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Effects of Moxibustion at ST36 on Body Temperature, Gut Microbiota, and Gut Microbiota in Healthy Females: A Pilot Study Exploring the Gut-Brain Axis

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Abstract

Background: In traditional East Asian medicine, moxibustion is widely used for thermal therapy and gastrointestinal regulation. While clinical evidence supports its efficacy, the underlying scientific mechanisms remain unclear. This study investigated the physiological, psychological, and microbiological impacts of pedal moxibustion. **Methods:** Ten healthy, moxibustion-naive female volunteers participated in a two-week winter study. Participants applied pedestal-type moxibustion cones to the bilateral *Zusanli* (ST36) acupoint daily. Physiological changes were evaluated via core (axillary) and cutaneous temperatures (forehead, hand, and foot). Psychological anxiety was assessed using the State-Trait Anxiety Inventory (STAI). Fecal samples were collected before and after the intervention for 16S rRNA gene-based gut microbiota analysis, alongside qualitative feedback on somatic conditions. **Results:** Due to pronounced individual variations, no statistically significant group-level differences were observed in STAI scores or body temperatures, although state anxiety showed a downward trend and axillary temperature tended to increase. In contrast, microbiota analysis revealed distinct shifts in microbial

profiles across all participants, including an increased relative abundance of the beneficial genus *Faecalibacterium* in half of the subjects. Furthermore, qualitative feedback indicated notable improvements in somatic symptoms: six participants reported increased bowel movement frequency, six noted altered stool consistency, three experienced longer sleep duration, and two reported reduced menstrual pain. **Conclusion:** A two-week pedal moxibustion intervention induced distinct, individualized shifts in gut microbiota profiles alongside qualitative improvements in bowel habits, sleep, and menstrual discomfort. These findings suggest that traditional moxibustion may influence host homeostasis through the gut-brain axis, potentially driven by mild thermogenesis. Large-scale, randomized controlled trials are required to validate these preliminary insights.

Keywords

Moxibustion, Body Temperature, Gut Microbiota

1. Introduction

In the East Asian cultural sphere, moxibustion has long been established as a traditional thermal therapy with a rich history spanning thousands of years, during which it has been widely integrated into daily healthcare practice. In recent years, modern molecular biological findings have begun to unveil its scientific basis, revealing the crucial involvement of transient receptor potential (TRP) channels—specifically thermo-TRP channels—in mediating cellular responses to such thermal stimulation. Building upon these cellular-level discoveries, several physiological studies have successfully reported the diverse systemic effects of moxibustion-like stimulation, particularly its regulatory impacts on central brain function and the enhancement of local muscular blood flow [1] [2]. Furthermore, ongoing research has been actively elucidating its complex mechanism of action on the autonomic nervous system by utilizing heart rate variability (HRV) as a non-invasive, quantitative index of sympathetic and parasympathetic activity [3] [4].

Clinically, moxibustion applied to specific acupoints on the lower limbs, most notably SP4 (*Sanyinjiao*), has been documented to elevate skin temperature not only locally but also remotely in the lateral and abdominal regions, thereby effectively mitigating menstrual pain and related discomforts [5]. Thus, it has been empirically and observationally recognized since ancient times that pedal stimulation contributes significantly to the regulation and homeostasis of the digestive system. However, despite its widespread utilization and perceived efficacy in modern clinical settings, the rigorous accumulation of robust scientific evidence and its underlying multisystemic mechanisms remain insufficient. Therefore, to bridge this gap between traditional empirical knowledge and modern science, this study aims to comprehensively elucidate the physiological and psychological im-

pacts of pedal moxibustion on the abdominal region. To achieve this, we employ a multifaceted approach that incorporates objective peripheral body temperature measurements, validated psychological testing via the State-Trait Anxiety Inventory (STAI), and an exploratory analysis of the gut microbiota.

2. Materials and Methods

2.1. Participants

Ten healthy female volunteers, who were recruited via a poster, participated in this study. The demographic characteristics of the participants were as follows: mean age, 43.7 ± 14.8 years (range: 20 - 70); mean height, 157.5 ± 4.5 cm (range: 150 - 163); mean weight, 52.6 ± 3.8 kg (range: 46 - 59); and mean Body Mass Index (BMI), 21.1 ± 1.0 kg/m^2 (range: 19.6 - 22.5). Informed consent was obtained from all subjects. This study was approved by the Medical Ethics Committee of Chuo University (Approval No. 2024-094(2)).

2.2. Moxibustion Points and Duration

Pedestal-type moxibustion (*Chosei-kyu*, Yanmasho, Shiga, Japan) [5] was utilized, with three cones applied to the ST36 (*Zusanli*) acupoint on each leg. Applications were performed daily for two consecutive weeks (**Figure 1(a)**). None of the ten participants had previously experienced moxibustion. Prior to obtaining informed consent, licensed moxibustion instructors explained the experimental procedure to each participant individually in a clear and accessible manner.

We asked all participants to keep a diary during the experimental period. A professional moxibustion instructor directly interviewed every subject regarding the implementation of the treatment (including missed sessions, moxibustion sites, and time of day); no burns, discomfort, or other adverse events were reported.

Data on bowel movements and stool consistency were collected through predistributed questionnaires and subsequently verified via interviews with all subjects. The experiment was conducted in December.

2.3. State-Trait Anxiety Inventory (STAI)

To evaluate psychological status (anxiety levels), the State-Trait Anxiety Inventory (STAI) was employed. This widely used self-report tool features two 20-item subscales: the STAI-S for current (state) anxiety and the STAI-T for long-standing (trait) anxiety.

2.4. Body Temperatures

Body temperatures were measured by moxibustion instructors according to previously established protocols [6]. Axillary body temperature was measured using a clinical mercury thermometer (FAVOR, Nihon Keiryoki Kogyo Co., Ltd., Chiyoda, Japan). Concurrently, cutaneous body temperature was measured using

a contactless digital thermometer (Microlife, IR 1DA1, Microlife, Switzerland) before and after the intervention at the following locations: *Yintang* (on the forehead, midway between the medial ends of the eyebrows), LI4 (*Hegu*; on the dorsum of the hand, radial to the midpoint of the second metacarpal bone), and LR3 (*Taichong*; on the dorsum of the foot, between the first and second metatarsal bones, in the depression distal to the junction of the bases of the two bones, over the dorsalis pedis artery) (**Figure 1(a)**).

2.5. Gut Microbiota Analysis

DNA extraction from fecal samples was performed by Metagen, Inc. (Tsuruoka, Japan). Fecal samples were freeze-dried for at least 24 hours using a VD-800R freeze-dryer (TAITEC, Japan). The samples were then subjected to vigorous shaking with 3.0-mm zirconia beads using a Shake Master (Biomedical Science, Japan) to rupture the cell membranes (1500 rpm for 10 min). After disruption, 10 mg of each sample was weighed, and 0.1-mm zirconia/silica beads were added. DNA extraction was performed as previously reported [7].

For 16S rRNA gene amplicon sequencing and bioinformatics analysis, amplification of the 16S rRNA gene was carried out using forward (27F-mod) and reverse (338 R) primers, and the amplified DNA was sequenced using a NextSeq 1000 system (Illumina, United States). The 16S rRNA gene-based microbiome analysis was performed using QIIME2 (version 2019.10) and DADA2. The amplicon sequence variants (ASVs) were assigned to taxa using the Silva database (version 132).

2.6. Statistical Analysis

The statistical significance of differences between values was evaluated using paired t-tests. P-values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics software for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Means (± standard deviations) were used to characterize the distribution of continuous variables.

2.7. Maintaining the Integrity of the Specifications

During the body temperature measurements and the collection of STAI questionnaires, the instructors gathered qualitative data regarding the participants' personal experiences, including menstrual or lower back pain, sleep quality, and bowel movements.

3. Results

3.1. STAI

The trait values of the STAI exhibited a mean of 43 ± 8.7 (range: 28 - 57). The state anxiety scores showed a decreasing trend in average values, moving from a pre-intervention mean of 41 ± 6.4 (range: 34 - 57; median: 38) to a post-intervention mean of 36 ± 8.6 (range: 21 - 46; median: 38). However, due to large individual

differences, the median remained unchanged, and no statistically significant difference was observed (Figure 1(b)).

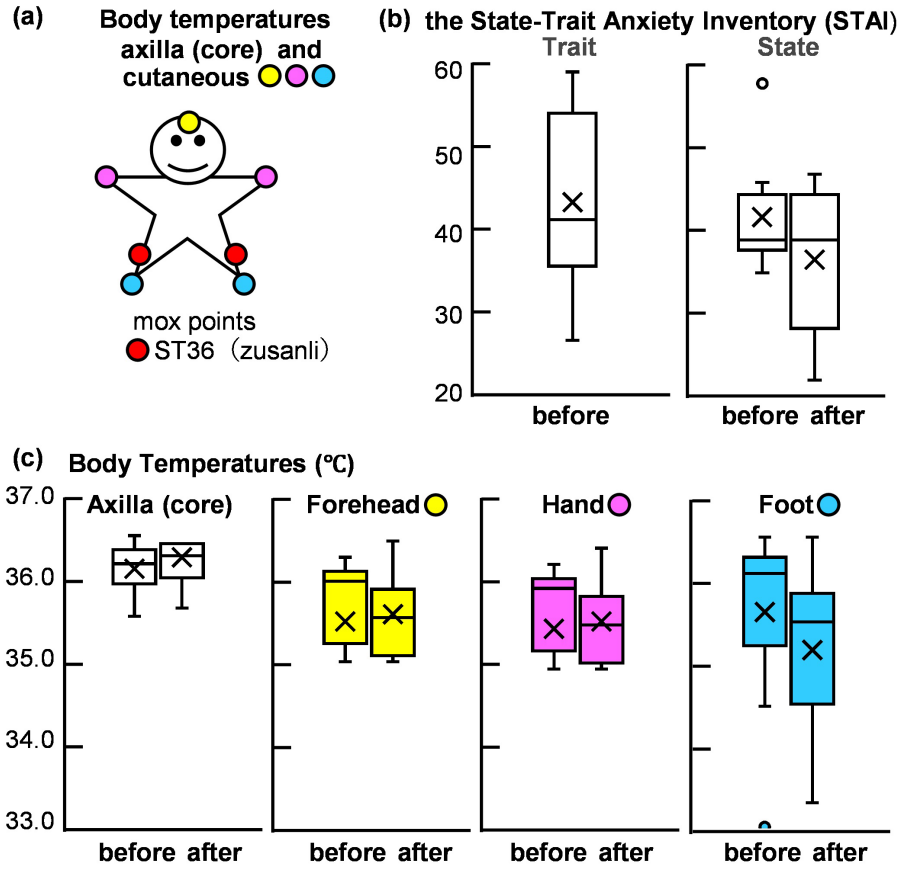

Figure 1. (a) Location of the measurement points (body temperature) and moxibustion sites; (b) State-Trait Anxiety Inventory (STAI) scores: comparison of trait conditions before and after the intervention; (c) Effects of moxibustion on body temperatures: axillary (core) and cutaneous (forehead, hand, and foot) temperatures.

3.2. Body Temperatures

Although the mean axillary body temperature showed an upward trend, substantial inter-individual variations were observed across all measurement sites (axilla, forehead, hand, and foot), and no statistically significant differences were detected. The pre- and post-intervention temperatures were as follows:

Axilla: 36.2˚C ± 0.3˚C (range: 35.6 - 36.6, median: 36.3) → 36.3˚C ± 0.4˚C (range: 35.7 - 37.2, median: 36.4);

Forehead: 36.5˚C ± 0.2˚C (range: 36.0 - 36.8, median: 36.6) → 36.4˚C ± 0.5˚C (range: 35.4 - 37.5, median: 36.5);

Hand: 35.5˚C ± 1.1˚C (range: 32.5 - 36.3, median: 36.0) → 35.6˚C ± 0.5˚C (range: 35.0 - 36.5, median: 35.6);

Foot: 35.7˚C ± 1.1˚C (range: 33.0 - 36.6, median: 36.2) → 35.2˚C ± 1.0˚C (range: 33.3 - 36.6, median: 35.6) (Figure 1(c)).

3.3. Gut Microbiota Analysis

The relative abundance of the genus *Faecalibacterium* increased in 5 out of the 10 participants. Although marked individual differences in the gut microbiota precluded the observation of statistically significant differences overall, distinct compositional shifts were observed in all subjects between the pre- and post-experimental stages (Figure 2).

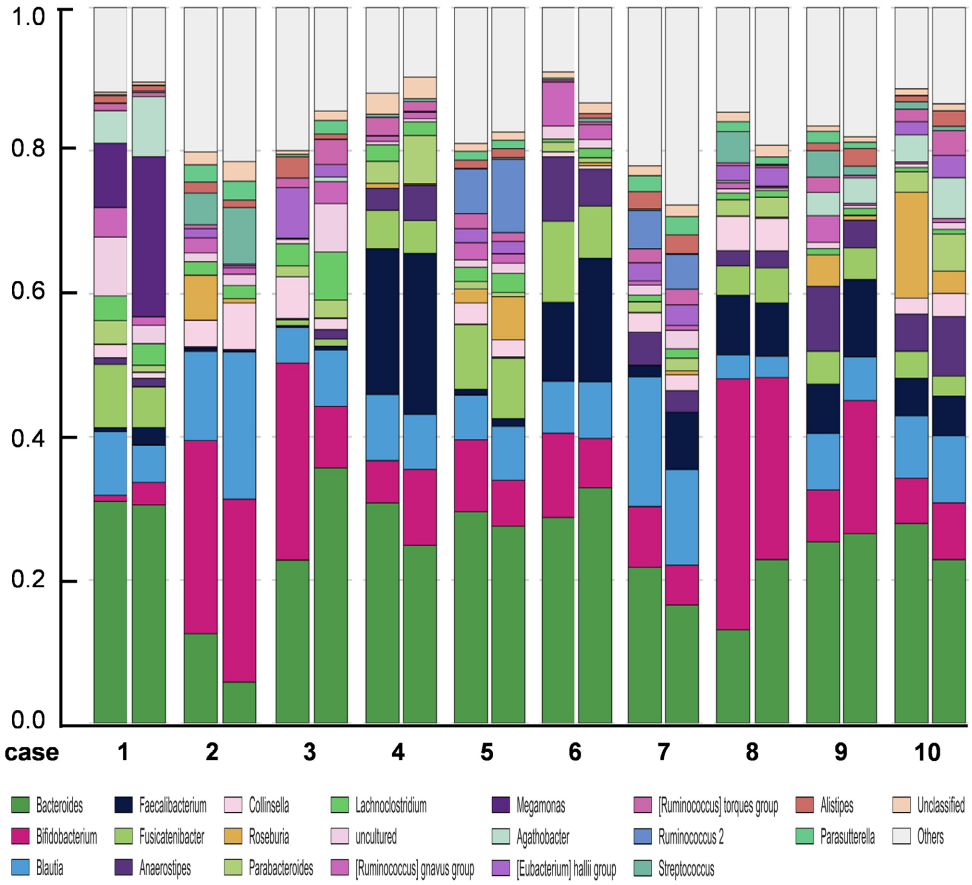

Figure 2. Comparison of gut microbiota before and after the intervention (proportions). Results are shown for ten participants. For each case, the left bar represents the pre-experimental state and the right bar represents the post-experimental state.

3.4. Participants' Personal Feedback

Among the ten participants, two reported reduced menstrual pain, three experienced an increase in sleep duration of one hour or more, and six reported an increased frequency of bowel movements. At that time, stool consistency altered in all of those six individuals.

However, these outcomes should be interpreted as qualitative participant reports rather than evidence of clinical improvement.

4. Discussion and Conclusion

It is suggested that *Yojo* (health cultivation) practices in traditional medicine po-

tentially influence host homeostasis through alterations in the gut microbiota and the gut-brain axis.

First, regarding the relationship between axillary body temperature elevation and the gut microbiota, Takeda *et al.* reported that an increase in body temperature during hot spring bathing leads to a significant increase in Bifidobacterium bifidum [8]. Although a tendency toward hypothermia is generally noted in patients with chronic diseases, including cancer [9] [10], our intervention revealed an increase in the relative abundance of the genus *Faecalibacterium* accompanying a trend toward elevated body temperature. Although this change did not reach statistical significance, these findings suggest a potential link between thermogenesis (body temperature elevation) and the proliferation of specific beneficial bacteria.

Second, a key characteristic of many traditional medical interventions is their direct or indirect action on the gastrointestinal tract. For instance, many Kampo medicines are administered as decoctions; they exert their pharmacological effects by passing through the intestinal tract, which houses approximately 70% of the body's immune cells and harbors a vast microbiota. Furthermore, regarding moxibustion—a therapy involving thermal and physical stimulation of the body surface—the acupoint Zusanli (ST36), frequently used to promote health, is known to activate gastrointestinal motility via the autonomic nervous system. In recent years, there has been a rise in research aimed at elucidating the mechanisms of traditional medicine from the perspectives of the immune system and gut microbiota. Yamashita *et al.* demonstrated that the tumor cell growth-inhibitory effect of *Inchinkoto* depends on the composition of the gut microbiota [11], while Shi *et al.* reported immunological effects of *Daikenchuto* administration, such as the activation of group 3 innate lymphoid cells (ILC3) and the amelioration of experimental colitis [12]. These findings suggest that traditional medical interventions have the potential to boost immunity through the intestinal tract.

Third, physical symptoms associated with vague complaints—such as sensitivity to cold, chronic lower back pain, and menstrual pain—often stem from psychosocial stress or negative emotions manifesting as abnormal peripheral sensations [13]. Recent research on the "brain-gut axis" suggests that functional changes in the serotonin (5-HT) system within the insular cortex are involved in these seemingly localized physical sensations [14]. The gastrointestinal tract, which produces approximately 90% of peripheral serotonin, is also a primary site where the gut microbiota modulates the immune system [15]. This implies that traditional medical therapies (such as moxibustion) may influence the close interrelationship between central emotional and cognitive networks by acting through the gut environment.

Based on this background, we hypothesized that a health-promoting practice (moxibustion) might alter the gut microbiota in a manner beneficial to the host by raising body temperature. To test this hypothesis, we conducted a multifaceted analysis incorporating body temperature measurements, psychological indicators,

and gut microbiota profiling.

This study employed a single-group, pre-post intervention design without a control group. Consequently, changes in the gut microbiota or symptoms cannot be definitively attributed to the moxibustion treatment. Furthermore, the findings are merely the results of exploratory, intra-individual observations.

This study has several limitations. First, the sample size was limited to ten female participants, and the study was conducted exclusively during the month of December. Second, the moxibustion treatment was self-administered at home, meaning the results may have been influenced by confounding variables, such as variations in dietary and lifestyle habits. Third, while the State-Trait Anxiety Inventory (STAI) was utilized to measure anxiety, it is necessary to consider the potential impact of anxiety specifically related to the moxibustion procedure itself among participants who were novel to the practice.

5. Conclusion

Nevertheless, it is noteworthy that changes in gut microbiota profiles were observed in all participants following the intervention. Future verification of this hypothesis will require larger-scale studies involving an increased number of participants and a control group, while strictly controlling for confounding factors such as dietary intake and lifestyle habits.

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Author Contributions

Conceptualization, M.W., K.O., T.M., T.Y., N.M. and C.T.; methodology, T.N. and M.R.; formal analysis, M.W., K.O., T.M. and N.M.; investigation, M.W., K.O. T.M., T.Y. and T.K.; data curation, M.W., K.O. T.M., T.Y. and T.K.; writing—original draft preparation, M.W., K.O. T.M., T.Y. and T.K.; writing—review and editing, T.N., M.R., T.N., N.M., C.T. and T.T.; visualization, X.X.; supervision, M.W.; project administration, M.W.; funding acquisition, M.W. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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