Trehalose is not associated with the emergence and spread of epidemic *Clostridium difficile* strains

The science around nutrition and food often requires an expert to interpret.
Unfortunately, we often see the media provide alarmist reporting on the latest research around food in attempts to gain attention and share news without confirming proper interpretation of scientific evidence first.

It is often difficult to tell what is accurate in the media when an entire body of evidence is not reviewed. For example, you may have seen some criticism of trehalose in recent media reporting based on a recent paper by Collins *et al.* (published in Nature), suggesting that trehalose is responsible for the spread of *Clostridium difficile*.

It is important to know that because of this paper, the media generated a significant amount of unnecessary fear around trehalose, sensationalizing the small-scale animal study. However, when you have an expert in the field compare the design and limitations of this study in a recent publication in EBioMedicine, you will see that these limitations not only put in to question, but actually disprove the researchers proposition that trehalose intake played a role in the emergence of epidemic and hypervirulent strains of *C. diff*.

We are going to outline limitations of this research and discuss why consumers need not fear trehalose – a commonly consumed disaccharide, both natural and synthetic, in our diet.

■ Overview

In a recent publication covered by the media, Collins *et al.* reported that a rise in trehalose consumption could explain increased rates of mortality from *Clostridium difficile* infections. Scientists were skeptical of the claims, and sought to clarify this important scientific question: is trehalose the cause of increasing *C. diff* virulence? A new publication led by Professor Mark Wilcox (MD) from the University of Leeds/Leeds Teaching Hospitals aimed to fill in the gaps of the previous research paper, and to test their claims under more robust settings. Prof. Wilcox's study in fact showed that trehalose is not responsible for increased virulent strains of *C. diff*. Prof. Wilcox presented his findings at the 2019 Trehalose

Symposium in Tokyo, Japan to a group of industry leaders and researchers interested in and working with trehalose, explaining crucial gaps in the Collins *et al.* paper, such as inaccurate conclusions about the correlation between trehalose consumption and epidemic strains of *C. diff*, whether bacteria with variant metabolism of trehalose are only commonly found in virulent strains, and if in fact, trehalose impacts *C. diff* growth or toxin production in a human gut model.

Key takeaways from Prof. Wilcox's speech at the Trehalose Symposium

- Trehalose is a naturally occurring disaccharide that is readily digested in our guts. A very small portion of the population may have impaired digestion of trehalose, but this is very uncommon.
- Many strains of *C. diff* carry the genetic mutation to metabolize trehalose, not just epidemic strains as claimed by Collins *et al.*
- A strain of *C. diff* that has the ability to metabolize trehalose does not result in more deaths than a strain of *C. diff* that cannot metabolize trehalose.
- There is no correlation between the importation of trehalose and the rate of epidemic strains of *C. diff*.
- Trehalose does not stimulate *C. diff* growth in an infected human gut model. In fact, supplementation with trehalose in an infected human model led to a reduced detection of *C. diff* toxin to undetectable levels.

■ What is trehalose?

Trehalose is a naturally occurring disaccharide made up of two glucose molecules. It occurs naturally in foods such as mushrooms, honey, shrimp, yeast, and soybeans. Because of its unique culinary properties, it is also a highly desirable disaccharide used in a variety of food service applications to enhance freshness and flavor.

Until the discovery of mass production in Japan, it was very difficult to produce trehalose. With the ability to produce this desirable sugar, it is now exported to a variety of different countries and used in many culinary applications.

■ Digestion of trehalose

In humans, trehalose is a highly digestible disaccharide – meaning it is broken down to 2 glucose molecules and absorbed into the blood stream, not reaching the colon. A very small portion of the

population (~8% of Greenlandic individuals) may carry a genetic abnormality that prevents digestion of trehalose and results in symptoms similar to lactose intolerance.

- **What is *Clostridium difficile (C. diff)?***

Clostridium difficile causes a type of hospital-acquired infection that is characterized by diarrhea and inflammation of the colon. This can pose serious consequences for patients, including prolonged hospitalization and increased mortality. *C. diff* infections are on the rise in some countries - both the number and severity of cases and can often be difficult to manage.
The change in the epidemiology of *C. diff* infection is in part due to emergence of certain strains such as ribotype-027 and 078, which have been associated with increases in morbidity and mortality. With the rise in *C. diff* virulence, researchers are working quickly to understand the cause and find solutions to this rapidly evolving disease.

- **Did trehalose play a significant role in the emergence of epidemic strains of *C. diff*?**

Collins *et al.* research claims that trehalose imports have played a significant role in the emergence of epidemic strains of *C. diff.* However, upon careful analysis, several concerning gaps in the research are identified by Wilcox *et al*. that actually disprove this theory.

We are going to outline limitations of this research and discuss why consumers need not fear trehalose, a commonly consumed disaccharide, both natural and added, in our diet.

- **Do certain strains of *C. diff* metabolize trehalose more effectively?**

Certain strains of *C. diff* carry a genetic mutation that allow them to, as Prof. Wilcox describes, "more effectively metabolize trehalose; breaking trehalose into glucose, and using the glucose as a growth food substance. And that if it can do that, the *C. diff* strain will be able to outcompete potentially, other *C. diff* strains and maybe other bacteria if we find *C. diff* in the human gut."

On this hypothesis, Prof. Wilcox and his team set out to determine if this mutation was common only to the epidemic strains, conferring a selective advantage as Collins *et al.* claimed, by reviewing the genetic code of over 10,000 strains of *C. diff* for the trehalose metabolizing variant/mutation.

Describing his findings, Prof. Wilcox states: "we see that actually the trehalose mutations, the gene mutations, are very common. They are spread through lots of different types of *C. diff*, not just epidemic types. And, because of the way they are distributed, we conclude that these mutations are ancient; very old mutations that happened hundreds or thousands of years ago. So the original claims that were made by Collins *et al.* about these mutations being specific to epidemic *C. diff* strains - 027, 078, 017 - are not

true."

This is important because it calls to question the claim that these genetic mutations are the driving force in the *C. diff* ribotype-027, 078, 017 epidemics, which leads up to the important question - does the ability of certain strains of *C. diff* to metabolize trehalose make a difference to patient outcomes?

Table1. Relationship between trehalose four-gene cluster presence and 30-day mortality from ribotype-15 (ST10/ST44) *C.difficile* infection. There was no evidence for a non-linear relationship between mortality and age using multiple fractional polynomials (p=0.80). There were no significant interactions (all p>0.05).

Factor	Alive, n (%) / median (IQR)	Died, n (%) / median (IQR)	Univariable OR (95% CI)	*p* value	Multivariable OR (95% CI)	*p* value
n	181	27				
Age, years OR per 10-year increase	73 (59-82)	84 (69-88)	1.42 (1.06-1.90)	0.02	1.45 (1.08-1.34)	0.01
Sex						
Female	110 (88%)	15 (12%)				
Male	71 (86%)	12 (14%)	1.23 (0.55-2.80)	0.61	1.27 (0.55-2.95)	0.58
ST						
ST44	103 (88%)	14 (12%)				
ST10	78 (86%)	13 (14%)	1.23 (0.55-2.76)	0.62	0.55 (0.15-2.00)	0.36
Four-gene cluster						
Absent	93 (85%)	17 (15%)				
Present	88 (90%)	10 (10%)	0.62 (0.27-1.43)	0.26	0.36 (0.09-1.34)	0.13

To see whether this genetic mutation is a causal factor in increased mortality, Wilcox *et al.* utilized *C. diff* ribotype-015; some of the strains in this ribotype carry the metabolic mutation for trehalose metabolism and some do not. This is unlike ribotype-027 strains, which all appear to have the mutation. This allowed researchers to test if those infected with a trehalose metabolizing strain of *C. diff* had worse outcomes than those infected with a non-metabolizing strain. Prof. Wilcox and his team found that "there is no association between possession of this trehalose mutant gene metabolism, four-gene cluster, and death, likelihood of death, in humans infected with these strains. The mutation does not appear to be responsible for increased risk of death in human infection."

Key takeaway: Many strains of *C. diff* carry the genetic mutation to metabolize trehalose, not just epidemic strains. A strain of *C. diff* that has the ability to metabolize trehalose does not result in more deaths than a strain of *C. diff* that cannot metabolize trehalose.

- **Does trehalose importation correlate with the incidence of *C. diff* infection?**

Interestingly, Collins *et al.* suggests correlation between the increasing importation of synthetic trehalose with a rise in *C. diff* infection, claiming that "the widespread adoption and use of trehalose in the diet coincides with the emergence of both ribotype-027 and 078 outbreaks". It is important to know that correlation does not equal causation, and in fact, when you look closer at trehalose importation and virulent *C. diff* out breaks, they do not correlate at all.

Collins *et al*. estimate that trehalose intake from naturally occurring sources is approximately 100 grams per capita annually. Between 2000 and 2006, imported levels of trehalose were <1 gram per capita per year in the USA and England, not substantially increasing trehalose intake.

He points out that while both strains ribotype-027 and 078 existed prior to 2001, that epidemic outbreaks did not occur until 2003, and include major outbreaks in both Quebec, Canada, and Pittsburgh, USA. He goes on to suggest a correlation of these major outbreaks with trehalose imports and use.

However, synthetic trehalose was not approved for use in Canada until 2005, and imports to the US did not start increasing until 2007, showing, as Prof. Wilcox described, that it's "not possible to claim that importation of trehalose was associated with the epidemic of ribotype-027." Trehalose imports postdate the start of the 2003 epidemic by 3 to 4 years and amounts of added trehalose per capita are extremely low compared with naturally occurring intakes of trehalose; this shows that there is no correlation between trehalose imports and the emergence of ribotype-027 and 078 outbreaks.

Key takeaway: There is no correlation with importation of trehalose and correlation of epidemic strains of *C. diff.* Increasing intake of added trehalose postdate the initial epidemics in question.

- **Does trehalose stimulate *C. diff* growth in a human model?**

Lastly, Prof. Wilcox and his team set out to describe how *C. diff* would grow in a human gut model (a

triple phase chemostat gut model) when trehalose is administered (as a potential food for the bacteria) in comparison to controls. Using a model that has been proven to simulate the human intestine and gut microbiota, the team infected several of these with *C. diff* and administered different substrates, including trehalose, glucose, or saline, to test this theory.

Interestingly, in a human model, trehalose suppressed the production of toxins, when compared with both the glucose and saline model, demonstrating that trehalose consumption is not associated with increased *C. diff* virulence in a human model.

As Prof. Wilcox explains:

"If trehalose is meant to be a stimulant, an inducer of infection in humans, as claimed in the Nature publication, why do we see no toxin production at all? Our conclusion again is that the data in the Nature publication are not accurate and the claims are not true about trehalose and virulent *C. diff.* The earlier experiments were done in mice, whereas ours were carried out in a model that simulates human *C. diff* infection. Notably, we have known for many years that *C. diff* infection, whether it occurs or not, is specific to the animal type."

Collins *et al.* state "the ability to metabolize trehalose at lower concentrations confers a competitive growth advantage in the presence of a complex intestinal community", however this was demonstrated in an animal model, which was not repeatable in a proven human model. In fact, supplementation with trehalose led to a reduced detection of *C. diff* toxin to undetectable levels, meaning that trehalose does not appear stimulate *C. diff* growth in humans.

> ***Key takeaway:*** Trehalose does not stimulate *C. diff* growth in an infected human model. In fact, supplementation with trehalose in an infected human model suppressed production of *C. diff* toxin to undetectable levels.

■ Summary

Through Prof. Wilcox's research and presentation at the Trehalose Symposium, it is clear that trehalose is not associated with the rise in epidemic strains of *C. diff*. Not only was Prof. Wilcox able to demonstrate that strains with a genetic mutation to metabolize trehalose have no impact on morbidity and mortality of *C. diff,* he was also able to demonstrate that trehalose had no bearing in a human model of *C. diff* infection.

In summary, we can say with confidence that there is no relationship between the importation and consumption of trehalose in humans and the emergence of epidemic strains of *C. diff*.

Acknowledgements
We are grateful for Professor Mark H. Wilcox for the support and proofreading.
We are grateful for Andrea Hardy, RD for preparation and suggestions.

The contents are prepared with the support of Andrea Hardy, Registered Dietitian in Calgary, Canada, in response to the following articles:

- Nature 553, 285-286 (2018) DOI: 10.1038/d41586-017-08775-4
- EBioMedicine 43 (2019) 347–355 DOI: https://doi.org/10.1016/j.ebiom.2019.04.038

with the information based on the speech of Prof. Mark H. Wilcox at The 23rd Trehalose Symposium held in Tokyo on 7th November, 2019.

For further information, please contact us at the below contact details.

Nagase Viita Co., Ltd.
Nihon-Seimei Okayama Bldg. II Shinkan
1-1-3 Shimoishii, Kita-ku, Okayama 700-0907, Japan
NVI800017@nagase.co.jp