L-carnitine tartrate supplementation in the fight against infectious disease

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The Lonza Group's Capsules & Health Ingredients (CHI) Division is a leading global provider of integrated nutraceutical solutions, which seeks to drive wellness and longevity through making advances in wholesome ingredients. Lonza's objective is to provide solutions for healthy living through innovative nutritional ingredients backed by sound science. This feature presents Lonza's recent research into L-carnitine tartrate, which has immunomodulatory effects that might influence the management of infectious diseases, including coronavirus disease 2019 (COVID-19).

L-CARNITINE IN IMMUNE FUNCTION

Inflammation is an essential part of the immune response to injury and infection[1,2]. It is intricately connected with immune function, and dysregulation between the two can lead to pathological conditions. Aging of the immune system is associated with reduced immune responsiveness[1]. Populations that are vulnerable to infectious diseases, such as COVID-19, including the elderly and people with chronic inflammatory diseases like obesity, diabetes, hypertension and cardiovascular illnesses, can also exhibit significantly impaired immune function[3]. Notably, these populations also exhibit lower levels of L-carnitine in their tissues compared to less-vulnerable healthy populations[4]. L-carnitine, which is a physiological molecule involved in lipid metabolism, has been shown to mitigate the dysregulation of an aging immune system[1]. Alongside its role in energy metabolism, L-carnitine has a function in viral infection possibly via its impact on key immune mediators[5]. Previous studies have demonstrated the antioxidant action of L-carnitine in the tissues of aged animals[1], and L-carnitine deficiency is associated with a higher rate of infections and failure to thrive[4]. Furthermore, in rodent models, L-carnitine has been shown to inhibit leukocyte apoptosis through its free-radical scavenging activities[6]. Similarly, L-carnitine supplementation enhanced immunity with vaccination in broiler chickens[7], and improved immune-cell functions, like chemotaxis and phagocytosis, in aged rats[2]. Comparable observations have been noted in humans, with exogenous L-carnitine supplementation having a positive impact on immune mediators, showing its beneficial effects in preventing infection[5]. Altogether, these observations suggest that L-carnitine plays a crucial role in immune-system functioning by alleviating inflammation and increasing antioxidant status.

L-carnitine accumulates mainly in muscle, heart and lung tissue[8]. Alongside the anti-inflammatory benefits, L-carnitine supplementation results in several improvements in health outcomes, which may influence susceptibility to viral infection. These health benefits include decreased insulin resistance and oxidative stress and improved immune function[8]. A recent meta-analysis showed that L-carnitine has numerous cardioprotective properties, which may be mediated through improved mitochondrial function, elevated antioxidant status and lowered oxidative stress[8]. Its proven anti-inflammatory effect (particularly in respect to heart health), the role of the renin–angiotensin system in heart health and the immune response in general, and the importance of inflammation in infection led Lonza to explore the impact of L-carnitine on COVID-19 outcomes.

INFLAMMATION-CENTRIC COVID-19

COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of April 2021, it had resulted in more than 2.9 million deaths worldwide[3]. COVID-19 is an airborne virus that mainly affects the lungs and the upper respiratory system, leading to lung injury, respiratory distress and, in severe cases, death[3]. Research has confirmed that the COVID-19 mortality rate sharply increases among seniors, with the majority of deaths reported among those aged 70 years and older[3], and among individuals who have pre-existing conditions such as frailty, obesity, diabetes and hypertension independent of age[3]. A potential unifying feature that enhances susceptibility among those with each of these conditions is an elevation in systemic inflammation, which leads to a greater viral load with the potential for a cytokine storm and increased mortality[3]. In addition, elderly, obese, hypertensive and frail populations have been reported to have lower plasma L-carnitine levels[9] (**Figure 1**).

COVID-19 infects humans through its attachment to the host-cell surface and the binding of its spike protein to the angiotensin-converting enzyme 2 receptor (ACE2) as an initial step[10]. Following its attachment, the SARS-CoV-2 spike protein is further cleaved to facilitate its entry into the host cell. The cleavage is mediated by furin peptidase at the S1/S2 site of the spike protein, and by transmembrane protease serine 2 (TMPRSS2) at the S2′ site within the S2 domain, as shown in human airway cells[10] (**Figure 2**). Heightened systemic inflammatory states appear to worsen COVID-19 outcomes by initiating a counter-regulatory response, which includes the upregulation of levels of the ACE2 receptor and its associated peptidases[11].

The binding of the SARS-CoV-2 spike glycoprotein with ACE2, which triggers the virus-mediated cellular uptake, can also cause a burst of inflammatory cytokine release (a cytokine storm) partially because of the decreased availability of functional ACE2. One cause of such a cytokine storm is an imbalance of the ratio between ACE1 (the pro-inflammatory mediator) and ACE2 (the anti-inflammatory mediator). This can lead to dysregulation of the renin–angiotensin–aldosterone system resulting in poor health outcomes and, in many cases, death[11]. Consequently, much interest has centred on therapies targeting systemic inflammation and COVID-19 receptor binding[10].

The physiological role of ACE2 is to lower blood pressure and counteract inflammation by decreasing the pro-inflammatory mediator angiotensin (1–7) (ref. 11). The conversion from angiotensin I to angiotensin II is mediated by ACE1. Increases in ACE1, or a decrease in ACE2 caused by SARS-CoV-2 in infected cells, may reflect an increased inflammatory state characteristic of severe COVID-19 (ref. 11). Therefore, therapies attempting to depress ACE2 should not impact the ACE1/ACE2 ratio[11].

L-CARNITINE AS AN IMMUNOMODULATOR THROUGH ANTI-INFLAMMATORY EFFECTS

Lonza hypothesized that L-carnitine tartrate supplementation may impact ACE2 expression and, consequently, its attachment to the SARS-CoV-2 spike protein through lowering inflammation. To test this, scientists used an exercise-challenge model in both rodents and humans to induce inflammation and evaluate whether the ability of L-carnitine tartrate to downregulate ACE2 expression could translate to an attenuation of SARS-CoV-2 infectivity in an *in vitro* human lung epithelial cell-based system[5].

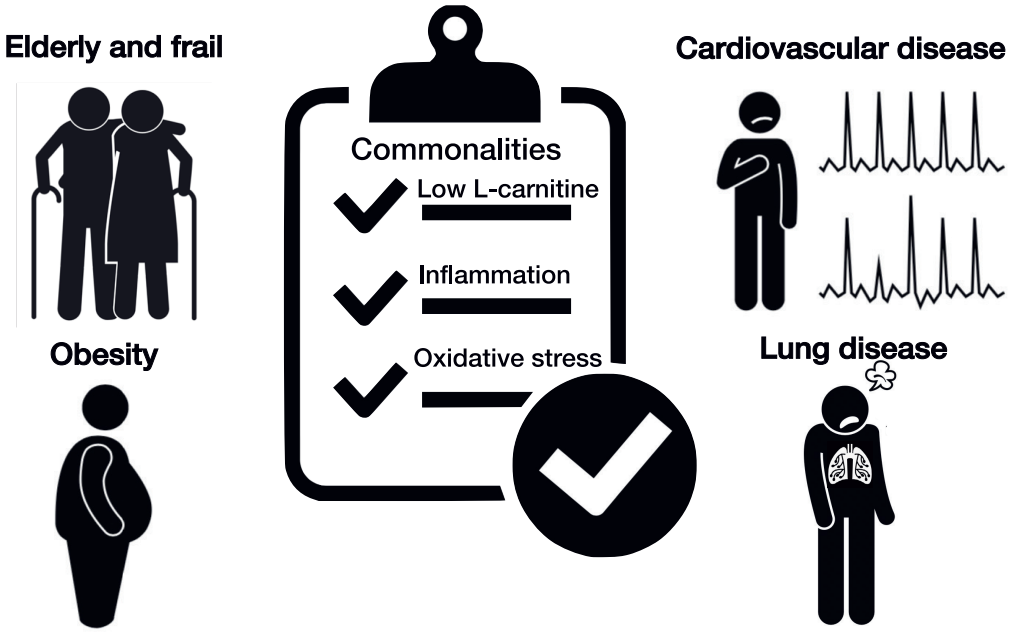

Figure 1. Commonalities among COVID-19 risk factors. General risk factors for COVID-19 include obesity, diabetes, lung disease, old age and cardiovascular disease. These risk factors are associated with low L-carnitine levels and high oxidative stress and inflammation.

EXERCISE AS A MODEL TO ELEVATE INFLAMMATION AND INCREASE ACE2

Both exhaustive aerobic and resistance exercises have been shown to increase short-term (<96 h) oxidative stress and mechanical damage in muscles, resulting in increased inflammation[12]. Strenuous exercise has resulted in increases in the inflammatory marker C-reactive protein (CRP) to levels comparable to those seen in cardiovascular disease states[12]. Recently, ACE2 has been shown to be elevated following strenuous exercise[13]. Collectively, these findings led Lonza to use exercise as a model to induce an inflammatory state and to upregulate ACE2 levels similar to those seen in chronic diseases.

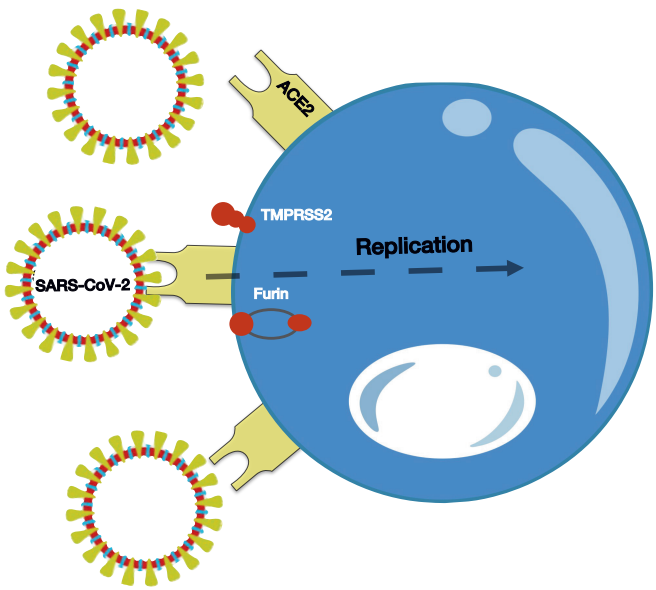

Figure 2. ACE2 receptors and viral infection. SARS-CoV-2 infects human airway cells via the binding of its spike protein to the ACE2 receptor. TMPRSS2 and furin then cleave the spike protein to allow the virus to enter the cell.

EFFECTS OF L-CARNITINE ON HOST-DEPENDENCY FACTORS AND INFLAMMATORY MARKERS DURING EXERCISE-INDUCED INFLAMMATION

Our initial study explored the dose-dependent response of L-carnitine tartrate at the tissue level, to determine whether it altered protein and mRNA levels of the host-dependency factors of SARS-CoV-2 infection, ACE1, ACE2, TMPRSS2 and furin, and the inflammatory markers serum CRP and interleukin-6 (IL-6). Rats were subjected to a high-intensity training exercise to induce inflammation[5]. L-carnitine tartrate was administered daily for 6 weeks at doses of 250–4,000 mg human equivalent dose (HED). In the exercise control group, which was not supplemented with L-carnitine tartrate, the protein and mRNA levels of ACE2, TMPRSS2 and furin in the

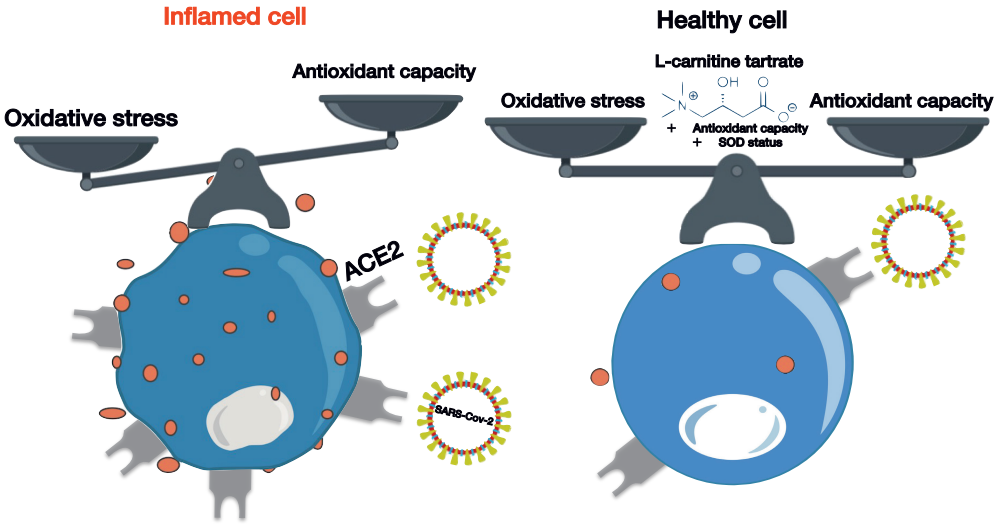

Figure 3. L-carnitine and oxidative and inflammatory status. L-carnitine helps regulate the balance between oxidative stress and antioxidant capacity in healthy cells. Lower L-carnitine levels may disrupt the balance and increase susceptibility to infections by viruses such as SARS-CoV-2 in inflamed cells.

lungs, liver and muscle tissues were increased (approximately 1.5–3-fold) and the levels of ACE1 decreased. The L-carnitine-supplemented group showed a dose-dependent reduction in the levels of ACE2, TMPRSS2 and furin with the maximum effect observed at 200 mg/kg/day, which translates to 2,000 mg HED. Despite the decrease in ACE2 levels, L-carnitine supplementation did not increase the ACE1/ACE2 ratio, which remained low compared to the control group, suggesting the prevention of a spike in inflammation. The blunted inflammation caused by L-carnitine was confirmed by decreased serum levels of CRP and IL-6 (ref. 5). These modulatory effects of L-carnitine tartrate were seen only in response to the exercise-induced inflammation challenge[5].

Serum ACE1 and ACE2 levels and the ACE1/ACE2 ratio are associated with several comorbidities that can worsen COVID-19 severity, including hypertension, type II diabetes, obesity and cardiovascular disease, and in those who smoke[14]. We therefore evaluated the effects of daily L-carnitine tartrate supplementation, yielding 2 g of elemental L-carnitine, on serum levels of ACE1, ACE2, furin, TMPRSS2 and CRP in humans[5]. The study group comprised 80 healthy subjects aged 21–65 years. Participants underwent a 5-week moderate exercise-training programme, which culminated in a high-volume resistance-training stimulus meant to induce muscle damage and inflammation. The exercise challenge resulted in a significant rise in serum ACE2, TMPRSS2 and furin levels, while ACE1 levels remain unchanged.

Intriguingly, when compared to the placebo arm, subjects supplemented with L-carnitine tartrate reported less muscle damage and inflammation as evidenced by lower levels of serum creatine kinase and CRP, respectively. The latter finding was consistent with research showing that antioxidant supplementation lowers CRP during exercise[12]. Notably, L-carnitine tartrate prevented the systemic rise in serum ACE2 without affecting ACE1. Previous research has suggested that an imbalance in the action of ACE1 and ACE2 is a primary driver of COVID-19 pathophysiology. The Lonza study in humans found that L-carnitine tartrate did not alter the ACE1/ACE2 ratio.

The ability of L-carnitine tartrate to reduce ACE2 is complex; however, our data suggest that it is related, at least in part, to its antioxidant and anti-inflammatory properties. In the rodent study, L-carnitine tartrate supplementation led to a significant increase in the antioxidant capacity, as demonstrated by an increase in superoxide dismutase (SOD) along with a decrease in inflammation[15].

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and levels of antioxidants such as SOD (**Figure 3**). Infection by SARS-CoV-2 or other viruses has been shown to substantially increase production of free radicals by the immune cells leading to oxidative stress. Similarly, exercise elevates metabolism, causing an increase in the use of oxygen and eventually resulting in oxidative stress[16,17]. Exercise-induced oxidative stress is known to alter cell structure and function, thereby contributing to inflammation and muscle damage[15]. Our rodent trial found that L-carnitine tartrate supplementation lowered oxidative stress, as indicated by a dose-dependent decrease in serum malondialdehyde[15] and, consequently, inflammation. These changes were paralleled by elevated antioxidant capacity, as shown by increases in several antioxidant enzymes including SOD, catalase and glutathione peroxidase. Collectively, these findings point to a correlation between the effects of L-carnitine on mitigating oxidative stress and inflammation and its effects on lowering ACE2, TMPRSS2 and furin; this suggests a mechanism for the observed effects of L-carnitine on inhibiting the infection of Calu-3 human lung epithelial cells[5]. Moreover, the fact that L-carnitine tartrate led to a decrease in the mRNA levels of ACE2 suggested that it may play a modulatory role through gene-level effects. This is notable given a recent report that L-carnitine can bind some of the transcription factors, leading to the modulation of their downstream genes such as ACE2 (ref. 18).

L-CARNITINE TARTRATE DECREASES SARS-COV-2 INFECTION OF CALU-3 CELLS BY DOWNREGULATING ACE2 RECEPTOR EXPRESSION

Immune responses, clinical presentations and severity of outcomes vary notably among patients infected by COVID-19, and include muscle weakness, fever and respiratory complications. Under extreme scenarios, patients experience a cytokine storm that targets the lungs[3]. Extensive lung transcriptome analysis established that individuals with high risk factors for severe disease outcomes expressed ACE2 at much greater levels than control subjects[19]. These findings led Lonza to explore whether L-carnitine tartrate could modulate the expression levels of ACE2 in cultured Calu-3 cells, thereby moderating SARS-CoV-2 infectivity[5]. The results indicated that L-carnitine tartrate reduced

ACE2 mRNA levels and dose-dependently decreased SARS-CoV-2 infection in Calu-3 cells[5].

Taken together, these findings demonstrate that L-carnitine tartrate treatment significantly decreases the susceptibility of Calu-3 cells to SARS-CoV-2 infection. These effects are probably mediated through a decrease in the expression of the host factors required by the virus to attach to and enter the cell (**Figure 4**).

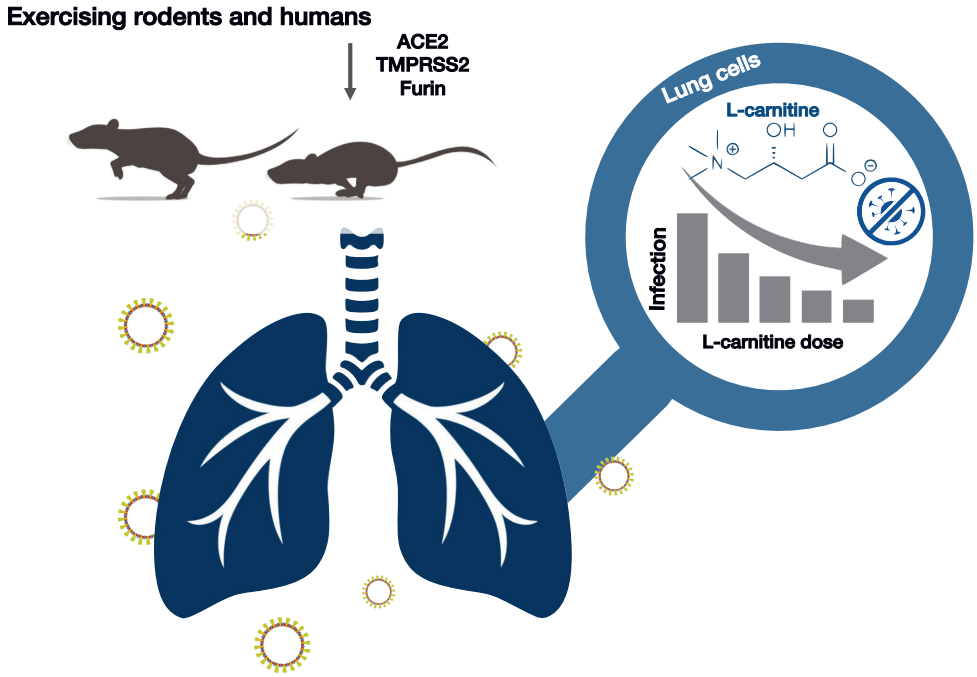

Figure 4. L-carnitine and lung-cell infection. L-carnitine reduces levels of ACE2, TMPRSS2 and furin in exercise-induced inflammation models in rats and humans, thereby decreasing susceptibility to SARS-CoV-2 infection.

CONCLUSIONS

Oxidative stress induced by acute viral infection helps to stimulate an increased inflammatory response. With the current pandemic, COVID-19 is a primary research target, and scientists and physicians are investigating a wide array of intervention strategies including the use of methods for blunting the inflammatory states that are characteristic of the virus. Medical presentations of COVID-19 include fever, dry cough and respiratory complications. These symptoms can escalate to life-threatening difficulties for individuals with comorbidities such as type II diabetes, frailty, heart disease and general aging. A unifying theme connecting these comorbidities is chronic inflammation and low endogenous L-carnitine levels. Lonza has shown that this nutritional ingredient can blunt systemic exercise-induced inflammation and oxidative stress in both humans and rodents. Supplementation reduced serum and tissue levels of ACE2, furin and TMPRSS2, which are considered host factors for SARS-CoV-2 entry into human cells. Reduction in expression levels of host factors was correlated with reduced virus infectivity of human epithelial lung cells in a cell-culture model. Lonza has established the safety and tolerability profile of L-carnitine tartrate in humans at a daily dose of up to 3 g (equivalent to 2 g elemental L-carnitine). Future investigations of the effects of L-carnitine tartrate in preventing SARS-CoV-2 infection and complications in human clinical trials are now warranted.

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