superoxide dismutase (SOD) enzyme, which converts superoxide anion into hydrogen peroxide, there is no uniform consensus on gender differences, but it has been suggested that there may be a difference in certain tissues. It was reported that brain and lung SOD activity levels were higher in female

Table 2
Sex differences in oxidative damage markers. CEL: carboxyethyl-lysine; CML: carboxymethyl-lysine; 8-OHDG: 8-hydroxy-2-deoxy Guanosine; 8-OHA: 8-hydroxyadenine; 8-iso-PGF2a: 8-isoprostaglandin F2α.

	Marker	Species	Cell type/Tissue	Sex-differences	References
Protein damage	**Protein hydroperoxide levels**	Rats	Plasma	♂> ♀	Kayali et al. (2007a)
		Rats	Heart	♂= ♀	Kayali et al. (2007b)
	Advanced oxidation protein products	Rats	Plasma	♂> ♀	Kayali et al. (2007a)
		Rats	Heart	♂> ♀	Kayali et al. (2007b)
		Humans	Plasma	♂> ♀	Tóthová et al. (2013)
	Protein carbonils	Rats	Plasma	♂= ♀	Kayali et al. (2007a)
		Rats	Heart	♂> ♀	Kayali et al. (2007b)
	Protein carbamylation	Humans	Plasma	♂> ♀	Carracedo et al. (2018)
	Advanced End Glycation (AGE) products	Rats	Kidney	♂> ♀ (CEL)	Wang et al. (2006)
		Rats	Kidney	♂= ♀ (CML)	Wang et al. (2006)
		Humans	Plasma	♂> ♀	Sternberg et al. (2010)
	Receptor Advanced End Glycation (RAGEs) products	Humans	Plasma	♂= ♀	Wu et al. (2021)
		Humans	Plasma	♂< ♀	Brinkley et al. (2017)
Lipid damage	**Malondialdehyde (MDA)**	Humans	Plasma	♂> ♀	Ide et al. (2002)
		Humans	Plasma	♂> ♀	Pinchuk et al. (2019)
		Humans	Plasma	♂> ♀	Carracedo et al. (2018)
		Humans	Plasma	♂> ♀	Ramos-Loyo et al. (2013)
DNA damage	**8-OHDG**	Humans	Lymphocytes	♂> ♀	Collins et al. (1998)
		Humans	Lymphocytes	♂= ♀	Proteggente et al. 2002
	8-OHA	Humans	Lymphocytes	♂< ♀	Proteggente et al. 2002
	FAPy Guanine	Humans	Lymphocytes	♂> ♀	Proteggente et al. 2002
	8-iso-PGF2a	Humans	Urine	♂> ♀	Ide et al. (2002)
	8-isoprostane	Humans	Urine	♂> ♀	Sakano et al. (2009)
	Single strand breaks	Humans	Lymphocytes	♂> ♀	Hofer et al. 2006
	Telomere length	Humans	Lymphocytes	♂< ♀	Cardano et al. (2022)
	Circulating cell free DNA (ccfDNA)	Humans	Plasma	♂> ♀	Yuwono et al. (2021)
		Humans	Plasma	♂= ♀	Zhong et al. (2007)
		Humans	Plasma	♂> ♀	Jylhävä et al. (2014)
		Humans	Plasma	♂> ♀	Kananen et al. (2023)

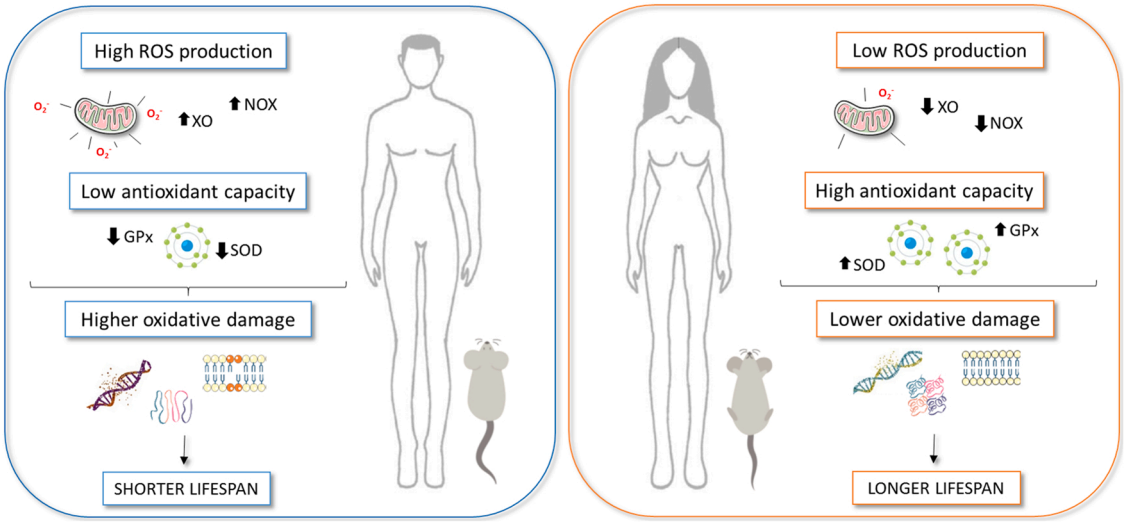

Fig. 1. Sexual differences in Reactive Oxygen Species (ROS) balance. Sexual dimorphism, in humans and mice, is evident in both the production and decomposition of ROS. First, males have a higher leakage of superoxide anion at the mitochondrial electron transport chain, the main site of ROS production, and higher activity of pro-oxidant enzymes, such as xanthine oxidase (XO) and NADPH-oxidase (NOX). In addition, they have a lower expression and activity of important antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). Both facts turn into a higher accumulation of oxidative damage over time in DNA, proteins, and lipids, impairing proper cellular and tissue function, which could account for their shorter lifespan.

mice, but there was no significant difference in SOD activity levels between male and female mice in the kidney or heart (Chen et al., 2011). In another study, female rats were found to have higher SOD activity levels in the heart than males (Barp et al., 2002). Remarkably, the SOD activity levels in both male and female rats were significantly decreased after castration compared to their respective controls (Barp et al., 2002), suggesting that there could be an association between sex hormones and SOD activity levels. In humans, one study found higher SOD activity in erythrocytes from women than in those from men (Semenova et al., 2022), and another one higher SOD in plasma from women than men (Mendoza-Núñez et al., 2010). However, a different study found higher SOD activity in men aged above 70 years old (Martínez de Toda et al., 2019). Finally, another report found that higher SOD activity is associated with lower all-cause mortality, in women but not in aged men (Mao et al., 2019), which further suggests gender differences in oxidative stress regulation with ageing.

Concerning catalase activity, which catalyzes the conversion of hydrogen peroxide to water, some studies showed no difference in catalase activity levels between males and females (Barp et al., 2002; Ide et al., 2002; Gómez-Pérez et al. 2011). In addition, it has been reported

that catalase activity is similar between genders in the brain, lung, and heart, but higher in the female kidney (Chen et al., 2011). In humans, higher mRNA levels of catalase were found in the muscles of women compared to men although no differences were found in protein levels (Maher et al., 2009). In erythrocytes, no gender differences were found in catalase activity in a population from 4 to 65 years old (Guemouri et al., 1991). However, in blood from elderly individuals, higher activities were observed in women than in men (Martínez de Toda et al., 2019). These data suggest that catalase activity and thus hydrogen peroxide degradation may not be affected by gender and sex hormones.

Another enzyme that detoxifies hydrogen peroxide into water and oxygen is glutathione peroxidase (GPx). With respect to its sex-related differences, they seem to be tissue-dependent. GPx activity was higher in the kidney and brain of female mice compared to males, whereas in the heart, male mice showed higher activity than females (Chen et al., 2011). Previous studies also found higher GPx activity in the female brain (Sobocanec et al. 2003) and in the female liver (Yamamoto et al., 2002; Liang et al., 2011). Other studies showed that the activity of GPx was more than double in hepatic mitochondria from female rats as compared with that in mitochondria from males of the same age (Viña et al., 2005) and that GPx activity in the liver from females was higher than in those from males (Erden Inal et al., 2003). In humans, adolescent girls showed higher GPx activity in blood than men (Alkazemi et al., 2021). Women showed higher GPx activity in blood than men between 20 and 25 years old (Kowalska and Milnerowicz, 2016) and greater activity at other ages as well (Mendoza-Núñez et al., 2010). Moreover, GPx activity appears to be regulated by sex hormones such as progesterone and testosterone. The activity of erythrocyte GPx was found to be higher in premenopausal than healthy postmenopausal women. Also, during the premenopausal period, female GPx activity is significantly higher than in age-matched men, but this difference was no longer observed between postmenopausal women and age-matched men (Massafra et al., 2002). In postmenopausal women, estrogen replacement therapy induced a significant increase in erythrocyte GPx activity. GPx correlated positively with serum estrogen levels in both premenopausal and postmenopausal women treated with estrogen replacement therapy, suggesting that estrogens induce the expression of GPx. Supporting this hypothesis, it has been reported that pre-menopausal females with total hysterectomy had a reduction in mRNA expression of SOD and GPx after surgery that recovered after estrogen replacement therapy, whereas catalase mRNA expression was not changed by surgery (Bellanti et al., 2013). This suggests that gene expression of SOD and GPx is estrogen-dependent, whereas gene expression of catalase is not (Viña et al., 2011). After menopause, the gender differences in GPx activity are no longer detected in an elderly population above 70 years old (Martínez de Toda et al., 2019).

The action of the enzyme glutathione peroxidase is joined by the enzyme glutathione reductase (GR), as the former oxidizes reduced glutathione (GSH) to oxidized glutathione (GSSG) to detoxify hydrogen peroxide, so this redox power has to be restored (Meister and Anderson, 1983). GR is responsible for maintaining the GSH/GSSG ratio by reducing GSSG generated during states of oxidative stress. The ratio of GSH/GSSG is a sensitive indicator of changes in a cell's thiol redox state and ongoing redox signaling (Jones, 2002) and the changes in GSH/GSSG ratio affect cell functions such as apoptosis, differentiation, and proliferation (Kirlin et al., 1999). GSSG production increases during the detoxification of reactive oxygen species (ROS) by GPx and the reactivation of oxidized GR, resulting in a decrease in the GSH/GSSG ratio (Aquilano et al., 2014). Since GSSG increases can be harmful to cells, the reduction of GSSG by GR is essential for restoring redox homeostasis (Maher, 2005; Wang et al., 2020).

Considering glutathione reductase (GR) activity, there are not many studies investigating sex-related differences although some data reported higher GR activity in the liver from female than male rats (Yamamoto et al., 2002; Erden Inal et al., 2003) meantime another study found no sex differences in the brain (Dukhande et al., 2009). In humans, women showed higher GR activity in blood than men between 20 and 25 years old (Kowalska and Milnerowicz, 2016) while after 70 years old of age, men show higher GR activity than elderly women in the blood (Martínez de Toda et al., 2019).

Regarding reduced glutathione (GSH) concentration, hepatic mitochondrial glutathione (GSH) levels in female rats are higher than in males. Nevertheless, when rats were ovariectomized their mitochondrial glutathione levels decreased to similar values to those in males. Estrogen replacement therapy completely prevented this fall in mitochondrial GSH (Borrás et al., 2003), which suggests that GSH levels might be under estrogen control. In humans, adolescent girls also showed a higher GSH/GSSG ratio in blood than men (Alkazemi et al., 2021).

With respect to oxidized glutathione (GSSG) concentration, men showed statistically significant higher values of oxidized glutathione (GSSG) and oxidized-reduced glutathione ratio (GSSG/GSH) compared to those obtained in women (Ferri et al. 2020). Moreover, premenopausal women that underwent a hysterectomy, after 30 days showed a reduction in GSH concentration and an increase in GSSG and GSSG/GSH ratio. Interestingly, estrogen replacement therapy restored glutathione levels to those before the hysterectomy, suggesting again the vital role of estrogens in the glutathione cycle (Bellanti et al., 2013).

2.3. Markers of oxidative damage

2.3.1. Protein damage

Protein carbonyls and advanced end glycation (AGE) products have been among the most successful markers of oxidative stress in proteins that have been associated with ageing (Frijhoff et al., 2015). One study found that levels of protein carbonyls and oxidation protein products were higher in the heart of male rats (Kayali et al., 2007a) and in male plasma (Kayali et al., 2007b) than in the female groups. In humans, carbamylated proteins in plasma were also higher in men aged between 60 and 79 years old than in women from the same age group (Carracedo et al., 2018). Accordingly, higher levels of advanced oxidation protein products were found in men from 20 to 23 years old of age comparing to women of the same age (Tóthová et al., 2013). With respect to AGE products, higher carboxyethyl-lysine (CEL) has been found in kidney from male rats comparing to females, whereas no sex differences were found for carboxymethyl-lysine (CML) (Wang et al., 2006). In humans, higher CEL and CML products have been reported in middle-aged men compared to women (Sternberg et al., 2010). In relation to AGE receptors (RAGE), one study showed no sex differences in plasma from elderly individuals (Wu et al., 2021), whereas another one reported higher RAGE in plasma from elderly women compared to men (Brinkley et al., 2017).

2.3.2. Lipid damage

Lipids are susceptible targets of oxidation because of their molecular structure abundant with reactive double bonds (Ho et al., 2013). One of the most well-studied markers of lipid peroxidation is malondialdehyde (MDA). In humans, one study found that the concentration of plasma MDA was higher in young men than in young women (Ide et al., 2002). More recently, also in humans, higher MDA concentration was described in men (Pinchuk et al., 2019). Moreover, MDA concentration in plasma was also higher in men aged between 60 and 79 years old than in women from the same age group (Carracedo et al., 2018), which correlates with another report showing that adult men display higher lipid peroxidation levels in serum than women of the same age (Ramos-Loyo et al., 2013).

2.3.3. DNA damage

Oxidative damage to DNA has often been used as a biomarker for oxidative stress and ageing. Indeed, the measurement of oxidative damage to DNA, particularly of 8-hydroxy-2-deoxy Guanosine (8-OHdG), has been postulated as a biomarker of ageing. In relation to differences between sexes, it has been found that levels of 8-OHdG from lymphocytes of healthy non-smoking women from Ireland were lower

than those observed in men (Collins et al., 1998). Furthermore, certain oxidative DNA modifications were higher in men, such as s 2,6-diamino-4-hydroxy-5-formamidopyrimidine guanine, whereas women had higher levels of 8-hydroxyadenine (8OHA) (Proteggente et al., 2002). Surprisingly, males had higher levels of single-strand breaks than females, but no difference in oxidative lesions (Hofer et al., 2006). Comparing metabolites in age ranges, levels of urinary 8-isoprostaglandin F2α were higher in young men than in young women (Ide et al., 2002) and in middle-aged Japanese adults (Sakano et al., 2009).

Oxidative damage to DNA can also cause telomere attrition, which has been also considered a biomarker of ageing. Nonetheless, the relationship between age, sex, and telomere length is complex but different meta-analysis studies demonstrated that, under physiological conditions, adult women have longer telomeres than men (Gardner et al., 2014). This association becomes stronger with increasing age and suggests that females may be more protected from telomere-shortening effects (Cardano et al., 2022). Indeed, estrogen-responsive elements have been found in hTERT promoter, and treatments with 17-b-estradiol result in the upregulation of telomerase activity (Mayer et al., 2006).

3. Sex differences in inflammatory markers

Inflammation can be defined as a general or local body reaction due to the action of different etiological agents (Milan-Mattos et al., 2019). It is mediated by immunocompetent cells and requires the participation of cytokines and intermediary factors in its progression (Garavelli et al., 2018). Physiological inflammation can be an acute response that stops completely when the damage ceases, leaving no sequelae or causing minimal harm associated with a healing process. However, the inflammatory response can become chronic, and prolonged over time. This chronic response shows similar characteristics to that observed in acute inflammation in terms of its initiation by inflammatory mediators but ultimately leads to tissue damage (Garavelli et al., 2018). The mechanisms of inflammation are heterogeneous, with changes related to age and sex. In general, it is believed that women exhibit increased cellular-mediated and humoral-mediated immune responses and a higher risk of autoimmune diseases compared to men (Dela Justina et al., 2021).

In the acute inflammatory process, there is a higher risk of morbidity and mortality in males compared to females (Mikkola et al., 2013). In contrast, in a chronic process, women have a worse prognosis and higher mortality, probably due to long-term inflammation (Hunter et al., 2017). In addition, it has been reported that autoimmune disorders are more frequent in women (Wang et al., 2015), which suggests that important gender differences must exist at the level of the immune system and the inflammatory response between both sexes. Therefore, the most important sex-related differences in several components of the inflammatory response are going to be disclosed and are summarized in Table 3 and depicted in Fig. 2.

3.1. Immune cell counts

In peripheral blood, the leukocyte cell count is similar between men and women (Choi et al., 2014). No sex differences have been reported in total numbers of monocytes and lymphocytes (Tollerud et al., 1989; Choi et al., 2014), although gender dimorphism has been described in specific lymphocyte subsets and natural killer cells. Thus, a slightly higher proportion of CD3-CD56 + natural killer cells and CD8 + T-cytotoxic lymphocytes have been reported in men compared to women. In contrast, women display a higher percentage of CD4 + T-helper lymphocytes and, consequently, they have an increased CD4/CD8 ratio compared to men (Jentsch-Ulrich et al. 2005; Choi et al., 2014; Valiathan et al., 2014). It has also been noted that with ageing older men have a greater accumulation of memory and senescent CD8 + T cells and therefore a lower ratio of CD4/CD8 cells than women of the same age group (Gubbels Bupp et al., 2018). This fact is of particular interest, given that a low CD4/CD8 ratio has been postulated as a marker of ageing and is predictive of increased mortality in very old individuals (Ferguson et al., 1995), and as such, could contribute to the different ageing trajectories between sexes.

3.2. Pattern recognition receptors

Cells of the innate immune system participate in the inflammation process, recognizing Pathogen-Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs) through Pattern Recognition Receptors (PRRs) such as Toll-like receptors (TLRs), NOD-like Receptor (NLR), C-type Lectin Receptors (CLRs) and RIG-I-like receptors (RLR) (Amarante-Mendes et al., 2018). These receptors are essential for the initiation of the inflammatory response and certain

Table 3
Sex differences in indicators of inflammation. NK: natural killer cell; Tc: cytotoxic T lymphocytes; Th: helper T lymphocytes; PRR: pattern recognition receptor; TLR: toll-like receptor; NLR: nod-like receptor; AIM2: absent in melanoma (interferon-inducible protein); RANKL: receptor activator of nuclear factor kappa-B ligand; PKD: Serine/threonine-protein kinase; IL: interleukin; TNF: tumour necrosis factor; IFN: interferon; pDC: plasmacytoid dendritic cell.

	Indicators	Species	Cell/sample type	Sex-differences	References
Immune cell counts	CD3-CD56 + NK	Human	Lymphocytes	♂> ♀	Jentsch-Ullrich et al. 2005;Choi et al. (2014); Valiathan et al. (2014)
	CD8 + Tc	Human	Lymphocytes	♂> ♀	Jentsch-Ullrich et al. 2005;Choi et al. (2014); Valiathan et al. (2014)
	CD4 + Th	Human	Lymphocytes	♂< ♀	Jentsch-Ullrich et al. 2005;Choi et al. (2014); Valiathan et al. (2014)
	CD4/CD8 ratio	Human	Lymphocytes	♂< ♀	Jentsch-Ullrich et al. 2005;Choi et al. (2014); Valiathan et al. (2014); Gubbels Bupp et al. 2018
	CD8 + T (senescent)	Human	Lymphocytes	♂> ♀	Gubbels Bupp et al. 2018
Toll-like Receptor (PRR)	TLR2	Human	Neutrophils	♂> ♀	Martínez-García et al. 2020
	TLR4	Human	Neutrophils	♂< ♀	Martínez-García et al. 2020
	TLR7	Human	Leukocytes	♂< ♀	Souyris et al. (2018)
Nod-like Receptor (PRR)	NLRP3 (RANKL; PKD3)	Human	Leukocytes	♂> ♀	Espinosa-Garcia et al. (2020);Ma et al. (2020);Alves et al. (2020);Srivastava et al. (2001); Borges et al. 2015
	NLRC4	Human	Leukocytes	♂> ♀	Habib et al. (2020)
AIM2-like receptor (PRR)	AIM2	Human	Leukocytes	♂> ♀	Habib et al. (2020)
Basal cytokines	IL-6	Human	Plasma	♂> ♀	Milan-Mattos et al. (2019)
	IL-6, IL-1β, TNFα	Human	Plasma	♂> ♀	Bernardi et al. (2020)
Cytokines produced after stimuli	TNFα	Human	Whole blood	♂< ♀	Moxley et al. 2002
	TNFα	Human	PBMCs	♂< ♀	Asai et al. (2001)
	TNFα	Human	Neutrophils	♂< ♀	Aomatsu et al. (2013)
	IFNα	Human	pDc	♂< ♀	Meier et al. (2009)
	IL-10	Mice	Splenocytes	♂> ♀	Liva and Voskuhl (2001)

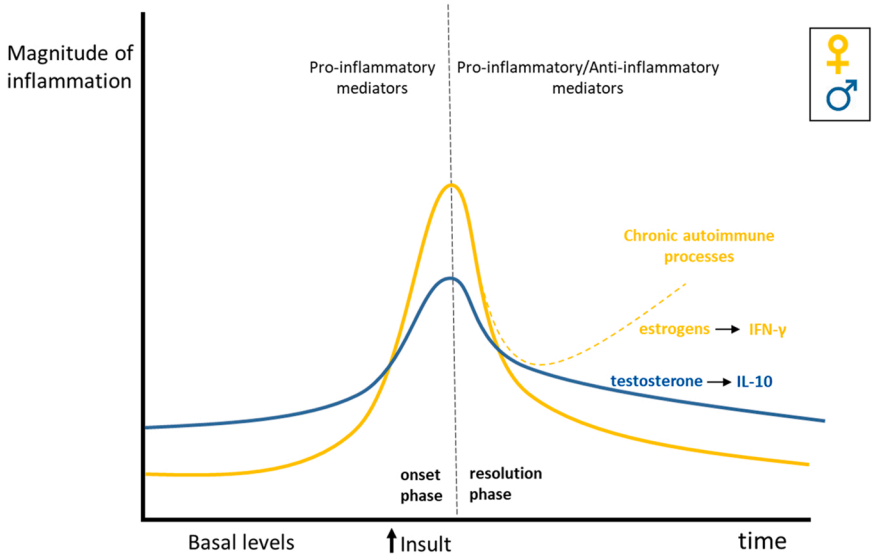

Fig. 2. Sex disparities in inflammation. There are sex differences in the mechanisms of inflammation. While males have higher basal levels of inflammatory mediators, they have a weaker response against a challenge. Nevertheless, they have a better resolution of the acute inflammation, possibly through the testosterone-mediated induction of IL-10, whereas females have a higher incidence of chronic autoimmune processes which could be mediated by the estrogen-mediated induction of IFN-γ.

differences in their expression levels have been described between sexes.

3.2.1. Toll-like receptors (TLRs)

TLRs are found on macrophages, dendritic cells, and B lymphocytes and activate the production of inflammatory cytokines. Intracellular ligands for the different TLRs include mitochondrial deoxyribonucleic acid (TLR9), single-stranded ribonucleic acids from viruses (TLR7), histones (TLR4), hyaluronate fragments (TLR4), and heat shock proteins (TLR2 y 4). Upon recognition of ligands, TLRs trigger an inflammatory response with activation of type I interferons (IFNs) and increased expression of co-stimulatory proteins, which serve to promote the innate immune response and activate the adaptive one in order to clear the infection. Concerning the sex differences in these receptors, it has been described that men have higher expression of TLR2 but lower of TLR4 in neutrophils than women (Martínez-García et al., 2020), while higher expression of TLR7 has been reported in female leucocyte populations (Souyris et al., 2018). Moreover, downstream of TLR activation, IFNα, and IFNβ, released by plasmacytoid dendritic cells (pDC), are the primary effector cytokines of the type I IFN response. Female pDC produce markedly more IFNα in response to HIV-1-encoded TLR7 ligands than male-derived pDCs, resulting in stronger secondary activation of CD8 + T cells (Meier et al., 2009). Thus, the higher expression of TLR7 together with the increased release of IFNα from pDCs in women could explain their stronger acute inflammatory response in response to an infection and their overall better prognosis in comparison to males (Mikkola et al., 2013).

3.2.2. NOD-like receptors (NLRs)

Additional interesting PRRs are the NLRs, a receptor family of 23 proteins encoded in the human genome. This family has been implicated in several pro-inflammatory signaling pathways, in fact, eight members of NLRs, with the ability to oligomerize in inflammasomes, have been identified: NLRP1, NLRP3, NLRP6, NLRP12, NLRP2, NLRC4, ALR (receptor type AIM2 or absent in melanoma 2), and the cytoplasmic sensor RIG-1. The NLRP3 inflammasome is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1β/IL-18 in response to microbial infection and cellular damage (Zheng et al., 2020). Appropriate inflammasome activation is vital for the host to cope with foreign pathogens or tissue damage, while aberrant inflammasome activation can cause uncontrolled tissue responses that may contribute to various diseases, including autoinflammatory disorders (Zheng et al., 2020). Numerous studies investigating sex-related differences in the activation of NLRP3 inflammasome have been conducted recently in the field of SARS-Cov-2 given the observed sex-related differences in terms of mortality and prognosis and it has been observed that sex hormones play a crucial role in regulating or controlling NLRP3 inflammasome overactivation (Zhang et al., 2021). Estrogens reduce activation of the NF-κβ ligand receptor (RANKL), which prevents RANKL-induced JNK activation (Srivastava et al., 2001), while the estrogen receptor binds directly to the promoter of the D3 protein kinase gene (PRKD3) to downregulate PKD3 expression (Borges et al., 2015), thus inhibiting the phosphorylation and the potential further activation of NLRP3. Furthermore, in women, progesterone also inhibits the activation of the NLRP3 inflammasome (Espinosa-Garcia et al., 2020). On the contrary, testosterone induces NLRP3 inflammasome activation (Ma et al., 2020) and excess testosterone production can induce mitochondrial reactive oxygen species (ROS) that indirectly activate the NLRP3 inflammasome (Alves et al., 2020). Thus, in general, both estrogen and progesterone prevent inflammasome overactivation while testosterone promotes it, which could explain the higher mortality and worse prognosis of men infected with SARS-Cov-2 (Zheng et al., 2020). Crucially, estrogen and progesterone levels decline significantly after menopause, which makes women no longer resistant to inflammasome overactivation (Liu et al., 2019). In fact, it has been reported that postmenopausal women with SARS-Cov-2 tend to share similar symptoms and prognosis to men, which is not the case in premenopausal women (Sha et al., 2021).

In addition to NLRP3, as a critical member of the inflammasome family, inflammasomes NLRC4 (NLR family CARD domain-containing protein 4) and AIM2 have also been found to be downregulated following estrogen and progesterone administration (Habib et al., 2020), which further supports the anti-inflammatory effect of estrogen and progesterone in the context of inflammasome persistent activation.

3.3. Immune soluble factors

Sex differences in the production of immune soluble factors such as cytokines and their involvement in many types of immune responses could explain, in part, the better prognosis of females against an acute challenge, such as an infection or sepsis, and conversely, their greater susceptibility, prevalence, and severity of chronic autoimmune diseases (Angum et al., 2020; Jacobsen and Klein, 2021). Although specific autoimmune diseases, such as type I diabetes and myocarditis have a higher prevalence in men, women are 2–10 times more prone to most autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, systemic rheumatoid arthritis,

myasthenia gravis, Hashimoto's thyroiditis, and Sjogren's syndrome (Keestra et al., 2021). This fact suggests that there may be differences in the release of pro-inflammatory cytokines between men and women.

Regarding sex differences in cytokines, it is needed to distinguish between cytokine levels in the absence of a challenge and cytokine production in response to different stimuli. The basal pro-inflammatory state in the absence of a stimulus has been referred to as "sterile inflammation" (Bauer and De La Fuente, 2016), and it is believed to be the result of persistent infections or accumulation of damage over time. With respect to this basal inflammation, it has been described that men have higher levels of pro-inflammatory cytokines (Milan-Mattos et al., 2019; Bernardi et al., 2020). As has been discussed in the previous section, this could be due to their higher levels of oxidative stress, given that oxidative stress is known to trigger and activate NF-κB transcription factor and, consequently, the synthesis of several pro-inflammatory cytokines (Wang et al., 2006; Vida et al., 2014). Nevertheless, there are conflicting results regarding other immune soluble factors such as copper (Cu) or ceruloplasmin (Cp). Cu and Cp, components of the acute phase, have been reported to be higher in women, independently of age (Piacenza et al., 2021). However, it is known that Cu can exert anti-inflammatory actions (Berthon, 1993) and that Cp scavenges oxygen-derived free radicals (Goldstein et al., 1982), which could, through neutralization of ROS, contribute to the lower pro-inflammatory status in women. In addition, the higher Cu and Cp in women have been shown to be dependent on the menstrual cycle (Michos et al., 2010). In addition, the menstrual cycle in women has been reported to affect other inflammatory mediators such as IL-6, which has been reported to be higher in the luteal phase than in the follicular one (Sikora et al., 2015). Thus, more research is needed investigating sex differences considering the different phases of the menstrual cycle.

This higher basal inflammation in men could also explain why they have a lower immune and inflammatory response when they are needed to do so, such as, when they are challenged with an infection. With respect to the sex differences in cytokine production in response to a stimulus, females have a stronger immune and inflammatory response than males (Klein and Flanagan, 2016; Ortona et al., 2019). Thus, different immune cells from women produce more TNF-α in response to a challenge than those from men (Asai et al., 2001; Moxley et al., 2002; Aomatsu et al., 2013) as well as IFNα (Meier et al., 2009). The higher production of pro-inflammatory mediators in response to a challenge of females has been linked to the action of estrogens. Thus, it has been demonstrated that physiological concentrations of estrogens enhance the production of IL-6, IL-1β, and TNF-α in response to a stimulus of human monocytes and murine macrophages (Klein and Flanagan, 2016). On the contrary, the removal of endogenous estrogen has also been shown to reduce the pro-inflammatory response of immune cells (Rettew et al., 2009). This stronger acute inflammatory response would explain why females generally display a better sepsis outcome compared to males (Shepherd et al., 2021).

However, this higher immune response of females makes them more reactive to both self and non-self antigens and therefore, could be responsible for their greater susceptibility to chronic autoimmune diseases (Angum et al., 2020). On the one hand, it is known that four DNA putative estrogen response elements are integrated in the interferon-gamma (IFNγ) promoter in lymphoid cells. Thus, elevated estrogen levels directly drive increased IFNγ production by T cells and predispose women to IFNγ-mediated autoimmune conditions (Fox et al., 1991). On the other hand, it has been suggested that testosterone may underlie why males are less susceptible to autoimmune diseases than females. Accordingly, several studies have described the protective effect of testosterone in autoimmune diseases. For example, castration of male mice has been linked to an increased prevalence of diabetes, thyroiditis, and adjuvant arthritis, whereas testosterone treatment was protective (Liva and Voskuhl, 2001). Furthermore, it has been demonstrated that testosterone binds to androgen receptors on CD4 + T cells and directly induces the release of the anti-inflammatory cytokine IL-10 (Liva and Voskuhl, 2001), a mechanism that could also underlie the observed gender differences in autoimmune diseases' prevalence.

4. Circulating cell-free DNA as a marker that links oxidation and inflammation

It is known that although both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) undergo oxidative damage, mtDNA is more vulnerable to oxidative stress, owing to its proximity to the respiratory chain, the lack of protective histones, and the less efficient DNA repair systems in the mitochondria (Richter et al., 1988). In addition, it has been stated that ROS can contribute to mtDNA mutations by affecting the mitochondrial polymerase decreasing its fidelity which results in increased somatic transition mutations. ROS may also act as a signaling molecule and influence mitochondrial biogenesis and/or mitochondrial turnover, which could in turn promote the clonal expansion of pre-existing mtDNA mutations (Ziada et al., 2020).

Interestingly, recent evidence has shown that oxidative damage to mitochondrial and nuclear DNA generates fragments that can be released to extracellular fluids and recirculate into the bloodstream, reaching other locations far from the tissue in which they were produced (Tuboly et al., 2017). Actually, they function as damage-associated molecular patterns (DAMPs) (Boyapati et al., 2017; Picca et al., 2021), that bind to pattern recognition receptors (PRR), and through activation of the nuclear transcription factor kappa b (NF-κB), they activate the expression of pro-inflammatory cytokines, boosting inflamm-ageing (Martínez de Toda et al., 2021).

When these fragments are outside the cell they are referred to as circulating cell-free DNA (ccfDNA). Nowadays, the most widely accepted hypothesis is that the main source of ccfDNA is dying cells (Jahr et al., 2001) although an active DNA release has also been reported in viable cells (Van der Vaart et al., 2008). Such active DNA release could be involved in pathogen defense as a component of NETs (neutrophil extracellular traps) (Papayannopoulos, Nov 1 et al., 2010), or participate, free or associated with exosomes or microvesicles, in cellular communication, lateral material transfer, immune system modulation, or homeostasis maintenance (Takahashi et al., 2017). Independently from its origin, ccfDNA has emerged as a promising non-invasive biomarker in multiple fields such as prenatal testing, organ transplantation, oncology, cardiovascular and autoimmune diseases (De Miranda et al., 2021) and, giving its correlation to both oxidative and inflammatory processes, it has potential to be an important biomarker of ageing.

Concerning sex differences, certain studies have evaluated the relation between ccfDNA and biological sex with different conclusions. Different results could be due to the lack of standardization in the assessment methods for quantifying ccfDNA, from blood collection to data interpretation, to obtain reproducible results. In a recent literature review, higher levels of ccfDNA were found in plasma from men in comparison to women in three cohorts (total of 584 subjects), while no sex differences were observed in a single cohort of 43 subjects (Yuwono et al., 2021). In another study, no significant differences were found between the levels of ccfDNA in plasma from age-matched men and women, while higher levels were found in the female group older than 60 years of age in comparison to the two younger female groups (Zhong et al., 2007). Further analysis showed the loss of estrogens could be the reason behind the increase observed in women (Meddeb et al., 2019). A different study analyzed 1337 participants (aged 46 from 77 years) in the Health 2000 Survey and found that men had significantly higher ccfDNA levels than women not using hormonal replacement therapy (HRT), and the women using HRT, either with an estrogen-only preparation or with a combination of estrogen and progestin, had significantly lower ccfDNA levels compared with the women not using HRT (Jylhävä et al., 2014). In addition, a more recent study analyzing ccfDNA in three different human cohorts (N = 5385; 17–82 years) also found higher

levels in men than in women (Kananen et al., 2023).

Thus, the differences observed between sexes in ccfDNA could be linked to the reported differences in oxidative stress and could be one of the contributors to the establishment of a low-grade chronic inflammation or "sterile inflammation" during ageing, an idea that has been illustrated in Fig. 3. According to the proposed hypothesis, males would have higher oxidative stress, higher ccfDNA concentration in plasma, and higher release of pro-inflammatory cytokines in the absence of a challenge. Supporting this theory, higher ccfDNA levels have been linked to elevated inflammation, such as higher C-reactive protein and IL-6 (Kananen et al., 2023).

Such a situation can potentially be exacerbated during aging, as old individuals and unhealthy centenarians show increased contributing tissues releasing ccfDNA (Teo et al., 2019), as well as in age-related diseases. Specific increasing concentrations of ccfDNA in human plasma correlate significantly with cancer, myocardial infarction, systemic lupus erythematosus, stroke or sepsis, type-2 diabetes, Parkinson, and Alzheimer's diseases (Ziegler et al., 2002; Jylhävä et al., 2014).

This hypothesis is supported by recent results analysing three different human cohorts (N = 5385) ranging from 17 to 82 years. ccfDNA quantification demonstrated that the total concentration of ccfDNA is higher in more frail older individuals, concluding that ccfDNA is also related to frailty (a multidimensional indicator of ageing-related accumulation of health deficits and a predictor of mortality) (Jylhävä et al., 2013; Kananen et al., 2023).

5. Implications of sex differences in redox and inflammatory markers for ageing

Ageing is characterized by the establishment of an oxidative stress situation resulting from a higher production of ROS and lower or less efficient antioxidant defense mechanisms, which translates into the accumulation of oxidative damage over time in all biomolecules, causing the functional decline of the organism and increasing morbidity and mortality. This age-related accumulation of oxidative damage has been described to occur at a slower rate in women from puberty to menopause, due to the antioxidant action of estrogens, as has been discussed in the previous section. However, the depletion of estrogens in women after menopause causes oxidative stress to increase, generating oxidative damage (Mendoza and Jiménez Zamarripa, 2013) and consequently accelerating the ageing rate in women at an elderly age. This could be the reason why in elderly populations, sex differences in oxidative stress markers are not as pronounced (Pinchuk et al., 2019). In fact, ovariectomy in female mice has been shown to increase oxidative damage in leukocytes, reaching similar levels to those of males (Baeza et al., 2011). Nevertheless, females display a higher lifespan than males, which suggests that the slower accumulation of oxidative damage that females experience across life could account for their observed longer lifespan.

In parallel, ageing is characterized by immunosenescence and inflamm-ageing, which translates into a higher incidence of infections, a dampened response to vaccines, and increased morbidity and mortality (Martínez de Toda et al., 2016). While both sexes experience age-associated changes in the immune system, men have been considered to experience them at a more dramatic or accelerated rate (Gubbels Bupp, 2015; Gomez et al., 2018). As has been mentioned in the previous section, women experience lower rates of infections during adulthood, an advantage attributed to stronger immune and vaccine responses and more efficient pathogen clearance (Gubbels Bupp, 2015; Márquez et al., 2020). On the other hand, women are more susceptible to autoimmune diseases than men also during the period from puberty to menopause. However, after the age of menopause, the incidence of autoimmune diseases in women decreases close to the numbers observed in men (Gubbels Bupp, 2015). The specific time windows of these changes, before and after menopause, point to the crucial role of sex hormones, estrogens, and androgens, in shaping immune ageing, although we can't rule out that are due to the differential expression of immunoinflammatory genes that are X chromosome encoded (Gubbels Bupp, 2015; Klein and Flanagan, 2016).

As stated above, men seem to experience immunosenescence to a greater extent than women. This could be due to women displaying a stronger immune and adaptive immune system during adulthood, such as higher basal immunoglobulin levels, higher CD4 +T cell counts, and an increased CD4/CD8 cell ratio compared to men (Gubbels Bupp, 2015; Gomez et al., 2018). Interestingly, a recent study conducted investigating genomic differences in peripheral blood mononuclear cells (PBMCs) between both sexes, demonstrated that sex differences in genetic expression increase after age 65, with men having higher innate and pro-inflammatory activity and lower adaptive activity (Hägg and Jylhävä, 2021). Older women had higher genomic activity for adaptive immune cells, while older men had higher activity for monocytes and inflammation, indicating greater inflamm-ageing in men (Márquez et al., 2020). In the same study, a life-course analysis of the timing of epigenomic regulation of chromatin accessibility showed that male immune cells are more strongly affected with ageing and that a decline in immune function occurs 5–6 years earlier in men than in women. The overall worse immune response that men show during adulthood, together with the earlier age-related decline in immune function, could be other important contributors to their shorter lifespan.

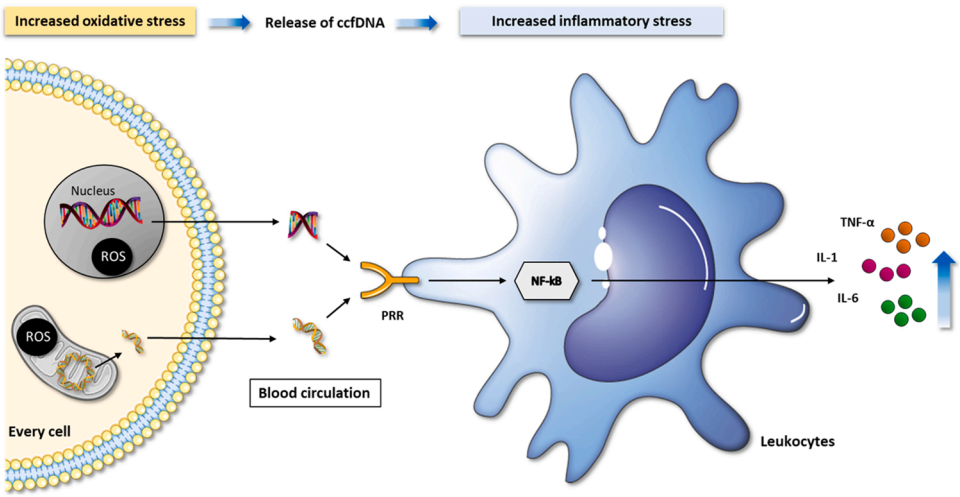

Fig. 3. Circulating cell-free DNA (ccfDNA) as a link between oxidation and inflammation. The loss of balance between reactive oxygen species (ROS) and antioxidant defenses, leads to the establishment of an oxidative stress situation that damages mitochondrial DNA (mtDNA) in the first place, and also nuclear DNA (nDNA) generating fragments that can actively or passively exit the cell. When these DNA fragments are outside the cell they are called circulating cell-free DNA (ccfDNA) and can trigger an inflammatory response in immune cells, acting as damage-associated molecular patterns (DAMPs) that bind to pattern recognition receptors (PRRs) and, through activation of the transcription factor NF-κB, induce the production and release of pro-inflammatory cytokines, providing another mechanistic link between oxidative and inflammatory stress by the interplay of immune cells.

6. Conclusions

The mechanisms of oxidation and inflammation have been proposed to be the roots of both ageing and age-related diseases, and consequently, they could explain the differences observed in ageing and lifespan between both sexes. In general, males seem to be more oxidized than women and this could be due to the antioxidant action of estrogens. Moreover, the male sex has more inflammation than women at the basal level, which could be the result of its increased oxidative damage accumulation, while they have a weaker pro-inflammatory response against an acute challenge. Although in the review only higher or lower terms have been used, it needs to be pointed out that the mentioned differences between sexes are indeed subtle. Still, small-scale differences can have a substantial impact. We propose that the observed sex differences in markers of oxidation and inflammation can underlie the differences in lifespan between sexes. In addition, we propose that the age-related increase in oxidation and inflammation can be linked by circulating cell-free DNA (ccfDNA), as ccfDNA increases as a result of the age-related increase in oxidative damage to cells, and at the same time, can induce a chronic activation of immune cells in the absence of a pathogen, contributing to the low-grade chronic inflammation observed with ageing, the so-called inflamm-ageing. Moreover, based on the connection that ccfDNA has to both age-related processes of oxidation and inflammation, we postulate its promising role as a valuable biomarker of ageing.

Nevertheless, more research is needed including sex as an essential variable in experimental designs to obtain conclusive results that could shed light on the different ageing mechanisms and trajectories between men and women. Moreover, understanding the grounds of the sex differences in ageing would lead to a better comprehension of ageing itself and to the development of personalized medicine approaches to address the age-related decline and age-related diseases more efficiently.

Declaration of interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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