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Role of Selenoproteins in Bacterial Pathogenesis

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Abstract

The trace element selenium is an essential micronutrient that plays an important role in maintaining homeostasis of several tissues including the immune system of mammals. The vast majority of the biological functions of selenium are mediated via selenoproteins, proteins which incorporate the selenium containing amino acid selenocysteine. Several bacterial infections of humans and animals are associated with decreased levels of selenium in the blood and an adjunct therapy with selenium often leads to favorable outcomes. Many pathogenic bacteria are also capable of synthesizing selenocysteine suggesting that selenoproteins may have a role in bacterial physiology. Interestingly, the composition of host microbiota is also regulated by dietary selenium levels. Therefore, bacterial pathogens, microbiome, and host immune cells may be competing for a limited supply of selenium. Elucidating how selenium, in particular selenoproteins, may regulate pathogen virulence, microbiome diversity, and host immune response during a bacterial infection is critical for clinical management of infectious diseases.

Keywords

Selenium; Selenoproteins; Bacteria; Pathogen; Immune Response; Microbiota

Introduction

Selenium is an essential micronutrient utilized by organisms across all three domains of life [1–3]. Selenium is co-translationally incorporated into the 21st amino acid selenocysteine [Sec] [4–7]. A vast majority of the biological effects of selenium are mediated by selenoproteins, proteins that contain one or more selenocysteines. These proteins make up an organisms' selenoproteome which vary from zero selenoproteins in some plants and fungi to

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more than 30 in some species of fish and algae [8]. In humans and other mammals, selenium has been shown to provide several health benefits such as decreasing cancer incidence, preventing cardiovascular disease, and improving overall immune function [9–11]. Conversely, selenium deficiency has been shown to lead to several disease conditions such as Keshan disease, white muscle disease, and Kashin Beck Disease [12, 13]. Interestingly, numerous chronic infectious diseases such as infections with Human Immunodeficiency virus, Hepatitis C virus, and *Mycobacterium tuberculosis* have been associated with selenium deficiency [9, 14–16]. Accordingly, nutritional intervention studies have shown a beneficial effect of selenium supplementation in treating patients with these illnesses [17–23].

Similar to eukaryotes, prokaryotes also express a diverse set of selenoproteins [8, 24]. It is estimated that approximately 20% of sequenced prokaryotic genomes encode at least one trait for selenium utilization [2, 3, 25]. Multiple selenium-dependent enzymes have been found in microorganisms such as numerous formate dehydrogenases, some hydrogenases, glycine reductase of *Clostridia*, and xanthine dehydrogenase [26]. Therefore, it is plausible that prokaryotes which possess selenoproteins may exhibit increased fitness in the presence of selenium similar to the benefits observed in humans and other mammals. This represents a unique challenge for the mammalian host upon infection with bacteria that possess the ability to utilize selenium with both host and parasite competing for the limited selenium resources. However, there is limited information available related to the role of selenium in bacterial physiology and virulence as well as overall bacterial pathogenesis.

Selenium Utilization by Bacteria

Prokaryotic Selenoprotein Synthesis and their Evolution

The mechanism of Sec insertion in prokaryotes has been most thoroughly described in *Escherichia coli* [27–30]. Unlike eukaryotes, Sec insertion is a one step process in prokaryotes. Briefly, Sec insertion in bacteria requires an in-frame UGA codon and unique $tRNA^{Sec}$ [SelC] [27–29]. The UGA codon [typically read as a stop codon] is reprogrammed to a Sec insertion codon by the presence of a Sec insertion sequence [SECIS], an mRNA stem-loop structure present in the selenoprotein mRNA immediately downstream of the Sec-encoding UGA codon [1, 28]. Reprogramming of the UGA stop codon also requires a Sec-specific elongation factor [SelB] which binds GTP, the SECIS element, and the $tRNA^{Sec}$ [SelC] [27, 28]. Conversely, in eukaryotes, the SECIS element is located in the 3'-untranslated region of selenoprotein mRNA and reprogramming of the UGA codon is carried out by two other proteins, EF-Sec and SECIS-binding protein [SBP2] [7]. Here, EF-Sec binds to GTP, $tRNA^{Sec}$, and SBP2 while SBP2 binds the SECIS element [1,7].

Sec synthesis is an important step in selenium metabolism and is required prior to the synthesis of selenoproteins. In bacteria, $tRNA^{Sec}$ is initially aminoacylated with serine via seryl-tRNA synthetase resulting in seryl-$tRNA^{Sec}$ [29]. Seryl-$tRNA^{Sec}$ is then converted into selenocysteyl-$tRNA^{Sec}$ by Sec synthase [SelA] [29]. SelA utilizes selenophosphate as a selenium donor which is provided by selenophosphate synthetase [SelD] [31]. Synthesis of Sec in eukaryotes follows a similar process to that in bacteria with the exception of an *O*-phosphoseryl-$tRNA^{Sec}$ intermediate which is generated by O-phosphoseryl-$tRNA^{Sec}$ kinase

[PSTK] phosphorylating the serine of seryl-tRNA^{Sec} [31,32]. Eukaryotic Sec synthase then converts *O*-phosphoseryl-tRNA^{Sec} to selenocysteyl-tRNA^{Sec} [32, 33]. tRNA^{Sec} generation in conjunction with the presence of the required Sec insertion machinery ultimately results in selenocysteine being transferred to the nascent polypeptide and the generation of selenoproteins.

In addition to selenocysteine and selenoproteins, some prokaryotes are able to utilize selenium from selenophosphate for the synthesis of a modified tRNA nucleotide, 5-methylaminomethyl-2-selnouridine [SeU] located at the anticodon wobble position of several bacterial tRNAs, including tRNA^{Lys}, tRNA^{Glu}, and tRNA^{Gln} [34–36]. SeU present on such modified tRNAs is believed to improve the translation accuracy and efficiency [34]. However, in order to replace a sulfur atom with selenium in 2-thiouridine within these tRNAs, a 2-selenouridine synthases [YbbB] is required [2]. Selenium has also been shown to be utilized in the form of a co-factor in molybdenum containing hydroxylases such as nicotinic acid hydroxylase and xanthine dehydrogenase [37, 38]. However, much like the sec insertion machinery and tRNA^{Sec}, SeU and YbbB are not ubiquitously expressed in all prokaryotes.

Numerous studies have investigated the evolution and distribution of these selenium utilization traits in various sequenced bacterial genomes [1–3, 31, 39]. Such studies suggest that the evolution of the Sec insertion system most likely arose once from a common ancestor and has been adjusted over time through speciation and differential gene loss with some contribution of horizontal gene transfer [1]. These studies also indicate a strong correlation between the ability to decode Sec and the presence of formate dehydrogenases which are important for anaerobic metabolism; thus, it is reasonable to believe that selenium may be important for respiration during restricted oxygen supply [1, 3]. Conversely, more recent studies involving larger data sets were unable to identify a trend between oxygen levels and Sec utilization [39]. However, they did note that the majority of selenoprotein rich organisms are anaerobic signifying that low oxygen levels may contribute to the evolution of new selenoprotein genes [39]. This study also demonstrated that bacteria with Sec and selenium-cofactor traits favor host-associated environments while SeU appears to favor more aquatic environments [39]. This may be due to the fact that host associated and aquatic environments may provide a more stable and abundant supply of selenium while terrestrial bacteria may be exposed to more diverse environmental selenium levels [39].

Selenoproteins of Bacteria

Formate Dehydrogenases

Selenium has been identified as a common component of formate dehydrogenases that are often identified in anaerobic organisms [40]. Formate dehydrogenase is an enzyme that supports the growth of multiple facultative and obligate anaerobic bacteria by catalyzing the reversible two electron oxidation of formate [40]. Because formate is produced during the fermentation of sugars, aromatic compounds, L-[+]-tartaric acid and oxalate, there tends to be a greater range of anaerobes which are able to metabolize formate [40].

E. coli is a facultatively anaerobic bacteria which possesses Sec within its formate dehydrogenase O, N, and H [6, 40, 41]. Axley *et.al.* investigated the role of this Sec residue by replacing the Sec contained in *E. coli's* formate dehydrogenase H with a cysteine resulting in a sulfur analog of the selenoprotein [42]. While both enzymes maintained similar overall structural properties and pH dependencies related to activity and stability, the mutant was found to bind formate with greater affinity than that of the Sec-containing wild type enzyme. However, the mutant was found to have a turnover rate (K_{cat}) two orders of magnitude (300 times) lower than that of the native enzyme due to a diminished reaction rate during the first step of the overall reaction. Thus, selenium containing formate dehydrogenases may provide an evolutionary advantage to facultative anaerobic bacteria allowing them to thrive under diverse conditions [42].

Campylobacter jejuni naturally resides in the intestinal tracts [specifically the cecum] of birds and is a known gastrointestinal pathogen of both humans and animals [43]. Due to its anaerobic location, *C. jejuni* metabolizes formate for energy which occurs via its formate dehydrogenase A [FdhA] which is known to contain a Sec. The bacterium utilizes its *fdhTU* genes for the selenium-controlled biosynthesis of formate dehydrogenase and inactivation of these genes results in the absence of FdhA. Likewise, inactivation of *C. jejuni's* selA and *selB* genes results in a lack of Sec insertion and the absence of formate dehydrogenase activity. Thus, selenium is essential for FdhA activity in *C. jejuni* which directly affects its pathogenic potential [43]. The *fdhTU* genes have been shown to affect *C. jejuni's* motility, chemotactic behavior, and play a role in invasion of host cells [44]. This poses a potential problem for the infected mammalian host which also requires selenium in order to mount an effective immune response to *C. jejuni*.

Glycine Reductases

Glycine reductases are composed of three proteins [protein A, B, and C] which catalyze the deamination of glycine and ammonia with simultaneous esterification of orthophosphate which reacts with ADP in order to form ATP [26]. These proteins are known to contain Sec and are expressed in a variety of anaerobes such as *Eubacterium acidaminophilum* and various *Clostridium* species [45]. Protein A of *Clostridium sticklandii* is an acidic, 18 kDa protein that contains a Sec residue and two Cysteine residues [46]. These three residues are highly reactive with oxygen resulting in an oxidized protein that is converted to the active, reduced form [26]. Further investigation of Protein A demonstrated a dependency on the amount of selenium present in the culture media for catalytic activity. However, the production of immunogenic material appears to be independent of the selenium level *in vitro.* Under selenium deficient conditions, cysteine is incorporated into the protein in the place of Sec resulting in an enzymatically inactive protein [46]. Therefore, selenium is an essential component of *Clostridium's* glycine reductase which allows the bacterium to produce ATP for energy. However, the role of selenium in different *Clostridium* species have not been elucidated.

Xanthine Dehydrogenases

Selenium has been shown to be beneficial in increasing the catalytic efficiency of various enzymes and while most selenoproteins utilize Se via incorporation of selenocysteine, it is also known to occur in enzymes as a cofactor [38, 47]. Enzymes known to require selenium in this form include xanthine dehydrogenases, nicotinic acid hydroxylase, and purine hydroxylase [48]. Each of these enzymes are complex and also contain a molybdopterin cofactor [49]. Xanthine dehydrogenase [XDH] is a flavoprotein which catalyzes the reaction of purines, hypoxanthine, and xanthine into uric acid via a complex mechanism [50].

Enterococcus faecalis is an opportunistic pathogen which produces biofilms during infection of the heart and bladder [49]. *E. faecalis* is a facultative anaerobe which is capable of utilizing selenium as a cofactor for XDH. Srivastava *et.al.* demonstrated that the density of biofilm was increased following the addition of uric acid to the culture media of *E. faecalis* [49]. However, this biofilm increase following the addition of selenium was only observed in the presence of molybdate in the media. Subsequent deletion of *selD* resulted in decreased biofilm formation following the addition of exogenous selenium. Likewise, disruption of the gene encoding the XDH resulted in diminished biofilm formation. It is known that enhanced biofilm proliferation correlates with increased extracellular production of peroxide following selenite addition. These data demonstrate that selenium dependent XDH is involved in the formation of biofilms presumably via oxidant production [49]. Therefore, selenium supplementation may increase the pathogenicity of *E. faecalis* which poses a potential risk for the selenium supplemented host during infection with this bacterium.

Selenoproteins in Host Responses to Bacteria

The host's micronutrient status is increasingly being linked to the response to infectious diseases as several micronutrients including selenium have been shown to play an important role in the immune system [16, 21, 51]. The primary method of selenium intake in humans/animals is via the diet; selenium rich foods include bread, cereals, meat, fish, eggs, as well as dairy products. In animal products, selenium status directly reflects the amount of selenium within the feed and thus that of the soil where the feeds were grown. It is recommended that humans consume a daily allowance of ~20 μg/day of selenium in order to prevent illnesses such as Keshan Disease, a form of cardiomyopathy [52]. However, intake of excess selenium [between 3,200 and 6,700 μg/day] is known to result in selenium toxicity. Thus, it is essential to consume selenium within the target range in order to prevent negative effects from selenium deficiency or over-supplementation. Absorption of selenium primarily occurs within the lower part of the small intestine. Both inorganic and organic selenium are generally absorbed with an efficiency between 70 and 90 percent under homeostatic conditions. Selenite, however, is only absorbed with an efficiency of 60 percent [53]. The selenium obtained via the diet is then utilized to form selenoproteins which are responsible for executing the biological functions of selenium.

The human selenoproteome consists of 25 selenoproteins which have been shown to play a variety of functions important for human health. These proteins are indispensable for the proper functioning of the immune system, male reproductive system, endocrine system,

muscular system, etc. [9, 11, 52, 54]. Selenoproteins expressed by the cells of the immune system are responsible for carrying out essential functions such as antioxidant functions, protein folding, play a role in cell signaling events, and other yet-to-be defined functions [55]. Therefore, selenium deficiency may result in a suppressed immune system while supplementation may improve overall immune function and aid in microbial clearance following infection. While more information regarding the influence of selenium on the immune system are reviewed elsewhere [55], the following sections will focus on the influence of selenium on immune responses during specific bacterial infections. A more specific relationship between selenoproteins and bacterial infections are listed in Table 1.

Mycobacterium tuberculosis infections

M. tuberculosis, the causative agent of Tuberculosis [TB], is a slow-growing, gram-positive, non-selenium utilizing, non-spore-forming bacteria responsible for infecting over 9,000 individuals in the United States in 2017. Today, TB remains one of the world's deadliest illnesses and is the leading cause of death in individuals who are co-infected with Human Immunodeficiency Virus [HIV] [56]. TB induces a Th1-type immune response characterized by CD4+ T cell production of IFN-γ, a cytokine which has shown to be crucial for the clearance of *M. tuberculosis* [51]. Due to the reduced CD4+ T cell numbers and overall impaired cell-mediated immune response, individuals with a pre-existing HIV infection are extremely susceptible to TB and pose an increased risk for developing overt tuberculosis resulting in death [21,51]. TB alone often results in decreased apatite, malabsorption of nutrients and micronutrients, and increased metabolic demands which collectively result in poor nutritional status and increased immunodeficiency [57]. It is unknown whether malnutrition leads to increased susceptibility to TB or if TB infection results in malnutrition due to the increased metabolic demands and reduced nutrient intake.

Numerous studies have demonstrated a reduction in serum selenium levels in patients with TB suggesting that selenium supplementation may be a beneficial therapeutic strategy for these individuals [21,58–60]. One such study conducted in Botswana showed that TB and HIV were both associated with decreased levels of micronutrients and supplementation with multivitamins and selenium reduced recurrence of TB as well as TB-related mortality in coinfected participants. Selenium supplementation also reduced the risk of developing TB in patients with a pre-existing HIV [18, 61]. Thus, selenium supplementation should be considered for the prevention of TB in high risk populations such as those with pre-existing HIV infections where TB is endemic. Other studies have demonstrated that micronutrient supplementation [including selenium] also significantly decrease the risk of TB recurrence in all TB patients regardless of HIV co-infection [62]. While it remains unknown how selenium reduces TB recurrence and mortality, it may be attributed to its antioxidant effects which prevent tissue damage and inflammation caused by the production of reactive oxygen species during *M. tuberculosis* infection [23, 62]. One study attempted to elucidate the molecular mechanism behind multi-micronutrient supplementation [including selenium] and TB. However, they found no significant differences in T cell proliferation following T cell mitogen challenge between the micronutrient supplemented group and control group [62]. While many studies have demonstrated the benefit of selenium supplementation on TB outcome, others have found no significant improvement following micronutrient

supplementation [22, 63]. However, these studies did not control for the varied selenium status of subjects. Therefore, additional studies with carefully monitored selenium status of test patients are needed to investigate the mechanism by which selenium alone functions during TB infection are necessary to understand the potential of selenium supplementation during host response to bacterial pathogenesis.

Helicobacter pylori infections

Helicobater pylori is a gram-negative, microaerophilic, helix-shaped bacterium which colonizes the gastric mucous layer or adheres to the epithelial lining of the stomach [64, 65]. This bacterium is present within ~50% of the human population worldwide and is responsible for causing 90% of duodenal ulcers and 80% of gastric ulcers [21,65]. *H. pylori* transmission most often occurs via fecal-oral and oral-oral exposures. Once acquired, those infected with *H. pylori* have an increased risk of developing gastric cancer and mucosal-associated-lymphoid type lymphoma [64, 65]. Treatment is currently available to those infected with *H. pylori* and includes a 10 to 14-day course of “triple therapy” which includes proton pump inhibitors, amoxicillin and clarithromycin [64]. Unfortunately, *H. pylori* has begun to develop resistance to clarithromycin resulting in decreased eradication rates. Thus, alternative treatments have been suggested which include proton pump inhibitors and amoxicillin for the first half of treatment followed by proton pump inhibitors, metronidazole, and clarithromycin for the second half of treatment [66].

Micronutrient homeostasis is often impaired during *H. pylori* infection, an effect which is restored following eradication of the bacteria [67]. Alteration of host micronutrient status is most likely due to lowered gastric acid secretion, atrophy of the gastric mucosa, and malabsorption [67]. While plasma selenium levels have not been shown to differ between patients with or without *H. pylori* caused inflammation, it has been shown that there are higher levels of selenium located in the antral mucosa in individuals suffering from *H. pylori* associated gastritis [68–70]. Moreover, the concentration of gastric tissue selenium tend to be increased with greater inflammation scores of the antral mucosa [68]. This is likely due to the strong relationship between *H. pylori* tissue damage and the generation of reactive oxygen species [ROS] with concomitant reduction in the levels of various antioxidants. Thus, an increase in the selenium concentration at the infected mucosa may be a protective response where selenium is acting as an antioxidant to prevent further damage caused by ROS [68, 71] or mediating resolution of inflammation. This is further supported by the decrease of gastric tissue selenium observed in patients after successful eradication of *H. pylori* [68]. It is important to note, that selenium deficiency has been shown to be a risk factor for the conversion of precancerous gastric lesions into carcinomas [68, 70, 72, 73]. This decrease in selenium may be due to long-lasting mucosal inflammation which results in an altered gastric microenvironment leading to gastric carcinogenesis [68]. These findings suggest that selenium supplementation may aid in preventing the onset of gastric carcinogenesis in chronically infected individuals and mortality in those whom already have gastric cancer [74, 75]. Furthermore, one study suggests that selenium status may be correlated to the location of gastric cancer [72]. More research is required to investigate why selenium levels drop prior to carcinogenesis and the mechanism by which this occurs.

Sepsis and Septic Shock

Clinically, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Such organ dysfunction is identified as an acute change in the total Sequential Organ Failure Assessment [SOFA] score due to the infection. A subset of sepsis, known as septic shock, occurs when underlying circulatory and cellular/metabolic abnormalities are so profound that they significantly increase the likelihood of mortality [76]. Sepsis, while incompletely understood, is a pathophysiologic process comprised of activation and dysregulation of pro-inflammatory/anti-inflammatory responses, complement and coagulation systems, metabolic alterations, hormonal changes, mitochondrial dysfunction, and epithelial and microcirculatory dysfunction. The organ injuries sustained during sepsis are generally caused by the patient's immune response to the pathogen[s] which results in significant oxidative cellular stress. Thus, early antibiotic intervention, re-establishment of cellular perfusion, and control of the source of sepsis are essential for a positive patient outcome [77]. A vast majority of sepsis is caused by bacteria, which are primarily gram-negative in nature.

Several studies have reported a relationship between selenium status and sepsis/septic shock. Sakr *et. al*, showed that plasma selenium concentrations were below the standard value 92 percent of the time in critically ill patients admitted to the surgical Intensive Care Unit [ICU]. All critically ill patients demonstrated a consistent decrease in plasma selenium concentrations throughout the course of their ICU stay. Moreover, the plasma selenium concentration was lower upon admission and decreased more significantly throughout the ICU stay in non-survivors compared to survivors. This affect is most likely due to selenium's role as an antioxidant and involvement with immune system function. Overall, lower plasma selenium levels were correlated with increased tissue damage, organ dysfunction/failure, and increased mortality [78].

In vitro models of sepsis have demonstrated that the selenium environment influences LPS/PepG-induced mitochondrial dysfunction. Mitochondrial dysfunction plays a significant role during the course of sepsis due to a loss of membrane potential and metabolic activity which ultimately results in decreased ATP production [79]. *In vivo* models have shown that the administration of selenium results in decreased septic alterations within lung tissue and improved glutathione peroxidase activity [specifically GPx3] which plays a role in preventing oxidative damage. Additionally, myeloperoxidase [MPO] activity [a marker for neutrophil accumulation and activity within the lung] is altered during sepsis. Following selenium supplementation, MPO activity significantly decreased indicating that selenium suppresses the severity of sepsis by decreasing MPO activity. This is thought to be due to decreasing neutrophil accumulation resulting in less tissue damage [80]. It should also be noted that few studies have demonstrated that MPO contributes to protection against endotoxemia [81]. Thus, the role of selenium may be more complex as the overall effect may be dependent on tissues, selenium status, causative agents, and immune status of the host.

Studies investigating the role of hepatic selenium metabolism during sepsis demonstrates that selenium metabolism is disturbed during sepsis which results in decreased serum

selenium due to decreased synthesis of Selenoprotein P [SelP], the selenoprotein responsible for transporting selenium. These data support the idea that SelP is the main determinant of the altered serum selenium status during sepsis as the expression of other selenoproteins rely on the availability of selenium for Sec biosynthesis within tissue specific locations. Moreover, SelP biosynthesis and selenium status may be under the control of the limiting trans-acting factors which are required for selenoprotein synthesis. This study demonstrated that redox status may control SBP2 trafficking and thus, may alter the rate of selenoprotein biosynthesis. However, how supplementation of selenium may reverse this condition has not been addressed. Thus, more research is required to determine the exact effect selenium exerts on various tissues during acute sepsis/septic shock and the mechanism by which selenium is regulated during these events.

Staphylococcus aureus Infections

Staphylococcus aureus is an opportunistic gram-positive commensal bacterium which may overcome the immune system gaining access to deep tissues. The resulting infections range from mild skin infections to more severe infections such as pyomyositis, necrotizing fasciitis, necrotizing pneumonia, and bacteremia [82]. Internalization of *S. aureus* by macrophages can occur via non-opsonic uptake [such as internalization via Pattern Recognition Receptors [PRRs]], complement dependent, and complement-independent uptake depending on the structure of the bacterium's capsule. Activation of PRRs result in the activation of the Nuclear Factor Kappa B [NFκB] signaling pathway which is thought to play a central role in inflammation leading to the activation of genes responsible for various cytokines and chemokines. The Mitogen Activated Protein Kinase [MAPK] signaling pathway has also been shown to be involved during inflammation and is able to induce pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [83]. Following internalization, macrophages produce Reactive Oxygen Species [ROS] as well as Reactive Nitrogen Species [RNS] to induce microbial killing. However, *S. aureus* produces a variety of virulence factors which protect them against microbicidal agents generated by phagocytosis allowing for their intracellular survival [84]. *S. aureus* pathogenicity is also related to its ability to produce arginase which can compete with inducible nitric oxide synthase [iNOS] for the shared substrate arginine leading to decreased NO production and reduced microbicidal activity by activated macrophages. Lower NO production results in bacterial expression of NO-inducible-L-lactate dehydrogenase improving *S. aureus* resistance to oxidative stress [85]. Because selenium is known to be an antioxidant and is required for optimal immune cell functioning, it is plausible that it will aid in the host response to infection of *Staphylococcus.*

In fact, upon internalization of *S. aureus*, macrophages supplemented with selenium produced a significantly reduced amount of NO with a concomitant increase in ROS production [specifically H_20_2]. Selenium supplementation also led to a decrease in the bacterial arginase activity, limiting the bacterial tolerance to oxidative stress. Moreover, selenium was shown to enhance phagocytosis of the bacterium and increase bactericidal capacity in a dose-dependent manner. Thus, selenium supplementation could result in an enhanced immune response to *S. aureus* [85].

Bi *et.al.*, demonstrated that RAW264.7 macrophages supplemented with selenium during *S. aureus* infection resulted in decreased inflammatory cytokine gene expression and protein levels (TNF-α, IL-1β, and IL-6]. Additionally, selenium supplementation appeared to inhibit the activation of both NF-κB and MAPK signaling pathways. This occurred via inhibition of the phosphorylation of IκBα and p65 as well as *Erk, Jnk*, and *p38* resulting in the suppression of NF-κB and MAPK signaling, respectively. Together, the inhibition of these pathways by selenium led to the attenuation of the overall inflammatory pathways [83].

S. aureus infection is also the primary cause of mastitis (mammary inflammation disease) in both humans and animals which results in inflammation injury to the mammary tissue [86]. Selenium has been shown to perform an immunoregulatory function on inflammation in mammary epithelial cells and glandular tissue, indicating it may play a role during S. aureus-induced mastitis [87]. Bacterial infection in selenium deficient mice resulted in mammary alveolus damage due to damaged and apoptotic epithelial cells while selenium supplementation improved the observed histological changes. Selenium deficiency was also shown to increase the levels of pro-inflammatory cytokines during infection with *S. aureus* [NO, IL-17, IL-8, IFN-γ]. Moreover, IL-10 expression was highest in the high selenium infection group indicating that selenium deficiency promotes pro-inflammatory cytokines while selenium supplementation promotes antiinflammatory cytokines. This study also demonstrated inhibition of NF-κB under selenium supplemented condition [86]. Additional work has demonstrated that selenium also plays a role inhibiting *S. aureus* infection of the uterus and reduces the activation of Toll Like Receptor-2 [TLR2] inflammatory signaling reducing Caspase activity [88]. Overall, selenium appears to benefit the host by limiting the inflammatory damage caused by *S. aureus.*

Escherichia coli infections

Escherichia coli is a versatile bacterium which is typically a part of the normal intestinal microflora of humans and various other mammals. While the commensal strains of *E. coli* are rarely involved in causing diseases in the healthy human host, there are numerous adapted *E. coli* strains which possess virulence attributes that allow them to cause disease. Infection with pathogenic *E. coli* are able to produce a variety of infections such as enteric/diarrheal disease, urinary tract infections, and sepsis/meningitis [89].

Citrobacter rodentium is a murine pathogen which mainly infects the colon and closely resembles enteropathogenic *E. coli* [EPEC] and enterohemorrhagic *E. coli* [EHEC] in humans. *C. rodentium* infections in mice have been shown to induce lesions similar to those caused by EPEC and EHEC making it an excellent model to study these human diseases. Furthermore, *C. rodentium* has been shown to alter gut homeostasis by causing crypt hyperplasia, epithelial cell proliferation, crypt dilation, mucosal thickening, and apical enterocyte surfaces. Mice fed diets deficient in both Vitamin E and selenium resulted in increased pathology, higher bacterial burdens, and elevated bacterial translocation to the spleen following *C. rodentium* infection. Moreover, the deficiency of Vitamin E enhanced the effects of selenium deficiency indicating that combined deficiency may exacerbate pathology. This is most likely due to a greater pro-inflammatory response to infection as demonstrated by increased chemokine and cytokine expression [90]. Thus, deficiency of

Vitamin E and selenium may result in increased oxidative stress leading to greater proinflammatory signaling which ultimately results in greater tissue damage during gastrointestinal tract disease.

Selenium-enriched probiotics have also been investigated as useful means to protect against pathogenic *E. coli* within the gut. Selenium-enriched probiotics demonstrated superior ability to increase the serum selenium levels over sodium selenite presumably due to the enhanced absorption of organic selenium compounds over that of inorganic selenium compounds. Four varieties of probiotic bacteria *[Candida utilis*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG, and *Streptococcus thermophilus]* were grown in the presence of selenium and were fed to mice. These bacteria adhered well to the intestine and resulted in the inability of other pathogenic bacteria, such as *E. coli*, to interact with potential binding sites. This demonstrates the enhanced ability of selenium-enriched probiotics to support the internal environment of the gut by increasing antioxidant performance, preventing pathogenic bacterial colonization, increasing immunity and preventing enteric illness [91].

Similar effects of selenium have been observed in an *E. coli* model of chronic bacterial prostatitis [CBP]. Currently, the primary method to treat this disease is the use of antibiotics. However, this requires small molecular weight antibiotics which are fat soluble in order for the antibiotic to diffuse on the prostate epithelial membrane. *Kim et. al.* demonstrated that selenium in addition to ciprofloxacin resulted in the most significant reduction of *E. coli.* Moreover, treatment with selenium lead to a significant reduction in inflammatory cell infiltration of the prostate. This suggests that selenium may play a role in the resolution of CBP especially when used in conjunction with antibiotics [92]. Overall, selenium supplementation may aid the host immune response ultimately preventing illnesses caused by pathogenic *E. coli.*

Clostridium Infections

Clostridium perfringens is a gram-positive, spore-forming bacteria which often colonizes the gastrointestinal tracts of humans and animals. This bacterium can be found in diverse environments such as soil, sewage, and food such as raw meat [93]. *C. perfringens* is often associated with severe systemic and enteric disease, food poisoning, and enterocolitis which is believed to be caused by the secretion of >20 identified toxins or enzymes [94].

In poultry, *C. perfringens* is known to colonize the intestines of chickens where it causes necrotic enteritis resulting in high morbidity and mortality; this disease results in an economic loss greater than 2 billion dollars. Broiler chickens which were supplemented with selenium and subjected to colonization of *C. perfringens*, demonstrated increased body weight gain, fewer intestinal lesions, improved antibody production to Net-B toxin, and upregulated transcript levels of IL-1β, IL-6, IL-8, iNOS, LITAF [Lipopolysaccharide induced TNF Factor], and TNSF15 [Tumor Necrosis Factor Superfamily, Member 15] compared to those which were not supplemented during infection. The upregulation of pro-inflammatory cytokines represents the initiation of the innate immune response which is important for protection against pathogens during the early phase of infection. The upregulation of these pro-inflammatory cytokines following selenium supplementation

suggests that they are essential during the host immune response to necrotizing enteritis [95]. These results support previous findings which demonstrated a beneficial effect of injected selenium into the amniotic cavity of developing eggs followed by exposure to *C. perfringens* in post-hatched chickens [96]. Further research is required to elucidate the direct mechanism by which selenium exerts its effects during necrotizing enteritis.

Vibrio cholerae Infections

Vibrio cholerae is a pathogenic bacterium which causes toxin-mediated diarrhea in humans. This waterborne pathogen leads to extreme dehydration which may result in death in untreated patients [97]. In order to cause infection within its host, *V. cholerae* relies on its motility, intestinal colonization, and the production of cholerae toxin [98]. Bhattaram *et. al.*, investigated the effect of a sub-inhibitory concentration of selenium [400 μg] on *V. cholera's* pathogenicity. They observed that selenium supplementation resulted in reduced motility, an important step in its pathogenesis which allows the bacterium to traverse the host's intestine. While they failed to elucidate the mechanism by which this occurs, prior literature focusing on other bacterial species has suggested that it may be due to an alteration of membrane integrity which affects flagellar structure [98]. Furthermore, Bhattaram and collogues demonstrated that selenium reduced intestinal cell-bacterial attachment and was effective at reducing the production of cholera toxin which causes profuse diarrhea by 95%. This effect was not the result of an alteration to the host but an alteration to bacterial virulence factors [98]. This case demonstrates an instance where selenium supplementation may benefit the host by increasing their immune response while concomitantly decreasing the virulence of the bacterial pathogen.

Other Bacterial Infections

Various additional studies have demonstrated a range of benefits from selenium supplementation during infection. *Listeria monocytogenes* is a gram-positive, food-borne bacterium that causes severe infections in immunocompromised patients. A study demonstrated that mice with a reduced resistance to infection were associated with reduced antioxidant activity and an overall decreased innate immune response. Moreover, the persistence of bacteria was greater in selenium deficient groups compared to those of the selenium adequate group. Selenium deficient mice also demonstrated reduced antioxidant activity compared to those in the selenium adequate group. Additionally, selenium deficient mice had a decreased natural killer cell response to infection. Thus, selenium deficiency results in a compromised response following infection with *L. monocytogenes* [99]. Such alterations of the immune response caused by selenium deficiency has also been demonstrated in ruminants with foot rot caused by *Dichelobacter nodosus.* While selenium did not prevent diseases in these animals, selenium supplementation was able to restore immune functions [100]. New studies are beginning to investigate the role of selenium during leprosy which have demonstrated that low selenium status occurs in patients with high bacterial load [101, 102]. While many studies indicate a benefit for selenium supplementation during infection by bacterial pathogens, there are studies which suggest there is no benefit of supplementation [103]. Thus, there is a need for more studies investigating the outcome of selenium supplementation following bacterial infections.

Selenium and Gut Microbiota

There is a growing body of evidence that links alterations of the host gut microbiome to various diseases such as colon cancer, Crohn's disease, inflammatory bowel disease, and obesity [104–112]. One strategy that may be valuable in preventing such alterations of the microbiome may involve modulating the diet specifically through alterations in micronutrient intake. Selenium supplementation in mice has shown to affect the composition of the existing microbiota as well as the establishment of the microflora. Significant changes were seen in the microbiota upon selenium supplementation as some groups increased in diversity while fewer groups exhibited decreases in diversity. The most prominent effect was seen in the decline of the genus *Parabacterioides* as well as several alterations in phylotypes of Firmicutes including *Clostridia.* Overall, selenium appeared to increase the diversity of the microbiome which is most likely due to the fact that selenium is able to be utilized in some bacteria while it remains toxic to others. Thus, the microbiome may sequester selenium for incorporation into bacterial selenoproteins. This may result in the microbiota competing with the host for dietary selenium [113]. A similar study investigating the effects of selenium nanoparticles in poultry also demonstrated positive effects in improving gut health by altering the microbiome's diversity [114].

Selenium supplementation has also shown to play a role in the intestinal barrier functions due to its effects on the microbiota. Selenium supplemented mice subjected to Dextran Sulfate Sodium [DSSj-induced colitis demonstrated enhanced survival, fewer symptoms of colitis, and decreased gut permeability. Moreover, fecal transplantation from selenium supplemented mice into deficient mice was able to alleviate colitis. This is thought to be attributed to the altered microbiota of supplemented mice which showed decreases in *Dorea* and increases in microbes with protective effects [115].

Conclusions

It is also important to recognize that many infectious diseases are associated with a reduced serum selenium level in the host. However, it is not clear if the low-selenium hosts are more prone to infectious diseases or infected hosts deplete selenium at a higher rate due to inflammation. It is also important to determine the levels of selenium in specific tissues during infections as the levels may vary between serum and infected organs. Lastly, more reliable biological markers that indicate the selenium status of hosts are required.

It is clear from the limited studies which have investigated the effect of selenium on bacterial infections that some bacterial species are able to benefit from the presence of selenium in their surrounding environment. Thus, when such bacteria are able to establish an infection in a mammalian host, there is a complex interaction that occurs between the host immune response, microbial pathogen, microbiota, and host selenium status. Many of the enzymes which utilize selenium allow the bacterium to survive in anaerobic conditions such as the mammalian intestine. When these infections occur, the bacterium may ultimately benefit from the selenium-supplemented host by leeching selenium in order to increase its virulence and pathogenicity. At the same time, the host may also benefit from the improved immune functions due to the beneficial effects of selenium on host immune responses. Host

microbiota may also differ in the presence of selenium that may prevent infection with selenium-dependent bacteria either by competing for selenium or by producing toxic metabolites that may be detrimental to the pathogenic bacteria. Conversely, it is known that selenium deficiency in the host may place them in an immunocompromised state where bacteria which do not require selenium may establish an infection and cause pathology. Microbiota in the absence of selenium may promote the establishment of infection as well. Because of this complex relationship, more research is required investigating the effects of selenium utilization among pathogenic bacteria, microbiota and hosts at various selenium status. Advances in next generation sequencing, availability of germ-free mice and specific immune deficient mice, in addition to the ability to grow and transplant monocultures of microbiota will allow better elucidation to the exact role of selenium in bacterial pathogenesis.

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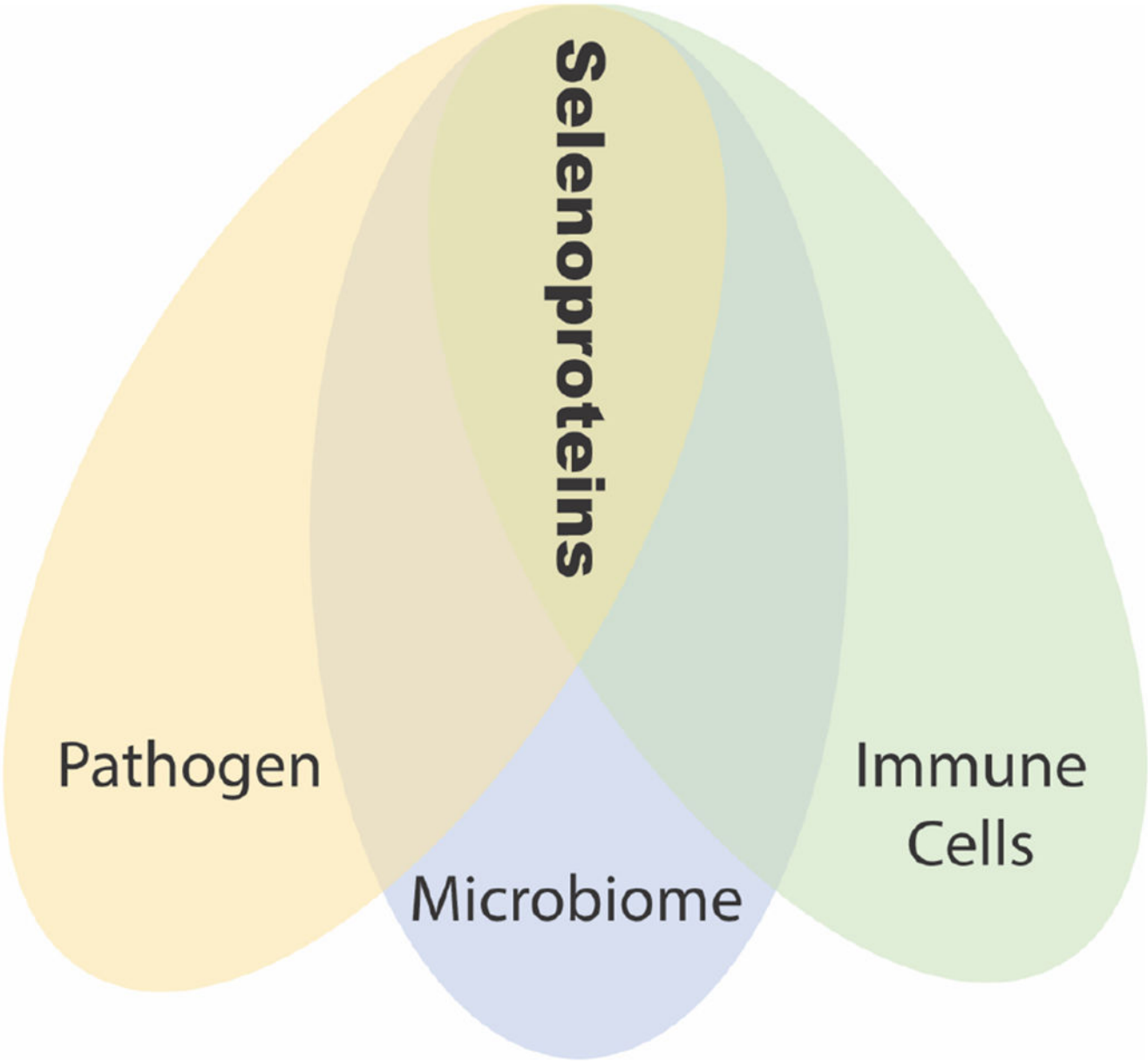

Figure 1. Schematic representing the close interactions of a pathogen, microbiome and immune cells in the context of requirement for selenium.

Several bacterial pathogens require selenium for adapting to host environment, particularly to the anaerobic environment. Host microbiome also requires selenium as selenium depletion leads to dysbiosis of microbiota. Host immune cells also require selenium for their optimum functions.

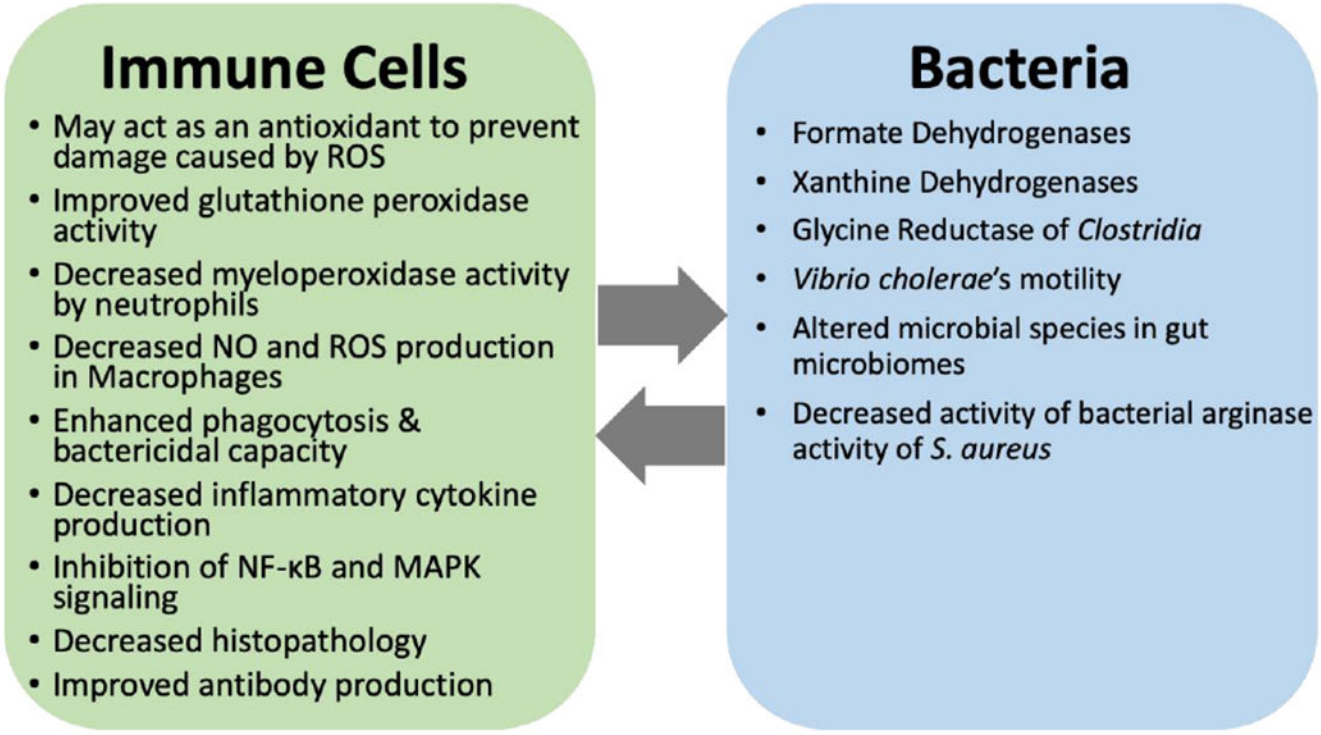

Figure 2. Involvement of selenium and selenoproteins in host-bacteria interactions. Several immune functions are dependent or modulated by selenoproteins.

Similarly, selenoproteins also contribute to the pathogen fitness in host environment. The dynamic interaction and consequent pathogenesis is thus regulated in part by the limited selenium availability.

Table 1.

Key findings related to the role of selenium in animal models of bacterial infections.

Study	Year	Model	Pathogen	Selenium*	Dose & Route**	Major Findings
Gao [116]	2016	Mouse	*S. aureus*	NaSe	0.03, 0.13, 1.5mg/kg diet	Deficiency lead to higher levels of proinflammatory cytokines, MPO activity, TLR2 signaling and NF-κB activation.
Smith [117]	2011	Mouse	*C. rodentium*	NaSe	0 or 0.2μg/g diet	Deficiency lead to increased cytokine levels, pathology and colonic hyperplasia. Maintenance of diet for 20 weeks verses 5 weeks exacerbated phenotype.
Smith [90]	2011	Mouse	*C. rodentium*	NaSe (Vit E)	0 or 0.2μg/g diet	Vitamin E and Se deficiency lead to increased bacterial burden reduced response and increased pathology.
Wang [99]	2009	Mouse	*L. monocytogenes*	Se	0.005 or 0.2mg/kg diet	Deficiency lead to increased bacterial burden, less infiltrating immune cells in the spleen and NK cells had lower activity.
Berg [118]	2005	Mouse	*E. coli LPS*	NaSe	0.05, 0.15, 2g/kg diet	Deficiency was marked by reduced levels of GPX in a dose dependent manner.
Altimira [119]	2000	Mouse	*L. monocytogenes*	Se	350 or <8μg/g diet	Deficiency leads to greater damage in CNS tissue.
Liu [88]	2016	Rat	*S. aureus*	MCS/MSA	1.5mg/kg Se i.p.	Deficiency leads to increased TRL-2 activation increasing caspase cleavage and apoptosis.
Kim [120]	2012	Rat	*E. coli*	Se	12μg/g water	Se with ciprofloxacin decreased bacterial burden.
Boyne [121]	1986	Rat	*S. typhimurium S. aureus*	Se	0.01 or 0.1 mg/kg	Deficiency had no effect on response to infection.
Sjunnesson [122]	2001	Guinea Pig	*H. pylori*	Se (Vit: A,C, E)	0.15 or 1 mg/kg diet	Dietary antioxidant levels increased with supplementation this protected against type-B gastritis. Data suggest there may be a correlation between bacterial load and gastric scores.

*Forms of Se: sodium selenite (NaSe), unknown or labeled as selenium (Se), selenium nanoparticles (SeNp), methylselenocysteine (MCS), methylseleninic acid (MSA)

**Intraperitoneal injection (i.p.)

Table 2.

Key findings related to the role of selenium in human clinical trials.

Study	Year	Patients	Selenium	Dose	Levels of Se/GPX	Mortality
Angstwurm [123]	1999	42	Selenite	535 μg/24hr/3d to 285 μg/24hr/3d to 155 μg/24hr/3d then maintained as control group on 35 μg/24hr/d	Increased	Total p=0.13 Post hoc p=0.0278
Berger [124]	2006	41	Selenite	copper 2.5-3.1 mg/d selenium 315-380μg/d zinc 26.2 - 31.4 mg/d	Increased	p=0.57
Angstwurm [125]	2007	249 (238, 189)	Selenite	1,000 μg/bolus followed by 1,000 μg/24hr/14d or placebo	Increased	p=.109 p=0.049
Forceville [126]	2007	60	Selenite	4,000 μg/24hr/1d to 1000 μg/24hr/9d or placebo	N/A *	p=0.691
Mishra [127]	2007	40	Selenite	474 μg/24hr/3d to 316 μg/24hr/3d to 158 μg/24hr/3d then maintained as control group on 31.6 μg/24hr/d	Increased	p=0.94
Andrews [128]	2011	502	Selenite	Parenteral glutamine 20.2 g/24hr/7d or selenium 500 μg/24hr/7d	N/A *	p=0.54
Manzanares [129]	2011	35	Selenite	1,000 μg/bolus followed by 1,000 μg/24hr/14d or placebo	Increased	p=0.55 p=0.95
Valenta [130]	2011	150	Selenite	1000μg/24hr/1d to 500μg/24hr/13d then maintained as controls group on 75μg/24hr/14d	Increased	p=0.367
Janka [131]	2013	72	Selenite	750 μg/24 h/6d or placebo	Increased	p=0.159

* N/A signifies information that is not available.