Sulforaphane: from death rate heterogeneity in countries to candidate for prevention of severe COVID-19

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To the Editor,

Sulforaphane [1-isothiocyanato-4-(methylsulfinyl)butane] is a clinically relevant nutraceutical compound present in cruciferous vegetables (Brassicaceae). It is used for the prevention and treatment of chronic diseases and may be involved in ageing.[1]Along with other natural nutrients, sulforaphane has been suggested to have a therapeutic value for the treatment of the coronavirus disease 2019 (COVID-19).[2] Sulforaphane is an isothiocyanate stored in its inactive form glucoraphanin.[3] The enzyme myrosinase, found in plant tissue and in the gut microbiome, is involved in the conversion of glucoraphanin to its active form sulforaphane.[4]

Heterogeneity of COVID-19 death rates in countries and relation to diet

When comparing death rates, large differences exist between and within countries (Figure 1). Although many factors are involved in COVID-19 mortality,[5-11] a recent hypothesis has proposed that differences between countries may partly be explained by diet: the diet of low-death rate countries often includes certain vegetables that are potent anti-oxidants.[12] Two studies have examined the association between the COVID-19 mortality rate in EU countries and diet. Fermented vegetables, head cabbage and cucumber have been associated with lower death rates.[13,14] Even though these are ecological studies, they support the association between diet and fatality. A healthy diet, rich in antioxidants, has been proposed as a therapeutic strategy to mitigate the cytokine storm that developed in COVID-19.[15] In Asia and the Middle East, a diet high in fermented cruciferous vegetables is common, and these countries have a lower COVID-19 mortality rate. Fermentation food processing is known to increase the antioxidant activity of foods.[16]Certain compounds found in the diet can interact with Nrf2 (nuclear factor erythroid 2 p45-related factor 2), a transcription factor involved in the protection against oxidative stress.[17]

Insulin resistance and COVID-19

COVID-19 is more severe in elderly adults and/or patients with comorbidities, such as diabetes, obesity or hypertension, suggesting a role for insulin resistance.[18] Although differences exist between countries, the same risk factors for severity were found globally, suggesting common mechanisms. A strong relationship

between hyperglycemia, impaired insulin pathway, and cardiovascular disease in type 2 diabetes is linked to oxidative stress and inflammation.[19] Lipid metabolism has an important role to play in obesity, in diabetes and its multi-morbidities, and in ageing.[20] The increased severity of COVID-19 in diabetes, hypertension, obese or elderly individuals may be related to insulin resistance, with oxidative stress as a common pathway.[21]

ACE2 and COVID-19

The angiotensin-converting enzyme 2 (ACE2) receptor is part of the dual system - renin-angiotensin-system (RAS) - consisting of an ACE-Angiotensin-II-AT_1R axis and an ACE-2-Angiotensin-(1-7)-Mas axis. AT_1R is involved in most of the effects of Ang II, including oxidative stress generation,[22] which in turn upregulates AT_1R [23]. In metabolic disorders and with older age, there is an upregulation of the AT_1R axis leading to pro-inflammatory, pro-fibrotic effects in the respiratory system, and to insulin resistance.[24]SARS-CoV-2 binds to its receptor ACE2 and exploits it for entry into the cell. The ACE2 downregulation, as a result of SARS-Cov-2 binding, enhances the AT_1R axis. [25]

Nrf2, a central mechanism in insulin resistance, and severity of COVID-19

Nrf2 is a pleiotropic transcription factor at the centre of a complex regulatory network that protects against oxidative stress and the expression of a wide array of genes involved in immunity and inflammation, including antiviral actions.[26] Nrf2 may be involved in diseases associated with insulin-resistance.[27,28] Nrf2 activity declines with age, making the elderly more susceptible to oxidative stress-mediated diseases. Nrf2-activating compounds downregulate ACE2 mRNA expression in human liver-derived HepG2 cells,[29] and in genes encoding cytokines including IL-6 and many others specifically identified in the "cytokine storm" observed in fatal cases of COVID-19.

Natural compounds derived from plants, vegetables, fungi and micronutrients (e.g. curcumin, sulforaphane, resveratrol and vitamin D) can activate Nrf2.[30] ACE2 can inhibit NF-ϰB and activate Nrf2.[31]

Sulforaphane: the most potent natural Nrf2 activator

A key mechanism of action of sulforaphane involves activation of the Nrf2-Keap1 signalling pathway.[32] Sulforaphane is the most effective natural activator of the Nrf2 pathway, and Nrf2 expression and function is vital for sulforaphane-mediated action.[33,34] It has been proposed that SARS-CoV-2 downregulates ACE2 and that there is an increased insulin resistance associated with oxidative stress through the AT_1R pathway. Cruciferous vegetables release glucoraphanin, converted by the plant or by the gut microbiome, into sulforaphane which activates Nrf2 and subsequently reduces insulin intolerance (Figure 2).

Complementing herd immunity with a sulforaphane-based diet

Although nutritional epidemiology has been criticized on several fronts, including the inability to measure diet accurately, and for its reliance on observational studies to address etiologic questions[35], there seems to be sufficient evidence to raise some conclusions that will require appropriate testing.

Great hope has been placed on herd immunity, cross-immunity and vaccination to provide protection against COVID-19. Unfortunately, herd and cross-immunity may not be very effective, and the development of vaccines is a long process. Until vaccines become available, a simple solution would be to complement the diet with food that may have positive metabolic regulatory and anti-inflammatory effects. It is clear that the ecological studies available in Europe should be confirmed by global studies and by adequate epidemiologic studies. However, proposing a modification of diet before scientific evidence is available is not harmful since these foods are usually, but not always, well-tolerated. An alternative approach would be to evaluate the value of sulforaphane in clinical trials, as recently proposed (Text box 1).[2]

In conclusion, many factors may play a role in the difference of severity and death rates in COVID-19 in different parts of the world. These factors include trained immunity, cross reactivities with different viruses and other coronaviruses as well as many different dietary measures such as vitamin D levels, short-chain fatty acids, omega 3-rich nutrition. [36,37] Here, the authors propose an additional dietary factor, a

suphoraphane-based diet. Further research is required to identify the synergistic effects between all of these anti-inflammatory dietary factors, especially those acting on insulin resistance and Nrf2.

Text Box:

Future research areas:

- Mouse models of COVID-19 to investigate the effects of diet, sulforaphane and similar compounds.
- Cellular experiments for the *in-depth* investigation of sulforaphane.
- Case control studies.
- Investigation of synergistic effects with other dietary factors and vitamins such as short-chain fatty acids, omega 3, and vitamin D.
- Multiple omics metabolome analyses of the different stages of COVID-19 patients.
- Clinical trials with sulforaphane to prevent severe COVID-19.
- Clinical trials with sulforaphane to reduce the severity of COVID-19.

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Figure 1: COVID-19 deaths per million inhabitants (May 20, 2020)

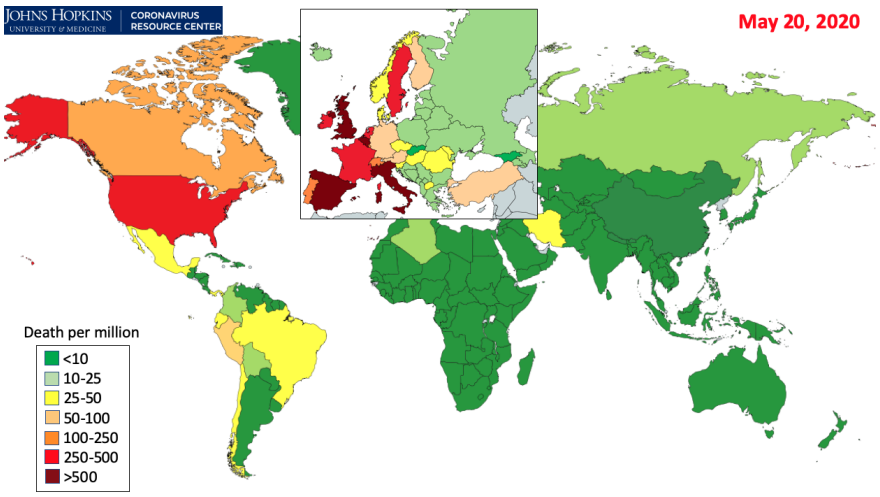

Figure 2: Putative mechanisms of *Brassica* (cruciferous) vegetables against COVID-19