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Obez Kadın Hastalarda Akupunktur Tedavisi ile Kilo Kaybının Gaita Mikrobiyota Kompozisyonu Üzerine Etkisi

The Effect of Weight Loss by Acupuncture Therapy on Fecal Microbiota Composition in Obese Female Patients

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ÖZ

Amaç: Bu çalışmanın amacı, akupunktur terapisinin obez kadınlarda antropometrik ve biyokimyasal parametreler ile bağırsak mikrobiyotası üzerindeki olası etkilerini araştırmak ve meydana gelen değişiklikleri tanımlamaktır.
Yöntem: Çalışmaya 15 obez (Vücut Kitle İndeksi≥30) kadın birey dahil edildi. Katılımcılara düşük kalorili diyet (diyet grubu, n = 6) veya akupunktur terapisi (12 seans, 12 hafta) ile kombine bir diyet tedavisi (akupunktur grubu, n = 9) uygulandı. Her iki grupta en az 10 kg kaybeden katılımcıların antropometrik ölçümleri, biyokimyasal parametreleri ve fekal mikrobiyota kompozisyonları, çalışmanın başında ve sonundaki (0 ve 12. haftalar) sonuçlara göre karşılaştırıldı. Tedavi öncesi ve sonrası değişiklikler, mikrobiyal genomu temsil etmek amacıyla hedef gen olarak 16S rRNA geni kullanılarak incelendi.
Bulgular: Diyet ve akupunktur grubunda tedavi ile antropometrik parametrelerde ve HOMA-IR düzeylerinde anlamlı düşüş gözlendi. Sadece diyet alan gruba kıyasla, akupunktur uygulanan grupta kilo ve BMI düşüşünün daha fazla olduğu izlendi. Ayrıca akupunktur tedavisi ile Bacteroidia, Prevotella, Butyricimonas, RF39, Coprococcus, Catenibacterium ve Tenericutes taksonları ön plana çıktı.
Sonuç: Bulgular, akupunktur terapisinin obez bireylerde vücut ağırlığı, yağ dokusu, lipid metabolizması ve bağırsak mikrobiyotası üzerinde etkili olduğunu gösterdi. Bu bilgiler ışığında akupunkturun metabolizmadaki rolünü ortaya koyacak daha kapsamlı çalışmalara ihtiyaç olduğu sonucuna varıldı.
Anahtar Kelimeler: Akupunktur, Kilo kaybı, Mikrobiyota, Obezite.

ABSTRACT

Objective: The aim was to investigate the effect of weight loss by acupuncture therapy on anthropometric measurements, biochemical parameters and fecal microbiota.
Method: The study included 15 obese (Body Mass Index ≥ 30) female individuals. Participants received a low-calorie diet (diet group, n = 6) or a diet combined with acupuncture therapy (12 sessions, 12 weeks), (acupuncture group, n = 9). The anthropometric measurements, biochemical parameters, and fecal microbiota compositions of participants from the two groups who lost at least 10 kg were compared regarding the results at the beginning and end of the study (0 and 12th weeks). Pre-and post-treatment changes were examined using the 16S rRNA gene as the target gene to represent the microbial genome.
Results: A significant decrease in anthropometric parameters and HOMA-IR levels was observed in the diet and acupuncture group with the treatment. In comparison to the group that only received diet, acupuncture was found to yield greater weight and BMI reductions. In addition, Bacteroidia, Prevotella, Butyricimonas, RF39, Catenibacterium, Coprococcus, and Tenericutes taxa came to the forefront with acupuncture therapy.

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Conclusion: The findings revealed that acupuncture therapy application has an impact on body weight, adipose tissue, lipid metabolism, and gut microbiota in obese individuals. In light of this knowledge, it was concluded that more comprehensive studies are needed to demonstrate the role of acupuncture in metabolism.

***Key words**:* Acupuncture, Weight loss, Microbiota, Obesity.

1. INTRODUCTION

Obesity, which is described as an energy-balance disorder that occurs with the accumulation of excess fat in the body, is a multifactorial disease induced by a number of genetic, environmental, and behavioral causes (1-3). Obesity has a detrimental effect on quality and length of life by paving the way for dyslipidemia, hypertension, diabetes, metabolic syndrome, cardiovascular disease, musculoskeletal disease, and malignancies (4-6).

Phylogenetics and the diet of organisms are important factors influencing differences in the composition of gut microbiota (7). Studies have shown that gut bacteria change significantly with dietary changes and suggest the presence of microbial dysbiosis in obese patients. Meanwhile, findings indicate that there are changes in the microbiota composition of obese patients who lose weight with various treatment methods and that these changes occur in a healthy way (8-9).

There are several options in obesity treatment, such as diet, exercise, lifestyle changes, medication, and bariatric surgery. Traditional complementary and alternative medicine practices, which are increasingly popular today, have taken their place among effective and reliable treatments for obesity (10). It is known that positive changes in anthropometric and metabolic parameters are observed with acupuncture therapy in obese individuals (11-13). However, it is yet unclear in human studies whether acupuncture has an effect on dysbiosis (8-14), which has been proven to be present in obesity, and if so, in what direction. The aim of this study was to investigate the possible effects of acupuncture therapy on anthropometric and biochemical parameters, as well as on gut microbiota in obese females and to describe any changes that occurred.

2. METHOD

This prospectively designed study was approved by the Necmettin Erbakan University Faculty of Medicine Ethics Committee for Research on Medicine and Non-Medical Devices, dated 11.04.2019 and numbered 2019/1820.

This study was performed on voluntary obese female individuals who applied to Necmettin Erbakan University Meram Faculty of Medicine GETAT Center Center between May 2019 and December 2019 to lose weight. The study was conducted with 15 individuals who displayed the following inclusion criteria: aged 34–45, female gender, body mass index (BMI)≥30 kg/m^2, not taking antibiotic medication in the last six months, no known systemic disease (diabetes mellitus, hypertension, chronic kidney disease, etc.), no smoking or alcohol use, not pregnant, not using supplements such as probiotics, antioxidants, vitamins, etc., and not using systemic drugs.

The participants were divided into two groups: the group that received diet therapy alone (diet group, n=6) and the group that received diet therapy and electroacupuncture therapy together (acupuncture group, n=9). The diet group participants received low-calorie (1400 kcal) diet8 15) therapy for 12 weeks. The acupuncture group participants received acupuncture therapy and a simultaneous low-calorie diet (15) therapy for a total of 12 sessions in 12 weeks on Shen-Men, hunger, larynx, stomach, kidney, and jerome on the ear and ST-24,25,36, Ren-5,7, H-7, Lu-9, PC-6, Du-20, and GV-21 regions on the body. Acupuncture therapy was administered by an acupuncturist with at least three years of experience. A total of 15 individuals who lost at least 10 kg by the end of the study were compared using anthropometric markers (body weight, BMI, waist circumference, waist/hip ratio), biochemical (glucose, insulin, HOMA-IR) measurements, and microbiota at the beginning (0 month) and the end (3rd month) of the study. Anthropometric measurements of the participants were recorded at the beginning and the end of the study (0 and 3rd months). The participants' weight and height were measured with a Tanita SC-330 body analyzer, and their waist and hip circumferences were measured with a tape measure. The measurements were repeated by the same nurse and with the same device. Fasting serum glucose and insulin values that were measured at the beginning (0 month) and the end (3rd month) of the study were recorded, and the HOMA-IR indexes were calculated with the following formula: HOMA-IR=Glucose (mg/dL)×Insulin (mU/L)/405. The fasting serum glucose levels were calculated using Roche Diagnostic Cobas 8000 (c702) systems and the hexokinase process, while the fasting serum insulin levels were determined using Roche Diagnostic Cobas 6000 (c501) systems and the electrochemiluminescence method.

Before the stool samples were taken at the beginning (0 month) and the end (3rd month) of the study, the individuals were informed about the collection of the samples. No laxatives were used. While taking the samples, the first part was eliminated, and the next part was examined. A mixture was made by scooping a small amount from the middle of the stool and transferring a spoonful (approximately 500 mg) to a plastic container with a spoon. Until the day of the DNA extraction procedure, samples were held at -80 ºC. An average of 50–60 ng of genomic DNA was obtained using the QuickGene (Kurabo) extraction kit. Quality and quantity controls of isolated DNA samples were carried out using the Nanodrop and Qubit fluorometer methods. Amplicon sequencing was performed on the Illumina HiSeq platform based on pair-end algorithms. The 16S rRNA V3-V4 region of the isolated DNAs was amplified by PCR. The primers targeting the V3-V4 region used were 341 F (5-CCTAYGGGRBGCASCAG-3) and 806R (5-GGACTACNNGGGTATCTAAT-3). The method was carried out in the form of library creation, the addition of adapter molecules, bridge PCR and clustering, and sequencing.

Reading pairs were separated with unique molecular barcodes. Paired-end reads were combined using FLASH (16). Analyses were performed with filtering according to QIIME quality control instructions (17). Chimeric strings were defined and removed by comparing them with the reference database using UCHIME (18). UPARSE was used to cluster operational taxonomic units (OTU) with a 97% similarity limit. For taxonomic classification, OTUs were mapped using an optimized version of the SILVA database that included the 16S V3-V4 region (19). MUSCLE was used to obtain the phylogenetic relationship of OTU representative sequences (20). Alpha diversity and beta diversity analyses were performed with the OTU tables created.

In order to compare anthropometric measurements and biochemical analyses, the difference between diet and acupuncture group measurements was analyzed using the Mann-Whitney U test, and the difference between the measurements of each group before and after treatment was analyzed using the Wilcoxon test and IBM SPSS Statistics 22 program. For study purposes, the $p<0.05$ level was considered statistically significant.

Alpha diversity analysis was performed to examine the microbiota differences within the group. Observed OTUs, Shannon, Pieloue's evenness, and Faith pd indexes were used for this purpose. Beta diversity analysis was conducted to evaluate the composition differences between the groups. Graphs were drawn using Jaccard, Bray Curtis, Weighted and Unweighted Unifrac metric, and Principal Coordinates Analysis (PCoA). The statistical significance of taxonomic biomarkers between two different situations was examined using linear discriminant analysis effect size (LEfSe) analysis. Adonis and Multi-Response Permutation Procedure (MRPP) analyses were performed, with a $p<0.05$ level considered statistically significant.

3. RESULTS

Table 1. Data on Pre-Treatment Age, Anthropometric and Biochemical Measurements of Obese Individuals.

	Acupuncture Group (n=9)	Diet Group (n=6)	*p* value
Age (Year)	40,00	38,00	0,145
Body weight (kg)	86,40	89,55	0,689
BMI (kg/m²)	33,71	34,04	0,456
Waist circumference (cm)	99,00	98,00	0,955
Waist/Hip Ratio	0,83	0,82	0,607
Glucose(mg/dL)	94,30	97,00	0,529
Insulin (mU/L)	11,20	10,95	0,776
HOMA-IR	2,46	2,59	0,955

All values are mentioned as median. BMI: Body Mass Index, HOMA-IR: Homeostatic model assessment for insulin resistance, SD: Standard deviation.

Table 2. Data on Comparison of Anthropometric and Biochemical Measurements of Groups Pre and Post Treatment.

	Acupuncture Group			Diet Group			
	0 month	3rd month	*p* value*	0 month	3rd month	*p*value*	*p** value*
Body weight (kg)	86,40	75,00	0,008	89,55	79,20	0,027	<0,001
BMI (kg/m²)	33,71	29,38	0,008	34,04	30,23	0,028	0,004
Waist circumference(cm)	99,00	91,00	0,007	98,00	92,25	0,027	0,723
Waist/Hip Ratio	0,83	0,81	0,114	0,82	0,81	0,066	0,858
HOMA-IR	2,46	1,77	0,008	2,59	2,15	0,046	0,480

All values are mentioned as median. BMI: Body Mass Index, HOMA-IR: Homeostatic model assessment for insulin resistance. *p*:* It represents the result of the statistical analysis of each group before and after treatment, ***p**:*** It represents the result of the statistical analysis obtained by comparing the post-treatment changes of acupuncture and diet group data.

The ages and anthropometric and biochemical measurements of a total of 15 individuals (acupuncture group, n=9; diet group, n=6) included in the study as a consequence of two different treatment methods are summarized in Table 1. There was no significant difference observed in pre-treatment (0 month) levels of age, body weight, BMI, waist circumference, glucose, insulin, and HOMA-IR of the groups ($p>0.05$). While a significant decrease was observed in body weight, BMI, and waist circumference measurements with three months of treatment in both groups who received acupuncture therapy and diet therapy alone ($p<0.05$), no significant change was observed in waist/hip ratio ($p>0.05$). Both groups' HOMA-IR levels fell dramatically as a result of therapy ($p < 0.05$), (Table 2). When the two groups were compared, acupuncture was found to be more effective in terms of weight loss ($p<0.001$) and BMI ($p=0.004$) changes.

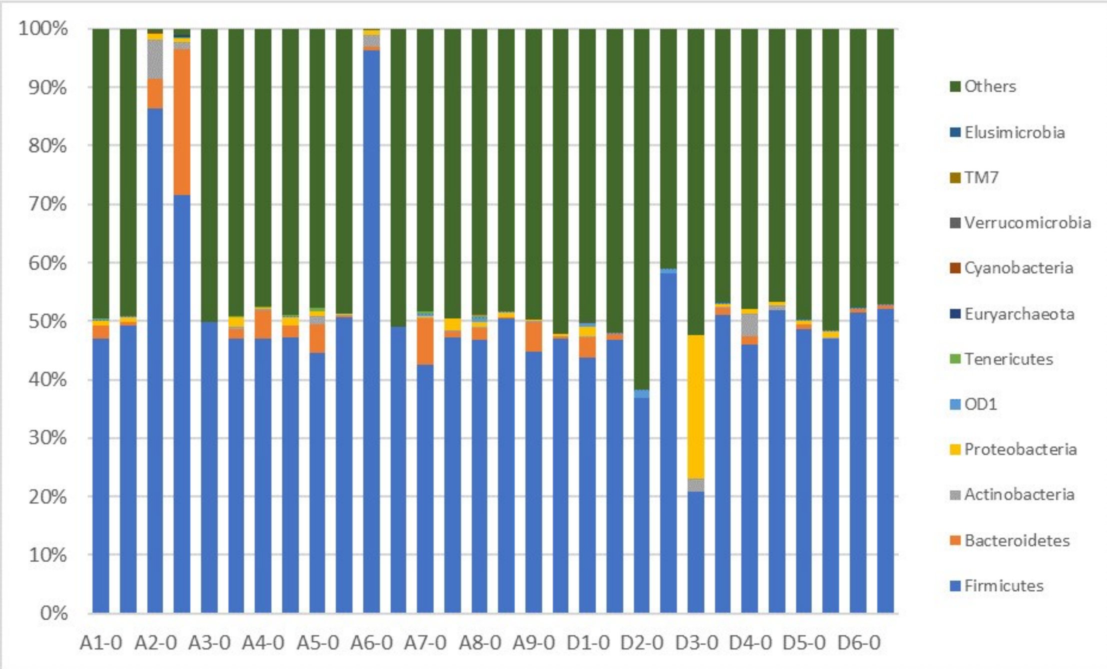


Figure 1. Relative abundance rates at phylum level in gut microbiota. The taxonomic relative abundance ratio of each participant at phylum level is shown as (0) for pre-treatment 0 month and as (3) for after treatment 3rd month. A: Acupuncture, D: Diet group. The most abundant 15 OTUs were represented, the remainders were added to the group others.

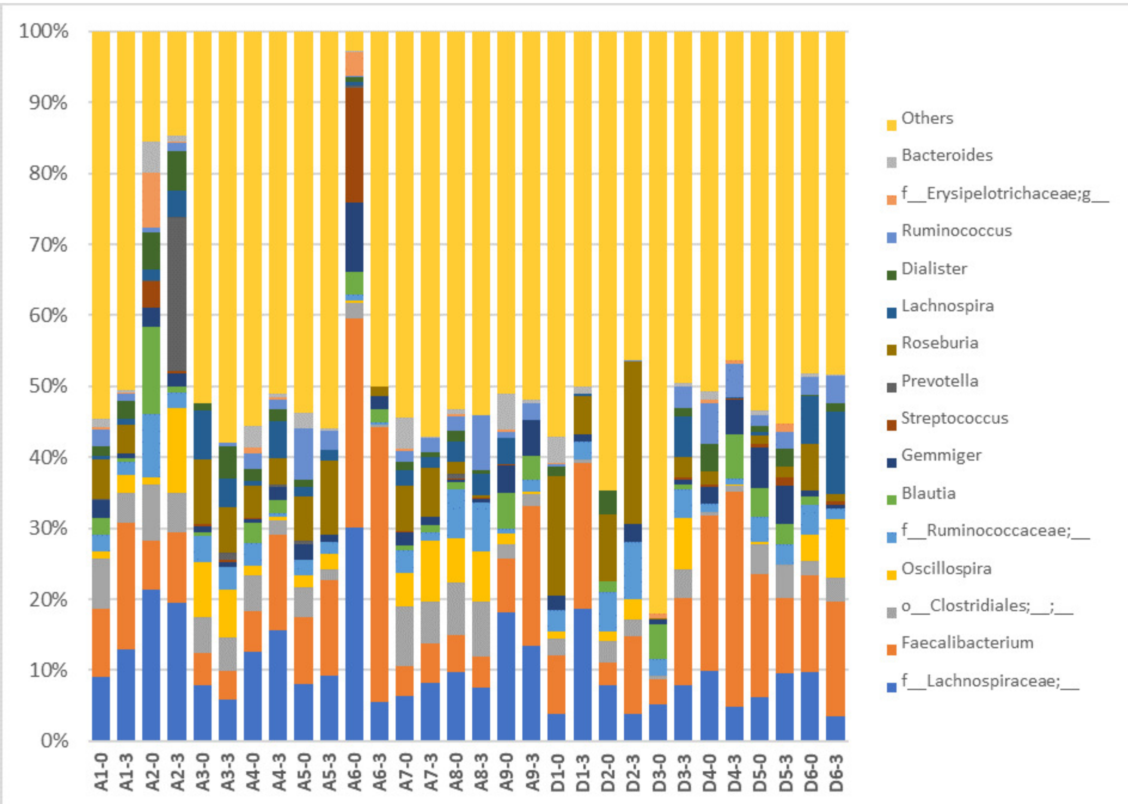


Figure 2. Relative abundance rates at genus level. The taxonomic relative abundance ratio of each participant at genus level is shown as (0) for pre-treatment 0 month and as (3) for after treatment 3rd month. A: Acupuncture, D: Diet group. The most abundant 15 OTUs were represented, the remainders were added to the group others.

In terms of changes in waist circumference, waist/hip ratio, and HOMA-IR levels, however, it was observed that the treatment methods did not differ (*p>0.05*). All except the 15 most common taxa were classified into the group *others* as a result of the readings obtained from 30 samples and the classifications made by QIIME. The taxa detected at the phylum level are shown in Figures 1. *Firmicutes* (61.99%), *Bacteroidetes* (5.44%), *Actinobacteria* (1.57%), and *Proteobacteria* (1.04%) were the most abundant phyla. In the samples before and after treatment of the individual coded as A2, it can be seen that the rate of the *others* group is low, and there is an increase in the relative abundance of *Prevotella* in the post-treatment sample. It can be seen that A6's rate of *Streptococcus* and *Gemmiger* was higher at the beginning of the treatment, and the rate of the *others* group was low (Figure 2). The most abundant taxa at the genus level were an unclassified genus from the *Lachnospiraceae* family (15.16%), *Faecalibacterium* (12.85%), an unclassified genus from *Clostridiales* (4.42%), and *Oscillospira* (3.55%) (Figure 2,3). It can be shown that the samples have a similar phylum relative abundance profile and that the rates of taxa combined in the *others* group in the samples before and after treatment in an individual coded as A2 are lower than the general rates. Again, the rate of taxa combined in the *others* group of individuals coded as A6 was low at the beginning of the treatment but increased to levels similar to the general rates after treatment.

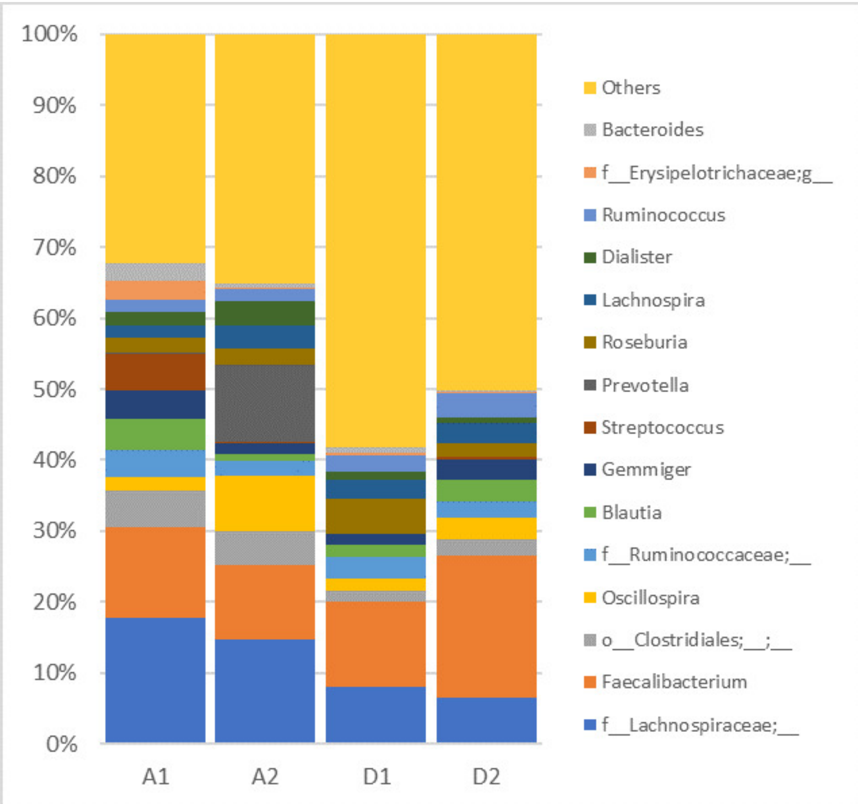


Figure 3. The most abundant taxa relative to the genus level for each group are shown in the figure. A1 (Acupuncture group 0 month; n = 9), A2 (Acupuncture group 3rd month; n = 9), D1 (Diet group 0 month; n = 6), D2 (Diet group 3rd month; n = 6)

Although the relative abundance rate of *Proteobacteria*, a group that comprises the majority of bacteria known as pathobiotics (21), is higher in the sample taken at the beginning of the treatment of D3, it can be observed visually that the rate regresses to similar rates to other samples after treatment (Figure 1). When the relative abundance rates at the genus level are analyzed, it can be seen that the microbiota profiles of the individuals have high heterogeneity, but there are not very large differences in general with treatment.

Bacterial alpha diversity analyses of the beginning, acupuncture, and diet groups were found to be similar before and after the treatment (p<0.05). Jaccard, Bray Curtis, Weighted Unifrac, and Unweighted Unifrac metric and PCoA analyses demonstrated that overall gut microbiota composition diversity was similar between the groups (Figure 3, 4). In those who received both acupuncture and diet, there was a substantial difference with the treatment in terms of composition variety before and after the procedure. However, this difference was found to be low (*p=0.002*, A=0.1037). When samples were analyzed before and after treatment in the group undergoing acupuncture therapy, the LEfSe analysis test indicated that there was a difference in abundance at different taxonomic levels after treatment. It was observed that *Bacteroidia, Prevotella, Butyricimonas, RF39, Coprococcus, Catenibacterium*, and *Tenericutes* taxa came to the fore with the treatment [Linear Discriminant Analysis (LDA) score>2] (Figure 5).

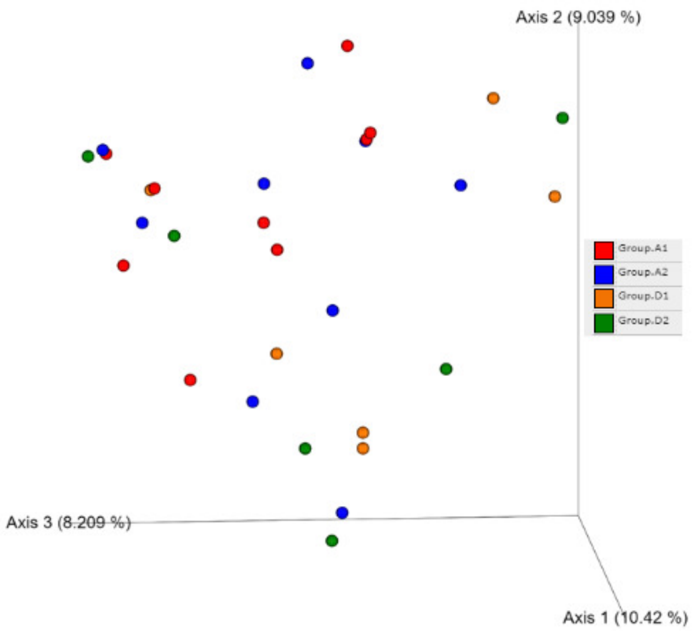
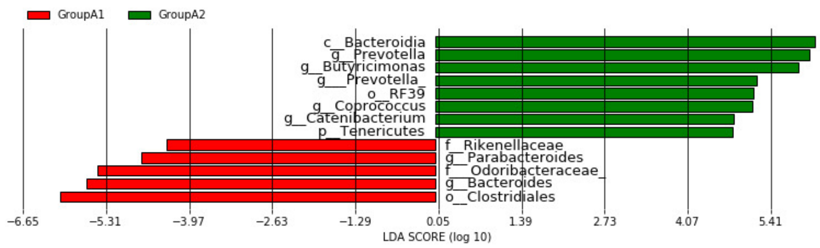


Figure 4. Beta diversity analysis results obtained by the Bray-Curtis method. Red points represent group A1 (Acupuncture group 0 month; n = 9), blue points group A2 (Acupuncture group 3rd month; n = 9), orange points



group D1 (Diet group 0 month; n = 6), green points represent group D2 (Diet group 3rd Month; n = 6). There is no difference observed between the groups.

Figure 5. Microorganisms that are prominent with LefSe analysis. LefSe identifies different statistically significant biomarkers between groups. LDA ((Linear Discriminant Analysis) scores for common taxa. It was shown in red on the negative side for group A1 (Acupuncture group 0 month) and in green on the positive side for group A2 (Acupuncture group 3rd month).

4. DISCUSSION

In the present study, it was observed that acupuncture therapy substantially decreased body weight and BMI levels in obese patients, but did not cause an effect on other anthropometric measurements (waist circumference, waist/hip ratio) and HOMA-IR levels when compared to diet therapy. Furthermore, it was observed that acupuncture and diet therapy together could cause low-level differences in terms of microbiome diversity, and *Bacteroidia, Prevotella, Butyricimonas, RF39, Coprococcus, Catenibacterium*, and *Tenericutes* taxa came to the fore with acupuncture therapy (Figure 5). These findings show that acupuncture therapy influences body weight, adipose tissue, lipid metabolism, and gut microbiota.

In studies performed by Abdi et al. and Zhang et al., it was found that acupuncture therapy provides greater reductions in anthropometric measurements compared to the control group in the treatment of obesity (12-22). The changes in body weight and BMI measurements in the present study are consistent with the literature (12-22). Our findings indicate that acupuncture has an impact on body adipose tissue metabolism and that it is a valuable alternative method for treating obesity that can be used in conjunction with diet. Mazzoni et al. reported that acupuncture had no significant impact on anthropometric parameters (23). This can be explained by the fact that the acupuncture points used in their analysis differed from those used in this study. Acupuncture has been shown to have beneficial effects on several metabolic parameters, including HOMA-IR, in randomized controlled groups in studies conducted by Li et al. and Garcia et al. (13-24). Results showing that acupuncture has no effect on HOMA-IR levels when compared to diet were also stated in the studies conducted by El-Mekawy et al. (25), which were similar to our findings. We believe that further and more detailed studies are needed, despite the fact that the studies indicate that acupuncture may have positive effects on glucose metabolism and insulin resistance.

In the present study, the heterogeneity observed in the microbiota abundance profile of each individual is expected due to the natural diversity that has been proven to exist due to many factors, such as genetics, birth method, and diet. However, among the taxa described in the present study, the most dominant phylum was *Firmicutes* and *Bacteroidetes*, which are known to be dominant in adult microbiota. Human and animal studies have reported that alterations in intestinal microbiota are associated with obesity and that acupuncture has beneficial effects by changing the composition of the intestinal microbiota (26-28). Acupuncture treatment improved anthropometric and metabolic parameters in obese rats but did not change microbial diversity. Instead, acupuncture increased the abundance of *Prevotella-9*, according to a study conducted on rats by Wang et al. (26). In a separate study on mice, Si et al. observed that the abundance of *Fusobacteria*, *Firmicutes*, *Spirochmycete*, *Thermotogae*, *Fibrobacteres*, and *Deferribacteria* increased in obese patients and that when acupuncture was applied, the abundance of these microorganisms gradually returned to levels comparable to non-obese patients (27). The routine culture method was chosen as the analysis method in another study by Xu et al., and it was shown that *Lactobacillus* and *Bifidobacterium* increased after acupuncture therapy in obese individuals compared to the control group (28). In this study by Xu et al., acupuncture was applied to CV12, CV9, BL24, BL26, ST28, ST25, ST36, and SP6 points by two separate practitioners, and *Bacteroides* and *Clostridium perfringens* were found to be reduced in one of the groups (28). In light of these findings, it has been suggested that even acupuncture practitioners may be one of the factors causing differences in results, even though the same points were used. In the present study, it was observed that *Bacteroidia, Prevotella, Butyricimonas, RF39, Coprococcus, Catenibacterium*, and *Tenericutes* taxa came to the fore with acupuncture therapy applied to the participants for a total of 12 sessions in 12 weeks on hunger, larynx, stomach, kidney, jerome for the ear, and ST-24,25,36, Ren-5,7, H-7, Lu-9, PC-6, Du-20, GV-21 regions for the body (Figure 5). Although the reported studies and our study's findings are consistent, the differences can be explained by the geographic location, the type of nutrients in the diet, the points on the body where acupuncture is applied, the application protocol, and the duration of the treatment. These findings suggest that acupuncture treatment may play a potential role in controlling body weight via the intestinal–brain axis and regulating lipid metabolism by affecting the gut microbiome structure. It can also be interpreted that acupuncture has positive effects on metabolic pathways and causes anthropometric parameters and the microbiome to change.

Limitations

The number of subjects participating in the study was small. It might be more beneficial to confirm the study's findings with further studies with a larger sample size.

5. CONCLUSION

According to the findings of the study, acupuncture has beneficial effects on anthropometric parameters in the treatment of obesity and can induce changes in the gut microbiota structure. Acupuncture can highlight *Bacteroidia, Prevotella, Butyricimonas, RF39, Coprococcus, Catenibacterium,* and *Tenericutes* taxa. In light of these changes in the gut microbiota, it can be thought that acupuncture has therapeutic effects on obesity and functions as a new mechanism underlying these effects. We believe that further comprehensive studies involving microbiome members, products, and related molecules are needed to assess the correlation between gut microbiota dysbiosis and obesity and to examine the clinical effects of acupuncture therapy in order to fully comprehend the effects of acupuncture on obesity, gut microbiota, and metabolism. Our study is about specifically identifying the microbiota of the effects of acupuncture on obesity, which is rarely mentioned in the literature. There are very few studies in the literature that specifically reveal the changes in microbiota and types of acupuncture used in the treatment of obesity. We hope that our study will contribute to the literature on this subject.

Ethical Consideration of the Study

This prospectively designed study was approved by the Necmettin Erbakan University Faculty of Medicine Ethics Committee for Research on Medicine and Non-Medical Devices, dated 11.04.2019 and numbered 2019/1820.

Conflict of Interest Statement

No potential conflict of interest was reported by the authors.

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