Gut microbiota and short-chain fatty acids profiles of normal and overweight school children in Selangor after probiotics administration

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Outline

• Introduction
• The study
• Discussion and Conclusion
• Future Research
Introduction

• Obesity is a major public health problem worldwide.
  • a key concern because it is linked to various health complications such as type 2 diabetes (Reilly et al., 2003).
• Prevalence of obesity among children in Malaysia (less than 18 years old) is 11.9% (Institute for Public Health, 2015).
• Apart from environmental and lifestyle factors, gut microbiota affects nutrient absorption and energy regulation.
Gut microbiota

- There are trillions of microorganisms in the gut
  - thousands of bacterial species with specific functions
  - *Firmicutes, Bacteroidetes, Actinobacteria* are the most common phyla

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**Table 1. Microbiota functions.**

<table>
<thead>
<tr>
<th>Function</th>
<th>Brief Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolite production</td>
<td>The fermentation of complex carbohydrates results in the production of short-chain fatty acids (SCFAs), which are involved in many cellular processes and metabolic pathways, in the enhancement of the gut barrier function and in the regulation of immune system and inflammatory responses.</td>
</tr>
<tr>
<td>Vitamin production</td>
<td>Microbiota synthesize essential vitamins that humans cannot produce (e.g., vitamin B12, vitamin K); a dysregulation results in metabolic pathologies such as obesity and type 2 diabetes mellitus.</td>
</tr>
<tr>
<td>Influence on epithelial homeostasis</td>
<td>Microbiota promote epithelial integrity by influencing the turnover of epithelial cells and modulating mucus properties.</td>
</tr>
<tr>
<td>Development of the immune system</td>
<td>Both intestinal mucosal defenses and the systemic immune system are modulated by microbiota, resulting in a greater protection against infections and against inflammatory diseases.</td>
</tr>
<tr>
<td>Influence on pathogen colonization</td>
<td>Microbiota compete with pathogens for attachment sites and nutrients, and they produce antimicrobial substances.</td>
</tr>
</tbody>
</table>

Brusaferro et al., 2018
Dysbiosis of gut microbiota

- Dysbiosis (modifications of the gut microbiota composition) can be associated with the development of both intestinal and extra-intestinal disorders:
  - irritable bowel syndrome
  - Inflammatory bowel disease
  - colorectal cancer
  - allergic diseases
  - arteriosclerotic diseases
  - metabolic syndromes - diabetes and obesity

Brusaferro et al., 2018
Gut microbiota and obesity

• Composition of gut microbiota is different among obese compared to a normal weight individual (Rouxinol-Dias et al., 2016).

• Studies have shown that faecal bacteria may have a role in modulating energy metabolism.

• Change in gut microbiota composition can be associated with increases or reductions of body weight and body mass index (Brusaferro et al., 2018)

• Pattern of gut microbiota in relation with obesity can be demonstrated by the release of microbiota-induced fermentation products
  • Eg. short-chain fatty acids (SCFAs)
Probiotics and gut microbiota

• Probiotics are live microorganisms with beneficial health effects if administered in adequate amounts (FAO/WHO, 2002)

• Manipulation of gut microbiota is possible through the consumption of probiotics.

• Commonly used microorganism for preparing probiotics-containing drinks is *Lactobacillus spp.*

• One example is a probiotic-fermented milk drink (Yakult) that contains *Lactobacillus casei* strain Shirota (LcS).
Limited Malaysian data

• Limited data on gut microbiota and faecal SCFAs in Malaysian children

• Similarly, limited data on probiotics administration and effects on gut microbiota and SCFA in Malaysian children.
Aim of the study

To investigate the gut microbiota and short-chain fatty acids profiles of normal and overweight children after probiotics administration
Study Design

- Crossover design
- Normal weight and overweight school children aged between seven to 10 years
- Probiotics for 4 weeks and 4 weeks washout period to
Probiotic administration

• A bottle (80ml) with 30 billions live LcS daily, for four weeks.
• The probiotic drink:
  • scientific research on human health
  • free of preservatives, colouring and stabilizers
The probiotic drink with LcS

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Per serving</th>
<th>Every 100ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>45</td>
<td>56</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>9.5</td>
<td>11.9</td>
</tr>
<tr>
<td>Total sugar (g)</td>
<td>6.5</td>
<td>8.1</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Natrium (mg)</td>
<td>16.7</td>
<td>20.4</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: https://www.yakult.com.my
Other factors

• Normal diet
• Normal routine physical activities
• Not on vitamin supplements, antibiotics and medication.
### Sociodemographic of study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal weight (n=19)</th>
<th>Overweight (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Total number of subjects (by gender)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>1.07</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>1.07</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.53</td>
<td>1.07</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>27.82</td>
<td>6.15</td>
</tr>
<tr>
<td>Height (m)</td>
<td>130.86</td>
<td>7.16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.07</td>
<td>2.34</td>
</tr>
</tbody>
</table>

BMI: body mass index, SD: standard deviation
a BMI is calculated based on the formula (body weight (kg)/height² (m²)).
Study protocol
Effects of LcS on gut microbiota in normal weight children

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intervention group</th>
<th>Control group</th>
<th>p-value $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (Log 10 copies)</td>
<td>Mean (Log 10 copies)</td>
<td>SD</td>
</tr>
<tr>
<td>Normal weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus spp.</em></td>
<td>1.68</td>
<td>0</td>
<td>2.18</td>
</tr>
<tr>
<td><em>Bifidobacterium spp.</em></td>
<td>1.63</td>
<td>0</td>
<td>2.12</td>
</tr>
</tbody>
</table>

SD: standard deviation  
$a$ Independent sample t test, $p<0.05$ indicated significant differences as compared between intervention and control groups
Effects of LcS on gut microbiota in overweight children

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intervention group (n=10)</th>
<th>Control group (n=9)</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Log 10 copies)</td>
<td>SD</td>
<td>Mean (Log 10 copies)</td>
</tr>
<tr>
<td>Overweight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus spp.</em></td>
<td>3.86</td>
<td>0.28</td>
<td>1.35</td>
</tr>
<tr>
<td><em>Bifidobacterium spp.</em></td>
<td>2.68</td>
<td>2.36</td>
<td>0</td>
</tr>
</tbody>
</table>

SD: standard deviation

*a* Independent sample t test, *p*<0.05 indicated significant differences as compared between intervention and control groups
Effects of LcS on fecal SCFAs concentration of normal and overweight children

<table>
<thead>
<tr>
<th>SCFA (µmol/g)</th>
<th>Intervention group (n = 10)</th>
<th>Control group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>At week 5</td>
</tr>
<tr>
<td>Total SCFAs</td>
<td>81.43</td>
<td>138.24</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>27.14</td>
<td>17.37</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>20.03</td>
<td>25.68</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>34.26</td>
<td>95.17</td>
</tr>
</tbody>
</table>

**Overweight**

<table>
<thead>
<tr>
<th>SCFA (µmol/g)</th>
<th>Intervention group (n = 10)</th>
<th>Control group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>At week 5</td>
</tr>
<tr>
<td>Total SCFAs</td>
<td>112.49</td>
<td>201.44</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>27.63</td>
<td>18.18</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>25.96</td>
<td>31.31</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>58.90</td>
<td>153.57</td>
</tr>
</tbody>
</table>
Discussion
Gut bacteria

- *Lactobacillus* spp. were higher in the overweight children who received probiotics intervention compared to normal weight children.
- *Bifidobacterium* spp. were higher among normal and overweight children from the intervention groups compared to control group.
  - suggests *Lactobacillus* probiotics encourage the proliferation of *Bifidobacteria*. 
Gut bacteria and body weight

• Evidence on the association between a bacterial genus or species and weight does not explain whether the microorganism is truly the cause of obesity or normal weight.

• Studies have shown that *Bifidobacterium* were more abundant in subjects with normal weight than in obese.

• For some genera, such as *Lactobacillus* conflicting results have been reported.
Probiotics and obesity

• Probiotics effect on body weight and metabolism has been reported to be strain specific.

• Dosage, duration of administration and long-term effects of the administration of the different strains are not known.
Short chain fatty acids

- The changes in fecal total SCFAs, butyrate and propionate were higher in overweight children than normal weight from the intervention group.
  - consistent with a cohort study among Swiss children in a 15 obese and 15 normal-weight of Swiss children aged 8 to 14 years old (Payne et al., 2011).
  - however, higher faecal SCFAs production among Japanese and Mexican lean children than their obese children (Nagata et al., 2017 & Murugesan et al., 2015).
- Gut microbiota dysbiosis, genetics, enviromental and diet may be factors that result in altered faecal propionic and butyric acids (Murugesan et al., 2015)
Short chain fatty acids after LcS

• A significant increased in the fecal propionic acid and total SCFAs after four weeks of intervention in overweight and normal weight participants (p<0.05).
  • Similar findings by Nagata et al. (2017) and Hemalatha et al. (2017)
  • However, studies among adults found reduced acetate and propionate after two weeks LcS administration (Matsumoto et al., 2006).
Propionic acid

• Propionic acid increment in the colon may act as appetite suppressor by activation of the G-protein-coupled receptors and increased secretion of gut hormones (Kobyliak et al., 2016 & Chambers et al., 2015).

• Elevated propionic acid in the colon promotes weight loss and reduced body adiposity among overweight adults, post Roux-enY gastric bypass and diet-induced obesity mice (Chambers et al., 2015 & Everard et al., 2013).

• However, the function of propionic acid in the metabolism and body weight regulation has not yet fully explained.

• Various factors may affect gut microbiota composition and body weight in children.
Conclusion

• This study indicates that consumption of LcS-fermented probiotic drinks by school children between aged seven to 10 years old potentially changes their gut microbiota composition of *Lactobacillus* *spp.* and *Bifidobacterium* *spp.*

• The total SCFAs and propionate levels of overweight children increased compared to a control group corresponds to increased gut microbiota.

• Findings need to be correlated with other factors and outcomes.

• More studies are needed for prescription of probiotics in obesity.
Future Research

• More sample size with other factors considered and other outcomes
  • multidisciplines
• Metagenomic data
• Consideration of dose, duration, individualized for probiotics administration.
Acknowledgement

• This study was supported by grants Exploratory Research Grant Scheme (ERGS) from the Ministry of Higher Education (MOHE), Malaysia [ERGS/1-2013/5527168].

• Research team:

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References


