

## Cholesterol in virus entry into host cell: diet, phytosterols and statins

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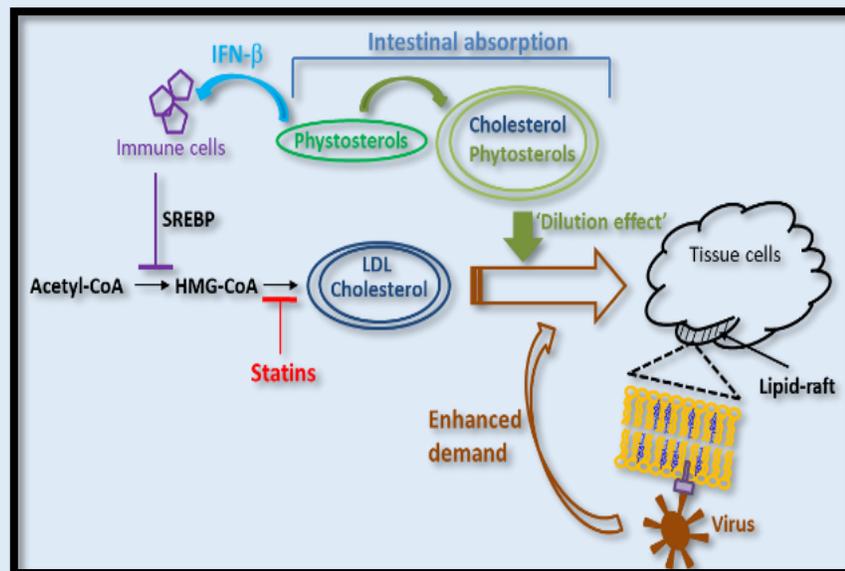
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### ABSTRACT

Cholesterol has been reported in a number of studies to play an essential role in the effectiveness of viral infection in mammal cells. The main characteristic of cholesterol that is involved in viral infection is its capability to afford negative curvature to the membrane. This faculty of cholesterol has to do with the necessary

steep curvature that the host cell membrane undergoes in the primary processes of viral infection. In particular, for RNA enveloped viruses, membrane curvature is involved in two mechanisms of the viral infection, which are virus and host cell membrane fusion for viral genome release and virus reproduction scaffold build-up. Low cholesterol diets have been shown to reduce or even suppress virus infection efficiency in murine models or cell cultures; but to what extent a diet-based lowering of the blood cholesterol level may help preventing virus infection still lacks enough scientific evidence. The use of statins in individuals with hypercholesterolemia has been recommended in the recent COVID-19 outbreak. The possibility of using sterols from natural sources in the



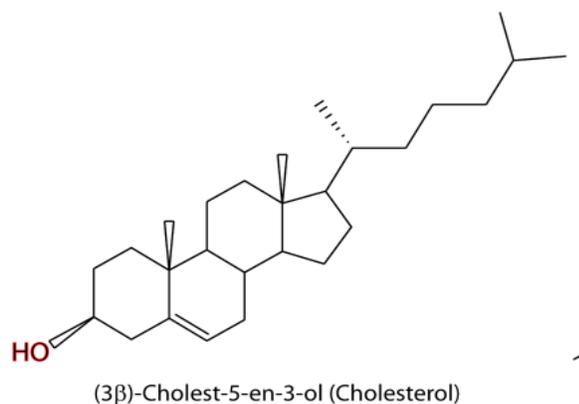
diet or in supplementary concentrates has been suggested to be an alternative to drop the circulating cholesterol. In this manuscript, the most relevant and recent bibliography on the aforementioned issues is reviewed.

**Keywords:** cholesterol, virus infection, membrane curvature, sterols, natural food sources

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## INTRODUCTION

In spite of contradictory results have been reported in some studies, in particular for older people [1,2], cholesterol is widely known at present to be involved in certain pathological processes that affect the human health, especially those regarding cardiovascular risk, [3–6]. However, cholesterol is an essential component molecule of the biological membranes in mammals, besides being the precursor of other relevant molecules of the mammal metabolism like steroid hormones, bile acids and vitamin D [7–9].



**Figure 1.** Schematic drawing of the chemical structure of the cholesterol molecule

The cholesterol molecule ((3-β)-Cholest-5-en-3-ol, PubChem CID 5997) is a very low polar lipid composed of four hydrocarbon cycles with a hydroxyl group and a dimethyl-hexyl chain bound to different positions of the cycles (Figure 1). The cholesterol

tetracyclic structure is embedded into the phospholipid acyl chain bulk of the membrane, whose fluidity, stability, through controlling the phospholipid packaging, and permeability regarding molecule flux across, the cholesterol contributes to regulate [10]. Every mammal cell is able to synthesise cholesterol through the mevalonate pathway, but about 80% of cholesterol is synthesised in the liver and the intestines, the remaining circulating cholesterol being acquired through the diet (Figure 2, scheme A). Because of the hydrophobic nature of the cholesterol, it is transported through the organism within plasma lipoproteins, namely very-low density lipoproteins (VLDL), low density lipoproteins (LDL) and the high density lipoproteins (HDL) (Figure 2, scheme B). VLDLP and LDL deliver the cholesterol cargo to the different tissues, whereas HDL is responsible to recycle the cholesterol in excess by transporting it backward to the liver; hence, they are commonly known as “bad cholesterol” (VLDL and LDL) and “good cholesterol” (HDL), respectively [11,12]. Under non-pathological conditions, the cholesterol metabolism is thoroughly regulated at the biosynthetic level in the liver (Figure 2, scheme A). However, cholesterol intake in excess may dysregulate its control mechanisms [6,9,13]. Recommended levels of cholesterol in blood are depicted in Table 1.

**Table 1.** Recommended healthy levels of cholesterol in blood in its different forms as reported by the National Library of Medicine of the National Institutes of Health(NLM-NIH, <https://medlineplus.gov/cholesterollevelswhatyouneedtoknow.html>)

Cholesterol form	Healthy level in blood	
	Men	Women
<b>Total</b>	125 – 200 mg/dL	125 – 200 mg/dL
<b>LDL</b>	< 100 mg/dL	< 100 mg/dL
<b>HDL</b>	≥ 40 mg/dL	≥ 50 mg/dL

A low energy diet is recommended to avoid obesity and the risk of heat strokes. This diet, which includes vegetables and fruits as main intakes, is also the main dietary style to reduce cholesterol. A vegetarian diet style has been reported to intake 140 mg/day less of cholesterol than a non-vegetarian diet [6].

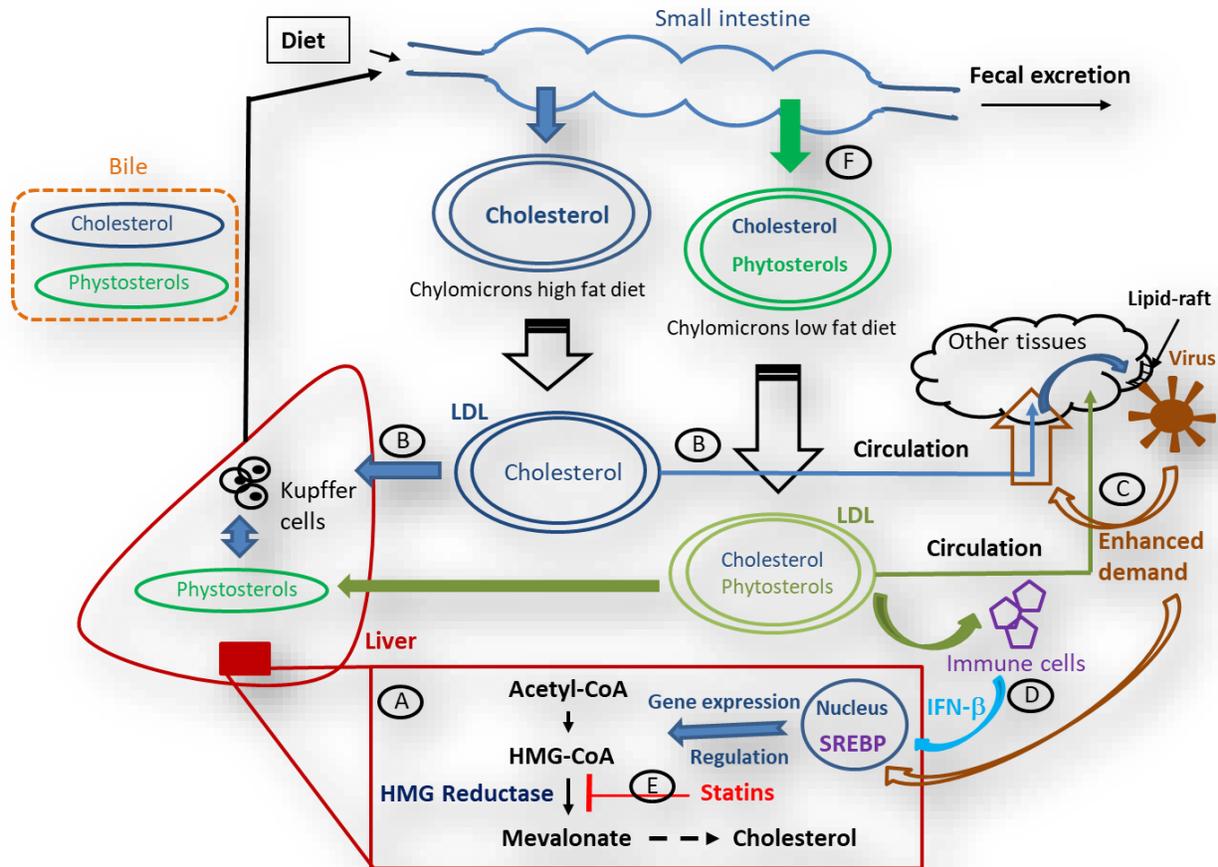
**Cholesterol in virus infection:** Because of cholesterol is an essential component of the animal cell membranes, the role played by cholesterol in virus infection has been matter of research over the last few decades [7–9]. In particular, enveloped RNA viruses have been found to hijack the host cell lipid metabolism to account for the cholesterol and other specific lipid demand for their replication scaffold build-up [14] (Figure 2, scheme C). Cholesterol is known to be a constituent of the virus membrane envelope, and transmembrane proteins inserted in specific domains of the host cell membrane known as lipid-rafts are preferred by the RNA viruses for docking to the infected cell during virus entry. Indeed, high cholesterol concentration in the micro-domain of the host membrane has been shown to be essential for viral and target cell membrane fusion in virus genome release into the cell, the cholesterol concentration acting as a pathway switch [15–17]. In

HIV-1 infection, the clustering of the virus envelope glycoproteins requires the interaction of the viral gp41 protein with cholesterol in the virus membrane, cholesterol acting as a molecular switch [18]. The angiotensin-converting enzyme 2 (ACE2), which is the binding protein for the severe acute respiratory syndrome coronavirus-1 and 2 (SARS-CoV-1 and 2) and other coronaviruses spike protein, was shown to be largely associated to lipid-rafts enriched in cholesterol [14,16,19,20]. Host cell cholesterol plays also an essential role in the infection by Flaviviruses [14,21].

The recent COVID-19 outbreak has prompted urgent research on the mechanisms used by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to infect the cells as well as the factors involved in such mechanisms. Different studies have shown that cholesterol content and metabolism pathways related to it are of primary relevance for virus entry into the cell. The C-terminal fragment of the sterol regulatory element-binding protein 2 (SREBP2), which is a transcription factor involved in cholesterol homeostasis, has been found to be present in blood of COVID-19 patients, and its presence is postulated to be a factor inducing the inflammatory response to infection that leads to the

cytokine storm [22]. The S1 subunit of the SARS-CoV-2 spike protein has been shown to bind cholesterol and other HDL components in order to facilitate virus

entry, this mechanism being mediated by the high-density lipoprotein (HDL) scavenger receptor B type 1 (SR-B1) [23]



**Figure 2.** Schematic drawing of the interaction of cholesterol metabolism with and its regulation by viral infection, and the positive intervention of statins and phytosterols to counteract the viral subversion of the cholesterol metabolism. Letter-indicated sections (A-F) of the figure are referred to in the text. A more detailed description of these mechanisms can be found in references [38] and [65].

Why do viruses need of cholesterol-enriched membrane micro-domains? The main reason, but not the unique one, for such requirement of cholesterol is considered to be that this molecule provides the curvature that is stipulated for efficient membrane fusion [24,25]. In influenza A virus (IAV), cholesterol-enriched domains in the host cell membrane were found to enhance virus entry efficiency, but not rate, in a receptor-independent manner, a feature that

leads the researchers to suggest that cholesterol improved membrane fusion by altering the membrane curvature [26]. Diverse studies have shown that lowering the cholesterol content in the cell, either by sequestering it or by means of synthesis inhibitory procedures, the virus entry is negatively affected, thus halting virus infection [14,24,27]. Viruses subverts the cholesterol biosynthetic pathway in their own benefit at the level of the sterol

regulatory element binding protein (SREBP), the S1 protein (S1P) or the cholesterol efflux regulatory protein (ABCA1), mechanisms that have been studied in different enveloped viruses like SARS-CoV, the hepatitis C virus (HCV), the human immunodeficiency virus (HIV), hantaviruses, the Dengue virus (DENV) and the West Nile virus (WNV). Therefore, drugs that drop the SREBP, S1P or ABCA1, enzymatic/transport activity have been suggested as potential treatments to ameliorate the virus infection pathology [20,27–31]. Another mechanism bound to cholesterol metabolism during viral infection is its delivery through lipid droplets that associate with autophagosomes, a process known as lipophagy, and which requires the activation of the AMP kinase/mTOR axis [32,33]. Some viral proteins have been identified to be implicated in this process through which the virus gets the required cholesterol to be delivered to the membranes of the replication complexes [33–36]. Additional pathways of the lipid metabolism like the fatty acid synthesis and fatty acid  $\beta$ -oxidation are also modified by the virus for its own profit.

**Cholesterol-related antiviral activity of the immune system:** Immediately after viral infection, the innate immune system reacts to halt the virus replication and budding. The lung injuries caused by the cytokine storm in SARS-CoV-2 infection has had deleterious and even lethal outcomes during the initial period of the COVID-19 outbreak. However, a softer and poorly deleterious antiviral reaction of the innate immune system is triggered through the interferon- $\beta$  (IFN- $\beta$ ). In positive single stranded RNA (ssRNA+) viruses, IFN- $\beta$  has been shown to antagonize the formation of the replication organelle membranes, thus avoiding virus replication [37]. The cholesterol biosynthesis is

downregulated by IFN- $\beta$  at the step of the protein SREBP2 of the mevalonate-isoprenoid branch (Figure 2, scheme D), where statins act as well [28]. A primary route through which IFN- $\beta$  interferes the mevalonate-isoprenoid branch of the sterol biosynthesis in the host cell has been shown to be inducing microRNAs that inhibit SREBP2 activity [38,39]. Viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible) mediates the antiviral activity after its induction by IFN- $\beta$  in macrophages through the interferon regulatory factor 3 (IRF3). The antiviral activity of viperin is related to the reduction of the cholesterol/sphingomyelin supply to the membranes, a mechanism in which upstream regulation by Toll-like receptor 4 (TLR4) is involved [40]. Accordingly, pharmacological treatment with interferon has been proposed in diverse studies to help ameliorating the viral infectivity.

Another interferon-related mechanism directly associated to cholesterol is that pointed out by Wang et al. in regard to SARS-CoV-2 [41]. These authors have shown that 25-hydroxy-cholesterol (25HC) may inhibit membrane fusion and, consequently, virus entry, through activation of the acyl-CoA:cholesterol acyl transferase (ACAT), which leads to cholesterol depletion in the plasma membrane. 25HC is overproduced in COVID-19 patients. The viral inhibitory capacity of 25HC has been demonstrated for a number of viruses, but the metabolic routes involved in the inhibitory effect remained elusive. 25-hydroxy-cholesterol is formed from cholesterol by the cholesterol 25-hydroxylase enzyme, which is induced after SARS-CoV-2 infection through interferon-stimulated genes (ISGs).

**Cholesterol reduction by statins as antiviral treatment:** The role that cholesterol plays in

cardiovascular disease was unveiled in the second half of the 20<sup>th</sup> century, and it was named the “lipid hypothesis”. The “lipid hypothesis” is well established nowadays as a major risk for heart attacks and other cardiovascular diseases even though scepticism still exists among certain groups and health professionals [6]. In order to struggle against this health risk, the Japanese Akira Endo started a research at the onset of the 1970<sup>th</sup> decade for development of a pharmacological treatment. The Akira Endo research team was able to isolate a compound from the fungus *Penicillium citrinum* that inhibited the mevalonate pathway at the step of the enzyme HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase), the key enzyme in the cholesterol and isoprenoid biosynthesis, and they named mevastatin such a compound. However, this compound had deleterious secondary effects on human health and was never marketed. Some years later-on, researchers of the pharma Merck & Co could isolate a compound, which they named lovastatin, from the fungus *Aspergillus terreus*, with not so negative secondary effects as mevastatin but which inhibited quite efficiently the HMG-CoA reductase (Figure 2, scheme E). Other statins have been developed afterwards, these including atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin, which may reduce LDL cholesterol content in blood by up to 50% [42] (see also Human Metabolome DataBase at <https://hmdb.ca/metabolites>). Only simvastatin is included in the World Health Organization (WHO) List of Essential Medicines. Nonetheless, in spite of their beneficial goals in lowering blood cholesterol, they have also negative effects on the human body and may result toxic above certain doses. These side effects are muscle pain or even severe damage to

muscle cells, increased risk of diabetes mellitus type 2, abnormal levels of circulating hepatic enzymes, and depressive mood alterations [43–45].

Given the capability of statins to inhibit the cholesterol biosynthesis, such type of drugs is currently used in research focused on viral infection mechanisms. At the COVID-19 onset, the use of statins, and other cholesterol modifying compounds, was proposed to reduce the SARS-CoV-2 infection capacity, encouraging active research on discerning the healing capacity of lipophilic and hydrophilic statins [46]. Specific statins are recommended for HIV patients in order to treat the disease-associated dyslipidaemia [47]. At present, the use of statins is still claimed for treating COVID-19 disease [12,48]. Lu et al. had shown for SARS-CoV-1 that depletion of cholesterol triggered the displacement of ACE2 from lipid-rafts to non-raft domains of the membrane, featuring in this way inefficient the viral entry mechanism [49]. Besides lowering cholesterol, the pleiotropic benefits of statins extend to anti-inflammatory, immunomodulatory, and antithrombotic properties, they likely playing a significant positive role regarding the amelioration of the cytokine storm and the atherothrombotic complications observed in those patients needing intensive care unit (ICU) admission [20]. Indeed, the COVID-19 pathology resembles to some extent and share traits with the cardiovascular disease [50]. In an *in silico* study, the antiviral effect of pitavastatin, and to a lesser extent of other statins, was found to be related to disruption of the viral protease, thus decreasing the maturing of viral glycoproteins and replication [51]. Nonetheless, it has been shown that the positive effect of statin treatment could depend upon the disease stage, such effect being more beneficial at the first stages of the disease.

**Phytosterols, diet and exercise:** Even though diet cannot prevent infection by SARS-CoV-2 or other viruses at all, an adequate nutrition may help facing the pathological outcomes of the COVID-19 and other viral diseases [52–56]. Regarding cholesterol, a diet that reduces the cholesterol and triglyceride intake while increasing the unsaturated fatty acid (PUFA) intake may help the body to counteract the required extra-energy and viral subversion of the lipid metabolism [57]. As pointed out above, viral hijacking of the lipid metabolism promotes increased synthesis of cholesterol and saturated fatty acids [14], a fact that provokes shortage of cell lipid requirements, and, consequently, replenishing the body's PUFA demand is of concern. In laboratory experiments, the main PUFA, that is arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have shown capability to inhibit enveloped virus proliferation during infection [58,59]. Additionally, adequate supply of micronutrients is essential for the proper activity of some enzymes, especially those involved in the functions of the innate immune cells [53,54,60,61]. Regular exercise is demonstrated to lower the LDL and to rise the HDL, even though with a variable effect between men and women, besides fostering the immune system response [62]. Therefore, a healthy style of life, this including appropriate nutrition and physical state, is likely to make one less susceptible to infection and to augment the survival probability after infection.

Provision to the body of micronutrients and phytochemicals with physiological action through plant extract intake is a current practice in Asian countries according to Herbal Medicine [63–65]. The use of plant derived compounds has been claimed to ameliorate the pathological effects of viral infection during the COVID-19 pandemic [63]. Regarding

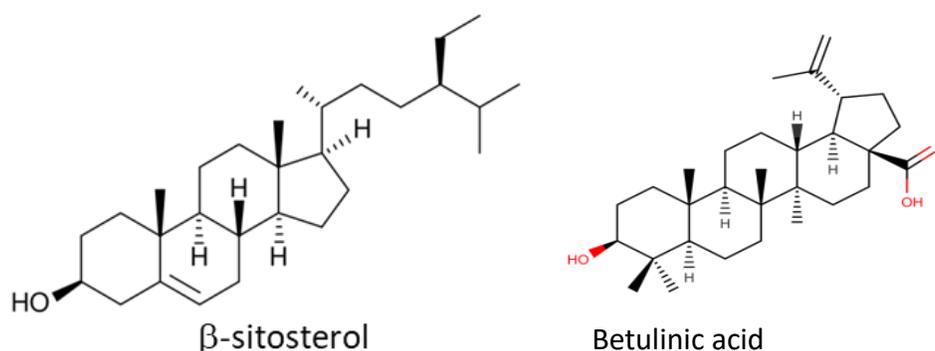
mechanisms to lower circulating cholesterol, intake of phytosterol and phytostanol concentrates is considered an acceptable dietary supplement (functional foods) to those ingested through vegetables, and compatible with natural practices [66–68]. They are also added to commercial processed foods worldwide and considered safe. Phytosterols act reducing the cholesterol absorption in the gut, that is through a “dilution effect” (Figure 2, scheme F). A daily dose of 3.4 to 5.2 g in esterified form is recommended by Food and Agriculture Organization of the United Nations (FAO) [66], even though it seems that a dose above 3 g/day has no a remarkable improvement in cholesterol reduction.

Phytosterols have a chemical structure similar to this of cholesterol, differing only in the aliphatic chain (Figure 3). Their function in plant membranes resembles that of cholesterol in animal membranes. However, phytosterols cannot replace cholesterol in animal membranes and, therefore, the dilution effect in the intestinal absorption is which helps reducing cholesterol levels. Phytostanols are rendered by hydrogenation of the phytosterol double bond. In nature (vegetable oils, nuts and cereals, for instance), they are present as free sterols, as sterol-glucosides and as sterol-esters. A typical western diet contains 300-400 mg/day of plant sterols [69–71], which is below the dose recommended ( $\geq 2$  g/day) to elicit a significant reduction (c.a. 10%) in the cholesterol level, mainly LDL cholesterol. However, it seems that above 3 g/day there is not higher cholesterol reduction, and the degree of the effect is likely to depend on both the sterol form and the food matrix in which phytosterols are supplemented [68–70]. In clinical nutrition, supplementary phytosterols are considered a safe practice to avoid raising the statin dose [64,71]. A mathematical model was developed

by Eussen et al. (2011) to assess the expected reduction in circulating (LDL-) cholesterol according to given physiological parameters under combined use of statins (atorvastatin) and phytosterols, the model forecasting 8-9% cholesterol reduction with 2 g/day phytosterol intake [72].

In addition to cholesterol lowering, a potential capacity of phytosterols to modulate the immune system response has been suggested, although contradictory results have been reported in different trials on this issue, in particular regarding markers of inflammation [69]. The sterol action on immune function is thought to be bound to the increase in T helper 1 (Th1) cell activity as assessed by the IFN- $\gamma$  concentration, whereas Th2 cell over-activity is observed in HIV patients and elderly people.

Nonetheless, the decrease in markers of inflammation might be a side effect of the cholesterol reduction. The influence of  $\beta$ -sitosterol (Figure 3), the most abundant form in human serum (about 6  $\mu$ m), on inflammation caused by influenza A virus (IAV) infection was recently reported by Zhou et al. [73]. These authors found that  $\beta$ -sitosterol positively diminished inflammation by down-regulating the retinoic acid-inducible gene 1 (RIG-I) signalling pathway and decreasing the IFN production. As a consequence of this action, expression of COX-2 and the proinflammatory prostaglandin PGE2 were also decreased. Hence, the authors conclude that pre-administration of  $\beta$ -sitosterol protected mice from lethal IAV infection.



[74]. In addition to phytosterols, other natural compounds from herbal medicine have been shown to have antiviral properties. For instance, emodin inhibits the 3a ion channel protein of SARS-CoV-1 [75]; cepharanthin and valinomycin are lipoxygenase inhibitors against SARS-CoV-1 infection [76]; and plant lectins were found to impede SARS-CoV-1 binding and budding [77]. Nonetheless, in order to assess whether phytosterols, and other natural plant derived compounds, may be considered functional foods against viral infection, more research on their activity beyond the "dilution effect" is devoted, in particular regarding the pathways through which the metabolism may be positively intervened, and the recent COVID-19 outbreak is expected to promote such research.

## CONCLUSIONS

Even though vaccines against SARS-CoV-2 have already been developed and they are clinically applied worldwide at present (January 2021), treatments to ameliorate the pathological effects of COVID-19 and possible further virus-driven pandemics are of concern. In this regard, the use of natural products to prevent or positively counteract the viral disease and post-infection side effects would be welcome for clinical intervention. Within a healthy style of life, phytosterols intake are amongst the compounds that might account for such purpose in addition to provide certain protection against cardiovascular diseases by reducing the cholesterol levels and their immunomodulatory effect.

**List of abbreviations:** COVID: coronavirus infectious disease, VLDL: very low density lipoprotein, LDL: low density lipoprotein, HDL: high density lipoprotein, NLM-NIH: National Library of Medicine of the National Institutes of Health, RNA: Ribonucleic acid,

HIV: human immunodeficiency virus, ACE2: angiotensin converting enzyme 2, SARS-CoV: severe acute respiratory syndrome coronavirus, SREBP: sterol regulatory element binding protein, SR-B1: scavenger receptor B type 1, IAV: influenza A virus, ABCA: ATP-binding cassette transporter or cholesterol efflux regulatory protein, HCV: hepatitis C virus, DENV: dengue virus, WNV, West-Nile virus, AMP: adenosine monophosphate, mTOR: mechanistic target of rapamycin (protein), IFN: interferon, Viperin: virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible, IRF: interferon regulatory factor, TLR: Toll-like receptor, 25HC: 25-hydroxy-cholesterol, ACAT: acyl-CoA:cholesterol acyl transferase, ISG: interferon stimulated gene, HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA (reductase), WHO: World Health Organization, PUFA: polyunsaturated fatty acid, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, FAO: Food and Agriculture Organization of the United Nations, Th: T helper (cell), COX-2: cyclooxygenase-2, PGE: prostaglandin E.

**Competing interests:** The author has a researcher position in the Spanish National Council of Research (Consejo Superior de Investigaciones Científicas, CSIC) of Spain. Its main research focus is UPLC-MS based Lipidomics, and he has collaborated with different groups in relation to the functional capacity of lipids.

**Authors' contribution:** Olimpio Montero has conceived, written the manuscript text and drawn the figures.

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