CHAPTER SEVEN

Vitamin C in Pneumonia and Sepsis

Anitra C. Carr

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INTRODUCTION

In the early literature, one of the most striking symptoms reported for the vitamin C deficiency disease scurvy was a marked susceptibility to infections, particularly pneumonia (reviewed in [1]). Autopsy findings from the 1920s indicated that pneumonia was one of the most frequent complications of scurvy and was the prevailing cause of death. Infantile scurvy was also observed to predispose children to infections, particularly of the respiratory tract. According to Hemilä [1], these findings supported observations of the disappearance of a pneumonia epidemic in Sudan when antiscorbutic treatment was given to the numerous cases of scurvy that occurred at the same time. Conversely, there have also been reports of scurvy following infectious epidemics, suggesting that infections can severely deplete vitamin C levels in the body [1]. Case reports indicate that children have developed scurvy symptoms after, or concurrently with, respiratory infections [2,3]. The authors stated that "possibly the increased metabolic needs associated with this infection unmasked a subclinical vitamin C deficiency" [2] and that "scurvy occurred as a result of their increased requirement of vitamin C due to stress of illness combined with poor dietary intake. It is therefore recommended that during illness one should be careful about the intake of vitamin C, keeping in mind that acute illness rapidly depletes stores of ascorbic acid. Those already malnourished are more prone to this development" [3]. Similarly, others have reported scurvy symptoms following confirmed or suspected respiratory infection, stating that "sepsis of either digestive or pulmonary origin, leading to sustained metabolic demand, might have acted as a precipitating factor" [4,5].

The anecdotal and epidemiological observations of a connection between vitamin C status and infections have been supported by animal studies using vitamin C–requiring guinea pigs and mice deficient in L-gulono-γ-lactone oxidase ($Gulo^{-/-}$), the rate-limiting enzyme in vitamin C synthesis. Identification of bacteria in the tissues of scorbutic guinea pigs in the early literature led to the erroneous hypothesis by some researchers that scurvy may in fact be an infectious disease [1]. Research has indicated an increased severity

of infections in scorbutic guinea pigs, with higher mortality observed in vitamin C–deficient animals infected with *Pseudomonas aeruginosa* compared with vitamin C–replete animals [6]. Vitamin C–deficient Gulo knockout mice were three times as likely as vitamin C–replete mice to die following infection with *Klebsiella pneumoniae* [7]. The lungs appear to be particularly susceptible to deficiency with vitamin C–deficient Gulo knockout mice exhibiting greater lung pathology following infection with influenza [8]. Conversely, infection of animals with *P. aeruginosa* and influenza A virus resulted in decreased vitamin C levels in tissues and fluids [6,9,10], possibly due to the inflammatory response and enhanced oxidative stress. Interestingly, infection by itself was found to decrease antioxidant capacity more than a vitamin C–deficient diet in guinea pigs, suggesting a high consumption of antioxidants during infection [6]. Although enhanced markers of oxidative stress have been observed in infected mice, there were no significant differences in the levels of the oxidation products in the vitamin C–deficient and –replete mice indicating that vitamin C may be acting via mechanisms other than oxidant scavenging [7]. Overall, the animal studies support a two-way relationship between vitamin C and infection.

PNEUMONIA AND VITAMIN C

Pneumonia is an acute infection of the lungs that can be caused by a range of microorganisms, including those of bacterial, fungal, or viral origin [11]. These microorganisms reach the lower respiratory tract and, dependent on microbial virulence factors, the host's immune defenses, and integrity of barriers, cause inflammation in the alveoli and consequently result in pneumonia. Diagnosis is usually determined through radiographic imaging, indicating shadowing of a lobe or segment of the lung, and the clinician's clinical assessment, and empiric treatment is through prompt antimicrobial intervention. Symptoms include cough, fever, aches, sweating, and shivering, and some patients may present with pleuritic chest pain and confusion [12]. Lower respiratory infections, such as pneumonia, are a leading cause of morbidity and mortality worldwide. In 2016, lower respiratory infections caused nearly 2.4 million deaths worldwide, making lower respiratory infections the sixth leading cause of mortality for all ages and the leading cause of death among children younger than 5 years [13]. This equated to more than 335 million episodes of lower respiratory infections and more than 65 million hospital admissions in 2016. Lower respiratory infection mortality is high in the elderly, and rates are increasing due to an increasing aging population, with the number of adults older than 70 years increasing by 50% between 2000 and 2016 [14].

Streptococcus pneumoniae is the leading cause of lower respiratory infection morbidity and mortality globally, contributing to more deaths than all other assessed etiologies combined [14]. Research has also indicated that increased pneumonia incidence is associated with higher deprivation and is particularly prevalent in developing countries where poverty is more prevalent [14,15].

Pneumonia has been reported as one of the most common complications and causes of mortality in individuals with scurvy, suggesting an important link between vitamin C status and lower respiratory infection [1]. This premise is supported by meta-analyses of three interventional studies that indicated that prophylactic administration of at least 200 mg/d vitamin C decreased the incidence of pneumonia in the study populations [16–19]. Furthermore, analysis of the vitamin C status of patients with pneumonia and acute respiratory distress syndrome has indicated significantly lower vitamin C concentrations in patients when compared with healthy controls, and levels appeared to inversely correlate with the severity of the condition (Table 7.1) [20,21]. Up to 40% of patients with pneumonia exhibited outright vitamin C deficiency (i.e., plasma vitamin C levels <11 μmol/L), and levels remained low for at least 4 weeks [22,23]. These studies indicate a higher utilization of, and potentially also a higher requirement for, vitamin C during lower respiratory tract infection.

An early report by Klenner indicated that administration of 2–4 g/d intravenous or intramuscular doses of vitamin C to patients with pneumonia decreased the symptoms of nausea, headache, temperature, and cyanosis [24]. Subsequent interventional studies have indicated that administration of oral or intravenous vitamin C decreased the severity of the respiratory symptoms, particularly in the most severely ill, and also decreased hospital length of stay in a dose-dependent manner (Table 7.2) [22,23]. A trend

TABLE 7.1
Vitamin C status of patients with pneumonia

Study Type	Cohort	Vitamin C (μmol/L)	References
Case control	20 Healthy volunteers	66 ± 3	[20]
	11 Pneumonia cases	31 ± 9	
Case control	28 Healthy participants	49 ± 1	[21]
	35 Lobular pneumonia		
	7 Acute—did not survive	17 ± 1	
	15 Acute—survived	24 ± 1	
	13 Convalescent cases	34 ± 1	
Interventional (placebo group)	29 Pneumonia/bronchitis		[22]
	Week 0	24 ± 5 (40%)[a]	
	Week 2	19 ± 3 (37%)	
	Week 4	24 ± 6 (25%)	
Interventional (control group)	70 Pneumonia cases		[23]
	Day 0	41	
	Day 5–10	24–23	
	Day 15–20	32–35	
	Day 30	39	

NOTE: Data represent mean and standard error of the mean (SEM).

[a] Percentage of patients with vitamin C deficiency. Vitamin C status categories: saturating (>70 μmol/L), adequate (>50 μmol/L), hypovitaminosis C (<23 μmol/L), and deficient (<11 μmol/L).

toward decreased mortality was observed in the interventional study by Hunt et al. [22], and a more recent case control study using a higher dose of 6 g/d intravenous vitamin C (in combination with thiamine and hydrocortisone) exhibited a significant (56%) decrease in mortality in patients with severe pneumonia [25]. Interestingly, Cathcart hypothesized that patients with severe respiratory infections and pneumonia had higher requirements for vitamin C based on the observation that they could tolerate more than 10 times the usual bowel tolerance doses of 4–15 g/24 hour [26]. Mochalkin assessed plasma vitamin C levels following intervention and observed that administration of 0.25–0.8 g/d vitamin C was insufficient to maintain the patients' initial vitamin C status; however, administration of 0.5–1.6 g/d vitamin C was able to maintain the patients' plasma vitamin C status for the duration of the study (30 days) [23]. However, these plasma concentrations were still inadequate (i.e., <50 μmol/L), suggesting a requirement of >1.6 g/d for saturating plasma status. Thus, patients with severe infections, such as pneumonia, have higher requirements for

TABLE 7.2
Vitamin C intervention in patients with pneumonia

Patients	Intervention	Outcomes	References
99 Severe pneumonia	IV vitamin C	↓ Hospital mortality	[25]
46 Controls	0 g/d		
53 Treatment	6 g/d		
57 Pneumonia/bronchitis	Oral vitamin C	↓ Respiratory symptom score in most severely ill	[22]
29 Placebo	0 g/d		
28 Treatment	0.2 g/d		
140 Pneumonia cases	Oral vitamin C	↓ Hospital length of stay	[23]
70 Control	0 g/d	24 days	
39 Low dose	0.25–0.8 g/d	19 days	
31 High dose	0.5–1.6 g/d	15 days	

ABBREVIATIONS: ↓ decrease; IV, intravenous.

vitamin C, and doses of vitamin C that provide adequate to saturating plasma vitamin C status in these patients appear to have beneficial effects on patient outcomes.

SEPSIS AND VITAMIN C

Sepsis is a condition of life-threatening organ dysfunction caused by a dysregulated host response to infection [27]. Sepsis is characterized by profound dysregulation of the circulatory, metabolic, and immune systems, and it is the primary cause of death from infection. Patients who develop septic shock can have hospital mortality rates of up to 50%. Management of sepsis involves fluid resuscitation for hypoperfusion and vasopressor drug administration for those in shock [28]. Global estimates indicate nearly 32 million sepsis cases and more than 5 million deaths annually [29]. Although sepsis mortality rates have been decreasing, particularly in developed countries such as the United States and Australasia, the incidence of sepsis continues to increase, likely due to an aging population [30–34]. Despite huge research efforts toward an attempt to identify effective sepsis therapies, to date most of these have proven futile [35]. Furthermore, patients who survive sepsis can often have long-term physical disabilities, cognitive dysfunction, or psychological issues, such as anxiety, depression, and posttraumatic stress disorder, which significantly affect their quality of life [36].

Case control studies have consistently indicated significantly lower vitamin C status in critically ill patients, particularly those with sepsis (Table 7.3). These critically ill patients have by far the lowest vitamin C status when compared with other common disease states [37,38]. Lower vitamin C status in these patients was associated with increased inflammation (C-reactive protein levels), increased severity of the illness (days in the intensive care unit [ICU]), and multiple organ failure [37,39,40]. Nearly 40% of patients with septic shock were deficient, and almost 90% had hypovitaminosis C, despite receiving recommended enteral and parenteral intakes [39]. Administration of 1 g/d vitamin C to critically ill patients was found to be insufficient to raise the patients' plasma vitamin C concentrations above the hypovitaminosis C cutoff, but 3 g/d resulted in saturating plasma status (i.e., ∼70 μmol/L) [41,42]. Recent pharmacokinetic data indicated that administration of 2 g/d vitamin C to critically ill patients, as either bolus or continuous infusions, resulted in plasma concentrations in the normal range, although hypovitaminosis C occurred in some patients following cessation of the intervention, suggesting sustained therapy may be required to prevent this from occurring [43]. Overall, these findings indicate that critically ill patients have vitamin C requirements that are approximately 10-fold higher than healthy individuals, whose plasma vitamin C typically saturates with intakes of 0.2 g/d [44].

TABLE 7.3
Vitamin C status of patients with sepsis

Study Type	Cohort	Vitamin C (μmol/L)	References
Observational	24 Septic shock patients	15 ± 2 (38%[a], 88%[b])	[39]
Interventional (baseline)	24 Severe sepsis patients	18 ± 2	[45]
Case control	6 Healthy controls 19 Severe sepsis 37 Septic shock	48 ± 6 14 ± 3 14 ± 3	[46]
Case control	14 Healthy controls 11 Septic encephalopathy	76 ± 6 19 ± 11	[47]
Case control	34 Healthy controls 62 ICU (injury, surgery, sepsis)	62 (55–72) 11 (8–22)	[37]

NOTE: Data represent mean and standard error of the mean (SEM) or median and interquartile range.

ABBREVIATION: ICU, intensive care unit.

[a] Percentage of patients with vitamin C deficiency.

[b] Hypovitaminosis C. Vitamin C status categories: saturating (>70 μmol/L), adequate (>50 μmol/L), hypovitaminosis C (<23 μmol/L), and deficient (<11 μmol/L).

Critically ill patients treated with ~3 g/d intravenous vitamin C (in combination with various other antioxidant vitamins and minerals) have shown improved outcomes, including decreased organ failure, ICU and hospital length of stay, mortality, inflammation, and infections/sepsis [42,48–51]. However, with combination interventions, it is difficult to know which component(s) are contributing to the various outcomes, particularly since baseline concentrations of the various components are not typically assessed in the patients prior to intervention. In 2014, the first phase I study investigating intravenous vitamin C as monotherapy in patients with sepsis was published [45]. In this study, 24 patients with severe sepsis were treated with 0, 50, or 200 mg/kg body weight intravenous vitamin C per day, which provided a dose-dependent decrease in systemic organ failure and decreased pro-inflammatory (C-reactive protein and procalcitonin) and tissue damage (thrombomodulin) biomarkers (Table 7.4). Although the study was not powered to detect a decrease

TABLE 7.4
Vitamin C intervention in patients with sepsis

Patients	Intervention	Outcomes	References
	Vitamin C Administered Alone		
100 Septic shock 50/Group	IV vitamin C: 0 or 6 g/d Duration: until ICU discharge	↓ Vasopressor duration ↓ ICU length of stay X Length of mechanical ventilation X Renal replacement therapy X ICU mortality	[53]
28 Septic shock 14/Group	IV vitamin C: 0 or 100 mg/kg/d Duration: 3 days	↓ Norepinephrine dose and duration X ICU length of stay ↓ 28-day mortality	[52]
24 Severe sepsis 8/Group	IV vitamin C: 0, 50, or 200 mg/kg/d Duration: 4 days	↓ Systemic organ failure ↓ C-reactive protein, procalcitonin, thrombomodulin levels	[45]
	Vitamin C plus Thiamine/Hydrocortisone Cocktail		
94 Severe sepsis 47/Group (retrospective)	IV vitamin C: 0 or 6 g/d + thiamine + hydrocortisone Duration: as little as 1 dose or up to 4 days	X ICU or hospital mortality X ICU or hospital length of stay X Renal replacement therapy for AKI X Time to vasopressor independence	[54]
1144 Septic shock 229 Treatment 915 Controls (retrospective)	IV vitamin C: 0 or 6 g/d + thiamine Duration: 1 day only	X 28-day or hospital mortality X ICU or hospital length of stay X Duration of mechanical ventilation X New renal replacement therapy	[55]
24 Septic shock 12/Group	IV vitamin C: 0 or 6 g/d + thiamine + hydrocortisone Duration: 4 days	↓ Vasopressin and noradrenaline requirements ↓ Procalcitonin levels X Systemic organ failure	[56]
94 Severe sepsis 47/Group (retrospective)	IV vitamin C: 0 or 6 g/d + thiamine + hydrocortisone Duration: 4 days or until ICU discharge	↓ Vasopressor duration ↓ Systemic organ failure ↓ Procalcitonin levels ↓ Renal replacement therapy X ICU length of stay ↓ Hospital mortality	[57]

ABBREVIATIONS: ↓ decrease; AKI, acute kidney injury; IV, intravenous; X, no change.

in mortality, fewer participants died in the treatment arms. Subsequently, a randomized controlled trial carried out in 28 patients with septic shock treated with 100 mg/kg body weight intravenous vitamin C per day showed a significant decrease in vasopressor requirements (dose and duration of norepinephrine) and a dramatic (78%) decrease in 28-day mortality [52]. No difference in ICU length of stay was observed. Another recent randomized study administering 6 g/d vitamin C or placebo to 100 septic shock patients also showed decreased requirements for vasopressors and decreased length of ICU stay [53]. However, no differences in ICU mortality, duration of mechanical ventilation, or renal replacement therapy were observed between the two groups.

Several recent studies have investigated the efficacy of administering a cocktail of vitamin C with thiamine (vitamin B1), with or without hydrocortisone (Table 7.4). A before-and-after study was carried out in which 47 patients with severe sepsis were treated with 6 g/d intravenous vitamin C, in combination with 0.4 g/d thiamine (vitamin B1) and hydrocortisone, and were compared with 47 retrospective controls, who also received hydrocortisone at the attending physicians' discretion [57]. This study also showed decreased vasopressor requirements, as well as decreased systemic organ failure and requirement for renal replacement therapy. Furthermore, a dramatic (79%) decrease in hospital mortality was observed in the group who received the intervention. A smaller randomized study administering the same cocktail of vitamin C, thiamine, and hydrocortisone to 24 cardiac surgery patients with septic shock showed decreased vasopressin and norepinephrine requirements and decreased procalcitonin levels in the treatment group, although no difference in sequential organ failure assessment (SOFA) scores was observed between the two groups [56]. A recent retrospective analysis of septic patients administered the same cocktail, however, showed no effect of treatment on any of the assessed outcomes (i.e., hospital and ICU mortality and length of stay, renal replacement therapy for acute kidney injury, or time to vasopressor independence). It should be noted that patients were included in the analysis if they received as little as one dose of the cocktail, and although a subgroup analysis of the 20 patients who did receive the full 4 days (or until discharge) also showed no significant outcome effects, these numbers would likely be too low to provide appropriate power. Another before-and-after study administering vitamin C and thiamine, but without hydrocortisone, to 229 septic shock patients found no effect on ICU or hospital mortality or length of stay when compared with 915 retrospective controls [55]. However, the treatment was administered for only 1 day, and the treatment group also had significantly higher baseline morbidity than the control group [58]. Despite this, in patients with the most severe organ dysfunction, the treatment did decrease mortality. Thus, most of these small studies have indicated that intravenous doses of ~6–7 g/d vitamin C administered for 3–4 days may improve the outcomes of patients with sepsis and septic shock, including a decreased requirement for vasopressors [59]. Currently, over a dozen registered randomized controlled trials are underway around the world to determine if these encouraging findings are reproducible.

ACUTE LUNG DYSFUNCTION

Acute respiratory infections and sepsis can result in the development of acute lung injury which, in its most severe form, is known as acute respiratory distress syndrome [60]. During acute lung injury, bronchoalveolar barrier function is compromised, resulting in abnormal capillary permeability and pulmonary edema [61]. Sepsis-induced acute lung injury is also associated with diminished expression and function of tight junction proteins in lung epithelium [62]. Furthermore, the iron pumps and channels that normally function to maintain continuous fluid clearance by the lungs can be affected early during sepsis [63]. In a murine model of sepsis-induced acute lung injury, Fisher et al. reported that concurrent administration of 200 mg/kg vitamin C to mice attenuated the resultant lung dysfunction [62]. The authors reported decreased lung water and alveolar epithelial permeability and increased alveolar fluid clearance in the animals that received vitamin C. Furthermore, increased expression of iron pumps and channels and increased expression of tight junction and cytoskeletal connector proteins were observed over and above both sepsis-induced and control levels, suggesting enhanced gene transcription in the presence of vitamin C (Figure 7.1). Other

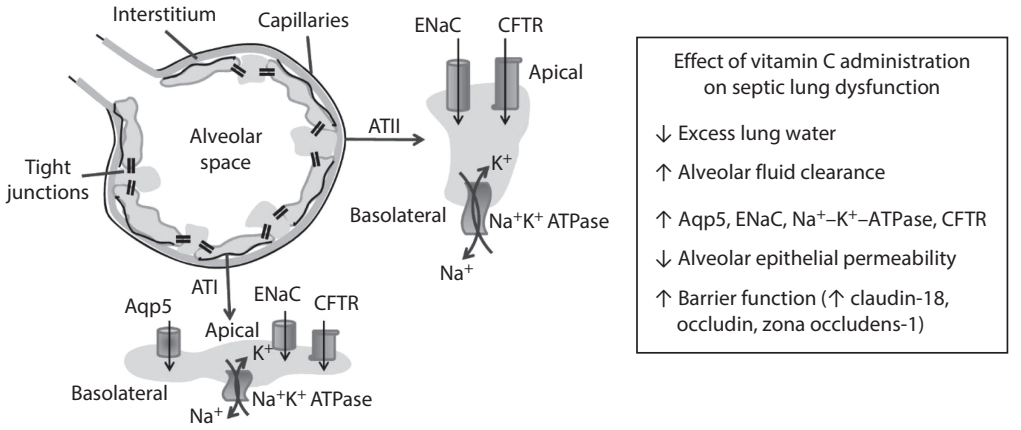

Figure 7.1. Effect of vitamin C administration on sepsis-induced acute lung injury in a mouse model. Vitamin C administration significantly increased expression of the key pumps and channels involved in alveolar fluid transport, including aquaporin 5 (Aqp5), cystic fibrosis transmembrane conductance regulator (CFTR), epithelial sodium channel (ENaC), and sodium-potassium-ATPase (Na^+-K^+-ATPase). Vitamin C administration also significantly increased expression of the tight junction proteins claudin-18 and occludin, as well as the cytoskeletal connector protein zona occludens-1. (↓ decreased, ↑ increased.) (The diagram is courtesy of R. Natarajan [personal communication], and the text box is a summary of findings from Fisher, B. J. et al. 2012. *Am. J. Physiol. Lung Cell Mol. Physiol.* 303, L20–L32.)

researchers have reported synergistic interactions between vitamin C and hydrocortisone in attenuating lipopolysaccharide-induced hyperpermeability of human lung microvascular endothelial cells [64]. The combination was found to normalize the expression and activation of proteins associated with actin stress fibers, which play an important role in the formation and maintenance of cell-cell adhesion, including tight junctions. Recently, case reports have been published that demonstrate dramatic lung clearance in septic patients with acute respiratory distress syndrome of bacterial and viral origins following treatment with intravenous vitamin C [65,66]. Vitamin C was administered to the patients at a dose of 200 mg/kg/d, and within 1–2 days there was observable clearance of the lung infiltrate upon chest x-ray (Figure 7.2). Similar lung clearance has been reported for vitamin C combination therapy in cases of noninfectious and aspiration-induced acute respiratory distress syndrome [67,68].

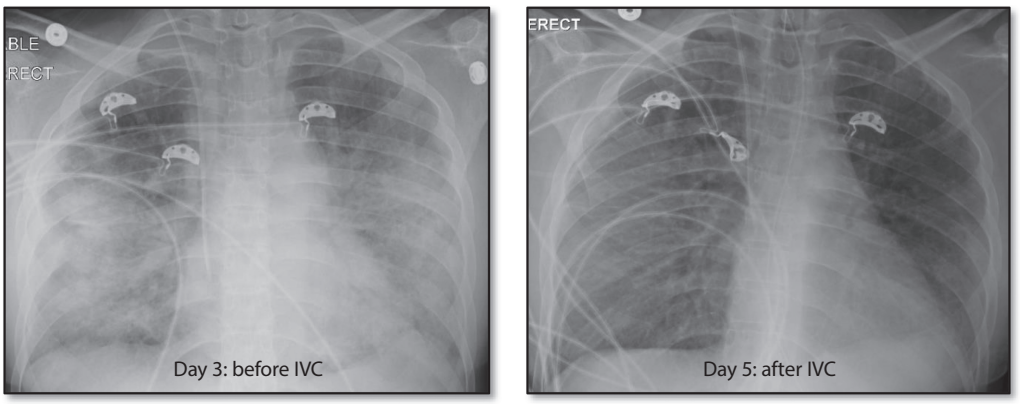

Figure 7.2. Chest x-rays of a septic patient with acute respiratory distress syndrome before and after intravenous vitamin C (IVC) administration. IVC (200 mg/kg/d) was initiated on hospital day 4, and chest x-ray on day 5 revealed significantly improved opacities. (Images from Bharara, A. et al. 2016 *Case Rep. Crit. Care.* Article ID 8560871, 2016, 4p. CC-BY.)

ROLE OF NEUTROPHILS

Infiltration and activation of neutrophils in lung tissue in response to infection represent a primary mechanism for sepsis-induced pulmonary dysfunction and injury [69]. Activated neutrophils release reactive oxygen species, proteolytic enzymes, and other pro-inflammatory mediators that can directly damage tissues and prolong the inflammatory process [70]. Spent neutrophils normally undergo a process of programmed cell death known as *apoptosis*, which facilitates clearance of the neutrophils from sites of inflammation by phagocytosing macrophages [71]. This process prevents excessive tissue damage and supports resolution of inflammation. Attenuated neutrophil apoptosis has been reported in patients with sepsis, and this appears to be related to disease severity [72–74]. Delayed apoptosis may be due to the regulatory effects of pro-inflammatory mediators as well as oxidative inactivation of the redox-sensitive caspase effector enzymes [75,76]. Vitamin C may be able to decrease pro-inflammatory mediator release during sepsis and also protect the oxidant-sensitive caspase enzymes in activated neutrophils, thereby protecting apoptotic cell death pathways [77,78]. In support of this premise, peritoneal neutrophils isolated from vitamin C–deficient Gulo knockout mice exhibited attenuated apoptosis and instead underwent necrotic cell death [79]. These vitamin C–deficient neutrophils were not phagocytosed by macrophages *in vitro* and persisted at inflammatory loci *in vivo*. Furthermore, Fisher et al. reported that administration of 200 mg/kg vitamin C to mice attenuated peritonitis-induced sequestration of neutrophils, and decreased myeloperoxidase mRNA expression, in the lungs of the treated animals [62]. This was also associated with a lower acute lung injury score and decreased mortality in these animals. The clearance of neutrophils from sites of inflammation could conceivably contribute to the enhanced lung clearance observed in patients following vitamin C infusions (Figure 7.2).

Neutrophils that fail to undergo apoptosis instead undergo necrotic cell death. The subsequent release of intracellular components, such as proteases, can cause extensive tissue damage [70]. One recently identified form of neutrophil death, termed *necroptosis*, occurs when caspases are inactivated [80]. Necroptotic signaling pathways can result in the release of "neutrophil extracellular traps" (NETs) composed of neutrophil DNA, histones, and enzymes [81,82]. Although NETs have been proposed as a unique method of microbial killing [83], they have also been implicated in tissue damage and organ failure [84,85]. NET-associated histones can act as damage-associated molecular pattern proteins, activating the immune system and causing further tissue damage [86]. Patients with sepsis, or who go on to develop sepsis, have significantly elevated levels of circulating cell-free DNA, which is believed to be a marker of NET formation [84,87]. Preclinical studies in vitamin C–deficient Gulo knockout mice indicated enhanced NETs in the lungs of septic animals and increased circulating cell-free DNA [88]. The levels of these markers were attenuated in vitamin C–sufficient animals or in vitamin C–deficient animals that were administered vitamin C 30 minutes after induction of sepsis. The same investigators showed that *in vitro* supplementation of human neutrophils with vitamin C attenuated phorbol ester-induced NET formation [88]. Administration of gram doses of vitamin C to septic patients over 4 days, however, did not appear to decrease circulating cell-free DNA levels [89]. The duration of treatment may have been too short, or initiated too late in the inflammatory process, to provide a beneficial effect. It should also be noted that cell-free DNA is not specific for neutrophil-derived DNA, as it may also derive from necrotic tissue; however, the association of neutrophil-specific proteins or enzymes, such as myeloperoxidase, with the DNA can potentially provide an indication of its source [84].

Patients with severe infection also exhibit compromised neutrophil chemotactic activity that can compromise the cells' ability to migrate to sites of infection [90,91]. This neutrophil "paralysis" is believed to be partly due to enhanced levels of immune-suppressive mediators released during the compensatory anti-inflammatory response observed following initial hyperstimulation of the immune system [92]. However, it is also possible that vitamin C depletion observed during severe infection may contribute. Support for this premise comes from studies in the 1980s and 1990s that indicated that the impaired leukocyte chemotaxis observed in patients with recurrent infections could be restored in response to supplementation with gram doses of vitamin C

[93–98]. Furthermore, vitamin C supplementation of neonates with suspected sepsis dramatically improved neutrophil chemotaxis [99]. Patients with recurrent infections can also exhibit impaired neutrophil bacterial killing and phagocytosis, which can be significantly improved following supplementation with gram doses of vitamin C, resulting in long-lasting clinical improvement [93,94,97,98,100].

Neutrophils accumulate vitamin C against a concentration gradient resulting in levels 50- to 100-fold higher than plasma concentrations [101,102]. Active uptake of vitamin C occurs via specialized sodium-dependent vitamin C transporters (SVCTs) and can also occur following activation of the neutrophil oxidative burst and accumulation of the oxidized form of vitamin C, dehydroascorbic acid, via glucose transporters (GLUTs) [103]. It is believed that the accumulation of millimolar vitamin C concentrations indicates an important role for the vitamin within these cells, and depleted levels may compromise vital functions [77]. Vitamin C levels in leukocytes have been reported to decrease by half within 24 hours of subjects contracting an upper respiratory tract infection, and these levels were restored to normal when the infection resolved [104]. The investigators found that administration of 6 g/d of vitamin C during the infection could attenuate the decline in leukocyte vitamin C, although 200 mg/d did not affect the drop in vitamin C levels over the first few days of the infection. However, a randomized controlled trial in patients with acute respiratory infections administered 200 mg/d vitamin C showed repletion of neutrophil and mononuclear cell vitamin C levels within 2 weeks, whereas cells isolated from the placebo group remained low [22]. Thus, repletion of neutrophil vitamin C status via vitamin C administration during infection may enhance vital neutrophil functions [77].

MECHANISMS OF ACTION

Vitamin C is a potent water-soluble antioxidant, able to scavenge a wide range of reactive oxygen species, thus protecting essential cellular structures, metabolic functions, and signaling pathways from oxidative damage [105,106]. Vitamin C also exhibits anti-inflammatory activity with inverse associations observed between vitamin C and pro-inflammatory cytokines and acute phase reactants such as C-reactive protein and procalcitonin [39,45,56,57,77]. Severe infection and sepsis are characterized by significant oxidative stress and overwhelming inflammatory mediators, sometimes referred to as a "cytokine storm" [107,108]. These stressors can contribute to the pathophysiology of sepsis, such as impaired microcirculatory flow, coagulopathy, capillary plugging, increased endothelial dysfunction and permeability, and multiorgan failure [109,110]. As such, the role of vitamin C in severe infection and sepsis has often focused on its antioxidant and anti-inflammatory functions and effects on signaling pathways [109,110]. However, less attention has been paid to its role as an enzyme cofactor.

Biosynthetic Functions

One of the primary roles of vitamin C in the body is to act as a cofactor for a family of metalloenzymes with various biosynthetic and regulatory roles [111–113]. These enzymes introduce hydroxyl groups into biomolecules and comprise two main categories: iron- and 2-oxoglutarate–dependent dioxygenases and copper-containing monooxygenases. Of the former category, vitamin C has long been known to act as a cofactor for the lysyl and prolyl hydroxylases required for stabilization of the tertiary structure of collagen, an essential component of the vasculature [114]. Vitamin C may also be able to stimulate the expression of collagen mRNA, perhaps through its gene regulatory mechanisms described later [77]. Similarly, vitamin C is a cofactor for the two hydroxylases involved in carnitine biosynthesis, a molecule required for transport of fatty acids into mitochondria for generation of metabolic energy [115]. Mitochondrial dysfunction and depleted ATP levels are observed in sepsis; thus, vitamin C may be able to contribute to metabolic resuscitation via both antioxidant and cofactor mechanisms [116,117].

Vitamin C is also known to facilitate the synthesis of the catecholamines dopamine, norepinephrine, and epinephrine within the sympathetic nervous system and adrenal medulla. These catecholamines are central to the cardiovascular response to severe infection; they increase arterial pressure through binding to α-adrenergic receptors on the smooth muscle cells of the vasculature and can promote increased cardiac contractility and heart rate through binding to β-adrenergic receptors on cardiac muscle [118]. Vitamin C is a cofactor for

the copper-containing monooxygenase dopamine β-hydroxylase that introduces a hydroxyl group onto dopamine to form norepinephrine (Figure 7.3) [119,120]. Epinephrine is subsequently synthesized in the adrenal glands via methylation of the amine group of norepinephrine. Recent research indicates that vitamin C may also stimulate the rate-limiting enzyme tyrosine hydroxylase via recycling the enzyme's cofactor, tetrahydrobiopterin, thus facilitating hydroxylation of L-tyrosine to form the dopamine precursor L-dopa (Figure 7.3) [121]. Some evidence also suggests that vitamin C may enhance the synthesis of tyrosine hydroxylase [121]; this is possibly through its gene-regulatory effects, as described below.

It is noteworthy that the tissues where the catecholamines are synthesized (i.e., the brain and adrenal glands) contain the highest levels of vitamin C in the body [122], indicating that the vitamin plays a vital role in these organs. Furthermore, animal models of vitamin C deficiency have shown significant retention of the vitamin in the brain during dietary depletion [123–125], supporting the importance of vitamin C in the central nervous system. Impaired adrenal hormone synthesis has been observed in critically ill patients and is probably a common complication in severe sepsis [126,127]. Interestingly, norepinephrine levels are decreased in vitamin C–deficient animal models, particularly in the adrenal glands [128–130]. Research has shown that vitamin C is also secreted from the adrenal glands as part of the stress response [131], which could conceivably result in adrenal vitamin C depletion under conditions of sustained stress. Thus, appropriate supplementation of vitamin C in sepsis may support endogenous synthesis of vasoactive catecholamines.

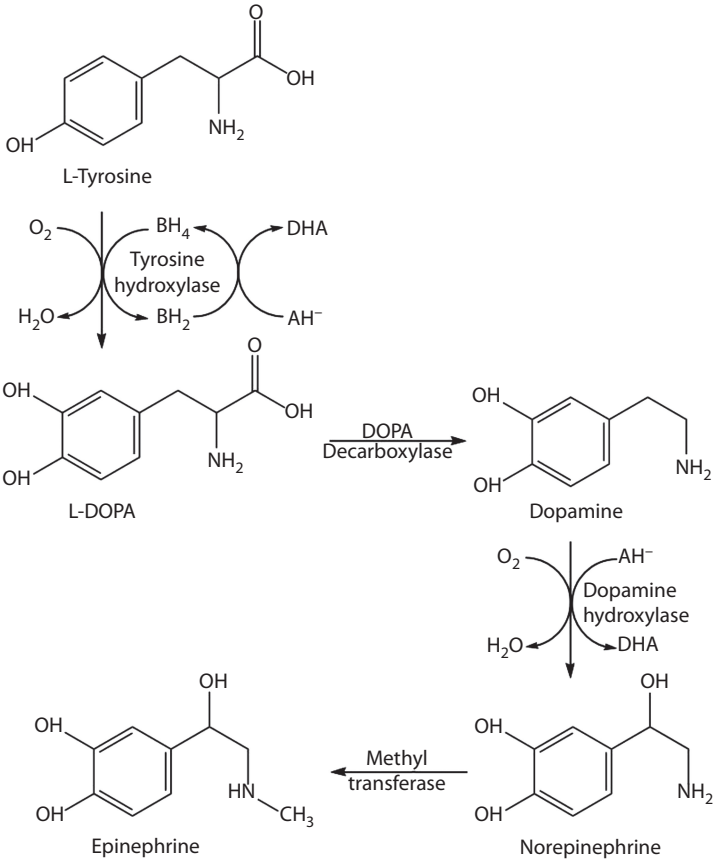

Figure 7.3. Vitamin C–dependent synthesis of the catecholamine vasopressors dopamine, norepinephrine, and epinephrine. Vitamin C acts as a cofactor for the metallo-enzyme dopamine β-hydroxylase and also recycles tetrahydrobiopterin, a cofactor for the rate-limiting enzyme tyrosine hydroxylase. (AH^- ascorbate, DHA dehydroascorbic acid, BH_4 tetrahydrobiopterin, BH_2 dihydrobiopterin.)

Vitamin C is also a cofactor for the copper-containing enzyme peptidylglycine α-amidating monooxygenase (PAM) that is required for the synthesis of amidated neuropeptide hormones [132]. The carboxy-terminal amine group of amidated peptides is essential for their biological activities [133]. One of these amidated peptide hormones is vasopressin, also known as arginine vasopressin (AVP) or antidiuretic hormone (ADH), which is synthesized in the hypothalamus, posttranslationally modified by PAM, and then stored in the posterior pituitary [134]. Vasopressin is secreted in response to decreased blood volume or arterial pressure or increased plasma osmolality. It interacts with specific receptors expressed by vascular smooth muscle cells and kidney collecting ducts to cause vasoconstriction and water retention, respectively [135]. The hormone is synthesized as a pre-prohormone that undergoes sequential cleavage steps to produce provasopressin and finally a glycine-extended precursor. The carboxy-terminal glycine residue of the vasopressin precursor subsequently undergoes posttranslational modification by the vitamin C–dependent enzyme PAM to generate the active carboxy-amidated hormone (Figure 7.4). Support for a connection between vitamin C and vasopressin biosynthesis comes from an animal study, whereby centrally administered vitamin C enhanced circulating levels of vasopressin and induced antidiuresis [136].

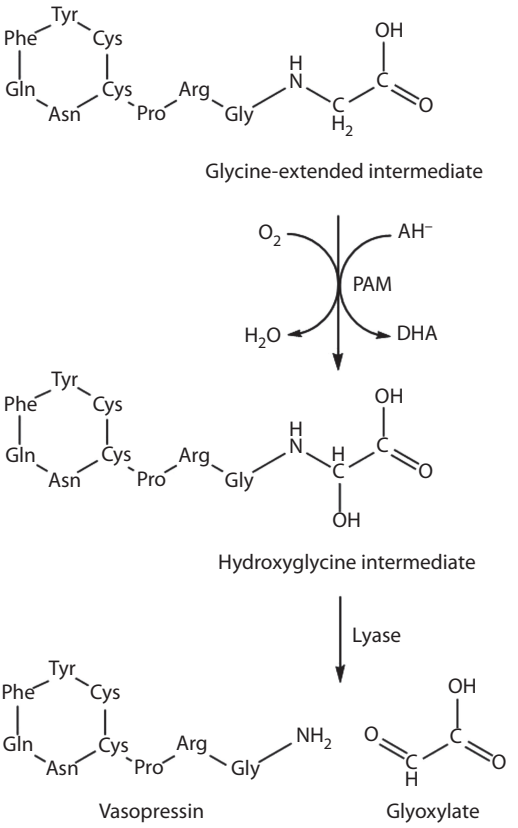

Figure 7.4. Vitamin C–dependent synthesis of mature carboxy-terminal amidated vasopressin. Vitamin C is a cofactor for the metallo-enzyme peptidylglycine α-amidating monooxygenase (PAM). The enzyme comprises two domains: a copper-dependent monooxygenase domain, which converts glycine-extended peptides into hydroxyglycine intermediates; and a lyase domain, which converts the hydroxyglycine intermediates into amidated products. (AH^- ascorbate, DHA dehydroascorbic acid.)

Circulating vasopressin levels increase dramatically during the initial phase of septic shock, but this is followed by a significant decline in the latter phase [137,138]. Patients in late-phase septic shock have significantly lower levels of circulating vasopressin compared with patients in cardiogenic shock, despite similar hypotension [138]. The decline in circulating vasopressin levels after the onset of septic shock is due to depletion of pituitary stores and possibly also impaired vasopressin synthesis [139]. It is of interest to note that the pituitary gland, where the enzyme PAM is abundantly expressed, has the highest levels of vitamin C in the body [122]. Thus, it is conceivable that the depleted vitamin C status of patients with sepsis could contribute to the observed decrease in vasopressin biosynthesis [137–139]. Furthermore, pro-vasopressin, which lacks the carboxyterminal amine of mature vasopressin, is significantly associated with mortality in patients with pneumonia and septic shock, with higher levels observed in nonsurvivors than survivors [140,141]. Although pro-vasopressin has enhanced stability in circulation, the higher ratio of pro-vasopressin to mature vasopressin could also be due to decreased posttranslational activation of the hormone by PAM because of limited cofactor availability. This premise is supported by the observation that other peptide pro-hormones that are substrates of PAM (e.g., pro-adrenomedullin and procalcitonin) are also elevated in severe infectious conditions, particularly in nonsurvivors [141–143]. It is interesting to note that elevated procalcitonin levels decrease following administration of vitamin C to septic patients [45,56,57].

Septic shock is normally managed through the administration of catecholamine vasopressors, primarily norepinephrine, to elevate mean arterial pressure to ≥65 mm Hg [144]. Vasopressin administration is also recommended in the Surviving Sepsis Campaign guidelines to raise mean arterial pressure to target or to decrease the norepinephrine dose [28]. Exogenous vasopressor administration to patients with septic shock, however, can result in adverse side effects such as tissue ischemia and resultant necrosis. Based on the evidence presented earlier, we hypothesized that administration of vitamin C to patients with septic shock may decrease the requirement for exogenously administered vasopressors, through acting as a cofactor for *in vivo* enzyme-dependent synthesis of norepinephrine and vasopressin [59]. Our hypothesis has been supported by four recent clinical trials that showed deceased vasopressor and norepinephrine requirements (both dose and duration) following administration of 6–7 g/d of intravenous vitamin C to patients with severe sepsis and septic shock [52,53,56,57]. Other trials are currently underway to confirm these findings.

Gene Regulatory Functions

Recent research has uncovered new roles for vitamin C in the regulation of transcription factor activity and epigenetic marks, thus influencing gene transcription and cell signaling pathways [112,113]. For example, the iron- and 2-oxoglutarate–dependent asparagyl and prolyl hydroxylases required for the downregulation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) utilize vitamin C as a cofactor [112]. HIF-1α is a constitutively expressed transcription factor that regulates numerous genes, including those involved in energy metabolism, angiogenic signaling, and vasomotor regulation [145]. Under normoxic conditions, HIF-1α is downregulated via hydroxylase-mediated posttranslational modifications that prevent coactivator binding and target HIF for proteosomal degradation [146]. During the hypoxia and ischemia observed with acute lung infections and sepsis, HIF is upregulated due to the absence of substrates (e.g., oxygen) and cofactors (e.g., vitamin C) required for hydroxylase-dependent downregulation. Although initially beneficial to the host, prolonged upregulation of HIF can result in pulmonary hypertension and edema [147]. HIF also facilitates neutrophil survival at hypoxic loci through delaying apoptosis [148]. In vitamin C–deficient Gulo knockout mice, upregulation of HIF-1α was observed under normoxic conditions, along with attenuated neutrophil apoptosis and clearance by macrophages [79]. HIF-1α has also been proposed as a regulator of NET release by neutrophils [149], thus providing a potential mechanism by which vitamin C could downregulate NET generation by these cells [88].

More recently, an important role for vitamin C has emerged in the regulation of DNA and histone demethylation by acting as a cofactor for iron- and 2-oxoglutarate–dependent enzymes that hydroxylate methylated epigenetic marks [113]. Vitamin C acts as a cofactor for the ten-eleven translocation (TET) dioxygenases that hydroxylate methylated cytosine moieties in DNA [150–152]. The hydroxymethylcytosine mark can be further oxidized and subsequently removed through both active and passive DNA repair mechanisms but may also represent an epigenetic mark in its own right [153]. Vitamin C is also a cofactor for several Jumonji C domain-containing histone demethylases (JHDMs) that catalyze histone demethylation [154]. Methylation of lysine and arginine residues on histones is closely associated with activation or silencing of transcription. JHDMs can hydroxylate mono-, di-, and trimethylated histone lysine and arginine residues, resulting in demethylation, and vitamin C is required for optimal catalytic activity and demethylation by JHDMs [155,156]. The involvement of vitamin C in JHDM-dependent histone demethylation was confirmed in somatic cell reprogramming [157,158]. It is likely that the gene regulatory functions of vitamin C play major roles in its immune-regulating functions. For example, preliminary evidence indicates that vitamin C can regulate T-cell maturation via epigenetic mechanisms involving the TETs and histone demethylation [159–161]. Supplementation of healthy volunteers with vitamin C was found to modulate *ex vivo* lipopolysaccharide-stimulated gene expression in mononuclear cells, specifically enhancing synthesis of the anti-inflammatory cytokine interleukin-10 [162]. Due to the thousands of genes regulated via both DNA and histone demethylation, epigenomic regulation by vitamin C likely plays a major role in its pleiotropic health-promoting and disease-modifying effects.

CONCLUSIONS AND FUTURE DIRECTIONS

Vitamin C has a myriad of functions that appear to contribute to beneficial outcomes in severe respiratory infections, such as pneumonia, and the potentially life-threatening condition of sepsis. These include acting as a scavenger of reactive oxygen species and a modulator of inflammatory mediators, as well as a cofactor for a variety of biosynthetic and regulatory enzymes, with the potential to modulate the transcription of thousands of genes and numerous cell signaling pathways. Observational studies indicate that patients with severe respiratory infections and sepsis have depleted vitamin C status during their illness, including a high prevalence of deficiency, despite recommended intakes. This suggests that these patients have higher requirements for vitamin C, which has been borne out in small interventional studies indicating that gram doses of vitamin C are required to normalize the vitamin C status of critically ill patients. Although optimal dietary intakes of vitamin C (i.e., 200 mg/d) may decrease the risk of developing a respiratory infection [16,163], it appears that successively higher amounts of vitamin C are required as infectious diseases progress in severity (Figure 7.5). A handful of small randomized controlled trials indicate that administration of gram doses of vitamin C to patients with pneumonia and sepsis improves multiorgan function, particularly the pulmonary, cardiovascular, and renal systems, as well as potentially decreases mortality rates. Currently, larger interventional studies are underway to confirm the effects of vitamin C on mortality. If these trials support the initially promising findings of the smaller studies, there would then be strong justification to introduce vitamin C administration into routine clinical practice for these conditions, as well as to assess the effects of vitamin C administration on related infectious conditions. Unlike many drugs that target only one specific biochemical pathway, vitamin C targets multiple pathways and hence can exhibit body-wide effects.

To date, there have been no studies that have assessed the effects of vitamin C intervention on the long-term quality-of-life outcomes of patients with pneumonia and sepsis (Figure 7.5). The reported vitamin C–dependent decreases in organ dysfunction, including decreased acute renal failure, which normally requires ongoing dialysis or a transplant, would be expected to significantly improve long-term patient quality of life. The human brain has a particularly high requirement for vitamin C, and both observational and interventional studies have shown inverse associations between vitamin C and cognitive dysfunction and psychiatric disorders such as depression and anxiety [165,166]. Furthermore, vitamin C may be able to modulate the stress response with inverse associations observed between vitamin C status and cortisol levels,

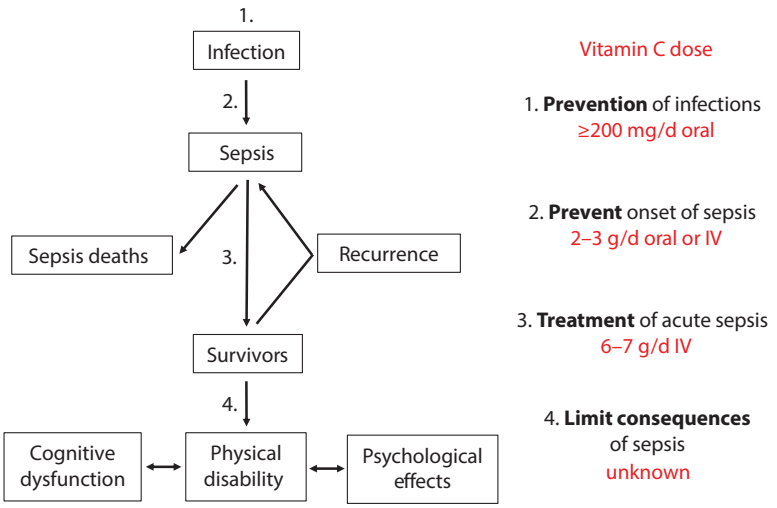

Figure 7.5. Vitamin C requirements during severe infection. The requirements for vitamin C increase during the progression from prevention of infection to treatment of acute sepsis. (Adapted from Carr, A. C. 2018. *Crit. Care* 22, 247.)

and decreased cortisol levels reported following vitamin C intervention [167]. Most of the vitamin C interventional studies carried out in critically ill patients have been for a total duration of 4 days, which may not be sufficient to have a dramatic effect on quality of life at 3 or 6 months later due to the rapid return of vitamin C levels to baseline following withdrawal of supplementation [43,168]. Because vitamin C is water soluble and is not retained by the body, sustained long-term quality-of-life effects will likely require ongoing vitamin C supplementation.

Vitamin C administration has been shown to improve the quality of life and decrease the symptoms of oncology patients, likely through repletion of inadequate vitamin C levels and decrease of the off-target toxicity and adverse side effects of chemotherapeutic drugs [169]. Animal studies have indicated that administration of drugs such as sedatives, analgesics, and muscle relaxants to vitamin C–synthesizing animals stimulates an increased synthesis/excretion of the vitamin, suggesting a higher requirement for vitamin C due to enhanced metabolism when drugs are administered [170,171]. Klenner also reported that penicillin had a retarding effect on the action of vitamin C in patients with pneumonia, and in one case, beneficial effects were not obtained until the penicillin was discontinued [24]. Therefore, it is conceivable that administration of additional vitamin C to patients with sepsis may counteract some of the adverse effects of the many drugs that are administered to these patients during intensive care. The effects of vitamin C on drug-related side effects and long-term patient quality of life should be assessed in further clinical trials of patients with severe respiratory infections and sepsis.

ACKNOWLEDGMENTS

Thank you to Dr. Paul Marik for critically reviewing the manuscript. The author is supported by a Health Research Council of New Zealand Sir Charles Hercus Health Research Fellowship.

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