Redox Experimental Medicine

Mini-Review

Redox medicine in viral infections: focus on AIDS and COVID-19

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Abstract

Despite the great progress and advancements in scientific knowledge, technology, and medicine, viral infections continue to put human health in trouble. Both acquired immunodeficiency syndrome (AIDS) and coronavirus disease-2019 (COVID-19), caused by HIV and SARS-CoV-2, two RNA viruses responsible for global pandemics respectively, have poor outcomes associated with increased oxidative stress, systemic inflammation, and immunopathology. Here, we have collected the current knowledge linking both viral infections, focusing on the role of oxidative stress and the redox balance. Furthermore, we provide information on some redox-active compounds, such as vitamins, thiol-based agents, and polyphenols, and their possible beneficial effects on both diseases. Thus, in this review, we aim to highlight the importance and impact of nutritional strategies to strengthen our immune system, especially to increase the effectiveness of pharmacological treatments, or when there are no effective treatments.
1. Oxidative stress-related mechanisms in HIV infection and treatment

1.1. A brief introduction to HIV and oxidative stress

HIV (human immunodeficiency virus) is a virus that attaches to the CD4 molecules and CCR5 (a chemokine co-receptor); then the virus surface and the cell membrane fuse (Negi et al., 2022) enabling the virus entry into a T-helper lymphocyte (Justiz Vaillant and Gulick, 2022). Thus, HIV targets the immune cells and weakens their ability to fight everyday infections and diseases. If left untreated, it can lead to AIDS (acquired immune deficiency syndrome) which is characterized, as its name indicates, by a deficiency of the immune system, which predisposes the patients to the development of certain cancers, infections, or other severe long-term clinical manifestations.

HIV first appeared in Central Africa during the first half of the 20th century. The global spread of the virus began in the late 1970s, and AIDS was first recognized in 1981 (World Health Organization, 2022). Since then, HIV infection continues to be a major global public health issue. Fortunately, effective prevention, early diagnosis, and highly active antiretroviral treatment (HAART) can make it a manageable chronic health condition (World Health Organization, 2022).

The mechanisms by which this virus spreads inside our immune system have been extensively described (Moir et al., 2011, Waymack and Sundareshan, 2022). In the following lines, we will focus on the relationship between HIV and oxidative stress. It has been generally acknowledged that HIV infection triggers massive ROS production, which in turn promotes HIV replication (Ivanov et al., 2016). The HIV tat (Trans-Activator of Transcription) gene is known to inhibit the expression of cellular superoxide dismutase (SOD). Reduced levels of this antioxidant enzyme cause a rapid depletion of sulfhydryl (SH) groups (also called thiol groups) and an increase in reactive oxygen species (ROS) levels which in turn activate the NF-κB signaling pathway involved in the transcription of HIV genome (Miesel et al., 1995). Finally, these redox alterations drive HIV pathogenicity, which includes exhaustion of CD4/CD8 T cells, organ cytotoxicity, and some side effects of antiretroviral therapy.

1.2. Azidothymidine treatment potentiates HIV-derived oxidative stress

Azidothymidine (zidovudine, AZT) was the first antiretroviral drug approved for the treatment of AIDS and, despite its numerous side effects, it remains one of the chemotherapeutic agents of choice approved by the Food and Drug Administration (FDA) (Kemnic and Gulick, 2022). AZT is a nucleoside analog that inhibits by competition the viral reverse transcriptase that HIV uses for its replication.

AZT has many side effects, including mitochondrial damage and toxicity, which could potentiate the already existing oxidative stress in HIV-infected patients. Indeed, a study analyzed the effects of AZT treatment in wild-type (control) mice and HIV-1 tat (transgenic) mice. The authors reported that AZT-treated control mice showed a 60% inhibition of the Mn-SOD activity, and AZT-treated transgenic mice displayed an 85%
inhibition of the enzyme’s activity. In both cases, the reduction of Mn-SOD activity was followed by increased protein carbonylation and reduced sulfhydryl groups, thereby suggesting that AZT treatment is an inducer of oxidative stress (Prakash et al., 1997). The mitochondrial toxicity of AZT is due to mitochondrial DNA damage caused by increased reactive oxygen species (Premanathan et al., 1997). An in-depth analysis performed by Butanda-Ochoa and colleagues demonstrated that AZT-mediated toxicity impairs mitochondrial function leading to increased ROS levels and oxidative stress in muscles and especially in the liver (Butanda-Ochoa et al., 2021). More recently, a study performed metabolic profiling of the sera of HIV-infected patients with an AZT-based antiretroviral treatment. The authors identified significant differences in metabolic features related to glutamine/glutamate metabolism; since glutamine is a precursor of glutathione, this finding suggests persistent oxidative stress in these patients (Sitole et al., 2022).

1.3. Antioxidant approaches to HIV infection

The control of imbalanced redox status by antioxidants might be beneficial for the quality of life of HIV patients. In the following paragraphs, we report data on some antioxidants (vitamins, NAC, and polyphenols) used as a therapeutic strategy for oxidative stress-associated disorders in HIV infection.

1.3.1. Vitamins

In vivo studies reported that AZT affected skeletal muscle, heart, liver, and neurons causing myopathy, cardiomyopathy, hepatotoxicity, and neurotoxicity respectively. A very promising solution to solve these side effects was the use of vitamins. This idea appeared when a study revealed that deficiencies in some vitamins were associated with an accelerated progression of HIV infection to AIDS (Tang and Smit, 1998). In particular, low cell/tissue amounts of vitamins A, B12, and especially, vitamin E, were related to increased levels of oxidative stress in HIV patients.

Indeed, it was demonstrated that vitamin E analogs could prevent NF-κB activation in an in vitro system (Staal et al., 1990), thereby hampering HIV transcription. Similarly, several studies revealed that mice infected with HIV had reduced levels of vitamin E in serum, which was accompanied by a dysregulated cytokine release and an altered immune system (Wang et al., 1994, Wang et al., 1995b, Wang et al., 1995a, Liang et al., 1996). Moreover, a longitudinal study and a randomized clinical trial in humans reported the same results (Tang et al., 1997, Allard et al., 1997). Interestingly, in both mice and humans, dietary intake of vitamin E was correlated with a slower progression of HIV infection.

Our group published some years ago a series of studies to determine the beneficial and protective effect of supranutritional doses of antioxidant vitamins (C and E) on skeletal muscle, liver, and heart from AZT-treated control mice. In our first study, we reported that AZT treatment caused an increase in peroxide production by skeletal
muscle mitochondria, which was accompanied by ultrastructural damage to mitochondria, increased mitochondrial lipid peroxidation, and oxidative damage to mitochondrial DNA (mtDNA), as demonstrated by high levels of 8-oxo-dG in urine. We found that antioxidant supplementation reverts this oxidative damage and the ultrastructural changes of muscle mitochondria caused by AZT (de la Asuncion et al., 1998).

Our next study focused on AZT-derived damage to liver mitochondria. We found similar results: increased peroxide production (over 240%) and oxidized mtDNA (40% more) in liver mitochondria from AZT-treated mice compared to untreated mice. This oxidative damage was also prevented by the dietary administration of vitamins C and E (de la Asuncion et al., 1999). Similarly, another study reported that 2 months of treatment with AZT significantly increased liver weights, plasma triglycerides, and total cholesterol. These effects, as well as oxidative stress and apoptosis, were mitigated by vitamin E administration compared to untreated rats (Adebiyi et al., 2015).

In another study, we aimed to test whether AZT treatment causes oxidative damage to heart mitochondria. As expected, we found increased mitochondrial lipid peroxidation and oxidation of mitochondrial GSH, as well as over 120% more 8-oxo-dG in the mitochondrial DNA of AZT-treated mice. Dietary supplementation with supranutritional doses of the antioxidant vitamins C and E protected against these signs of mitochondrial oxidative stress (de la Asuncion et al., 2004). Another study on rats investigated whether AZT treatment’s effect could impair cardiac function by affecting intercellular junctions. Rats were treated for 8 months with AZT, vitamin C, or a combination of both. Their results proved that AZT treatment induced ROS-mediated damage to cardiac intercalated discs that was prevented by vitamin C (Belloni et al., 2009).

Taken together, vitamin supplementation seems to prevent AZT-derived myopathy, hepatotoxicity, and cardiomyopathy (Figure 1).

Lastly, we also investigated the effect of vitamins on another common side effect of AZT: leukopenia. Our results demonstrated that AZT-derived leukopenia in control mice was abrogated by the administration of vitamins C and E. These vitamins diminished peroxide levels in myeloid precursors in the bone marrow and oxidized glutathione levels in blood (Garcia-de-la-Asuncion et al., 2007).

### 1.3.2. Thiol-based agents: N-acetyl-L-cysteine (NAC) and GSH

As mentioned above, another characteristic of HIV-infected patients is that they exhibit low levels of cysteine and reduced GSH in plasma. Glutathione is synthesized from cysteine, glutamate, and glycine by a series of reactions catalyzed by the action of γ-glutamylcysteine synthase and glutathione synthase. Thus, cysteine is required for the synthesis of GSH and is the rate-limiting factor.

The liver transforms methionine into cysteine through the transulfuration pathway, which is mediated by γ-cystathionase. We reported that liver samples...
obtained from AIDS patients displayed reduced levels and activity of this enzyme, thereby resulting in low levels of both cysteine and GSH (Martin et al., 2001).

GSH deficiency leads to ROS-induced activation of the NF-κB signaling pathway, which in turn activates HIV gene expression. In vitro studies showed that N-acetylcysteine (NAC) raises intracellular GSH levels and inhibits HIV-1 replication in persistently infected cultured cells (Mihm et al., 1991, Staal et al., 1993).

A randomized, placebo-controlled pilot trial (NCT01962961) assessed the effect of PharmaNAC (at 900 mg twice daily for 8 weeks) on HIV-infected adults (above 50 years old) who were receiving antiretroviral treatment. They found that PharmaNAC effectively increased the levels of oxidized glutathione (GSH) but decreased the levels of reduced glutathione (GSSG) in red blood cells, thereby leading to non-significant increased ratios of GSH:GSSG compared to placebo (Gupta et al., 2016). More recently, another clinical trial (NCT02348775) reported that patients with HIV display premature aging and develop geriatric comorbidities; the authors hypothesized that these effects were derived from GSH deficiency in these patients. Indeed, they assayed the effect of an 8-week treatment with glycine and NAC (GlyNAC) on several parameters including GSH concentrations, mitochondrial function, autophagy, oxidative stress, and inflammation. They concluded that GlyNAC treatment improved all these parameters related to premature aging (Kumar et al., 2020). Similarly, GlyNAC has proven to rapidly improve health-related quality of life and lower the perception of fatigue in HIV-infected patients (Sekhar, 2021).

NAC treatment for 8 weeks replenished GSH levels in the plasma of HIV patients (De Rosa et al., 2000). But, it has also been reported that the intracellular GSH levels in lymphocytes from HIV-infected patients treated with NAC are not significantly higher than placebo (Nakamura et al., 2002). A phase II randomized clinical trial (RIPENACTB) analyzed NAC treatment concomitantly administered with anti-tuberculosis treatment in patients with tuberculosis and HIV coinfection since tuberculosis causes significant mortality in HIV-infected patients. The authors reported that NAC co-administration was safe and entailed a significant increase in GSH levels and total antioxidant status (Safe et al., 2020).

### 1.3.3. Polyphenols

Polyphenols have also antioxidant properties and therefore, they could also be suitable candidates for HIV treatment.

Indeed, a study analyzed the effects of tannic acid on AZT-derived hepatotoxicity in HIV-infected mice. AZT treatment increased the plasma levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase, thereby supporting its hepatotoxic activity. Treatment with tannic acid lowered AZT-derived hepatotoxicity thus restoring the levels of ALT, AST, and alkaline phosphatase. Moreover, tannic acid also promoted an increase in GSH levels and a decrease in malondialdehyde levels in AZT-treated mice, thereby decreasing oxidative stress and damaging (Tikoo et al., 2008).
Another study using primary human cardiomyocytes evaluated the effects of pretreatment with resveratrol before AZT treatment on mitochondrial ROS generation. The authors reported that resveratrol attenuated AZT-induced cardiomyocyte death through modulation of caspase-3 and -7 activity and poly (ADP-ribose) polymerase (PARP) activation. AZT also increased mitochondrial ROS generation in a concentration-dependent manner, which was prevented by resveratrol pre-treatment (Gao et al., 2011). Another in vitro study using established HIV-1 transcription and latent cell models assayed the mechanism by which resveratrol stimulates HIV-1 gene transcription. They reported that resveratrol promoted HIV-1 Tat protein levels, which were dependent on AKT/FOXO1 signaling (Feng et al., 2021). Similarly, pretreatment with resveratrol increased intracellular NAD+ levels and sirtuin 1 (SIRT1) protein expression after Tat plasmid transfection and attenuated Tat-induced HIV-1 transactivation in an in vitro cellular model (Zhang et al., 2009). Thus, resveratrol could be a promising cotreatment to eradicate HIV-1 reservoirs. Lastly, it has also been suggested that HIV-1 decreases nuclear factor erythroid-derived 2 (Nrf2) activity and inhibits the antioxidant response element (ARE) leading to immune dysfunction in individuals with HIV infections (Staitieh et al., 2017). Since resveratrol is a well-known activator of Nrf2/ARE (Farkhondeh et al., 2020), it would be of utmost importance to test its efficacy against HIV infection.

More recently, silibinin, another polyphenol obtained from Silybum marianum, also proved to be an efficient treatment against AZT-derived oxidative stress. In the following studies, the authors evaluated the alleviating properties of silibinin against AZT-induced hepatotoxicity and oxidative stress in rats. AZT treatment increased ALT, AST, and alkaline phosphatase levels in serum thus suggesting that the oral dosage was effectively hepatotoxic. In parallel, oxidative stress was observed by increased lipid peroxidation and total carbonyl content, as well as reduced SOD and catalase activities, and protein thiol levels in liver homogenates. Simultaneous treatment of silibinin prevented liver hepatotoxicity and oxidative stress induced by AZT (Raghu et al., 2015, Raghu and Karthikeyan, 2016).

Lastly, coffee consumption, which has anti-inflammatory and hepato-protective properties, has also been evaluated on patients co-infected with HIV and hepatitis C virus (HCV). This study proved that 3 cups of coffee a day for 5 years was associated with a 50% reduced risk of all-cause mortality in these patients (Carrieri et al., 2017).

2. Oxidative stress-related mechanisms in SARS-CoV-2 infections

2.1. Role of oxidative stress in SARS-CoV-2 infection

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11th, 2020. Although the pathogenesis of this viral
infection is still poorly understood, the main signs and symptoms include respiratory and cardiovascular problems, which can progress to severe organ failure and death.

Oxidative stress is related to all the main changes observed in other similar infectious diseases and has therefore been investigated as one of the links that connect all these events during SARS-CoV-2 infection.

Experimental animal models showed increased ROS levels and an altered antioxidant defense during SARS-CoV-2 infection (Huang et al., 2020). These observations have been validated in human samples from infected patients. A study reported significantly higher levels of superoxide anion radicals and lower levels of nitric oxide in severe cases of SARS-CoV-2 compared to healthy individuals (Cekerevac et al., 2021). Similarly, other studies found reduced antioxidant defenses (SOD, CAT, GPx) that were correlated with the disease severity in hospitalized patients with COVID-19 (Karkhanei et al., 2021). Moreover, the antioxidant defenses collapse was also confirmed by the evidence of very low levels of vitamin C, GSH, protein thiols, α-tocopherol, and β-carotene in blood samples obtained from critically ill COVID-19 patients (Pincemail et al., 2021). Also, serum-free thiol concentrations have been analyzed and correlated with the disease severity: hospitalized subjects had significantly lower levels of serum-free thiols compared to non-hospitalized subjects and healthy controls (van Eijk et al., 2021). Lastly, oxidative damage to lipids has also been detected in plasma samples from patients at admission (Zendelovska et al., 2021). Indeed, physiological serum levels of 7-ketocholesterol and 7β-hydroxycholesterol in SARS-CoV-2 positive subjects were significantly increased compared to healthy subjects (Marcello et al., 2020). Taken together, there is enough evidence suggesting that excessive ROS levels and a limited antioxidant system play a central role in SARS-CoV-2 infection, progression, and severity of the disease (Delgado-Roche and Mesta, 2020).

According to the current knowledge, the underlying mechanism seems to be as follows: SARS-CoV-2 virus uses the angiotensin-converting enzyme 2 (ACE2) receptor as the entry site into human respiratory epithelial cells (Hamming et al., 2004). ACE2 receptor is a metallopeptidase located in the cell membrane that promotes the conversion of Angiotensin II (potent vasoconstrictor and ROS producer) into Angiotensin 1-7 (potent vasodilator and ROS inhibitor) (Suhail et al., 2020). Importantly, once the virus has entered to cell, ACE2 expression is downregulated (Hoffmann et al., 2020). As a consequence, Angiotensin II accumulates and activates NADPH oxidases (through AT1-TLR4), leading to ROS overproduction and increased release of inflammatory molecules (Wieczfinska et al., 2022). After the virus enters the airways, the immune innate response begins with the activation of monocytes and macrophages that release IL-1, IL-6, IL-8, and TNF (Kozlov et al., 2021). In their turn, neutrophils release ROS to promote the cell death of infected cells. Moreover, SARS-CoV-2 infection has been associated with the inhibition of the Nrf2 signaling pathway and the activation of the NF-κB signaling pathway, leading to inflammation and oxidative damage (Cecchini and Cecchini, 2020, Delgado-Roche and Mesta, 2020).
Taken together, COVID-19 is characterized by a cytokine storm, but also by an oxidative stress storm with all the derived deleterious effects, such as oxidative damage to lipids, proteins, and DNA leading to hyalinization of pulmonary alveolar membranes (Xu et al., 2020) with lethal respiratory distress (Ntyonga-Pono, 2020). The severity and mortality risk of this disease have been associated with age, as aging is associated with increased oxidative stress and inflammation, which is exacerbated by the SARS-CoV-2 infection.

2.2. Antioxidant approaches in SARS-CoV-2 infection

Oxidative stress appears to be a promising target to fight some of the effects of COVID-19 infection. Indeed, some of the antioxidant agents that have been used are NAC and GSH, vitamins, and polyphenols. However, more research is still needed in this area.

2.2.1. Vitamins

Several studies have reported low levels of vitamins C and D in SARS-CoV-2 infection (Kalyanaraman, 2020, Arvinte et al., 2020), and a strong correlation has been established between these vitamin levels in the organism and COVID-19 severity and mortality rate (Daneshkhah et al., 2020, Rhodes et al., 2021).

Vitamin D deficiency was suggested as a predictor of poor prognosis (Carpagnano et al., 2021). Vitamin D at low levels promotes the over-activation of the renin-angiotensin-aldosterone system (RAAS), leading to increased levels of Angiotensin II, and vice versa (Ferder et al., 2013). On the other hand, vitamin D increases the expression of antioxidant enzymes, such as glutathione reductase. Hence, vitamin D supplementation might ameliorate COVID-19 disease symptoms by exerting an antioxidant effect, but also through RAAS inhibition (de Las Heras et al., 2020). Indeed, bolus vitamin D supplementation in COVID-19 patients was correlated to less severe disease progression and enhanced survival rate (Annweiler et al., 2020). Therefore, vitamin D supplementation has been recommended for COVID-19 patients (Albergamo et al., 2022), however, the existing clinical trials analyze heterogenous patient populations and different vitamin D dosages and administration routes, which make it difficult to establish a proper treatment (Abdrabbo et al., 2021, Szarpak et al., 2021). Moreover, it should be considered that vitamin D also enhances ACE2 expression, which in turn could result in enhanced SARS-CoV-2 binding and an aberrant immune response (Cereda et al., 2021) (Figure 2).

Regarding vitamin C, unsuccessful results have been reported in a randomized clinical trial with COVID-19 patients, where the authors reported no significant improvements when patients were administered vitamin C (Thomas et al., 2021).

Taken together, more research is needed to determine whether vitamins C or D could exert therapeutic effects on SARS-CoV-2 infection.
2.2.2. Thiol-based agents: N-acetyl-L-cysteine (NAC) and GSH

It has been reported that GSH deficiency is correlated with increased susceptibility to SARS-CoV-2 infection in the elderly and patients with pre-existing medical conditions, such as diabetes (Polonikov, 2020). Based on the ability of GSH to alleviate oxidative stress, reduce interleukins circulating levels, and modulate viral load, it has been proposed that glutathione supplementation could be a therapeutic agent for COVID-19 patients (Guloyan et al., 2020). Indeed, increased levels of GSH lower viral load and viral infection, reduce oxidative stress and pro-inflammatory cytokines release, and boost immune function (Kalyanaraman, 2020).

Drugs with a functional thiol group (“thiol drugs”) might cleave cystines to hamper SARS-CoV-2 entry into the cell. To test this hypothesis, Khanna and colleagues analyzed the effects of cysteamine delivered intraperitoneally to SARS-CoV-2-infected hamsters. Although the reached concentrations of cysteamine in the lung were not sufficient for antiviral effects, they were sufficient for anti-inflammatory effects (Khanna et al., 2022). Accordingly, several in vitro and in vivo studies have suggested that NAC, which reduces the disulfide bonds (-S-S) to sulfhydryl groups (-SH) thus increasing intracellular GSH levels, improves T cell response, inhibits the NLR family pyrin domain containing 3 (NLRP3) inflammasome pathway, and inhibits viral replication (Poe and Corn, 2020). However, a double-blind randomized clinical trial using high doses of NAC in SARS-CoV-2 infected patients did not show any significant beneficial effect when compared to patients that received a placebo (de Alencar et al., 2021). More studies are needed to determine if NAC treatment could offer a benefit for COVID-19 patients (Forcados et al., 2021).

2.2.3. Polyphenols

Resveratrol is a polyphenol with demonstrated anti-viral and anti-inflammatory properties that might mitigate the signs and symptoms of COVID-19 disease. Indeed, it has been shown that resveratrol hampers the entry of the virus inside the cell; it disrupts the spike protein-ACE2 complex, through sirtuin 1 (SIRT1)-dependent mechanisms (Horne and Vohl, 2020). There is a clinical trial reporting that resveratrol-treated patients have a lower incidence of hospitalization, COVID-related ER visits, and pneumonia, compared to the placebo group (McCreary et al., 2021). Unfortunately, no parameters related to oxidative stress, inflammation, or virus load were measured. Another study performed a computational approach and discovered that resveratrol could inhibit the RNA-dependent RNA polymerase of SARS-CoV-2 since the estimated binding affinity was better than control compounds such as remdesivir (Wu et al., 2021).

Similarly, curcumin has been assayed as a possible treatment for COVID-19. An in silico study established that curcumin could inhibit Omicron Spike protein and Omicron S-hACE2 complex (Nag et al., 2022). Several clinical trials administer curcumin to COVID-19 patients to determine possible therapeutic effects regarding inflammatory mediators, disease progression, and severity (Miryan et al., 2020, Ahmadi et al., 2021,
Saber-Moghaddam et al., 2021, Valizadeh et al., 2020, Tahmasebi et al., 2021a, Tahmasebi et al., 2021b, Pawar et al., 2021). The main conclusion of these clinical trials is that curcumin supplementation reduced pro-inflammatory cytokines and increased anti-inflammatory cytokines, thus restoring the balance and leading to a significant decrease in common symptoms, hospitalization, and deaths (Vahedian-Azimi et al., 2022).

2.2.4. Oxysterols

Other “redox molecules”, named oxysterols, are known to have antiviral properties (Lembo et al., 2016). These molecules are cholesterol oxidation products that can be formed enzyme-dependent or -independent. The formers, 24-, 25- and 27-hydroxycholesterol (HC), are good ligands of several cell membrane receptors, thus modulating downstream signaling pathways (Poli et al., 2022). Indeed, oxysterols have recently been suggested to substantially inhibit SARS-CoV-2 viral replication and propagation in cultured cells (Ohashi et al., 2021).

It is known that enveloped viruses use cholesterol-rich regions of the cell membrane to enter the host cell through a variety of mechanisms including membrane curvature formation, receptor clustering, and binding to viral fusion proteins (Foo et al., 2022). Indeed, 25HC and 27HC have proven to efficiently inhibit SARS-CoV-2 entry and replication (Yuan et al., 2020, Marcello et al., 2020) inside target cells using in vitro models.

The exact mechanism to hamper the virus entry relies on the fact that oxysterols alter the membrane fusion process through the modification of lipid membrane properties. Indeed, in vitro studies have suggested that oxysterols drive a cholesterol remodeling of the cellular plasma membrane upon infection through Acyl-CoA cholesterol acetyltransferase (ACAT) activation (Zu et al., 2020, Zang et al., 2020, Wang et al., 2020). Other described mechanisms suggest that 25HC and 27HC reduce the expression of the cation-independent mannose-6-phosphate receptor (MPRci) and junctional adhesion molecule A (JAM-A), both molecules needed for the virus entry (Civra et al., 2020). Moreover, 27HC has been shown to inhibit SARS-CoV-2 replication by reducing the formation of double lipidic membrane vesicles (Ohashi et al., 2021).

Also interestingly, 27HC, but not 25HC, was found at lower levels in serum samples of SARS-CoV-2 infected patients when compared to control healthy subjects (Marcello et al., 2020).

3. Concluding remarks and future perspectives

HIV and SARS-CoV-2 are both enveloped, RNA viruses, and their entry into the cell is a two-step process involving virion binding to cell-surface receptors and fusion of the viral envelope with cell membranes. Fenouillet et al., described in detail how both
viruses require a specific thiol content to trigger their entry (Fenouillet et al., 2007). Thus, one can conclude that the thiol-disulfide balance is of utmost importance for the viral entry inside the cell.

Moreover, several studies have unraveled the strategy of these viruses to alter the redox balance of a cell to survive (Cecchini and Cecchini, 2020). These findings confirm that oxidative stress is a key factor in the success or failure of the host’s response to viral infection. Hence, vitamins, thiol-based agents, and polyphenols have demonstrated promising results towards oxidative stress-related complications in AIDS and COVID-19 diseases.

Taken together, both AIDS and COVID-19 may offer new possibilities to improve the diagnosis and treatment of redox-related diseases.

Declaration of interest
Cristina Mas-Bargues is an author of this manuscript and a member of the editorial board. She has had no role in the peer review process of this manuscript.

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Author contribution statement
C.M.-B. was in charge of conceptualization, and writing-original draft preparation. C.B. and J.V. were in charge of writing and editing the review and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Figure legends

**Figure 1. Vitamin supplementation to prevent AZT-derived myopathy, hepatotoxicity, and cardiomyopathy.** AZT: Azidothymidine, HIV: human immunodeficiency virus, SOD: superoxide dismutase.

**Figure 2. Dual role of vitamin D supplementation to prevent COVID-19 disease.** SRAA: system renin-angiotensin-aldosterone, RONS: reactive oxygen and nitrogen species, ACE: Angiotensin-converting enzyme.

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**Conflict of interests**

Cristina Mas-Bargues is an author of this manuscript and a member of the editorial board. She has had no role in the peer review process of this manuscript.

**References**

531 HUELSBECK, A. C., LABERGE, B., LEAVENS, C. M., LE, C. N., LINDQUIST, A. C.,
532 LUDWIG, R. K., REYNOLDS, J. H., SEVERSON, N. J., SHERMAN, B. A., SILLMAN, H.
533 W., SMITH, M. A., SMITH, M. A., SNORTHEIM, M. J., SVAREN, L. M., VANDERPAAS,
535 Vitamin D and COVID-19: A review on the role of vitamin D in preventing and
537 ADEBIYI, O. O., ADEBIYI, O. A. & OWIRA, P. M. 2015. Naringin Reverses Hepatocyte
538 Apoptosis and Oxidative Stress Associated with HIV-1 Nucleotide Reverse
539 Transcriptase Inhibitors-Induced Metabolic Complications. *Nutrients*, 7, 10352-
540 68.
541 AHMADI, R., SALARI, S., SHARIFI, M. D., REIHANI, H., ROSTAMIANI, M. B., BEHMADI, M.,
543 Oral nano-curcumin formulation efficacy in the management of mild to
544 moderate outpatient COVID-19: A randomized triple-blind placebo-controlled
545 clinical trial. *Food Sci Nutr*, 9, 4068-4075.
546 ALBERGAMO, A., APPRATO, G. & SILVAGNO, F. 2022. The Role of Vitamin D in Supporting
549 peroxidation during n-3 fatty acid and vitamin E supplementation in humans.
551 ANNWEILER, C., HANOTTE, B., GRANDIN DE L’EPREVIER, C., SABATIER, J. M., LAFAIE, L.
552 & CELARIER, T. 2020. Vitamin D and survival in COVID-19 patients: A quasi-
554 ARVINTE, C., SINGH, M. & MARIK, P. E. 2020. Serum Levels of Vitamin C and Vitamin D
555 in a Cohort of Critically Ill COVID-19 Patients of a North American Community
556 Hospital Intensive Care Unit in May 2020: A Pilot Study. *Med Drug Discov*, 8,
557 100064.
558 BELLONI, A. S., PETRELLI, L., RUGA, E., MILANESI, O., RICATO, S., CAVALLI, M.,
559 CARGNELLI, G. & BOVA, S. 2009. AZT dilates rat cardiac intercalated discs, and
562 oxidative damage in the liver. *Toxicology*. Elsevier.
563 CARPAGNANO, G. E., DI LECCE, V., QUARANTA, V. N., ZITO, A., BUONAMICO, E.,
566 CARRIERI, M. P., PROTOPOPESCU, C., MARCELLIN, F., ROSELLINI, S., WITTKOP, L.,
567 ESTERLE, L., ZUCMAN, D., RAFFI, F., ROSENTHAL, E., POIZOT-MARTIN, I.,
569 effect of coffee consumption on all-cause mortality of French HIV-HCV co-
570 infected patients. *J Hepatol*, 67, 1157-1167.
571 CECCHINI, R. & CECCHINI, A. L. 2020. SARS-CoV-2 infection pathogenesis is related to
573 CEKEREVAC, I., TURNIC, T. N., DRAGINIC, N., ANDJIC, M., ZIVKOVIC, V., SIMOVIC, S.,
574 SUSA, R., NOVKOVIC, L., MIJAILOVIC, Z., ANDJELKOVIC, M., VUKICEVIC, V.,
575 VULOVIC, T. & JAKOVLJEVIC, V. 2021. Predicting Severity and Intrahospital


Figure 1. Vitamin supplementation to prevent AZT-derived myopathy, hepatotoxicity, and cardiomyopathy. AZT: Azidothymidine, HIV: human immunodeficiency virus, SOD: superoxide dismutase.

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Figure 2. Dual role of vitamin D supplementation to prevent COVID-19 disease. SRAA: system renin-angiotensin-aldosterone, RONS: reactive oxygen and nitrogen species, ACE: Angiotensin-converting enzyme.