THE POSSIBLE EFFECT OF PREBIOTIC OLIGOFRUCTOSE ON GUT MICROBIOTA AND METABOLIC ENDOTOXEMIA PRODUCED BY HIGH FAT DIET IN ADULT MALE RATS

By

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ABSTRACT

Background: Gut microbiota is the complex community of microorganisms that live in the GIT of humans and other animals. Oligofructose (OFS) is one of prebiotics which modulates gut microbiota.

Objective: Assessing the potential effects of the prebiotic OFS on gut microbiota and metabolic endotoxemia in high fat diet (HFD) fed rats.

Material and methods: Forty adult male albino rats were divided into 2 groups: Group I (10 rats) fed on a standard rat chow for 14 weeks. Group II (30 rats) fed on HFD for 8 weeks. In the next 6 weeks, rats of group II were divided into 3 equal subgroups: Group II 1 (control B) continued feeding on HFD. Group II 2 continued feeding on HFD with administration of OFS. Group II 3 continued feeding on standard rat chow instead of HFD with administration of OFS. At the end of 14 weeks, blood and fecal samples were collected for biochemical analysis to gut microbiota (Firmicutes and Bacteroidetes phyla), lipopolysaccharide (LPS), and tumor necrosis factor alpha (TNFα).

Results: OFS produced increase in Bacteroidetes phylum in comparison with HFD fed group (control B). On the other hand, OFS produced decrease in Firmicutes phylum, LPS, and TNFα in comparison with HFD fed group (control B). There was better improvement when OFS was given in HFD fed rats.

Conclusion: OFS induced improvement in gut microbiota composition, endotoxemia, and inflammatory biomarkers. There was better improvement when OFS was fed with standard diet in HFD fed rats.

Keywords: Prebiotics, oligofructose, gut microbiota, metabolic endotoxemia, high fat diet.

INTRODUCTION

There is a relationship between HFD and gut microbiota composition (Lau et al., 2016). Gut microbiota (Gut flora) is the complex community of microorganisms that live in the digestive tracts of humans and other animals (Kobyliak et al., 2016). These bacteria play an important physiological role in vital processes such as digestion, vitamin synthesis, development of microvilli, and metabolism (Houghton et al., 2016).

The gut microbiota is estimated to comprise over 10^{14} bacteria from more than 1000 different species. Recent studies described more than 70 bacterial phyla with four constituting the majority of mammalian intestinal microbiota (Bacteroidetes, Firmicutes, Actinobacteria...
and Proteobacteria), and only two predominating in the intestinal tract: the Bacteroidetes and the Firmicutes (Fujio- Vejar et al., 2017).

Previously, Aguirre and Venema (2015) mentioned that the balance between Bacteroidetes and Firmicutes plays a crucial role in health and disease. In healthy individuals, Bacteroidetes bacteria representing the majority of the colon microbiota when compared to Firmicutes. Individuals fed high fat diet (HFD) favor the balance towards Firmicutes when compared to Bacteroidetes.

HFD changes gut microbiota composition and increases intestinal permeability. The altered intestinal barrier (due to increased intestinal permeability) and the subsequent translocation of toxic bacterial products, mainly lipopolysaccharide (LPS) to the circulation, produce a state of “metabolic endotoxemia” (Yang et al., 2015).

Host cells recognize LPS by specific receptors (Toll-like receptor 4) which is followed by downstream inflammatory events that contributes to the development of obesity and other metabolic disorders (Jiang et al., 2016).

Prebiotics are non-digestible food and plant ingredients. Oligofructose (OFS) is one of prebiotics which is not hydrolyzed and absorbed in the upper parts of the GIT. OFS is considered the main nutrient for beneficial bacteria (Bacteroidetes) which leads to modulation of gut microflora (Barczynska et al., 2015).

On contrary, Boulange et al. (2016) and Duranti et al. (2017) recorded that how external factors, such as diet affect the gut microbial composition, and the effectiveness of microbial functions in rodents and humans is still unclear.

In 2018, Zhang et al. reported that application of OFS reduces the relative abundance of Firmicutes and increases the abundance of Bacteroidetes with improvement of endotoxemia.

There are few researches which demonstrate the effect of prebiotics on gut microbiota and endotoxemia. So, we need further researches to study this relationship (Catinean et al., 2018).

The aim of the present study was to assess the potential effects of the prebiotic OFS on gut microbiota and metabolic endotoxemia in HFD fed rats.

**MATERIALS AND METHODS**

**Animals**

The present study was conducted on 40 adult male albino rats of local strain weighing 110 – 130 g. They were obtained from Nile Pharmaceuticals Company (Cairo, Egypt). They were kept in suitable cages which were 40x25x25 cm in size, 5 rats per cage.

All rats were allowed to adapt to the prevailing environment for one week prior to the beginning of the experiment. Animals were housed under appropriate conditions of controlled humidity. They were maintained at constant room temperature and suitable illumination conditions (light/dark cycle of 12/12 h). Rats were allowed to ordinary rat chow and fresh tap water ad-libitum.

**Experimental design**

After the period of accommodation, rats were divided into 2 groups:
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Group I (G I) (10 rats): Normal control fed on a standard rat chow for 14 weeks.

Group II (G II) (30 rats): High fat diet (HFD) fed group fed on HFD which consists of 20% buffalo fat and 80% standard rat chow (Abozid and Mariah, 2016) for 8 weeks (Jiang et al., 2016).

For the next 6 weeks, rats of group II were divided into 3 equal subgroups:

Group II 1 (G II 1) (10 rats) Control B, continued fed on HFD.

Group II 2 (G II 2) (10 rats) continued feeding on HFD with ingesting prebiotic [oligofructose (OFS)] through oro-gastric gavage. The dose of OFS was 8 g/kg body weight (Koleva et al., 2012) dissolved in potable water (Bustamante et al., 2015).

Group II 3 (G II 3) (10 rats) continued feeding on a standard rat chow instead of HFD with administration of OFS through oro-gastric gavage. The dose of OFS was 8 g/kg body weight dissolved in potable water.

Diet

1. Standard rat chow (Giza, Egypt) was composed of 7-10% fat, 68-70% carbohydrate, 18-20% protein, 1-2% vitamins and minerals (Altunkaynak, 2005).

2. High fat diet: buffalo fat was melted by heating, then the chow (in powder form) was mixed with 20% melted fat until it became homogenous in a dough-like consistency. It was prepared as blocks and let to dry then used for feeding (Selim, 2013).

3. Prebiotic [oligofructose (OFS)] (D-26434 Wangerland, Germany) provided in powder form. According to the manufacturer, OFS used in this study was a mixture of oligosaccharides extracted from chicory root.

Sampling

1-Blood Samples

At the end of the experiment (at the end of 14 weeks), all the animals were fasted for 12 h before scarification. Animals were anesthetized by using diethyl ether. Blood samples were taken from retro-orbital sinus by capillary tubes. The blood was collected in a centrifuge tubes. It was allowed to clot for an hour at room temperature, and then centrifuged at 3000 rpm for 15 minutes (using cooling centrifuge, Micro 22R, Germany). The sera were separated and stored at -80°C (using Arctiko deep freezer, Denmark) until the time of use (Simmons and Brick, 1970).

2. Fecal matter samples

Fresh fecal samples were collected directly from the cecum and colon of all animals. These fecal samples were stored at −80 °C until further analysis (Choo et al., 2015).

Determination and quantification of gut microbiota

The work was done using qRT-PCR device (applied biosystem Foster city, USA). It includes DNA extraction, PCR amplification of target microbiota (Bacteroidetes and Firmicutes) and quantification using system software.

DNA extraction procedure: According to the instructions of the manufacturer (Qiagen, Hilden, Germany), DNA was extracted from stool using QiaAmp (Qiagen amplification) DNA Mini Kit (Mirsepas et al., 2014).

Blood Parameters

Serum LPS and TNFα concentrations were determined using reagent kits obtained from MyBiosource in accordance with the manufacturer’s instructions.
Statistical Analysis

Statistical analysis was done using statistical package for the social science (SPSS) for windows, version 20. The obtained data were presented as means ± standard error of mean (SEM). Statistical analysis of variance between mean values of different groups was performed using one way ANOVA followed by Bonferroni’s post hoc test. Differences were considered significant at $p \leq 0.05$ (Kang et al., 2017).

RESULTS

Effect of HFD and OFS on different parameters (Table 1)

1. Administration of HFD produced increase in Firmicutes phylum and decrease in Bacteroidetes phylum versus normal control group A (G I), and increase in LPS and TNFα versus normal control group A (G I).

2. OFS with HFD induced significant decrease in Firmicutes phylum and significant increase in Bacteroidetes phylum versus control B (G II 1). These parameters did not return to normal. On the other hand, OFS with standard diet induced significant decrease in Firmicutes phylum, and significant increase in Bacteroidetes phylum versus control B (G II 1). These parameters showed insignificant change versus normal control group A (G I) and G II 2.

OFS with HFD induced significant decrease in LPS and TNFα versus control B (G II 1). These parameters were still significantly higher than that in normal control group A (G I). On the other hand, OFS with standard diet induced significant decrease in LPS and TNFα versus control group B (G II 1). However, there was an insignificant change versus G II 2. LPS and TNFα in G II 3 were still significantly higher than that in normal control group A (G I).

Table (1): Effect of high fat diet (HFD) and prebiotic [oligofructose (OFS)] on different parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>G I</th>
<th>G II 1</th>
<th>G II 2</th>
<th>G II 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal control</td>
<td>G II</td>
<td>control group B</td>
<td>HFD+OFS</td>
<td>Standard diet +OFS</td>
</tr>
<tr>
<td></td>
<td>group A</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmicutes phylum x10^5</td>
<td></td>
<td>4.75±0.52</td>
<td>9.35±0.58</td>
<td>6.48±0.28</td>
<td>5.78±0.46</td>
</tr>
<tr>
<td>Bacteroidetes phylum x10^5</td>
<td></td>
<td>5.71±0.43</td>
<td>2.12±0.26</td>
<td>4.05±0.11</td>
<td>4.92±0.12</td>
</tr>
<tr>
<td>Lipopolysaccharide (LPS) (ng/mL)</td>
<td></td>
<td>18.50±0.97</td>
<td>189.43±10.44</td>
<td>83.88±7.59</td>
<td>59.11±6.74</td>
</tr>
<tr>
<td>Tumor necrosis factor α (TNFα) (pg/mL)</td>
<td></td>
<td>25.18±3.77</td>
<td>122.61±4.58</td>
<td>75.85±5.77</td>
<td>59.41±4.93</td>
</tr>
</tbody>
</table>

Values were represented as means ± SEM and statistically evaluated using one way ANOVA followed by Bonferroni’s post hoc test

a: statistically significant compared to corresponding value in G I (normal control group A).

b: statistically significant compared to corresponding value in G II 1 (control group B).
DISCUSSION

In the present study, administration of HFD produced significant increase in Firmicutes phylum and significant decrease in Bacteroidetes phylum compared to normal control group A (GI). Anitha et al. (2016) and Jiang et al. (2016) agreed these findings. Also, Nie et al. (2015) and Duranti et al. (2017) agreed these findings and attributed changes in gut microbiota composition to increases bile acid secretion with HFD. HFD increases bile acid secretion which would exert strong selective pressure on the gut microbiota. However, some bacteria, as Firmicutes, are bile acid-tolerant. So, they proliferate even in the presence of bile acids. Therefore, bile acids in the intestine have negative effects on Bacteroidetes, while they exert beneficial effects on Firmicutes.

In this study, administration of HFD produced significant increase in serum LPS compared to normal control group A (GI). Kim and Kim (2017) agreed these findings and reported that circulating LPS are sensed by a cell-surface-receptor compound that contains toll-like receptor 4 (TLR4), and its co-receptors cluster of differentiation 14 (CD14) and myeloid differentiation protein-2 (MD-2). TLR4 is present on the membrane surface of immune cells (monocytes, macrophages and Kupffer cells) and other cells (adipocytes, hepatocyte and endothelial cells). In response to LPS-binding, the intracellular domain of TLR4 activates several signal transduction responses that lead to the production of pro-inflammatory cytokines such as TNF-α and interleukin-6 (IL-6).

In this study, administration of OFS induced decrease in Firmicutes phylum, and increase in Bacteroidetes phylum versus control B (GI I). These parameters returned to normal when OFS was administrated with standard diet. Vieira et al. (2016) agreed these findings and purposed that metabolic products of anaerobic fermentation of prebiotics, such as short chain fatty acids (SCFAs), can change the gut environment especially its pH, creating a more acidic environment. It has been shown that pH exerts a strong influence on the microbiota composition. Holscher (2017) confirmed that, at pH 6.5, gram-negative Bacteroides predominates, but at pH 5.5, gram-positive Firmicutes have an advantage. Subsequent experiments also showed that pH exerts important control over the competition between bacteria from different phyla or families with varying abilities to consume similar prebiotics. Thus, pH is considered
an important factor in prebiotic use because it has a strong influence on competition between bacteria. However, we must consider that in vitro studies pH may differ from the situation in vivo, in which the absorption and turnover of fermentation products are very dynamic.

In this study, administration of OFS induced decrease in LPS versus control B (G II 1). This parameter remained above normal. Bomhof et al (2014) and Arana et al. (2017) agreed these findings and attributed improvement in intestinal permeability and metabolic endotoxemia to increase in glucagon-like-peptide 2 (GLP-2) with prebiotic OFS. GLP-2, a peptide that is co-secreted with GLP-1 from enteroendocrine L cells in the small and large intestine. GLP2 increases the rate of crypt proliferation, villus elongation and reduces apoptosis; contributing to an enhanced gut barrier function which decrease translocation of LPS to circulation.

In this study administration of OFS induced decrease in TNFα versus control B (G II 1). This parameter remained above normal. Viladomiu et al. (2013) agreed these findings and mentioned that administration of prebiotics as OF Smay decrease effect or responses and pro-inflammatory cytokine (e.g. TNF-α) expression through the production of short chain fatty acids (SCFAs); products of fermentation of OFS. These SCFAs and mainly butyrate induce the activation of peroxisome proliferator activator receptor γ (PPARγ). PPARγ is a nuclear receptor and transcription factor involved in lipid metabolism and glucose homeostasis. Interestingly, PPARγ was shown to be efficacious in suppressing intestinal inflammation. In addition, the activation of PPARγ was shown to reduce pro-inflammatory pathways, such as the signal transducer and activator of transcription (STAT), activator protein 1 (AP-1), and NF-κB pathways.

CONCLUSION

Administration of HFD induced impairment in gut microbiota composition, endotoxemia (LPS) and inflammatory biomarkers (TNFα), while administration of OFS induced improvement in gut microbiota composition which return to normal when OFS was administrated with standard diet. OFS induced improvement in endotoxemia and inflammatory biomarkers. These parameters remained above normal.

Endotoxemia biomarkers and gut microbiota analysis are applicable methods. So, it is recommended to perform serum LPS and stool analysis as routine investigations to follow up metabolic endotoxemia and metabolic disorders.

REFERENCES


الدور المحتمل للبريوبيوتك أوليجوفركتوز على بكتيريا القناة الهضمية والسموم المشتقة من البكتيريا بالدم الناتجة عن الغذاء عالى الدهون في ذكور الجرذان البالغة

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خلفية البحث: تعد بكتيريا القناة الهضمية مجتمعاً مفعماً من الكائنات الدقيقة التي تعيش في القناة الهضمية للإنسان والحيوان. والبريوبيوتك هو أحد البريوبيوتكس والذي يعمل على تحسين بكتيريا القناة الهضمية. هناك قليل من الأبحاث التي تدرس تأثيره على بكتيريا القناة الهضمية والذي مازال غير واضح.

الهدف من البحث: صنعت هذه الدراسة لتوضيح التأثير المحتمل للبريوبيوتك أوليجوفركتوز على بكتيريا القناة الهضمية وإرتفاع السموم المشتقة من البكتيريا بالدم وذلك في الجرذان التي تتغذى على غذاء عالي الدهون.

مواد وطرق البحث: تم تنفيذ الدراسة الحالية على أربعين من ذكور الجرذان البالغة، وتم تقسيمهم إلى مجموعتين:

المجموعة الأولى (10 جرذان) مجموعة طبيعية ضابطة: أنتغذى على غذاء الجرذان القباسي لمدة 14 أسبوع.

المجموعة الثانية (30 جرذان) تنتغذى على غذاء عالي الدهون لمدة 8 أسابيع.

وفي الأسابيع الستة التالية تم تقسيم جرذان المجموعة الثانية إلى 3 مجموعات فرعية متساوية:

المجموعة الثانية 1: مجموعة ضابطة ب انتغذى على غذاء عالي الدهون.
المجموعة الثانية 2: انتغذى على غذاء عالي الدهون مع إعطاء البريوبيوتك أوليجوفركتوز.
المجموعة الثانية 3: انتغذى على غذاء الجرذان القباسي بدلاً من الغذاء عالي الدهون مع إعطاء البريوبيوتك أوليجوفركتوز.

في نهاية الأسبوع الرابع عشر تم جمع عينات الدم والبراز وذلك لإجراء التحاليل الكيميائية وتم إجراء تحليل بكتيريا القناة الهضمية (شبيهة الفرميكيوتس والبيكرتوديتز) والليوبوليسكاريد وعامل نخر الورم ألفا.

النتائج: نجع عن إعطاء الأوليجوفركتوز زيادة في شعبة البكتيريوتين مقترنة بالمجموعة ضابطة ب التي تنتغذى على غذاء عالي الدهون. وعلى الجانب الآخر نجع عن إعطاء الأوليجوفركتوز نقصاً في شعبة الفرميكيوتس والليوبوليسكاريد وعامل نخر الورم ألفا مقترنة بالمجموعة ضابطة ب التي تنتغذى على غذاء عالي الدهون. كما كان هناك نقص أكبر عندما تم إعطاء الأوليجوفركتوز مع غذاء الجرذان القباسي.

الاستنتاج: نجع عن إعطاء الأوليجوفركتوز تحسين في بكتيريا القناة الهضمية ومؤشرات السموم المشتقة من البكتيريا بالدم والإلتهاب. كما كان هناك تحسناً أكبر عندما تم إعطاء الأوليجوفركتوز مع غذاء الجرذان القباسي.