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Original article

The weight and ghrelin changes of fecal microbiota transplantation in rats

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Summary. *Background:* Fecal microbiota transplantation is a promising method to solve obesity. Our study's aim was to investigate the changes of weight and ghrelin levels in obese rats receiving fecal microbiota transplantation from lean rats. *Methodology:* Twenty-one rats were divided into three equal groups: Group 1: Obese control group; group 2: Obese recipient group; and group 3: Lean donor group. Feces which was collected from donor group was transferred to the rats in recipient group, orally by gavage, 3 times every other day. The weight and ghrelin levels were measured from each rat at the beginning and end of the study. *Results:* There was statistically significant weight gain in donor group (p:0.001), but there were no statistical significant weight chances was detected in control and recipient groups (p:0.82, p:0.12, respectively). There was an increase in donor and control groups, but a decrease was observed in the recipient group at ghrelin levels. However, there was no significant difference at ghrelin levels in any groups (p:0.05, p:0.2, p:0.4, respectively). There was a significant relation in control group in weight and ghrelin changes (p:0.007), but no significant relation was detected in either recipient or donor groups (p:0.29, 0.53, respectively). *Conclusion:* Metabolism changes of obese rats were observed after fecal microbiota transplantation, and it was the only group that decreasing ghrelin levels.

Key words: animal experimentation, body weight changes, ghrelin, microbiota, transplantation

Introduction

Obesity is a major global public health problem that is becoming increasingly common. In 2014, the World Health Organization (WHO) reported that 38% of male and 40% of female adult individuals worldwide were overweight, with obesity ratios of 11% and 15%, respectively (1). Obesity is a multifactorial problem related to genetics, a lack of physical activity, unhealthy eating, as well as other diseases such as diabetes, hypertension, and metabolic syndrome, all of which are also major risk factors (2). There are many treatment modalities for obesity, from lifestyle changes to surgery; however, success cannot be achieved at the desired rate due to reasons arising from patients or treatment methods (1-3).

The human intestinal microbiota begins to develop from birth, depending on personal and environmental factors. The colon contains microorganisms, with a total concentration of ~10^{11}-10^{12} CFU/g, which are in a favorable symbiotic relationship with their human hosts (4). A possible role of intestinal microbiota has been suggested in obesity pathogenesis. Human and animal studies of obesity have shown that the

gut microbiota in obese individuals was significantly changed and the bacterial diversity was reduced (5-7). Similar studies have reported that *Bacteroidetes* strains were reduced, while *Firmicutes phylum* was increased proportionately (8-11).

In recent years, fecal microbiota transplantation (FMT) has garnered significant interest and attention. In particular, several case series have been published about *Clostridium difficile* (CD) infections and inflammatory bowel disease. The success ratios for CD infections are above 90% (12-16). In studies performed on germ-free obese mice, treatment with FMT led these mice to gain weight (17).

Ghrelin was discovered after a rat gastric extraction in 1999 (18). In subsequent studies of ghrelin, it was demonstrated that ghrelin is functionally active and effective in many areas including sleep, weight control, glucose metabolism and the control of stress and anxiety (19). Ghrelin levels change depending on many factors. To date, ghrelin has been the focus of research across numerous fields, and the data concerning ghrelin levels are available for many diseases such as obesity, anorexia nervosa, cachexia, chronic obstructive pulmonary disease, chronic heart failure and after obesity surgery (19). However, from our review of the literature, we did not find any studies concerning ghrelin hormone levels or weight changes in animal models following FMT. Our aim in this study was to investigate how changes in ghrelin hormone levels after FMT impact the pathophysiology of weight control.

Materials and Methods

This study was performed at the Experimental Animal Production and Research Laboratory and was approved by the local Animal Ethics Committee. All protocols were performed in accordance with the regulations governing the care and use of laboratory animals in the Declaration of Helsinki.

Twenty-one Wistar female albino rats {14 obese rats (mean weight was 454 g and mean age was 11 months) and 7 lean rats (mean weight was 283 g and mean age was 3 months)} were divided evenly into three groups. According to a power analysis using 0.05 accuracy and a power of 0.95, seven rats were assigned to each of the groups. The groups were defined as follows: Group 1: Obese control group (n:7); group 2: Obese recipient group (n:7); and group 3: Lean donor group (n:7). During the entire experiment, the rats were kept alive in standard laboratory mice and rat cages; the bases and sides were made of plastic, and the tops were covered with iron wire netting. The laboratory settings included a 12-hour light/12-hour dark cycle, and the rats were fed a pellet-type fabricated feed specially produced for small experimental animals.

At the beginning of the study, both the obese and lean rats (n=21) were weighed. After weight measurements, all rats were administered general anesthesia with xylazine (Rompun® Bayer Co.; 5 mg/kg body weight), and 1 ml blood samples were obtained from each subject through the intra-cardiac entry and then centrifuged at 3000 g at 10 minutes. The plasma was stored at -80°C. A fecal solution was applied to recipient rats via oral gavage, three times every other day. After the FMT application, all rats were weighed on the same day weekly. After four weeks, a 1 ml blood sample was taken from all rats under general anesthesia intracardially (Rompun® Bayer Co.; 5 mg/kg body weight). This blood sample was then centrifuged at 3000 g for 10 minutes, and the harvested plasma was stored at -80°C.

The ghrelin levels in the plasma obtained from the blood samples taken at the beginning and end of the study were measured with enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich RAB0207, St. Louis, US).

Fecal microbiota transplantation

Immediately after excretion by the donor lean rats, the fecal samples were put into transfer tubes (which contained pre-reduced sterile phosphate buffered saline with 0.05% cysteine-HCl, 2 mL/g). Each fecal sample was blended with the help of a mixer until homogenization. The mixtures were filtered with the help of a cloth filter, and the solid particles were separated. One milliliter of the filtered fecal solution was then given to the recipient obese rats via oral gavage.

Statistical analysis

The statistical analyses were performed using IBM SPSS Ver. 21.0. In addition to the descriptive statisti-

cal methods (mean and standard deviation), we used the Paired t Test for comparing in-group variations in weight change. Additionally, we used the Wilks' Lambda for in-group variable comparisons of ghrelin changes. The correlation between the changes in weight and ghrelin, for all three groups, was analyzed with Spearman's rho test. The results were evaluated at the 95% confidence interval and $p<0.05$ significance level.

Results

In this study, the weight changes, ghrelin levels of the groups before and after the study were compared. The weight changes in rats before and after this study are given in table 1. When the weight changes in the groups over time were compared, the mean weight of the group three was 283.28±27.63 g at the beginning of the study and 319.14±27.17 g at the end of the study and this was the significant weight changes in groups ($p<0.05$); however in group two, while there was weight reduction over time, it was not statistically significant ($p>0.05$). Also there was no weight changes in group one ($p>0.05$).

The ghrelin levels before and after the study are given in table 2. We observed an increase at ghrelin levels in the rats of groups 1 and 3, while the ghrelin values of the rats in group 2 decreased. Despite these observed trends, none of these were found to be statistically significant (p:0.05, p:0.2, p:0.4, respectively).

Next, the correlation between the weight changes and ghrelin changes of all three groups was analyzed. No significant relation was found between the changes in weight and ghrelin in groups 2 and 3 (p:0.29 and 0.53, respectively), however, there was a significant relation detected in group 1 (p:0.007).

Table 1. Mean weight changes in the groups over time.

	Group 1 (g ±SD)	Group 2 (g ±SD)	Group 3 (g ±SD)
Week 0	447.00±39.21	462.57±55.26	283.28±27.63
Week 4	446.71±39.36	454.57±55.99	319.14±27.17
p value (Paired t test)	0.82	0.12	0.001

Table 2. Mean ghrelin changes in the groups over time (ng: nanogram, ml: milliliter)

	Ghrelin (ng/ml) before the study ± SD	Ghrelin (ng/ml) after the study ± SD	p values (Wilks' Lambda)
Group 1	55.71±18.71	69.34±21.46	0.05
Group 2	45.91±11.38	38.98±9.21	0.2
Group 3	47.52±18.47	51.87±16.93	0.4

Discussion

Fecal microbiota transplantation is one of the current treatment methods used for treating *C. Difficile* and inflammatory intestinal infections (20,21).

It is well known that the fecal microbiota of obese individuals is different from those found in non-obese individuals and that FMT in obese individuals changes their fecal microbiota. Previous studies in animal models and humans have shown that obesity increases *Firmicutes* levels and decreases levels of *Bacteroidetes* in the respective host's fecal microbiota (17, 22). Fecal microbiota transplantation was associated with favorable changes in gut microbiota, including greater bacterial diversity and a 2.5-fold increase in butyrate-producing bacteria (17,23-25). A double-blind randomized controlled trial of nine middle-aged patients with metabolic syndrome who experienced a fecal microbiota transfer from lean individuals via a nasoduodenal tube reported similar findings. Six weeks after FMT, the study reported a 75% increase in their insulin sensitivity, increased the diversity of gut microbiota, and increased levels of the butyrate-producing bacteria *Roseburia intestinalis* in the obese patients with metabolic syndrome post-fecal microbiota transfer (26).

There are several studies that address the effect of FMT on insulin resistance and weight change. One such study utilized germ-free rats and found that the germ-free rats gained more weight after fecal microbiota transplantation from obese rats when compared with transplantation from lean rats (17). More recently, some scientists inoculated the germ-free mice with gut microbes from four pairs of female twins, in which one of the twins was obese and the other had a healthy weight. The mice that received the obese humans' microbes gained more body fat, put on more weight, and

showed increased levels of markers for metabolic disorders (27). In one case report, a woman successfully treated with FMT developed new-onset obesity after receiving stool from a healthy, but overweight donor (28).

Germ-free mice do not become obese, nor do they develop insulin resistance after exposure to a high-fat diet. The transfer of gut microbiota from a laboratory-raised mouse into germ-free mouse results in an increase in body fat content and insulin resistance, despite reduced food intake (29). As shown, animal studies were generally performed on germ-free animals. On the other hand, our study evaluated the outcomes of FMT application on rats with developed microbiota. According to results of our study, despite the minor weight loss observed, there was no statistically significant difference in weight between the beginning and the end of the study. This effect could have been due to the short duration of our study as well as the multifactorial causes effecting both the development of obesity and fecal microbiota.

Some studies have accepted ghrelin as a possible weight loss mechanism, which may be a consequence of sleeve gastrectomy and gastric bypass surgery that are applied to treat obesity (30). In the majority of the studies that investigate the ghrelin levels after sleeve gastrectomies, a decrease in ghrelin levels is observed, whereas in the studies in which the ghrelin levels were measured after gastric bypass report that there can be a decrease, an increase, or no change in the ghrelin levels (30-32). In our review of the literature, we found many studies about the changes in microbiota with FMT; however, we found no study that evaluated the relation between fecal transplantation and ghrelin levels. However, we did find two studies similar to our own in certain ways. In the first study, fecal microbiota changes and ghrelin levels were investigated in cats fed different foods and a positive relation was observed between *Bifidobacteria* and blood ghrelin levels (33). This finding supports our previous statement mentioning that the differences in microbiota can change ghrelin levels. In the second study, Oxyntomodulin, which is released from intestinal L-cells, was found to be effective in weight loss, but the iv application of its synthetic analog can be difficult. Therefore, significant weight loss was observed in obese rats fed with a probiotic, namely *Bifidobacterium*, and an integrated human Oxyntomodulin gene, compared to rats fed normal feeds without the gene transfer. The Oxyntomodulin levels in the blood were found to be high, while ghrelin levels were found to be low (34). This finding showed us that weight loss could occur with increased amounts of Oxyntomodulin, which may reverse the effect of ghrelin, and that decreased amounts of ghrelin and gut hormones, such as ghrelin, can have an effect on weight changes after transplantation.

Although it was not statistically significant, we observed a decrease in the levels of ghrelin in rats with weight loss after FMT application and an increase in ghrelin levels in lean rats who gained weight and obese rats to whom FMT was not applied. These results show that there can be changes in the metabolism of obese rats after FMT and that the metabolism of FMT applied rats can behave differently from rats in the control group. It is possible that ghrelin may play a similar role in weight loss as noted in previous studies with FMT.

There were some limitations in this study. First, fecal microbiota analysis was not controlled before or after FMT application. The reasons for this limitation were that looking for fecal microbiota is an expensive procedure and there were many studies in the literature about microbiota changes with FMT (17,23-25). And our study had a short follow-up time.

Conclusion

Although FMT is a treatment method growing in popularity and its success has been demonstrated in many studies of certain diseases, its use in studies of obesity remains limited. Although it is a known fact that FMT can cause changes in the fecal microbiota, there was no study of its effect on intestinal hormone levels, namely on ghrelin levels, in relation to weight changes in animals and humans. Although the results of our study did not show any statistical significance, ghrelin levels decreased only for the fecal transplantation applied group and only these group lost weight. This finding made us think that there can be changes in the metabolism of obese rats after FMT. The metabolism of FMT applied rats can behave differently than

for rats in the control group, and fecal transplantation can cause weight loss via changing levels of ghrelin. As this study is the first to evaluate ghrelin levels after FMT, we think the differences can be brought to statistically significant levels with future studies that include the use of more animals, longer observation periods and different transplantation protocols.

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