DIABETES CARE AND EDUCATION PRACTICE GROUP AND SPONSORED BY: THE CALORIE CONTROL COUNCIL (CCC) AND THE INTERNATIONAL FOOD ADDITIVES COUNCIL (IFAC) PRESENTS:

“Low-Calorie Sweeteners, Food Additives and the Microbiome: What We Know Now”

Thank you for joining us.
Please remember to Mute your sound. Thank You.
Our program will begin shortly.
Special Thank You to

DCE Leaders
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  ➢ DCE Industry Chair
➢ Constance Brown-Riggs, MSEd, RD, CDE, DCN  
  ➢ DCE Chair
➢ Paula Kellogg Leibovitz, MS, RDN, CDE, CDN  
  ➢ Chair-Elect
➢ Anna Parker, DCN, MS, RD, CDE  
  ➢ Professional Development

Workgroup Members
➢ Andrea Hebert MS, RD, LD, CDE
➢ Sarah Williams, RD, LD, CDE
➢ Adam Reppert, RD, CDE

Contact us:  DCEwebinars@gmail.com
PARTICIPATION INFORMATION

- Handouts of the presentation will be sent out before the presentation or on the day or 2-3 days after the presentation
- A recording of the webinar will be available on the DCE website after the webinar.
Next DCE Webinar

“Hope Warshaw and Kirstie Canene-Adams, will discuss Allulose, its benefits and the science that supports the safety and efficacy of the sweetening ingredient, and provide tools to assist diabetes educators."

*Watch for more details in DCE Updates*
Diet and the Human Gastrointestinal Microbiota

Hannah D. Holscher, PhD, RD
Assistant Professor of Nutrition
Department of Food Science and Human Nutrition
Division of Nutritional Sciences
Institute of Genomic Biology
National Center for Supercomputing Applications
University of Illinois

Nutrition & Human Microbiome Laboratory
# Speaker Disclosure

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Learning Objectives

1. Recognize complexities of studying how diet affects the gastrointestinal (GI) microbiota.

2. Identify dietary components that escape digestion and differentially affect GI microbiota.

3. Discuss the importance of digestion, treatment doses, and the study designs in microbiota trials.
Learning Objectives

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**Diet: Digestion, Dose, and (study) Design**
Definitions and Overview

**Microbiome** - a collection of microbial genomes

**Microbiota** – a collection of microbes

- As many bacteria as host cells in human body\(^1\)
- > 150x more bacterial genes than our human genome\(^2\)

Diet, GI Physiology, & Microbiota

The microbiota varies throughout GI tract

Colon
$10^{10} - 10^{12}$ CFU/mL
- Bacteroides
- Prevotella
- Faecalibacterium
- Ruminococcus
- Roseburia
- Clostridium
- Bifidobacteria
- Collinsella
- Desulfovibrio
- Bilophila
- Akkermansia
- Methanobrevibacter

Stomach & Duodenum
$10^1 - 10^2$ CFU/mL
- Helicobacter
- Streptococcus

Jejunum & Ileum
$10^4 - 10^8$ CFU/mL
- Bacteroides
- Streptococcus
- Lactobacillius
- Bifidobacteria
- Fusobacteria
GI Microbiota Metabolic Functions

- Ferment nondigested food
  - Dietary fiber
  - Resistant starch
  - Protein

- Synthesize secondary bile acids

- Synthesize vitamins
  - B vitamins
  - Vitamin K

The composition of the diet impacts digestion, absorption, and transit time.

Humans digest and absorb the majority of nutrients in the diet.

Diet provides a source of nutrients for us and the GI microbiota.
**Digestion:** diet affect GI secretions & transit time

- **Stomach:**
  - Acidic pH
  - Oxygen

- **Duodenum:**
  - Neutral pH
  - Rapid Transit
  - Bile Acids

- **Jejunum & Ileum:**
  - Bile Acids
  - Reduced Oxygen
  - Mucin Layer

- **Colon:**
  - Neutral pH
  - Slow Transit
  - Minimal Bile Acids
  - Anaerobic
  - Thick Mucin Layer

- **Colon & Intestines:**
  - Digestion: diet affect GI secretions & transit time

**Diet, GI Physiology, & Microbiota**
Diet, GI Physiology, & Microbiota

We **digest** and absorb most nutrients

*Stomach*
- Protein

*Duodenum*
- Carbohydrates
- Protein
- Fats

*Jejunum & Ileum*
- Carbohydrate
- Protein
- Fats

*Colon*
- Resistant Starch
- Fiber

~ 40 g dietary carbohydrates, 12-18 g protein, & 5 g fat

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Gut microbes metabolize nondigested dietary substrates.
Microbes Ferment Carbohydrates, Fibers, & Resistant Starch

Dietary Fiber

Resistant Starch

Acetate, Propionate, Butyrate
(Short-chain fatty acids)

Microbes Ferment Proteins

Diet affects GI microbiota composition

Colon
$10^{10} - 10^{12}$ CFU/mL
Bacteroides
Prevotella
Facaelibacterium
Ruminococcus
Roseburia
Clostridium
Bifidobacteria
Collinsella
Desulfovibrio
Bilophila
Aktermansia
Methanobrevibacter

Stomach & Duodenum
$10^1 - 10^2$ CFU/mL
Helicobacter
Streptococcus

Jejunum & Ileum
$10^4 - 10^8$ CFU/mL
Bacteroides
Streptococcus
Lactobacillus
Bifidobacteria
Fusobacteria

Diet, GI Physiology, & Microbiota
A Deeper Dive Into Diet & Microbiota
Diet Impacts GI Microbiota

- **Habitual diet** is related to the composition of the GI microbiota.¹

- **Acute changes** in macronutrient composition can rapidly (within 2-4 days) change the composition and function of gut microbes.²

- Individuals that consume **more plants** have greater GI bacterial diversity.³

- **Dietary fiber and prebiotic** intake differentially impacts GI microbiota composition and function.⁴

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- **Dietary fiber and prebiotic** intake differentially impacts GI microbiota composition and function.⁴

Diet rapidly impacts GI microbiota

- Cross-over design: *ad libitum* 5-day consumption of diets composed entirely of animal or plant products
- 6 male + 4 females; 21-33 yr, BMI 19-32 kg/m²

**Diet Composition**

**Fiber:**
- Plant Based: 25 g per 1000 kcal
- Animal Based: 0 g per 1000 kcal

**Fat:**
- Plant: 20% of kcal
- Animal: 70% of kcal

**Protein:**
- Plant: 10% of kcal
- Animal: 30% of kcal

Diet rapidly impacts GI microbiota

Plant-based: increased short-chain fatty acids, acetate and butyrate.
Animal-based: increased branch-chain fatty acids, isovalerate and isobutyrate.

Bile acids tended to increase on animal-based diet.

Diet rapidly impacts GI microbiota

- **Plant-based**: increased short-chain fatty acids, acetate and butyrate

- **Animal-based**: increased branch-chain fatty acids, isovalerate and isobutyrate

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Diet Impacts GI Microbiota

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Diet & GI Microbiota

- Cross-sectional analysis of > 10,000 fecal samples from participants in the US, UK, and Australia
- Individuals completed health status and dietary questionnaires

Plants & Microbiota Diversity

**INDIVIDUALS THAT CONSUMED MORE PLANTS HAD GREATER GI BACTERIAL DIVERSITY**

Observed molecular features vs. bootstrap depth for individuals who consumed more plants per week compared to those who consumed less than 10 or more than 30 plants per week.
Diet & GI Microbiota

Image: U.S. Department of Agriculture myPlate
Diet & GI Microbiota

- Wheat
- Rye
- Oats
- Barley

- Agave
- Avocado
- Banana

- Broccoli
- Potatoes
- Onions
- Celery

- Legumes
- Nuts

Image: U.S. Department of Agriculture myPlate
Diet & GI Microbiota

Fructans
Pectins
Resistant Starch

B-glucan
Resistant Starch
Pectins
Hemicellulose
Cellulose

Hemicellulose
Cellulose

Image: U.S. Department of Agriculture myPlate
Human vs. Microbial Enzymes

**Amylose**: $\alpha$-1,4 glucosidic bonds

**Cellulose**: $\beta$-1,4 glucosidic bonds

**$\beta$-Glucan**: mixed $\beta$-1,3 and $\beta$-1,4 glucosidic bonds

Image adapted from Linus Pauling Institute, OSU
Design: Feeding trials allow for the assessment of the effect of diet on the GI microbiota
**Inulin Type Fibers**

**Plant Sources**
- Wheat
- Bananas
- Garlic
- Onion
- Agave
- Chicory root

**Food Sources**
- Bars
- Cereals
- Yogurt
- Ice cream

---

Inulin Type Fibers

- **Structures**
  - Fructose polymer linked by $\beta$-2,1 linkages
  - Varying degrees of polymerization (2-60)
  - Fructooligosaccharides (FOS) → Inulin

**Study Design**: Agave Inulin

- Randomized, double-blind, placebo-controlled crossover trial with three 21-day treatment periods
- Healthy adults (n=30)
- Daily food and GI tolerance records
- 3 fecal specimens were collected on days 16 – 20
- **Dose**: 5 & 7.5 g/d

Agave Inulin dose dependently increased *Bifidobacterium*

**Graph**: 
- **X-axis**: Treatment (Agave inulin g/day) 
- **Y-axis**: Bifidobacterium (% of sequences) 
- **Legend**: 
  - **Orange bars** represent 7.5 g/day 
  - **Light pink bars** represent 5.0 g/day 
  - **Dark blue bars** represent 0 g/day 

**Notes**: 
Positive relationship between treatment dose and *Bifidobacterium*

\[ r = 0.43, p < 0.01 \]

Responders vs. Non-responders

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**LEGEND (\% change)**
- 0 to 0.9% increase
- 1.0 to 4.9% increase
- 5.0 to 9.9% increase
- 10 to 14.9% increase
- >15% increase
- 0 to 0.9% decrease
- 1 to 5% decrease

Diet & GI Microbiota

Well designed dietary trials with adequate doses of non-digested nutrients provide evidence that diet impacts the GI microbiota.

### Key Takeaways

1. **Diet** impacts the human GI microbiota.

2. Consider the **digestibility** of dietary component.

3. Critically assess the study **designs** and treatment **doses**.
Dietary exposures to emulsifiers & LCS and their impact on the gut microbiota: Is there a concern?

Ashley Roberts, PhD
President
AR Toxicology Inc
Disclosure

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<td>Preparation of publication</td>
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<td>Scientific &amp; regulatory consulting on Steviol glycosides and</td>
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AGENDA

• The role of emulsifiers and LCS in the diet
• Why the concern regarding food ingredients and the microbiome?
• Effects reported only within in vitro and animal feeding studies
• Limitations in study design (focusing on both emulsifiers and LCS)
• Review of clinical studies
• What is the potential for interaction with the microbiome?
• Summary & conclusions
The Role of Emulsifiers And LCS in The Diet

- Emulsifiers and low and no sweeteners are commonly used food additives for the technological purpose of altering the sweetness/flavor or to improve the texture of foods.

- Commonly used emulsifiers include carboxymethylcellulose (CMC), Polysorbate 80, arabinogalactan, gum arabic and carrageenan.

- Food uses include; ice cream, margarine, breads, cakes, salad dressings, pasta, jam etc.

- Commonly used sweeteners include Acesulfame K, Aspartame, Saccharin, steviol glycosides and sucralose.

- Due to their ubiquity they are consumed daily, although at low levels in the diet.
Why The Concern?

- It has been postulated that both LCS and emulsifiers alter the gut microbiota leading to metabolic disease (glucose intolerance).
- A high-profile article published in a premier journal concluded that “artificial sweeteners alter the gut microbiota”.
- Likewise Nature also has linked Emulsifiers with impacting metabolic syndrome.

Both Nature articles resulted in immediate widespread negative media attention.

However, does the Science really support these accusations?
Animal Studies: Reporting on Effects of Emulsifiers & LCS on the Gut Microbiome
Animal Studies Investigating The Effects of Emulsifier Consumption on The Gut Microbiota

Concerns regarding impact on the gut microbiota is solely related to animal and in vitro studies conducted with carboxymethylcellulose (CMC) polysorbate 80, gum arabic, carrageenan and arabinogalactan

**Arabinogalactan**

An *in vitro* study model impacted the relative abundance of various bacterial groups in the microbiome

**Carrageenan**

Was shown to impact the abundance of bacteria and the *Bacteroides/Firmicutes* ratio and the abundance of *Akkermansia muciniphila*. Which was considered to be a potent anti-inflammatory commensal bacterium in the gut

**Gum Arabic**

1 healthy human female showed an impact on anaerobic viable count between start and end of study.

**Polysorbate 80 (P80)**

The effect of P80 on composition of the gut microbiome was investigated in several studies. The results provided varying outcomes but in general showed an impact on the abundance of various bacteria resulting in perceived promotion of metabolic syndrome.

**Carboxymethylcellulose (CMC)**

The effect of CMC on composition of the gut microbiome was investigated using the same experimental systems as P80 above. The authors observed similar effects in the CMC studies as those observed following treatment with P80.
Previous Animal Studies Indicate a Lack of Association Between Emulsifier Consumption & Changes in The Gut Microbiota

The JECFA (FAO/WHO) have reviewed the safety of certain emulsifiers in accordance with standard regulatory guidelines.

**Carboxymethylcellulose**
- JECFA assigned an ADI of “not specified” thus indicating no safety concerns of CMC on glycemic response or gut microbiota:

**Polysorbate 80**
- JECFA established an ADI of 0-25 mg/kg bw/day for P80 based upon all available animal toxicity data

**Carrageenan**
- Likewise the JECFA has established an ADI of “not specified” for carrageenan

There were no significant adverse effects on gastrointestinal or animal health
Animal Studies Investigating The Effects of LCS Consumption on The Gut Microbiota

- Concerns regarding impact on the gut microbiota primarily related to studies conducted in animals (rats, mice and piglets)

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<th>Sweetener</th>
<th>Study (details)</th>
<th>Microbiome-associated changes</th>
<th>Study Confounders</th>
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<td>Aspartame</td>
<td>Palmnäs et al., 2014</td>
<td>Normal rats: ↑ fecal C. leptum</td>
<td>Food, water intake was not comparable between aspartame and control</td>
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<tr>
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<td>(Normal rats, 7 mg/kg/d; obese rats, 5 mg/kg/d; 8 weeks Water Control)</td>
<td>Obese rats: ↑ fecal total bacteria, Bifidobacterium spp., C. leptum, Enterobacteriaceae, Roseburia spp.</td>
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<td>Cyclamate</td>
<td>Matsui et al., 1976</td>
<td>Compared 1 cyclohexylamine “converter” to 3 controls</td>
<td>Small sample size</td>
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<td>(Monkey, 250 mg/kg/d; 30 days)</td>
<td>No change in total fecal bacteria and several bacterial families and genera</td>
<td>Control and cyclamate exposed monkeys were not the same species</td>
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<td>Saccharin</td>
<td>Anderson &amp; Kirkland, 1980</td>
<td>↑ number of aerobes in cecum</td>
<td>Dose in excess of ADI (~2000x)</td>
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<td>(Rats, 10 – 14 g/kg/d, 10 days)</td>
<td>↓ cecal anaerobe:aerobe ratio</td>
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<td>Daly et al., 2014; 2016</td>
<td>↑ fecal Lactobacillus OTU4228 [NHDC component responsible]</td>
<td>Food intake not reported</td>
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<td>(Piglets, 0.015% SUCRAM, 2 weeks)</td>
<td>↓ fecal Veillonellaceae, Ruminococcaceae</td>
<td>Saccharin dose unknown</td>
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<td>Sucrose (SUCRAM)</td>
<td>Daly et al., 2014; 2016</td>
<td>↑ diversity fecal Lactobacilli (high dose only)</td>
<td>High dose in excess of ADI (~10x)</td>
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<td>Li et al., 2014</td>
<td>(Mice, 5.5 or 139 mg/kg/d, 4 weeks)</td>
<td>Food consumption not reported</td>
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ENHANCED GLYCEMIC RESPONSE REPORTED IN LCS-TREATED MICE

Experiment 1

- Suez et al. (2014)
- Control groups: Water, sucrose or glucose
- LCS groups: Commercial formulations of aspartame, saccharin or sucralose
- N = 20 mice per group
- ROA = Drinking water
- 11 weeks

Reproduced from Suez et al., 2014; Figure 1a & b.
LCS Doses Were at Least 30-fold Higher Than The ADI

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<th>Approx liquid intake/day (mL)</th>
<th>Daily exposure (mg/kg/day)</th>
<th>ADI (mg/kg/day)</th>
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<tbody>
<tr>
<td>Water</td>
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<td>Aspartame</td>
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<td>Saccharin</td>
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<td>Sucralose</td>
<td>10</td>
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Limitations in Animal Study Design When Assessing The Affects on The Gut Microbiota

- Human dietary relevance is limited because:
  - Studies utilize emulsifier & sweetener doses that are significantly greater than the ADI
  - For emulsifier studies the test doses were upwards of 2 orders of magnitude greater than the EDI
- Generally small numbers of animals evaluated
- Lack of isocaloric control groups to account for differences in caloric intake between sweetener and control groups
  - Food consumption and body weight monitoring essential
- For LCS studies conducted with the commercial product which contains low levels of the sweetener and high levels of a carrier agent (e.g. Maltodextrin)
- Subtle changes in gut microbiota composition as a toxicological end point is not supported by previous safety conclusions of International food safety authorities
- EFSA recently indicated within their draft protocol for the assessment of hazard identification and characterization of sweeteners that they would only evaluate human microbiome studies (calls into question the usefulness of high dose animal studies)
Clinical Studies:

- No randomized clinical studies currently exist looking at the impact of emulsifiers on the gut microbiome
- Limited information regarding LCS and the human gut microbiota
Suez et al., 2014:
• 381 non-diabetic individuals participating in clinical nutrition study
• Completed food frequency questionnaire
• Subjects were divided into 2 groups:
  • High LCS/NAS consumers (40 subjects)
  • Non-LCS/NAS consumers (236 subjects)
• Results: “significant positive correlations between LCS consumption and several metabolic syndrome-related clinical parameters”

Concerns:
• Observational study – cause and effect cannot be assumed
• No information on types of LCS consumed, amount, or other components of diet
• No criteria provided to determine “High” LCS consumption → 105 subjects were arbitrarily excluded from the study
• Inappropriate statistical analysis of glycosylated hemoglobin levels
Small Clinical Study With Saccharin Inconclusive

**Suez et al., 2014**

- Saccharin, 5 mg/kg/day, 1 week (no control)
- Subjects (N=7) did not normally consume LCS
- Fecal samples collected daily for 16S rRNA analysis
  - OGTTs conducted daily (used to classify subjects as “non-responders” or “responders”)

**Concerns:**

- Number of study subjects too small
- Already a difference on baseline Day 1
- Confounding factors not controlled for – no control group, diet not controlled
- “Responders” and “Non-Responders” analysis not scientifically justified.
- Data provides no meaningful response overall.
Impact of Sucralose on Metabolic Control And The Gut Microbiome in Healthy Adults

Thomson et al. 2019

• First Prospective clinical study looking at dosages within the ADI on glycemic and insulinemic responses in a double blind placebo controlled study
• Sucralose 780mg/day (approx. 13 mg/kg bw/day) for 7 days
• Subjects N=17 per group
• Glucose and insulin response assesses following an OGTT (75g)
• Gut Microbiome evaluated using 16S rRNA sequencing

Reproduced from Thomson et al. 2019; Figures 1 & 2

• Consumption of high doses of sucralose for 7 days does not alter glycemic control or insulin resistance or the gut microbiome in healthy adults
Previous Clinical Data Indicate a Lack of Association Between LCS Consumption & Changes in The Gut Microbiota

**Saccharin**
- Clinical studies in diabetic and non-diabetic patients have not observed any effects of saccharin on glycemic response or gut microbiota:
  - Diabetic patients consuming up to 4.8g/day for 5 months were not observed to have any adverse effects

**Aspartame**
- Numerous clinical studies demonstrate that chronic consumption does not affect glycemic response in diabetics

**Sucralose**
- Consistent clinical evidence shows that chronic administration does not affect glycemic response in both diabetics and non-diabetics
- Consumption of 1 g/day for 12 weeks had no effect on glucose response
Similarity in Emulsifier Structures Lead to Similarity in Digestion/Hydrolysis

• Food emulsifiers are complex carbohydrates that are not digested by endogenous enzymes along the human gastrointestinal tract
• Digestion is reliant on the commensal bacteria present in the large intestine
• Potential therefore exists for emulsifiers to modulate the gut microbiome
  • However not considered likely at the daily low dietary levels of exposure.
## Differences in Molecular Structures Lead to Differences in Metabolism and Absorption

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>ADME</th>
<th>Sucrose sweetness equivalence</th>
<th>ADI (mg/kg bw/d)</th>
<th>Max daily mg intake based on 70kg person</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame K</td>
<td>Not metabolized. Rapidly absorbed. Excreted unchanged in urine.</td>
<td>200 x</td>
<td>15</td>
<td>1050</td>
</tr>
<tr>
<td>Aspartame</td>
<td>Rapidly metabolized to amino acids &amp; methanol that are absorbed in small intestine.</td>
<td>200 x</td>
<td>40</td>
<td>2800</td>
</tr>
<tr>
<td>Saccharin</td>
<td>Not metabolized. Rapidly absorbed. Excreted unchanged in urine.</td>
<td>400 x</td>
<td>5</td>
<td>350</td>
</tr>
<tr>
<td>Sucralose</td>
<td>Not metabolized. Poorly absorbed. Excreted unchanged in feces.</td>
<td>600 x</td>
<td>15</td>
<td>1050</td>
</tr>
</tbody>
</table>
Summary & Conclusions

• The majority of effects on the microbiome for emulsifiers and LCS have been reported in animal studies at high dietary exposure levels (not relevant to the human situation)

• The studies currently present in the scientific literature provide no significant evidence that either emulsifiers or LCS alter the gut microbiota in humans at currently permitted human intake levels

• Limitations in the experimental designs of the animal studies and selectivity in both the reporting and analysis of results call into doubt the results and conclusions from these experiments

• Consistent clinical evidence continues to support the safety of these food ingredients for their intended uses

• The safety of emulsifiers is supported through animal toxicity studies conducted in accordance with standard regulatory guidelines

• Numerous clinical trials and long-term animal studies show no effect of permitted LCS on blood glucose
Summary & Conclusions

- Differences in structure/absorption/metabolism and excretion means these different sweeteners cannot be grouped together for effects on the microbiome.

- No randomized control clinical trials have been conducted with emulsifiers to ascertain if the effects noted in animal studies at high dosages are extrapolatable to humans.

- No adverse health effects (metabolic syndrome) mediated by gut microflora changes can be assumed based upon the currently available data.

- No evidence to suggest that emulsifiers or LCS as a group or individually pose any safety concerns at currently approved levels, which is a viewpoint endorsed by all the major International regulatory authorities.
QUESTIONS!!! THANK YOU!

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