Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

S3QELs

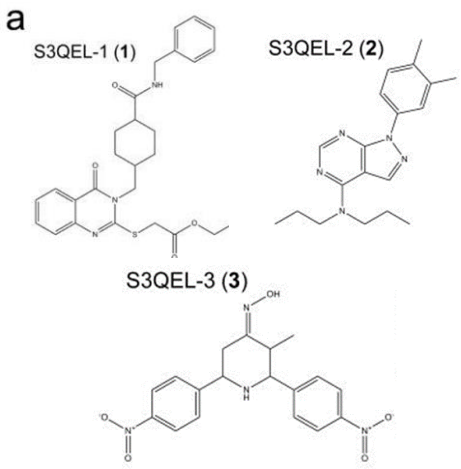
Evidence Summary

By reducing the production of damage-inducing oxidative species, S3QELs may help preserve cell integrity and function. Their relevance to specific chronic conditions *in vivo* needs to be determined.

Neuroprotective Benefit: S3QELs may help mitigate oxidative stress-induced neuronal death in the brain, but evidence is limited as to which conditions may preferentially benefit.

Aging and related health concerns: S3QELs may mitigate the oxidative stress damage related to overnutrition which contributes to obesity-related complications. They may also have utility in some types of cancer, but clinical studies are needed.

Safety: The *in vivo* safety profile of S3QELs needs to be established. It is projected to be safer than other attempted antioxidant strategies due to its narrow mechanism of action, but it could potentially impact immune defense against pathogens.

Availability: Research use	**Dose**: Not established	(Orr et al, 2015) 
Half-life: Not established	**BBB**: Not established	
Clinical trials: None	**Observational studies**: None	

What is it?

Suppressors of the site IIIQo electron leak (S3QELs) are small molecules that suppress the production of the free radical superoxide from one of the electron leak sites in mitochondrial complex III, the outer quinone binding site (IIIQo) [2]. The maintenance of the redox status of the cell is critical for its proper functioning, such that when there is an imbalance it can lead to cell damaging oxidative or reductive stress [3]. In the context of oxidative stress, there is an excess of reactive oxygen species (ROS) due to increased production and/or reduced removal. ROS can be generated from multiple sources, including from NADPH oxidases (NOX enzymes) and from mitochondrial electron leak, producing cellular and mitochondrial ROS, respectively. Endogenous antioxidant enzymes are involved in their removal. ROS have signaling roles in cells, and ROS generated from different sources can play different roles, due to their differential impact on these signaling cascades. As a result, targeting ROS from different sources can have different effects. Additionally, the mechanism underlying the excess ROS for a given condition needs to be taken into account when determining an antioxidant therapeutic strategy. In general, targeting excess ROS production is thought to be superior to an approach that mops up the excess ROS in the cell, as the former strategy can prevent ROS-induced damage, while the latter simply mitigates it. In the process of generating energy via the electron transport chain, sometimes electrons leak out, leading to the formation of mitochondrial ROS. When a single electron leaks out and reacts with oxygen, it forms superoxide, which can be converted to hydrogen peroxide, a more stable ROS, via superoxide dismutase. There are at least eleven different leak sites in the mitochondrial electron transport chain,

and depending on the location, the ROS can end up in different compartments, and have different impacts on the cell [4]. S1QELs act on the complex 1 quinone site, which can generate ROS in the mitochondrial matrix. S3QELs act on the complex III outer quinone site, which generates ROS on either side of the mitochondrial matrix, thus it can influence ROS levels on both the matrix and cytosolic sides. As a result, IIIQo-generated ROS can also impact levels of ROS in the cytoplasm, and are generally thought to have a broader cellular impact. Additionally, unlike many other mitochondrial ROS-targeted agents, including S1QELs, the antioxidant capacity of S3QELs does not depend on the metabolic state of the cell, as some of these agents show pro-oxidant activity under certain cellular metabolic modes [3; 5]. This suggests that S3QELs have the potential for broader therapeutic utility. S3QELs are currently in preclinical development, primarily for conditions with intestinal barrier disruption and neurodegenerative diseases.

Neuroprotective Benefit: S3QELs may help mitigate oxidative stress-induced neuronal death in the brain, but evidence is limited as to which conditions may preferentially benefit.

Types of evidence:

- A few laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Redox stress, stemming from an imbalance between oxidative and reductive chemical reactions is a common feature of aging and neurodegenerative disease, and is thought to be a driver of disease progression [6]. Neurons are particularly vulnerable to oxidative stress damage due to high metabolic demand, high levels of readily oxidizable polyunsaturated fatty acids in their membranes, and low levels of antioxidant enzymes in the brain. As a result, interventions that mitigate redox stress are expected to slow rates of both age-related and neurodegenerative disease-related cognitive decline. If S3QELs can meaningfully reduce levels of pathological reactive oxygen species (ROS) and associated oxidative stress damage, then they would be hypothesized to mitigate cognitive decline, but human testing is needed to validate this theoretical benefit.

Human research to suggest benefits to patients with dementia:

S3QELs have not yet been tested in humans.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Ferroptosis: Ferroptosis is a form of programmed cell death that is mediated by iron-dependent lipid peroxidation. Brain iron accumulation is a common feature of aging, and there is increasing evidence to suggest a prominent role for ferroptosis in neurodegenerative diseases [7]. The glutathione antioxidant system helps protect against lipid peroxidation and thus the induction of ferroptosis. The synthesis of glutathione is limited by the availability of cysteine, thus cysteine starvation is an established method of inducing ferroptosis in cell culture. S3QELs were shown to protect cells (Hepa 1-6, HeLa, and mouse embryonic fibroblasts) from cysteine-starvation induced cell death [8]. Notably, S1QELs were not protective in this context, suggesting that ROS generated through the IIIQo site, rather than through other leak sites, play a specific role in the induction of ferroptotic cell death.
Lysosome dysfunction, driven in part by changes to lysosomal membranes is a common feature of several neurodegenerative diseases [9]. The sequestration of ferric iron in lysosomes protects cells, but lipid peroxidation can make these membranes more fragile, which can release iron into the cytoplasm, which drives further lipid peroxidation and ferroptosis [10]. More studies, especially *in vivo* studies, are needed to determine the extent to which S3QELs can prevent ferroptosis and overall neuron loss in neurodegenerative diseases.

APOE4 interactions: Not established

Aging and related health concerns: S3QELs may mitigate the oxidative stress damage related to overnutrition which contributes to obesity-related complications. They may also have utility in some types of cancer, but clinical studies are needed.

Types of evidence:

- Several laboratory studies

Lifespan: MAY PRESERVE LIFESPAN-ASSOCIATED INTESTINAL BARRIER INTEGRITY (Preclinical)
Due to the presence of bacteria that live in the gut, the preservation of the intestinal barrier is critical to limiting the passage of microbes, both resident and pathogenic, and their associated endotoxins into the blood and rest of the body [11]. Reductions in intestinal barrier integrity, leading to increases in permeability, have been seen in various animal models in the context of aging. The maintenance of intestinal barrier function appears to contribute to the prolongation of healthspan and lifespan [12]. A study in humans found that there were not significant differences in intestinal permeability between healthy young and healthy aged participants, but that permeability was increased with age in individuals with chronic gastrointestinal disorders [13]. Obesity, particularly in combination with other chronic conditions, such as fatty liver disease, is associated with both increased intestinal permeability and shortened lifespans [14; 15]. This suggests that individuals with chronic inflammatory and metabolic conditions may be more prone to age-related reductions in intestinal barrier function, which in turn, exacerbate the chronic illness, and reduce healthspan and lifespan.
A common mechanism of increasing intestinal permeability is through a high-nutrient diet [12]. In flies, a high-nutrient diet increases intestinal permeability and reduces median lifespan, while a low protein diet (less than 2.5%) reduces intestinal permeability and increases median lifespan [11]. These are associated with changes in the levels of intestinal cell oxidative stress damage. In high-nutrient fed flies, supplementation with S3QELs halved the incidence of intestinal permeability and increased the median lifespan by 20-30%, such that the effect on lifespan was mediated by the degree of intestinal permeability mitigation [11]. Similarly, male wildtype (C57Bl/6J) mice fed S3QELs (S3QEL1.2 and S3QEL2.2) showed protection against high-fat diet-induced intestinal permeability, but the S3QELs did not impact glucose tolerance [11]. Overall, these studies suggest that by mitigating oxidate stress damage in the intestine, S3QELs may help preserve intestinal barrier integrity in the context of high-nutrient diets, and thus mitigate the negative impacts of overnutrition to healthspan and lifespan.

Diabetes: MAY PRESERVE PANCREATIC BETA CELL FUNCTION (Preclinical)
Oxidative stress damage plays a key role in the loss of pancreatic β-cells in type 2 diabetes. Due to their high utilization of oxygen and low endogenous antioxidant capacity, β-cells are thought to be particularly vulnerable to oxidative stress damage [16]. Insulin resistance is strongly associated with markers of oxidative stress in human studies [16]. The production of insulin from β-cells primarily occurs in response to the sensing of elevated glucose levels. Persistently high glucose levels can put insulin biosynthetic strain on the β-cells, and increase mitochondrial activity, resulting in the elevated production of ROS [17]. High levels of ROS can, in turn, disrupt insulin biosynthesis, leading to a state of

insulin resistance. Mitigation of ROS production and oxidative stress is then expected to help preserve β-cell function.
S3QELs have been shown to reduce ROS levels in INS-1 β-cells subject to tunicamycin-induced ER-mitochondrial stress and in INS-1E cells in high glucose conditions [2; 5]. Additionally, S3QELs improved cell function and survival in primary pancreatic islets derived from adult male rats [2].

Obesity: MAY HELP PROTECT AGAINST OBESITY (Preclinical)
Excess nutrient intake is a major inducer of mitochondrial ROS production [18]. Oxidative stress vulnerability appears to be a key determinant of an individual's propensity for becoming obese and/or developing obesity-related comorbidities. Rodents resistant to high-fat diet-related weight gain were found to have higher antioxidant capacity and better mitochondrial function, which allowed them to maintain redox homeostasis for a longer period of time in response to the high nutrient challenge [19]. Susceptible rodents lost redox homeostasis relatively quickly in response to the high-fat diet, suggesting that minimizing mitochondrial ROS production in response to high nutrient intake may help protect against obesity.
However, there are numerous mechanisms regulating cellular redox, including at least 11 mitochondrial electron leak sites [3]. ROS generated from different sites appear to play different roles in the cell, and the contribution may vary by cell type and metabolic state [4]. Although the site targeted by S3QELs, the IIIQo site, is implicated in a broad range of ROS-mediated signaling [2], its contribution to different pathologies is likely to be variable, and thus, different antioxidant strategies may be needed for different conditions. In general, the contribution of mitochondrial ROS (superoxide and hydrogen peroxide) originating from site IIIQo to cellular ROS levels ranges from 11-17% based on cells from seven different tissue types, thus it is not the dominant source of cellular or extracellular ROS [20].
Preclinical studies suggest that S3QELs may help preserve pancreatic β-cell function in the context of high-nutrient stress [5], but they were ineffective in reducing hepatic steatosis in mice lacking the antioxidant enzyme superoxide dismutase 2 (SOD2) [21]. In the latter case, S1QELs were effective in reducing ROS production, suggesting that the contribution of ROS from site IQ was greater/and or more relevant than from site IIIQo, in this context. It is currently unclear whether S3QELs on their own would be effective in reducing obesity and obesity-related conditions.

Cancer: MAY LIMIT GROWTH OF SOME TUMOR TYPES (Preclinical)
High levels of mitochondrial ROS production can support the survival and proliferation of some types of cancer cells, depending on the metabolic conditions of the tumor [22]. Inhibiting the production of

mitochondrial ROS can lead to the induction of reductive stress in the cancer cells. The JNK signaling cascade is commonly dysregulated in cancer. ROS can activate JNK, which in turn translocates to the mitochondria, resulting in further mitochondrial ROS production and impaired respiratory capacity [23]. In hepatocellular cancer cell lines (HepG2, Huh7), S3QELs prevented JNK-mediated mitochondrial dysfunction [23]. This suggests that S3QELs could potentially be useful as adjuncts to sensitize cancer cells to anti-cancer therapies, particularly in cancers where activated JNK plays a role in disease progression.

Inflammation/Immunity: POTENTIAL MIXED CONTEXT-DEPENDENT EFFECTS (Preclinical)
ROS are involved in signal transduction cascades and impact metabolic state and cell function in immune cells [24]. Elevated ROS can be an important mechanism used by immune cells to combat pathogens, but also promotes the formation of pro-inflammatory mediators, which can harm the cells of the host. As a result, immune cells are likely to be the most sensitive to ROS-targeted interventions. By reducing mitochondrial ROS production, S3QELs may impact the metabolic rewiring of immune cells which could be protective or maladaptive, depending on the context [4]. Several experimental conditions where S3QEL administration was maladaptive have been reported in preclinical models.
In flies, supplementation with S3QELs, but not with S1QELs, reduced the proliferation of intestinal stem cells following infection, as mitochondrial ROS from site IIIQo supports the activation of these cells [25]. Similarly, reducing mitochondrial ROS production may increase susceptibility to pathogens. ROS from the IIIQo leak site have been implicated in the antigen presentation capacity of plasmacytoid dendritic cells, which are important for activating T cells at the site of inflammation [4]. Treatment with S3QELs inhibited the cross-presentation capacity of splenic plasmacytoid dendritic cells, and their ability to elicit clonal CD8 T cell expansion [26]. Mitochondrial ROS also play a role in the process of mitohormesis, whereby mitochondria undergo metabolic adaptations to a small amount of stress which serves to protect them from future stress [27]. Mitohormesis appears to contribute to some forms of immune tolerance, whereby the inflammatory response is dampened upon repeat exposure. The mitigation of macrophage inflammatory responses to the pro-inflammatory stimulus LPS following an initial insult involves signaling by mitochondrial ROS. Treatment with S3QELs during the initial LPS priming event, prevented the metabolic remodeling and induction of tolerance by the macrophages [27].
On the other hand, ROS production promotes the metabolism of lipids on immune cells, many of which serve as inflammatory mediators [24]. Additionally, ROS-mediated lipid peroxidation also promotes inflammatory processes. It is currently unclear, however, which of these processes are specifically sensitive to ROS from the IIIQo leak site. Although the NLRP3 inflammasome can be activated in

response to ROS, mitochondrial ROS from the IIIQo leak site do not appear to play an essential role, as its activation was not found to be prevented by S3QELs [28].
More work is needed to determine which aspects of immune cell signaling and function are impacted by S3QELs. The net benefit to harm profile will likely depend on the specific context.

Safety: The *in vivo* safety profile of S3QELs needs to be established. It is projected to be safer than other attempted antioxidant strategies due to its narrow mechanism of action, but it could potentially impact immune defense against pathogens.

Types of evidence:

- Several laboratory studies

S3QELs have not been tested in humans, and there is currently limited data from animal and cell models regarding their safety. In cell culture, S3QELs were found to be non-toxic, and respiration was not impacted when treated at levels at least 20 times the IC_{50} for suppression of site IIIQo [2]. A S3QEL that was formulated for *in vivo* (i.p. administration) use (S3QEL941) had no impact on body weight or survival in Sod2-/- mice [21]. Feeding S3QELs (S3QEL1.2, S3QEL2.2, and S3QEL3) to flies and mice did not impact their food consumption [11].

Overall, the safety profile of S3QELs to date appears to be superior to other mitochondrial ROS targeting therapies, and several other antioxidant approaches [3]. S3QELs do not impact/impair respiration and can remain potent regardless of redox state because they are not consumed in redox reaction [4]. Importantly from a safety perspective, S3QELs are highly selective in only impacting ROS generated through the mitochondrial complex IIIQo leak site, thus important signaling ROS generated through other sites and processes are unaffected. The key outstanding question regarding safety stems from whether mitochondrial ROS generated by the IIIQo site participate in any essential processes, in certain cellular contexts.

Drug interactions: Potential interactions have not been established, but due to their potential effects on immune cell function, they may not be suitable for immunocompromised individuals.

Sources and dosing:

S3QELs are not approved for human use. Research grade S3QELs can be purchased from commercial suppliers.

Research underway:

S3QELs are currently in preclinical development, including the optimization of molecules suitable for *in vivo* use. Development for intestinal barrier disruption is primarily being done by Michael Brand at the Buck Institute for Research on Aging, who holds a patent for the use of S3QELs to protect against intestinal permeability. Much of this work has been supported by Calico.

Search terms:

Pubmed, Google: S3QELs

- Alzheimer's, neurodegeneration, aging, lifespan, cancer, diabetes, obesity, immunity

Websites visited for S3QELs:

- PubChem

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