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Review Article

Lactoferrin for Treating and Preventing COVID-19: A Review

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Abstract

After nearly two years of living with the COVID-19 pandemic, much has been learned. Today we know that the virus that causes COVID-19 is transmitted in the air, treatments have improved, and prevention of contracting it is possible through vaccination. This opens an opportunity for natural ingredients and bovine lactoferrin is considered to be one of the best options for treating and preventing COVID-19. Lactoferrin has been studied extensively before the identification of severe acute respiratory syndrome coronavirus (SARS-CoV-2), which causes COVID-19. It was found to be an anti-viral agent against other viruses, and now new research has shown it to be equally effective against the virus that causes COVID-19. In addition, lactoferrin has the potential to boost the body's natural immune function without over-reacting. Clinical studies in COVID-19 patients, who took oral bovine lactoferrin, improved faster and tested negative for SARS-CoV-2 virus sooner compared to those who were not supplemented. Besides helping treat COVID-19 patients, lactoferrin holds promise for prevention against getting it. In one clinical trial, healthy subjects, who received supplemental lactoferrin, did not develop COVID-19 despite living with family members who had it. From the current body of evidence, it is possible to determine the right amount of lactoferrin to use if you contract COVID-19 and what you need to prevent it. For treatment, use of 500 mg to 1,000 mg daily of lactoferrin is recommended until symptoms associated with COVID-19 have improved or have gone away completely. For prevention, taking 250 mg to 500 mg daily is recommended. Lactoferrin is a well-tested, safe, and natural compound with the potential to help many during the COVID-19 pandemic.

Keywords: Lactoferrin; Dietary Supplements; Novel Coronavirus; COVID-19 and SARS-CoV-2; Prevent COVID-19; Treat COVID-19

Introduction

The Severe Acute Respiratory Syndrome coronavirus (SARS-CoV-2 virus), which causes coronavirus disease (COVID-19), has lead to a pandemic. Just about everyone on the planet knows someone who has contracted COVID-19 or died from the illness. Pharmaceuticals are available to treat this illness, but they are limited and are administered based on the severity of the disease [1]. In the U.S., three vaccines are available to those over 11 years of age, and one has been approved for those starting at 5 years of age. These vaccines reduce the risk of contracting COVID-19, or if it is contracted, lessen the severity of illness.

New drugs to treat any condition are slow in developing, taking more than 10 years typically [2]. And the failure rate is more than 90%. Finding natural compounds to treat and prevent COVID-19 may hold promise and certainly be faster. For example, in April 2020, the U.S. Food and Drug Administration gave approval to two academic centers to study the effect of a medicinal mushrooms (turkey tail, *Trametes versicolor*; and agarikon, *Fomitopsis officinalis*) and a herbal blend (modified Qing Fei PaiDu Tang, which is a combination of 21 herbs from 4 Chinese herbal formulations) in patients with acute SARS-CoV-2 virus [3].

Other researchers explored more than 1,400 FDA-approved compounds for their anti-viral effect on the SARS-CoV-2 virus [2]. Seventeen compounds were effective anti-viral agents, but lactoferrin was the best. It was shown to inhibit the SARS-CoV-2 infection in all cell models, by multiple modes of action (including pre-

venting the virus from attaching to the body's cells; enhancing the body's immune function; and minimizing an overactive immune response). These authors encouraged further exploration into using lactoferrin as a therapeutic option for managing COVID-19, due to its effectiveness and high safety profile [2]. This sentiment was echoed by others, who averred that lactoferrin may represent a potent new prevention against COVID-19, because of its ability to block the binding of the SARS-CoV-2 virus to the body's cells, thereby creating a natural host-defense [4].

Lactoferrin is immune-boosting, and an antioxidant, anti-inflammatory, and anti-viral natural ingredient [5]. This review covers: definitions of COVID-19 and lactoferrin; how lactoferrin works against the SARS-CoV-2 virus; review of human studies using lactoferrin as a treatment and preventative compound against COVID-19; liposomal vs. non-liposomal lactoferrin; and proposed amounts of lactoferrin to use to treat and prevent COVID-19.

What is COVID-19?

COVID-19 is an acute disease characterized by pneumonia and acute respiratory distress syndrome (ARDS) [5]. It is caused by the virus, SARS-CoV-2, which is highly contagious and adept at preserving its own survival by spreading and replicating easily in the human body.

The SARS-CoV-2 virus gets transmitted through contact of aerosol droplets in the air from an infected person. The virus enters the nasal passage and continues down into the lungs, where it binds to cells [5,6]. The SARS-CoV-2 virus can also later retreat to the gastrointestinal tract and replicate there [6,7]. Once inside the body, the SARS-CoV-2 virus suppresses a person's own antiviral and immune response capabilities. This can cause acute lung injury and pneumonia [5]. Symptoms usually appear two to fourteen days after exposure to the virus [8].

People who develop COVID-19 usually experience an asymptomatic phase with or without detectable virus, followed by a symptomatic phase with a high viral load. It is possible that people are more contagious during the asymptomatic stage than when they experience symptoms [9]. What is perplexing is that clinical presentation of COVID-19 can range from having no symptoms all the way up to severe, life-threatening cases.

How the SARS-CoV-2 virus progresses depends upon someone's immune status, age, and the presence of underlying medical conditions [5]. Based on the literature, typical clinical symptoms of COVID-19 patients include dry cough (67%), fever (88%), fatigue (38%), muscle aches (14.9%), and shortness of breath (18.7%) (5). Other symptoms, according to the Centers for Disease Control and Prevention (CDC), can occur including, headache, sore throat, runny nose, and gastrointestinal problems (e.g., diarrhea, vomiting and nausea) (Table 1) [10]. Pneumonia is the most severe manifestation of this viral infection. Elderly individuals and those with underlying conditions, such as cardiovascular and lung diseases, may suffer from more severe symptoms and mortality [9]. Obesity and having type 2 diabetes have also been identified as risk factors for COVID-19.

People with these symptoms may have COVID-19, but does not include all possible symptoms	Fever or chills Cough Shortness of breath or difficulty breathing Fatigue Muscle or body aches Headache New loss of taste or smell Sore throat Congestion or runny nose Nausea or vomiting Diarrhea
If someone is showing any of these signs, seek emergency medical care immediately.	Trouble breathing Persistent pain or pressure in the chest New confusion Inability to wake or stay awake Pale, gray, or blue-colored skin, lips, or nail beds, depending on skin tone

Table 1: Symptoms of COVID-19 from the Centers for Disease Control and Prevention*

*Based on reference 10.

What is lactoferrin?

Lactoferrin is an iron-binding, defense glycoprotein that is naturally occurring and non-toxic with a molecular weight of 70-80kDa [5,9]. It is a simple polypeptide chain with two symmetrical lobes that can bind metal atoms like iron. Lactoferrin is found in most

bodily fluids and expressed and secreted by glandular cells [6,11]. For example, it is present in several body fluids besides milk such as saliva, tears, semen, vaginal fluid, gastrointestinal fluids, and urine [6]. It is also found in nasal and bronchial secretions, and therefore, uniquely becomes the primary defense against respiratory microbial infections like the SARS-CoV-2 virus. Some blood cells like neutrophils and acinar cells in the panaceas, which allow for digestion of foods, also make lactoferrin [6].

Lactoferrin appears in especially high concentrations in mammalian milk, such as from humans and bovines. The concentration of lactoferrin is highest in colostrum and declines over time [9]. For example, typical human breast milk has about 1 gram per liter of lactoferrin, while colostrum can have up to 7 grams per liter [6].

The effectiveness of bovine-derived lactoferrin has been compared to human lactoferrin. Laboratory tests on human coronaviruses, like those that cause the common cold, showed that both forms of lactoferrin – bovine- and human-derived – exhibited antiviral activity [12]. Interestingly, the human lactoferrin was three to eight times less potent than the bovine form.

Paradoxically, in the presence of an infection like the SARS-CoV-2 virus, the body's ability to make it decreases [11,13]. Thus, the use of bovine lactoferrin to treat or prevent COVID-19 could be a useful strategy.

Mechanism of actions of lactoferrin on the sars-COV-2 virus that causes COVID-19

Lactoferrin is of intense scientific interest as a treatment and preventative natural ingredient against SARS-CoV-2 virus that causes COVID-19 [11,14]. Lactoferrin confers unique roles in protecting an individual from getting COVID-19, and recovering from it, if contracted. Mechanisms of action of bovine lactoferrin on SARS-CoV-2 virus are discussed below (Table 2).

Mechanism of action	Explanation
Stimulating the body's immune system	
Immune modulation	Increasing the numbers of immune cells that can fight COVID-19 (e.g., increase cells that destroy the virus) [9]. Reducing other cells that may foster viral replication (e.g., inflammatory cells) [9]. Directing more immune-fighting cells to the site of an infection and activate them to destroy viral particles [9]. Stimulating natural killer cell activity [6].
Iron binding and viral replication	Binding to iron by chelation, thereby rendering it unavailable for replication of a virus like SARS-CoV-2 virus [7]. Reducing ferritin levels and removing excess iron out of the body, leading to less viral replication [13]. Inhibiting creation of reactive oxygen species, thereby reducing oxidative stress. The antioxidant effect impairs the ability of SARS-CoV-2 virus to replication [13,15].
Anti-viral activity	Competing with SARS-CoV-2 virus for receptors sites that allow entry into cell where replication occurs (i.e., heparan sulphate proteoglycan and angiotensin converting enzyme-2 [6,9,11,12,14]. Working synergistically with the anti-viral drug, remdesivir [12].
Preventing the body's immune system from over-reacting to a viral invasion	
	Controlling the cytokine storm [6,7,11,13, 14,16]. Reducing the cytokines interleukin-6 and ferritin, which are associated with higher disease severity during COVID-19 [6,7,13,14,16]. Increasing interferon, leading to a reduction in SARS-CoV-2 viral replication [5,9]. Reducing platelet aggregation, by regulating plasminogen, which is released in the presence of SARS-CoV-2 virus [13,14,15].
Protecting the gastrointestinal tract	
	Lessening diarrhea [6]. Exerting an anti-inflammatory effect in the gastrointestinal tract by balancing the local microbiota and reducing the intestinal damage induced by the SARS-CoV-2 virus [6]. Increasing the good micro-flora-such as bifidobacterial-and decreasing the bad bacteria, such as E. coli, streptococcus, and clostridium [6]. Binding to ACE-2 and HSPG receptors, disallowing SARS-CoV-2 virus from attaching and replicating [7]. Offering beneficial effects in the gastrointestinal tract and recommended as preventive measure against COVID-19 [15].

Table 2: Mechanisms of action of bovine lactoferrin on SARS-CoV-2 virus.

Stimulating the body's immune system to counteract a pathological viral invasion

Before the discovery of SARS-CoV-2 virus, lactoferrin had been studied extensively for its ability to enhance the body's immune system after invasion from a viral or bacterial infection [11,13]. This is important because the presence of the SARS-CoV-2 virus dampens one's own immune system, just when it is needed to fight off this virus.

Below are several ways that lactoferrin works to enhance the body's immune system to combat SARS-CoV-2 virus.

Immune modulation

Lactoferrin is a true immune-modulator as it increases the numbers of immune cells that can fight SARS-CoV-2 virus (e.g., increase natural killer cells that destroy the virus) and reduces inflammation that may foster viral replication (e.g., enhancing the antigen expression ability of B cells and regulating the function of T cells) [9]. The immune-modulatory effects of lactoferrin, in essence, allow the body to defend itself against infections, while at the same time, controlling excessive inflammation (discussed in the next section, "Preventing the body's immune system from over-reacting to a viral invasion").

Lactoferrin is an attractive, natural immune enhancer because many immune cell surfaces house receptors that recognize lactoferrin [9]. These cells can use lactoferrin to fight off infections such as the virus that causes COVID-19. Lactoferrin can also direct more immune-fighting cells such as natural killer cells and type 1 interferons to the site of an infection and activate other cells to destroy viral particles.

Lactoferrin also plays a unique role in stimulating the body's ability to make virus-fighting cells called natural killer cells [9]. Oral administration of lactoferrin can enhance natural killer cell activity against virus-infected cells by facilitating the production of another cytokine, interleukin-18 [9]. Cytokines are proteins secreted by cells as part of the body's immune response. It is believed that lactoferrin stimulates natural killer cell activity in the presence of the SARS-CoV-2 virus [6].

Iron-binding and viral replication

Any bacterial or viral infection that has invaded the body is looking for iron to replicate [13,14]. Lactoferrin has the ability to bind to iron by chelation, thereby rendering it unavailable for replication of a virus like SARS-CoV-2 virus [7]. Paradoxically, in response to an inflammatory insult like an invading virus, the body releases more iron in the form of iron-containing proteins like ferritin. Lactoferrin can also reduce ferritin levels, thereby removing excess iron out of the body, which leads to less viral replication and reduces inflammation [13].

The ability of lactoferrin to bind iron leads it to behave like an anti-oxidant by controlling oxidative stress [13,15]. The binding of iron is associated with inhibition of reactive oxygen species formation, the hallmark of oxidative stress. This antioxidant effect impairs the ability of SARS-CoV-2 virus to replicate.

Anti-viral activity

Lactoferrin has anti-viral effects, which generally occur in the early phase of infection, preventing viral particles from replicating [4,7,9,11]. It is possible that the way lactoferrin behaves as an anti-viral is that it binds directly to SARS-CoV-2 virus, and prevents the virus from replicating [6,11]. However, based on how lactoferrin behaves against other viruses, this seems unlikely, according to others who have argued that lactoferrin doesn't bind to a virus like SARS-CoV-2 [12].

Lactoferrin has a long history of being a broad-spectrum, natural, anti-viral natural compound. It has demonstrated abilities to counteract a wide range of human and animal viruses including rotavirus, respiratory syncytial virus, herpes viruses, hepatitis C, Chikungunya, cytomegalovirus, human papillomavirus, and the human immunodeficiency virus [6,9,11]. All of these viruses and SARS-CoV-2 virus share a common molecule, heparan sulphate proteoglycan (HSPG), which is an attachment factor that sits on the hosts' cell membranes [12].

Any virus, including SARS-CoV-2 virus, needs to attach to the host's cells and then bind to receptors on the cell surfaces to make someone ill [9]. SARS-CoV-2 virus uses its spike protein, which sits on the viral cell membrane, to attach to HSPG and then to the host cell's receptor, angiotensin converting enzyme-2 (ACE-2) [12,14]. Receptors are like doors that enable movement of biological chemicals in and out of the cells. They require "keys", which only allow certain compounds like SARS-CoV-2 virus' spike protein to attach and enter a cell if they have the right match (i.e., the right key).

Two receptors are of interest because both SARS-CoV-2 virus and lactoferrin possess the "right key". One receptor is called HSPG

and the other is ACE-2 [14]. Once a virus attaches to these doors (receptors), it takes over a cell's regular operation, and begins replicating itself. If lactoferrin binds to these receptors first, then SARS-CoV-2 virus can no longer attach to the cell and commandeer operations.

These ACE-2 and HSPG receptors are on cells in the respiratory system, where SARS-CoV-2 virus first takes hold. Taking lactoferrin immediately after testing positive for COVID-19 may be advisable to prevent SARS-CoV-2 virus attaching to these receptors and replicating. In addition, over time, SARS-CoV-2 virus can progress to the gastrointestinal tract, where these same receptors are also present [9]. The gastrointestinal tract is a site where the body internalizes SARS-CoV-2 virus, allowing for viral replication to occur leading to active infection. Abundant lactoferrin remains on the lining of the gastrointestinal tract immediately after ingestion, thereby protecting the body's cells against SARS-CoV-2 virus. Thus, it seems prudent to continue taking lactoferrin for the entire time that the test for COVID-19 is positive, potentially preventing further viral replication and infection.

Another reason to continue with lactoferrin supplementation is that it has been shown to work synergistically with a commonly prescribed anti-viral treatment drug, remdesivir [12].

Preventing the body's immune system from over-reacting to a viral invasion

Any invasion from a foreign body like a virus or bacteria causes the body to mount an immune response to attack it, including releasing cytokines. A modest response is beneficial, but far too often, and in the case of COVID-19, the body over-reacts and releases too many cytokines [15]. This over-reaction is referred to as a cytokine storm, which some consider the cause of death associated with COVID-19, rather than the virus itself [11]. In addition to causing a violent immune response, the cytokine storm is also associated with increased thrombosis from excessive platelets clotting, and with intestinal microflora dysbiosis [15]. These changes related to a cytokine storm are thought to be associated with increased morbidity and mortality seen in COVID-19 patients [11,14,15].

Lactoferrin does three things related to preventing the cytokine storm during COVID-19:

- Controls the release of all cytokines [16]
- Enhances the release of "good", less inflammatory cytokines such as interleukin (IL)-4 and IL-10 [6]
- Reduces the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), IL-6 and IL-1 beta, and down-regulates nuclear factor-kappa B [6,13,14].

The immune system has been shown to over-respond to the SARS-CoV-2 virus but this can be dampened with lactoferrin. Lactoferrin helps control the immune response to a pathological virus and restore homeostasis [7,11]. This milk protein is able to limit tissue damage in the body by controlling the release of cytokines.

Acute respiratory distress and subsequent death from COVID-19 is not only caused by the SARS-CoV-2 viral infection itself, but also by the hyper-inflammation from the cytokine storm [6,7,11,14,16]. A cytokine profile in severe COVID-19 cases is characterized by an increase in cytokines like IL-6, TNF-alpha, and by acute-phase reactants such as ferritin. With COVID-19, higher levels of IL-6 and ferritin are associated with higher disease severity [13].

Lactoferrin has been shown to reduce IL-6 and ferritin in both experimental models and human studies of SARS-CoV-2 virus [6,7,13,14,16]. In experimental studies, TNF-alpha was also decreased in cells treated with lactoferrin. These findings suggest that lactoferrin can modulate an over-active immune and control the inflammatory response, which, if left unchecked, causes respiratory failure and possibly death. Thus, lactoferrin represents a non-toxic health supplement that could be a candidate as an adjunct treatment in treating severe cases of COVID-19 [7,11,14].

Another cytokine of interest is interferon. The presence of the SARS-CoV-2 virus reduces the body's ability to produce interferon, which is normally made to fight off infections [5]. Interferon production is increased with lactoferrin, leading to a reduction in the SARS-CoV-2 viral replication [9].

Not directly related to the immune system over-reacting but equally important is the ability of lactoferrin to serve as an anti-coagulant. Lactoferrin has the ability to regulate plasminogen, which is a protein that is released in the presence of the SARS-CoV-2 virus. Over production of plasminogen causes the body to make too many platelets, resulting in excess blood clotting [13,14,15]. The ability to lessen how aggressively the blood clots is as important as con-

trolling how fast the SARS-CoV-2 virus replicates. Both are major causes of organ failure and possibly death.

Protecting the gastrointestinal tract

Although the SARS-CoV-2 virus starts to replicate in the respiratory system, it eventually makes its way to the gastrointestinal tract where it continues replicating [9]. This is known because viral shedding from the stool occurs in a substantial portion of patients with COVID-19 [7].

Evidence of the SARS-CoV-2 virus entering the gastrointestinal tract is also based on a human study in which 94% of the patients developed diarrhea [6]. When oral lactoferrin was given to these COVID-19 patients, it lessened diarrhea within five days. It is thought that lactoferrin may exert an anti-inflammatory effect in the gastrointestinal tract by balancing the local microbiota and reducing the intestinal damage induced by the SARS-CoV-2 virus. Lactoferrin can increase the good micro-flora-such as bifidobacterial-and decrease the bad bacteria, such as E. coli, streptococcus, and clostridium.

Using a line of cells from the intestine called Caco-2, investigators looked at the effect of lactoferrin blocking SARS-CoV-2 virus from entering these cells [7]. These cells have two receptors (i.e., ACE-2 and HSPG) that are available for both SARS-CoV-2 virus and lactoferrin. If lactoferrin has been orally consumed, then it is able to bind to these receptors, disallowing SARS-CoV-2 virus from attaching and replicating. These investigators found that lactoferrin boosted the anti-viral immune response in these human gastrointestinal cells, partially inhibiting SARS-CoV-2 infection [7]. Thus, lactoferrin appears to be an anti-viral treatment in the gastrointestinal tract against SARS-CoV-2 virus. Lactoferrin is also considered to be a preventative measure, offering beneficial effects in the gastrointestinal tract [15].

Human studies

Despite the promising *in vitro* studies showing positive effects of lactoferrin on neutralizing the harmful effects of SARS-CoV-2 virus, clinical studies are lacking. Three studies were identified on PubMed (https://pubmed.ncbi.nlm.nih.gov/) in which lactoferrin was given to people who had COVID-19. A summary of the three studies is presented on table 3.

Giving lactoferrin to patients with mild-to-moderate COVID-19 shortened the time to a negative polymerase chain reaction (PCR) test, compared to patients not receiving lactoferrin.
Lactoferrin speeds up recovery by improving symptoms related to COVID-19 like having a dry cough, muscle aches, fatigue, headaches, and being able to taste food.
Beneficial effects of supplemental lactoferrin begin within five days of use and continue improving symptoms after 10 days.
Lactoferrin helps patients with COVID-19, but also seemed to prevent family members living with these patients from contracting the virus.
Lactoferrin, provided as a liposome, was studied in two of the three studies and was found to be effective at treating COVID-19. One study used the non-liposomal form of lactoferrin and did not find any benefit, but perhaps this was due to the low dose provided (200 mg and 400 mg) and the short duration of the study, which was seven days. It is not clear whether the lactoferrin needs to be provided as a liposome based on these three clinical trials. All in vitro studies on SARS-CoV-2 virus revealed important benefits from non-liposomal lactoferrin.
Lactoferrin should be considered as an adjunctive treatment to patients with COVID-19. It was shown to be effective when combined with usual care consisting of things like supplemental oxygen, anti-biotics, anti-viral medications, and other nutrients like vitamin C and zinc.
Liposomal lactoferrin can be provided orally and as a nasal spray. The SARS-CoV-2 virus begins in the nose and in some cases migrates to the gastrointestinal track. Hence, this dual approach of delivering lactoferrin may be more beneficial than just providing it orally.
No adverse side effects of lactoferrin were reported. Lactoferrin is safe for children, adults, and pregnant women.

Table 3: Summary of outcomes from three clinical studies in patients with COVID-19 who received lactoferrin*.

*Based on references [6,13,17].

Detailed review of three human studies using lactoferrin in treatment of COVID-19.

Study 1

The first reported use of lactoferrin in patients with documented COVID-19 was conducted by a group of researchers from Sesderma Laboratories [6]. Seventy-five, home-bound patients with documented COVID-19 entered a study to receive liposomal lactoferrin, zinc, and vitamin C. Patients were monitored twice daily for ten days and then followed up one month later. Another group of family members (n = 256), who were living with these COVID-19 patients, received the same compounds but at half the doses of these compounds.

COVID-19 patients in this study took four to six doses of liposomal lactoferrin daily that provided between 256 and 384 mg daily; in this range, the participants also received about 60 mg of vitamin C. Liposomal zinc, 10 mg twice daily was also provided. Those who started the study with a headache, dry cough, and nasal congestion were also treated with liposomal lactoferrin nasal drops and mouth spray. Others with breathing difficulty were provided lactoferrin in an aerosolized form.

The most important finding was that the major symptoms associated with COVID-19 mostly cleared up after five days with liposomal lactoferrin, liposomal zinc, and vitamin C. Sixty-one percent started off with a dry cough and 39% had it at day-5. Similarly for muscle aches, 67% started off with this, yet only 2% complained of this symptom at day 5. Ninety-four percent started off complaining of feeling tired, but by day 5, only 28% had this complaint. Headaches, that were present in 56% of the subjects at the start of the study, went away completely at day 5. For those 77% who lost their sense of taste, on the fifth day of follow-up, 44% still had this problem, but which completely resolved by day 10. This showed that liposomal lactoferrin was helpful in treating COVID-19 patients. For the healthy family members who got half of the lactoferrin dose (128 to 192 mg) and other nutrients, no one contracted COVID-19, indicating that this regimen may be helpful as a preventative agent.

The results of this study were considered to be limited because there was no control group. However, the promising results showed that lactoferrin was simple to take, safe, and potentially could be used as a treatment for and prevention against COVID-19. And lactoferrin can be used by the young, elderly, and pregnant women.

Study 2

A later clinical study in patients with COVID-19 [13], who received lactoferrin and usual care, found similar benefits to the earlier study [6]. Ninety-two individuals were recruited for the study of which 67 presented with mild-to-moderate symptoms and 25 were asymptomatic. Each was assigned to one of three treatment groups: 1st, oral and intranasal liposomal lactoferrin (32 total, of which 14 were hospitalized but not in the ICU, and 18 were at home), 2nd, usual care of antibiotics (28 hospitalized patients), or 3rd, home-based patients not taking medications or lactoferrin (28 patients). Another group of 32 COVID-19-negative subjects were untreated, healthy, and served as a control group. Average ages for the three patient groups ranged from 40 to 50 years.

The 32 patients assigned to receive daily liposomal lactoferrin received capsules taken orally containing 100 mg of lactoferrin each, and a nasal spray containing 8 mg of lactoferrin per mL. The scheduled oral treatment was 1 gram daily of lactoferrin for 30 days (10 capsules per day) and the intra-nasal spray was administered 3 times daily (a total of about 16 mg of lactoferrin per each nostril).

The primary endpoint was the length of time to become negative for SARS-CoV-2 virus using a PCR test. The group receiving lactoferrin were the first to become negative at day 14 (mean time to negative test; $P < 0.0001$), compared to non-significant findings from those who received standard medical management (mean time to negative test; day 27), and no treatment COVID-19 patients (mean time to negative test; day 33). This suggests that liposomal lactoferrin may shorten the time that someone is contagious.

The secondary endpoint was the duration of time to recover from COVID-19 symptoms. Those receiving lactoferrin had faster recovery compared to the standard care group and to non-treated patients. Fatigue was the most common remaining symptom of those who were still symptomatic at day-30 (21%), which was down from its high of 50% of subjects at day-15. Other symptoms of a cough and joint pain improved, but the lack of a sense of smell did not return.

Tertiary endpoints were related to inflammatory markers. After 30 days in the lactoferrin-supplemented group, the inflammatory cytokine, IL-6 was significantly reduced compared to baseline ($P < 0.05$). As previously mentioned, this cytokine goes up with the se-

verity of illness, indicating that lactoferrin may reduce it, and thus the burden of the virus on the body. Similarly, elevated ferritin is associated with worse outcomes from COVID-19, and the lactoferrin group experienced a significant decrease in this marker ($P < 0.04$).

Thus, the findings of this study suggest that liposomal lactoferrin is a safe and effective adjunctive treatment of patients with COVID-19, who have mild-to-moderate symptoms or are asymptomatic.

Study 3. A third study lasting seven days [17] using a non-liposomal lactoferrin showed improvement in symptoms, but these did not achieve the statistically significant as seen in the previous two studies in patents with COVID-19 [6,13]. The participants (n = 54) had confirmed, mild-to-moderate cases of COVID-19 [17]. All participants received usual care which included: oxygen, hydroxychloroquine, vitamin C at 1 g daily, zinc at 600 mg daily and acetylcysteine at 200 mg three times daily. Two groups, each with 18 subjects, received lactoferrin - one at 200 mg once daily, and the other at 200 mg twice daily for seven days. Another group served as a control and did not get lactoferrin.

The findings showed no statistically significant difference among the three groups regarding recovery of symptoms or laboratory tests improvements. The two lactoferrin groups improved over seven days for COVID-19 symptoms including fever, dry cough, diarrhea, headache, loss of sense of taste and/or smell and tiredness, but not significantly more than the control group. It is possible that the amount of lactoferrin provided, the use of non-liposomal lactoferrin, or the short duration of study lead to these null findings.

Digestion and absorption of lactoferrin through the life cycle

Orally ingested lactoferrin passes from the stomach and travels into the blood or lymphatic system, depending upon different periods of life [14,18,19]. The major challenge that hinders the wide application of lactoferrin is related to its poor *in vivo* stability, due to rapid degradation by proteolytic enzymes associated with aging.

Human babies and newborns of other mammals enjoy the full benefits of lactoferrin with milk consumption that is essential for their development [18]. Breast-fed babies or infants, who are given a formula fortified with added lactoferrin, ingest high amounts of lactoferrin that directly induces the growth and proliferation of enterocytes [19]. In these cases, limited gastric degradation of lactoferrin occurs. Lactoferrin plays an important role during infancy and has been shown to reduce the risk of sepsis in pre-term infants, according to a meta-analysis including ten randomize controlled trials involving 3, 679 infants [20].

As infants grow, less lactoferrin is ingested and the maturation of the digestive system allows for a more complete digestion of it. Lactoferrin is broken down into several large fragments by the gastric digestive enzyme, pepsin [19]. Since lactoferrin receptors are found in the intestinal mucosa and in the cells of the lymphatic tissue of the intestine, it is important that lactoferrin maintains its structural integrity to bind its receptors at these two sites [19].

By adulthood, orally consumed lactoferrin will be largely digested into small molecules in the stomach, and thus, it will no longer be intact. However, gastric degradation of lactoferrin in humans still allows for 60 - 80% of what is ingested to enter the small intestine intact after oral ingestion [21]. In addition, protein degradation also has positive aspects as some peptides produced by its digestion, such as lactoferricin, a 25-residue peptide, and lactoferrampin, a 20-residue peptide, display potent defensive activity [19]. Lactoferricin, in some cases, displays more potent antibacterial, antimicrobial, and anti-inflammatory properties, and greater anti-fungal activity than intact lactoferrin. In addition, lactoferrampin shows wide antimicrobial action against bacteria, viruses, yeasts and parasites. Additional studies are needed to identify all biological activities of these bioactive peptides derived from the digestion of lactoferrin.

Encapsulating lactoferrin

Some have argued that encapsulating or enterically-coating orally ingested lactoferrin will prevent gastric degradation [14,19,21]. Some methods include PEGylation, which appears to be one of the best; iron saturation; chitosan and chitosan derivatives; and shells comprising alternate layers of bovine serum albumin and tannic acid [18,19]. However, clinical studies are lacking to show the effectiveness of these methods.

Liposomal lactoferrin

Creating a lactoferrin liposome is another method that may offer protection in the stomach and allow for the lactoferrin to be taken up by the lymphatics rather than going through the portal circulation, as is typical with most ingested protein [14]. A group

of Japanese investigators prepared a lactoferrin liposome using multi-lamellar layers composed of egg yolk phosphatidylcholine and phytosterols [21]. Using an *in vitro* artificial gastric digestion model, this liposomal form of lactoferrin withstood gastric degradation better than a non-liposomal form but a certain level of the non-liposomal form remained intact. This study showed that oral lactoferrin can be considered to enter the intestinal lumen and have the biological functions, regardless of whether it is liposomalized or not.

The only human study testing liposomal lactoferrin (300 mg) on immune function was performed in healthy males [22]. The liposomes were made using multi-lamellar vehicles and prepared by hydrating the food grade lecithin, but this method was not identical to other preparations [21]. Multiple measures of immune function were evaluated but only one, interferon-alpha, increased in the liposomal but not non-liposomal form of lactoferrin. Other markers of immune function were not significantly different between the two forms of lactoferrin (e.g., natural killer cell activity, IL-6, TNF-alpha). This finding contradicts another study in which both interferon and natural killer cells increased in response to a non-liposomal form of lactoferrin [9]. Another human study [6] used a different preparation method for liposomal lactoferrin to the one in this study [22], but did not measure blood, so no comparison can be made about the efficacy of liposomal lactoferrin on immune markers.

It seems that liposomal lactoferrin is an appealing way to deliver this protein, but clinical data are lacking on whether it is superior to the non-liposomal form. Both seem to work in a variety of ways to boost immunity. No human studies have confirmed that liposomal lactoferrin remains intact and enters the lymphatic system and other unresolved issues remain (Table 4).

Issue related to lactoferrin	Resolved/unresolved
Clinical studies	
Liposomal form of lactoferrin	Showed efficacy against COVID-19 symptoms [6,13].
Non-liposomal form of lactoferrin	Did not show efficacy [17]. Unresolved: the non-liposomal did not show efficacy because of its form, inadequate dose, or short duration of the study [17].
Digestion/absorption	
Changes with aging	Less lactoferrin is digested by gastric enzymes in infants and more of the intact form reaches the intestine [18,19]. 60 - 80% of orally consumed lactoferrin enters the intestine intact [21]. Degradation of lactoferrin in the stomach produces other bio-active proteins [19]. Unresolved: providing more non-liposomal lactoferrin may obviate the need to create a liposome
Encapsulation	
Multiple methods	Many methods exist, including liposomal form [18,19,21]. Liposomal methods used in clinical studies [6,13,22] were not identical to others that confirmed entry into the lymphatics [21]. Unresolved: all liposomal forms of lactoferrin are not the same.

Table 4: Liposomal or non-liposomal lactoferrin: what is known and what is unresolved.

Liposomal vs non-liposomal lactoferrin for COVID-19 treatment and prevention

Whether it is necessary for lactoferrin to be in a liposomal form to be effective against COVID-19 is unknown. Serrano., *et al.* [6] argue that the use of soybean phosphatidylcholine to make the liposomal lactoferrin enhances absorption and bioavailability. They argue that the newly formed liposomes are thought to be protected from gastric acid degradation, allowing more lactoferrin to arrive in the duodenum. This would enable the liposomal lactoferrin to remain in the circulation longer compared to a non-liposomal form.

Based on the three clinical trials identified in this review [6,13,17] using lactoferrin to treat or prevent COVID-19, only studies using the liposomal form had significant beneficial findings [6,13]. One of the two liposomal lactoferrin studies [6] also provided 60 mg of vitamin C and 20 mg zinc, which have been shown to have anti-viral properties. However, based to two extensive reviews, neither nutrient has conclusive evidence to be effective against SARS-CoV-2 virus [23,24]. The amounts of vitamin C and

zinc provided to these COVID-19 patients [6] were lower than the amounts proposed by others [23,24]. In the review articles, the authors suggest giving daily amounts of vitamin C at 2-8 grams [23], and zinc, based on 15 clinical studies in COVID-19, of at least 50 mg [24]. Hence, it is unlikely that the inclusion of vitamin C and zinc had any meaningful impact on the benefits seen in recovery from COVID-19, and all benefits were likely attributable to the liposomal lactoferrin [6].

The study using non-liposomal lactoferrin for COVID-19 patients [17] provided the participants with daily amounts of vitamin C (1 gram) and zinc (600 mg). The amount of vitamin C is closer to what has been proposed by others to fight SARS-CoV-2 virus [23]. However, the amount of zinc provided was well in excess of what is typically recommended or used in clinical studies in patients with COVID-19 [24]. It is possible that excessive zinc (more than 300 mg per day) can adversely affect the immune response by impairing lymphocyte and neutrophil function, and on the anti-viral side, by reducing interferon-gamma levels and interferon regulatory factor 1 expression [24]. Whether the excess zinc used in this study [17] adversely impaired the ability of the non-liposomal lactoferrin to have significant benefits against COVID-19 is unknown.

It seems paradoxical that the non-liposomal form of lactoferrin had no significant benefits for the treatment of COVID-19. In many other *in vitro* studies that did not use the liposomal form of lactoferrin, numerous benefits were observed (e.g., antibacterial, antiviral, antitumor, antifungal, anti-inflammatory, immunomodulatory), including in fighting SARS-CoV-2 virus [9,11,12,13]. Furthermore, this assumption contrasts another clinical study, which showed significant benefits of non-liposomal lactoferrin in reducing the number of common colds ($P < 0.001$) and of associated symptoms ($P < 0.05$) compared to a placebo group [25].

Proposed amounts of lactoferrin to use for treatment and prevention of COVID-19

Lactoferrin has been studied extensively in the laboratory setting for its benefits in reducing the harmful effects of the SARS-COV-2 virus. It is clear that this milk protein works to reduce viral replication, enhance the immune system, and control the immune system in response to this virus. Only three published clinical studies are available from PubMed (https://pubmed.ncbi.nlm.nih.gov/). Other articles have put forth recommendations as well. By staying within these recommendations, lactoferrin has been shown to be safe. Several investigators have stated that lactoferrin has two potential therapeutic roles: [1] to serve as a treatment to control tissue damage induced during COVID-19 disease; and [2] to function prophylactically to prevent getting COVID-19 [11,14,15].

Treatment

Optimal intake summary

At least 245 mg daily of lactoferrin is required to see COVID-19 patients convert to a negative PCR test and see improvements in symptoms associated with this conation. Taking up to 1 gram daily is safe and effective. Using only 200 mg and 400 mg daily was not effective at improving symptoms.

Bottom line

500-1,000 mg daily of lactoferrin until symptoms associated with COVID-19 have improved or gone away completely.

Scientific support

One group recommended taking 64-96 mg of liposomal lactoferrin every six hours to treat COVID-19 (245-384 mg daily) [6]. Doses can be increased to 128 mg every six hours (512 mg per day) as needed. Others suggest taking 1 gram of liposomal lactoferrin daily for 30 days along with an intranasally spray administered 3 times daily (a total of about 16 mg/nostril) [13]. Another group showed no benefit of 200 mg or 400 mg of non-liposomal lactoferrin for significantly improving symptoms [17]. Safety and use of lactoferrin in other conditions has been confirmed in a range of 100 mg to 4.5 grams daily [11].

Prevention

Optimal intake summary

Lactoferrin taken between 200 mg and 1,000 mg daily is required.

Bottom line

250 mg to 500 mg of lactoferrin daily.

Scientific support

Oral lactoferrin in the range of 100 mg to 1,000 mg daily for humans was effective at reducing the incidence of getting sick from viral infections [11]. Another group recommended taking 64 mg,

two to three times daily of liposomal lactoferrin to prevent COVID-19 (128-192 mg/d) [6].

Conclusions

COVID-19 is here to stay. Much has been learned over the past two years about how it is transmitted, treated, and prevented. Now is the time to consider natural products that can work with available treatments and preventative agents like vaccines. Of those natural compounds screened for efficacy, bovine lactoferrin, a milk protein, rises to the top as being the best at supporting the body to protect itself against the SARS-CoV-2 virus. In addition, the effective amounts of lactoferrin have been deemed safe. Based on human studies in patients with COVID-19 who received lactoferrin, the amount needed for treatment is between 500 mg and 1,000 mg and for prevention is 250 mg to 500 mg daily.

Disclaimer

Lactoferrin is a dietary supplement and is generally recognized as safe (GRAS) with no contraindications by the United States Food and Drug Administration. However, before using any dietary supplement, it is advisable to speak with your healthcare professional first.

Conflict of Interest

Lindsey Ormond is an employee of Milk Specialties Global, a manufacturer of dairy ingredients including lactoferrin.

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