

# Evaluating the Psychological and Physiological Effects of Hippotherapy on Young Individuals with Autism Spectrum Disorder: A Study Utilizing POMS2 and Salivary Amylase Markers

Osamu Nakata<sup>1</sup>, Hisatugu Sasai<sup>2</sup>

<sup>1</sup>Department of Rehabilitation, Naragakuen University, Nara, Japan

<sup>2</sup>TOMOMO, a Child Development Support and After-School Day Services Center, Kyoto, Japan

Email: otnakata@nara-su.ac.jp, coccinelle2022tomomo@gmail.com

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## Abstract

Autism Spectrum Disorder (ASD) is characterized by deficits in social and communication skills, emotional dysregulation, and challenges in adaptive functioning. Hippotherapy, a therapeutic intervention utilizing interactions with horses, has shown promising psychological and physiological benefits across various conditions. This study aimed to evaluate the effects of hippotherapy on young individuals with ASD using validated tools: the Profile of Mood States-Second Edition (POMS2) and salivary amylase activity as objective measures of stress and mood regulation. A total of 23 participants diagnosed with ASD were recruited from a child development support center in Kyoto Prefecture, Japan. Each participant underwent a single-session hippotherapy intervention, which included riding a horse on a 1500-meter course under the supervision of an occupational therapist. Pre- and post-intervention POMS2 scores and salivary amylase activity were compared to assess changes in mood states and stress levels. Results indicated significant improvements in certain POMS2 subscales, suggesting potential benefits of hippotherapy in enhancing emotional regulation and social adaptability. However, no significant changes were observed in overall salivary amylase activity, although individual analyses revealed reduced stress in participants with high pre-intervention stress levels. For individuals predisposed to tantrums, initial sessions occasionally increased stress levels, highlighting the need for tailored interventions. This study provides preliminary evidence for the psychological and physiological benefits of hippotherapy in ASD populations and underscores the importance of utilizing standardized assessment tools for evaluating therapeutic interventions. Future research should focus on longitudinal designs

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and larger sample sizes to comprehensively examine the sustained effects of hippotherapy and its potential as a therapeutic strategy for enhancing the well-being of individuals with ASD.

## Keywords

Autism Spectrum Disorder, Hippotherapy, POMS2, Salivary Amylase Markers

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## 1. Introduction

Autism Spectrum Disorder (ASD) is characterized by significant impairments in social and communication skills, as well as restricted and repetitive patterns of behavior. Individuals with ASD often struggle with regulating emotions, such as anger and anxiety, which poses considerable challenges in their daily lives. Difficulties in social and communication abilities frequently lead to complex interpersonal situations [1] [2], underscoring the importance of managing emotions like anger, confusion, anxiety, and tension in these contexts. Furthermore, research has shown that stress and trauma can have profound effects on the psychological well-being of individuals with ASD [3].

Some scholars suggest that behaviors often perceived as “unusual” among individuals with ASD are not simply attempts to seek attention but are instead mechanisms to fulfill fundamental needs, such as safety, affection, and acceptance [4]. However, despite progress in formal inclusion within educational settings, many individuals with ASD still do not receive adequate support in practice [5]. These systemic gaps exacerbate the emotional and psychological challenges faced by individuals with ASD, significantly impacting their overall well-being.

Hippotherapy, a supplementary therapeutic approach, leverages interactions with animals to provide therapeutic benefits for a variety of dysfunctions and disabilities. It has been demonstrated to improve motor functions in children with cerebral palsy [6] [7], enhance cardiac functions [8] [9], increase physical capabilities in elderly individuals [10] [11], and aid gait recovery in stroke patients [12]. Additionally, psychological benefits have been observed in individuals with PTSD [13] and those with neurodegenerative diseases [14]. These findings highlight the diverse therapeutic applications of hippotherapy, indicating its potential relevance to the ASD population.

In the context of ASD, several studies have explored the relationship between hippotherapy and the social behaviors of children with ASD [15]-[17]. However, most assessments rely on subjective evaluations from parents or instructors. While Ward *et al.* have noted that the benefits of hippotherapy diminish when the intervention is discontinued [18], Coman *et al.* reported that the positive effects persisted for up to eight weeks post-intervention [19]. These discrepancies may stem from differences in intervention protocols, participant diversity, and evaluation methods. This highlights the need for research employing standardized and

validated psychological and physiological assessment tools. Furthermore, unlike typically developing children, ASD youth exhibit independent development of executive functions and emotional intelligence, which can impede their ability to effectively adapt when confronted with stress [20]. As a result, objective investigations into the effects of hippotherapy on stress management in young individuals with ASD are warranted.

To address these gaps, the present study aims to comprehensively evaluate the psychological and physiological effects of hippotherapy on young individuals with ASD. Utilizing validated assessment tools, namely the Profile of Mood States-Second Edition (POMS2) and salivary amylase activity as a physiological stress marker, this study seeks to provide reliable evidence for the effectiveness of hippotherapy. The findings are expected to contribute valuable insights for the development of treatment and support programs tailored to individuals with ASD.

## 2. Methods

### 2.1. Participants

Participants were recruited from TOMOMO, a center providing Child Development Support and After-School Day Services in Kyoto Prefecture, Japan. The hippotherapy sessions were conducted at an equestrian facility affiliated with the center.

The inclusion criteria were: 1) a diagnosis of ASD, 2) an interest in hippotherapy and a willingness to participate, 3) the ability to cooperate and follow verbal instructions, and 4) no physical disability that would prevent horseback riding. The exclusion criteria were: 1) inability to understand verbal instructions, 2) known allergies to horses, and 3) significant fear of horses.

### 2.2. Hippotherapy

The hippotherapy in this study was conducted by an occupational therapist who is experienced in horse handling. Under the therapist's guidance, participants rode at a normal pace for approximately 10 minutes along a 1500-meter course with inclines and declines, ensuring safety throughout. Depending on their preferences, some participants opted to be led by the therapist or chose to ride on a flat course without inclines.

### 2.3. POMS2

The Japanese version of Profile of Mood States Second Edition-Youth Short (POMS 2<sup>®</sup>-Y Short) was used to assess subjective mood changes before and after horseback riding.

Developed by McNair *et al.*, the Profile of Mood States (POMS<sup>®</sup>) is designed to measure mood and consists of 65 items corresponding to six distinct mood subscales: Tension-Anxiety (T-A), Depression-Dejection (D-D), Anger-Hostility (A-H), Vigor-Activity (V-A), Fatigue-Inertia (F-I), and Confusion-Bewilderment (C-B), and the Total Mood Disturbance (TMD) score is calculated from the scores of each subscale as follows:  $(T-A + D-D + A-H + F-I + C-B) - V-A$  [21]. Finally,

the obtained values are converted using a standard conversion table to derive the T-scores, enabling their subsequent comparison. The reliability and validity of the Japanese version of POMS have been verified [22], and it is reported to reflect patients' emotions and moods as they change with the progression of various diseases [23].

POMS was updated in 2012 to include a new subscale, Friendliness (F), and was re-released as POMS2. POMS2 consists of four self-report measures: Profile of Mood States Second Edition-Adult, Profile of Mood States Second Edition-Adult Short, Profile of Mood States Second Edition-Youth, and Profile of Mood States Second Edition-Youth Short [21]. Each version has a different number of items tailored for two distinct populations. According to Konuma *et al.*, the correlation coefficients between the six subscales of POMS and the corresponding subscales of POMS2 were high in both the full-length and shortened versions, and then scores on the new subscale, Friendliness, showed the strongest correlation with the Vigor-Activity subscale of POMS in both versions [24].

From this point forward in the study, the Japanese version of the Profile of Mood States Second Edition-Youth Short (POMS 2\*-Y Short) will be referred to as POMS2.

#### 2.4. Salivary $\alpha$ -Amylase Activity

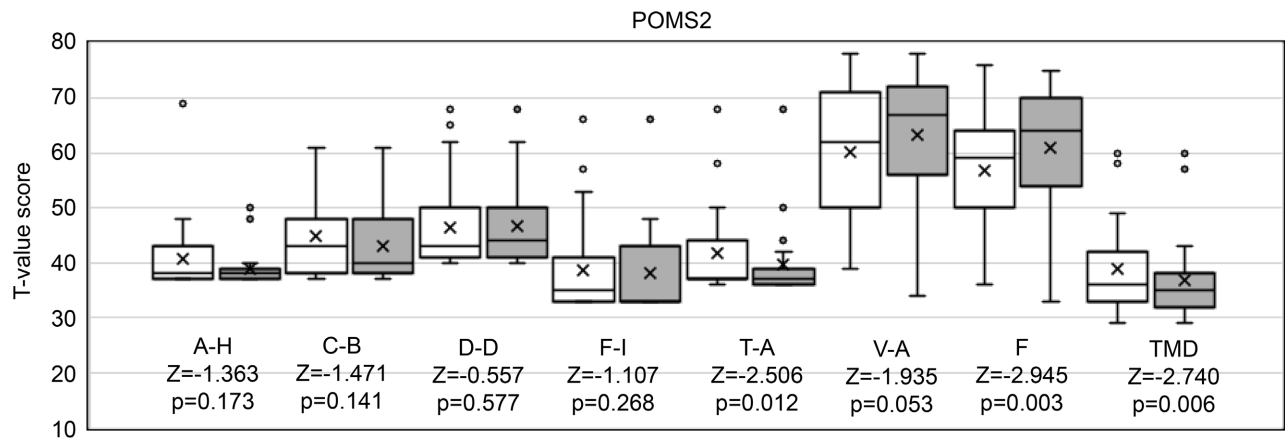
To assess the subjects' stress levels before and after horseback riding, salivary  $\alpha$ -amylase activity (SAA), a recognized stress marker [25], was measured. The primary trigger for salivary amylase secretion is sympathetic nervous activity, and psychological stress also enhances its secretion [26]. Measurements were conducted using a salivary amylase monitor from Nipro Corporation. This device is reputed for its ability to easily measure SAA on-site, being minimally invasive and highly reproducible [27]. To measure SAA, first place a saliva collection paper under your tongue and gather saliva for about 30 seconds. Then, insert the saliva collection paper into the measuring device, which will analyze the SAA in approximately one minute. The measurement results are evaluated as follows: 0 - 30 KU/L indicates no stress, 31 - 45 KU/L indicates slight stress, 46 - 60 KU/L indicates moderate stress, and 61 KU/L or more indicates considerable stress [28].

#### 2.5. Statistical Analysis

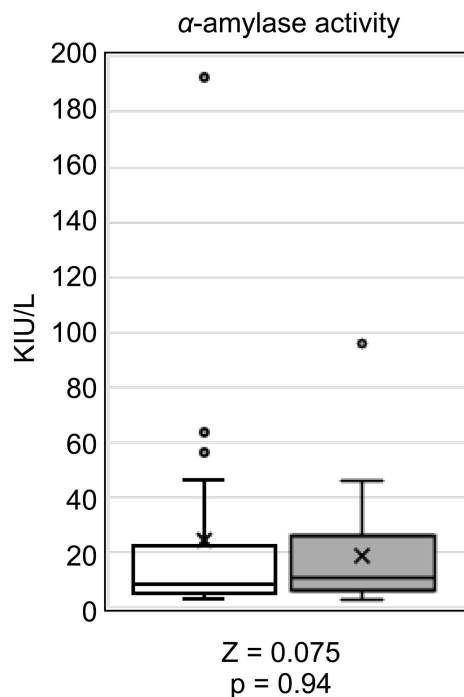
To validate the physiological and psychological impacts of horse riding, we compared SAA and POMS2 scores before and after the activity using the Wilcoxon signed rank test. Statistical analyses were conducted with SPSS version 2, and a significance level of 5% was applied.

#### 2.6. Ethics

This study received ethical approval from the ethics committee of the institution affiliated with the first author. Informed consent was obtained from all participants prior to their involvement in the study.



**Figure 1.** The box-and-whisker plot displays the results of the POMS2, as analyzed by the Wilcoxon Signed-Rank Test, comparing T-values obtained before and after hippotherapy. The white box represents the pre-hippotherapy T-values, while the gray box corresponds to the post-hippotherapy T-values. The upper and lower boundaries of each box denote the third quartile (Q3) and first quartile (Q1), respectively, while the bold horizontal line within the box signifies the median. The upper whisker extends to 1.5 times the interquartile range (IQR), and the lower whisker represents the smallest observed value within the IQR. Data points outside the IQR are marked as individual dots, representing outliers. Additionally, each “X” symbol denotes the mean value. A-H: anger-hostility; C-B: confusion-bewilderment; D-D: depression-dejection; F-I: fatigue-inertia; T-A: tension-anxiety; V-A: vigor-activity; F: friendliness. The TMD score was calculated using the following formula:  $([T-A] + [D-D] + [A-H] + [F-I] + [C-B] - [V-A])$ . Statistical markers include the Z-value (Z) and p-value (p). Statistical significance is denoted as follows: \* $p < 0.05$ ; \*\* $p < 0.01$ .



**Figure 2.** The box-and-whisker plot illustrates the  $\alpha$ -amylase activity results analyzed using the Wilcoxon Signed-Rank Test, comparing pre- and post-hippotherapy values. The white box represents pre-hippotherapy  $\alpha$ -amylase activity, while the gray box corresponds to post-hippotherapy values. The boundaries of each box indicate the first quartile (Q1) and third quartile (Q3), with the bold horizontal line inside the box marking the median. The upper whisker extends up to 1.5 times the interquartile range (IQR), and the lower whisker represents the smallest value within the IQR. Outliers beyond the IQR are depicted as individual dots, while “X” symbols denote the mean values. Statistical indicators, including the Z-value (Z) and p-value (p), suggest no significant difference between the  $\alpha$ -amylase activity levels before and after hippotherapy.

### 3. Results

This study included 23 children and adolescents aged 7 to 18 years who had been diagnosed with ASD. The participants had a mean age of  $11.8 \pm 2.7$  years and a median age of 12 years. Hippotherapy sessions, along with pre- and post-intervention measurements, were conducted between June 2022 and February 2025. Of the 23 participants, 14 were male and 9 were female. All participants successfully completed SAA measurements and responded to POMS2 questionnaire before and after undergoing hippotherapy.

No significant differences were observed in SAA levels before and after hippotherapy (**Figure 1**). Similarly, no significant differences were found in the POMS2 T scores (**Figure 2**) for Anger-Hostility (A-H), Confusion-Bewilderment (C-B), Depression-Dejection (D-D), Fatigue-Inertia (F-I), and Vigor-Activity (V-A). However, a significant decrease was detected in Tension-Anxiety (T-A) ( $p < 0.05$ ), while a significant increase was observed in Friendliness (F) ( $p < 0.01$ ). Additionally, Total Mood Disturbance (TMD) demonstrated a significant decrease ( $p < 0.01$ ).

### 4. Discussion

The increase in POMS2 Friendliness scores and the decrease in Tension-Anxiety and Total Mood Disturbance scores observed in this study suggest that the hippotherapy intervention fosters a tendency toward friendliness and a calmer mood in young individuals with ASD. These findings may be attributable to mechanisms such as hormonal regulation and the promotion of emotional stability through interactions with horses. Evidence from diverse populations underscores the multifaceted impact of hippotherapy; for example, studies involving elderly individuals have demonstrated hormonal regulation effects, such as serotonin and cortisol adjustment to optimal levels [29], while research focusing on veterans [30] has highlighted improvements in self-efficacy. Additionally, studies involving at-risk adolescents [31] point to emotional stabilization achieved through interactions with horses. These results collectively indicate that hippotherapy can offer beneficial effects across varied demographics and contexts.

ASD youth often face challenges in emotional regulation, exhibit reduced use of adaptive emotion regulation strategies [32], and show a heightened focus on stress-inducing factors [33]. It is hypothesized that hippotherapy, by inducing calming effects, may support the development and adoption of adaptive emotion regulation strategies in response to stressors. The observed improvements in POMS2 scores may reflect an overall enhancement in mood and positive energy. These changes could have further implications for emotional regulation and stress-coping mechanisms, potentially strengthening self-regulatory capacities. Previous studies have highlighted that children with ASD, even those with relatively high functioning, often experience significant impairments in self-regulation, leading to reduced social interactions in school and peer environments [34]. Since improvements in self-regulation are closely tied to the development of social skills

and adaptability in educational settings, the observed changes in POMS2 scores may signal meaningful progress in these areas. If such changes are recognized as markers of enhanced self-regulatory abilities, they could inform the design of additional support programs to further nurture this capability.

The newly introduced Friendliness (F) subscale in POMS2 has been reported to strongly correlate with the Vigor-Activity subscale, indicating positive mood states [24]. An increase in F scores suggests a positive shift in mood, which prior research links to improved emotional regulation [35]. Emotional regulation is a critical factor for social adaptation in children with ASD [36], and hippotherapy may contribute to improvements in social functioning, potentially mediated by its impact on emotional regulation.

Regarding physiological stress markers, salivary amylase activity was assessed, with values below 30 KU/L classified as indicating no stress, and levels above 30 KU/L reflecting varying degrees of stress [28]. Among the participants, seven individuals exhibited salivary amylase levels above 30 KU/L (Table 1). A Wilcoxon signed-rank test comparing pre- and post-intervention salivary amylase levels for these participants revealed no significant differences ( $Z = -0.524$ ,  $p = 0.600$ ). However, further analysis showed that participants P21 and P22, whose salivary amylase levels increased after the intervention, exhibited a higher tendency for tantrums when exposed to situations that did not align with their expectations.

**Table 1.** Changes in salivary amylase activity pre- and post-hippotherapy.

	P2	P5	P14	P17	P20	P21	P22
Before	31	56	192	46	63	15	8
After	34	45	4	20	7	46	96

The values in the table represent salivary amylase activity, expressed in KIU/L. Out of the 23 participants, seven individuals with values exceeding 30, indicative of stress, were selected for analysis. Among these, P2 showed little to no change, while P5, P14, P17, and P20 exhibited decreases. Conversely, P21 and P22 demonstrated increases.

For some individuals with ASD, factors such as unpredictability, sensory overstimulation, or hypersensitivity may act as irritability triggers. While prior studies have suggested that hippotherapy may alleviate such tendencies [37], it is important to consider that riding a horse and guiding its movements—particularly during the first session—can present significant challenges. In this study, the potential stress induced by the initial session, especially for participants prone to tantrums in unpredictable situations, underscores the importance of carefully adapting hippotherapy to minimize stress during early sessions and to optimize its therapeutic benefits.

The findings of this study demonstrate the potential of hippotherapy to enhance emotional regulation and mood among children with ASD, as evidenced by increased Friendliness scores and reduced Tension-Anxiety and Total Mood Dis-

turbance scores. Ward *et al.* identified positive associations between therapeutic horseback riding and improvements in social communication and sensory responses in children with ASD [18]. Similarly, Gabriels *et al.* explored the relationship between repetitive behaviors and sensory abnormalities, revealing that sensory-based therapies could mitigate such challenges [37]. These findings align with the notion that hippotherapy fosters emotional stability and social adaptability in individuals with ASD, thereby underscoring the novelty and potential implications of this study.

Further examination is required regarding the lack of significant changes observed in the Vigor-Activity subscale, despite improvements in the Friendliness subscale. One possibility is that the T-value score for the Vigor-Activity subscale in this study approached the threshold for statistical significance ( $p = 0.053$ , **Figure 1**), suggesting that additional data collection may confirm significant changes. Alternatively, initial apprehension or sensory overload during the first session might have influenced the Vigor-Activity outcomes. Participants who exhibited elevated salivary amylase levels tended to experience higher stress, indicating that individualized interventions may be necessary to optimize the therapeutic benefits of hippotherapy. Future studies should consider incorporating gradual adaptation protocols to account for these individual differences, thereby enhancing the effectiveness of intervention. Further research is essential to gain a more comprehensive understanding of the emotional and physiological impacts of hippotherapy on the well-being of children with ASD.

## 5. Study Limitations

The sample size of this study was limited to 23 participants. Future research should aim to include a larger sample, ideally exceeding (e.g., 50 or 100 participants), to enhance statistical validity and generalizability of the findings. Moreover, variability in participant characteristics, such as age, severity of ASD symptoms, and prior exposure to similar interventions, may have affected the outcomes and should be considered in future studies.

The present study utilized a single-session pre- and post-intervention comparison design. While this approach provided valuable preliminary insights into the potential psychological and physiological effects of hippotherapy on young individuals with ASD, it limits the ability to assess long-term outcomes and sustained effects of the intervention. To develop more effective programs aimed at enhancing the well-being of young individuals with ASD, future research should incorporate longitudinal designs and multiple intervention sessions, along with appropriate control groups. This would allow for a comprehensive understanding of the cumulative and lasting benefits of hippotherapy.

Finally, environmental factors such as the setting of the hippotherapy sessions and differences in horse behavior may have influenced participant responses. Future studies should aim to standardize these variables to ensure consistency and better interpret the impact of hippotherapy.

## 6. Conclusions

To investigate the psychological and physiological effects of hippotherapy on young individuals with ASD, we compared pre- and post-intervention POMS2 scores and salivary amylase activity. Significant differences were observed in some subscales of POMS2, suggesting the potential psychological benefits of hippotherapy, including improvements in self-regulation abilities and social skills. While no significant changes in salivary amylase activity were observed overall, individual analysis indicated that, in cases with relatively high pre-intervention stress levels, hippotherapy may help reduce stress. However, for individuals with ASD who have a predisposition toward tantrums, the initial session might exacerbate irritability, potentially increasing stress levels.

Based on these findings, future efforts should focus on developing programs designed to enhance stress reduction and improve self-regulation skills, ultimately contributing to the well-being of young individuals with ASD.

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## Conflicts of Interest

The authors indicated no potential conflicts of interest.

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