

Differential Effects of Cultural Performing Arts on Anti-Aging Biomarkers and Well-Being: A Non-Randomized Pre–Post Comparative Study of Traditional Noh/Kyogen and Comedy

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Abstract

This study examined how cultural performing arts affect psychological well-being and stress-related biomarkers, comparing traditional Japanese Noh and Kyogen (TNKP) with live comedy (CLP). Participants aged 18–64 years were assessed before and after attending performances (TNKP: $n = 41$; CLP: $n = 119$). Salivary biomarkers (cortisol, dehydroepiandrosterone [DHEA], and their ratio), psychological outcomes (State-Trait Anxiety Inventory), and well-being (PERMA framework) were measured pre- and post-show.

Laughter frequency during performance was higher in the CLP than in the TNKP. Cortisol levels decreased significantly in both groups. However, a significant decrease in the cortisol/DHEA (C/D) ratio was observed only in the TNKP, driven by cortisol reduction while DHEA remained stable. State anxiety decreased significantly in the CLP. Both groups showed significant improvements in positive emotion, accomplishment, and negative emotion. The CLP also showed significant improvements in overall well-being, meaning, relationship, engagement, and loneliness, with no significant changes in these domains in the TNKP.

Both performances reduced stress and improved some aspects of well-being. Comedy led to faster, broader psychological gains. Noh and Kyogen improved neuroendocrine balance, suggesting anti-aging benefits. Each art form offered distinct health effects.

Introduction

The beneficial effects of laughter on human health have been widely documented, and public awareness and interest in this topic have steadily increased[1, 2]. Laughter is an essential component of human life and communication; it reduces negative emotions and improves well-being. Moreover, interventions involving laughter have been shown to reduce stress, as evidenced by decreases in biomarkers such as salivary amylase and cortisol[3, 4].

To further explain the mechanisms underlying these effects, it is important to consider individual characteristics. Dispositional optimism—defined as a general tendency to expect positive outcomes—has been shown to be associated not only with psychological adaptation and mental health, but also with physical health[5, 6]. In a prior experimental study conducted by the present research group[2], participation in a live comedy performance significantly increased positive outlook while reducing stress levels, pessimism, and anxiety. These effects were especially pronounced among individuals who regularly engaged in laughter in their daily lives[2].

In general, people aspire to maintain good health, remain youthful, and experience satisfaction and happiness in their daily lives. Cultural activities represent an important factor supporting well-being. These activities provide enjoyment, emotional enrichment, psychological comfort, and a sense of purpose, thereby contributing to meaningful life experiences and enhanced psychological well-being[7–9]. Laughter itself can be considered a form of cultural performance that improves individuals' lives.

Previous studies have demonstrated that viewing humorous films can increase salivary dehydroepiandrosterone (DHEA) levels, revealing a possible anti-aging effect[10, 11]. DHEA is recognized as a protective factor against the adverse effects of cortisol and stress[12, 13]. In particular, the cortisol/DHEA ratio has attracted attention, with lower values being associated with more favorable health outcomes[14]. Importantly, differences have been observed between simulated laughter (e.g., laughter yoga) and spontaneous, natural laughter in relation to hormones associated with aging; natural laughter appears to promote more favorable neuroendocrine responses and reduce stress more effectively[10, 11].

Although cultural interventions may influence not only stress and optimism but also wider aspects of quality of life (QOL), such as subjective well-being and aging, research in this area remains limited[15]. This issue is particularly relevant in regions such as Aomori Prefecture, Japan, which has the shortest average life expectancy in the country, along with higher mortality rates from cancer and diabetes, as well as higher suicide rates. Therefore, exploring culturally grounded interventions may strengthen mental and psychological support in this region.

The present study aims to clarify the effects of cultural performing arts—specifically, live comedy performances and traditional Japanese Noh and Kyogen—on psychological well-being, including life satisfaction and QOL, as well as on indicators of anti-aging.

Results

Socio-demographic characteristics

The TNKP group had 41 valid responses (valid response rate: 93.2%), comprising 6 males and 35 females, while the CLP group had 119 valid responses (valid response rate: 99.2%). No significant difference in sex ratio was found between the two groups. The mean age was 36 ± 16 years in the TNKP group and 41 ± 13 years in the CLP group (Mean $\pm$ SD), with no significant difference between the groups. In the TNKP group, participants in their 20s were the most prevalent, accounting for 21 participants (47.7%), followed by those in their 50s (11 participants, 25.0%) and 60s (7 participants, 15.9%). In contrast, the CLP group was predominantly composed of participants in their 50s (34 participants, 28.6%), followed by those in their 40s (30 participants, 25.2%) and 20s (26 participants, 21.8%) (Table 1). The proportion of participants in their 20s was notably higher in the TNKP group than in the CLP group, whereas those in their 40s were significantly less represented in the TNKP group.

Table1 Background of Participants (n=160)

personality	items	TNKP (n = 41)		CLP (n = 119)		Chi-Square test
		number	%	number	%	
sex	Male	6	13.6	23	19.3	p = 0.64
	Female	35	85.4	96	80.7	
age	10s	0	0	3	2.5	p = 0.002
	20s	21*	47.7*	26*	21.8*	
	30s	2	4.5	19	16.0	
	40s	3*	6.8*	30*	25.2*	
	50s	11	25.0	34	28.6	
	60s -	7	15.9	7	5.9	
Occupation	Student	17*	38.6*	20*	16.8*	p = 0.02
	Employee	13*	29.5*	65*	54.6*	
	Independent business	2	4.5	2	1.7	
	Farming and Fishery	0	0	2	1.7	
	Houseworker	2	4.9	10	8.4	
	Not employed	1	2.4	2	1.7	
	Other	6	14.6	18	15.1	
Performance Attendance History	First-time attendee	24	58.5*	112	94.1*	p < 0.001
	Repeat attendee	17	41.5*	7	5.9*	

Overall, the age distribution differed significantly between the two groups ($\chi^2 = 18.3$, $p = 0.002$).

*Adjusted residual $\geq |2.0|$, indicating a significant difference from expected frequency.

In terms of occupational distribution, students were the most prevalent in the TNKP group (17 participants, 38.6%), followed by company employees (13 participants, 29.5%) and others (6 participants, 14.6%). In contrast, company employees accounted for the largest proportion in the CLP group (65 participants, 54.6%), followed by students (20 participants, 16.8%) and others (18 participants, 15.1%). The occupational distribution differed significantly between the two groups ($\chi^2 = 13.8$, $p = 0.02$), with a higher proportion of students in the TNKP group and a higher proportion of company employees in the CLP group.

Regarding Performance Attendance History, 24 participants (58.5%) in the TNKP group and 112 participants (94.1%) in the CLP group reported being first-time attendees. Repeated attendees were reported by 17 participants (41.5%) in the TNKP group and 7 participants (5.9%) in the CLP group. First-time attendees were significantly more prevalent in the CLP group than in the TNKP group ($\chi^2 = 30.3$, $p < .001$).

Regarding habitual laughter frequency, the most common response in both groups was laughing many times a day reported by 25 participants (68.3%) in the TNKP group and 75 participants (63.1%) in the CLP group (Table 2). This was followed by those who laughed at least once per day, with 9 participants (22.0%) in the TNKP group and 37 participants (31.1%) in the CLP group. Those who laughed once per week accounted for 4 participants (9.8%)

in the TNKP group and 6 participants (5.0%) in the CLP group. No significant difference in habitual laughter frequency was found between the two groups.

Table2 Usual frequency of laughing (n=160)

	TNKP (n = 41)		CLP (n = 119)	
	number	%	number	%
Laugh many times a day.	25	68.3	75	63.1
Laugh at least once a day	9	22.0	37	31.1
Laugh once a week	4	9.8	6	5.0
Laugh once a month	0	0	1	0.8
I don't laugh at all	0	0	0	0

A significant difference in laughter frequency during the performance was found between the TNKP and CLP groups, with significant differences observed at all frequency levels between the two groups (Table 3).

Table3 Frequency of laughing with TNKP or CLP (n=160)

	TNKP (n = 41)		CLP (n = 119)	
	number	%	number	%
I laughed out loud many times	0	0	109	92.4
I laughed out loud a couple of times	6	14.6	2	1.7
I laughed frequently	13	31.7	6	5.1
I laughed occasionally	12	29.3	1	0.8
I did not laugh much	5	12.2	0	0
I did not laugh at all	5	12.2	0	0

Frequency of laughter was analyzed using the Chi-square test. $\chi^2 = 129.1$, $p < 0.001$

All frequency categories showed significant differences between groups (all adjusted residuals $\geq |2.0|$).

Salivary cortisol (sCort) and DHEA

The concentration of salivary cortisol, a known stress biomarker, was quantified. A comparison between the groups revealed that the TNKP exhibited higher cortisol levels than the CLP at both the pre- and post-performance time points. However, both groups demonstrated a significant decrease in cortisol levels post-performance (Figure.1). Because cortisol is subject to diurnal variation[16], pre-performance cortisol concentrations were compared between the morning and afternoon sessions within the TNKP; however, no significant differences were observed (Figure.2). Therefore, it was confirmed that the post-performance decrease in cortisol observed in both groups was not attributable to diurnal variation.

Regarding DHEA concentrations, although the TNKP showed a tendency toward higher pre-performance levels compared to the CLP, this difference was not statistically significant. In contrast, post-performance DHEA levels were significantly higher in the TNKP than in the CLP (Figure.3). Similar to cortisol, DHEA is known to exhibit diurnal variation[17]; however, no significant differences were observed in pre-performance concentrations between the morning and afternoon sessions within the TNKP group (Figure.4).

As for the cortisol/DHEA (C/D) ratio, the TNKP exhibited significantly higher values than the CLP at both the pre- and post-performance time points. Furthermore, within the TNKP, the C/D ratio decreased significantly post-performance compared to the pre-performance baseline (Figure.5).

State Anxiety

In the CLP group, state anxiety scores decreased significantly from pre- to post-intervention (42.4 ± 5.5 to 28.9 ± 5.5 , $p < 0.001$), indicating a marked reduction in anxiety following the comedy live performance.

In the TNKP group, state anxiety also showed a numerical decrease (35.2 ± 7.6 to 32.5 ± 6.3); however, this change did not reach statistical significance.

Meaning

Both groups demonstrated significant improvements in meaning scores following the intervention (TNKP: 6.6 ± 1.9 to 6.8 ± 1.9 ; CLP: 6.4 ± 1.7 to 6.9 ± 1.7 , $p < 0.001$), suggesting that both types of performing arts contributed to an enhanced sense of purpose and meaning.

Relationships

The CLP group demonstrated a significant improvement in relationship scores (6.8 ± 1.6 to 7.2 ± 1.6 , $p < 0.001$), whereas no significant change was observed in the TNKP group (6.7 ± 1.8 to 6.8 ± 2.0).

Engagement

The CLP group demonstrated a significant improvement in engagement scores (7.1 ± 1.4 to 7.5 ± 1.4 , $p < 0.001$), while no significant change was observed in the TNKP group (7.3 ± 1.3 to 7.6 ± 1.3).

Accomplishment

Both groups showed significant improvements in accomplishment scores. The CLP group demonstrated a significant increase (6.2 ± 1.5 to 6.7 ± 1.5 , $p < 0.01$), and the TNKP group also showed a significant improvement (6.5 ± 1.8 to 7.0 ± 1.8 , $p < 0.001$), suggesting that both types of performing arts experiment contributed to an enhanced sense of achievement.

Negative Emotion

Both groups showed significant reductions in negative emotion scores following the intervention. The CLP group demonstrated a highly significant decrease (4.4 ± 1.6 to 3.8 ± 1.6 , $p < 0.001$), and the TNKP group also showed a significant reduction (4.1 ± 1.5 to 3.7 ± 1.6 , $p < 0.05$), indicating that both comedy live performance and traditional performing arts were effective in alleviating negative emotional states.

Health

The CLP group demonstrated a significant improvement in engagement scores (CLP: 6.4 ± 1.9 to 6.8 ± 1.9 , $p < 0.001$), while no significant change was observed in the TNKP group (TNKP: 6.8 ± 2.0 to 7.1 ± 2.0).

Loneliness

The CLP group showed a significant reduction in loneliness scores following the intervention (3.3 ± 2.4 to 2.9 ± 2.1 , $p < 0.05$), whereas no significant change was observed in the TNKP group (3.4 ± 2.5 to 3.1 ± 2.3).

Table4. Pre- and post-intervention scores for State Anxiety and the Japanese PERMA-Profiler in the TNKP and CLP groups. (n=160)

	TNKP (n = 41)		CLP (n = 119)	
	Pre	Post	Pre	Post
State Anxiety(STAI)	35.2 (7.6)	32.5 (6.3)	42.4 (5.5) ^{***}	28.9 (5.5) ^{***}
Japanese PERMA-Profiler				
Overall	7.7 (1.4)	7.9 (1.5)	7.6 (1.7) ^{***}	7.9 (1.5) ^{***}
Positive Emotion	6.9 (1.6) [*]	7.2 (1.7) [*]	6.9 (1.5) ^{***}	7.4 (1.5) ^{***}
Meaning	6.6 (1.9)	6.8 (1.9)	6.4 (1.7) ^{***}	6.9 (1.7) ^{***}
Relationship	6.7(1.8)	6.8(2.0)	6.8 (1.6) ^{***}	7.2(1.6) ^{***}
Engagement	7.3 (1.3)	7.6 (1.3)	7.1(1.4) ^{***}	7.5 (1.4) ^{***}
Accomplishment	6.5 (1.8) ^{**}	7.0 (1.6) ^{**}	6.2 (1.5) ^{***}	6.7(1.5) ^{***}
Negative Emotion	4.1 (1.5) [*]	3.7 (1.6) [*]	4.4 (1.6) ^{***}	3.8 (1.6) ^{***}
Health	6.8 (2.0)	7.1 (2.0)	6.4 (1.9) ^{***}	6.8 (1.9) ^{***}
Lonely	3.4 (2.5)	3.1(2.3)	3.3 (2.4) [*]	2.9 (2.1) [*]

Note

Meaning and Relationship showed significant within-group changes in the CLP group ($p < 0.001$); these sections have been corrected from the original draft to reflect Table 1 values.

Discussion

This study aimed to clarify the effects of cultural performing arts, specifically live comedy performances (CLP) and traditional Japanese Noh and Kyogen (TNKP), on psychological well-being, including life satisfaction and quality of life (QOL), as well as on indicators of anti-aging.

Consistent with previous findings demonstrating that comedy and laughter promote optimism and reduce pessimism, anxiety, and stress[1, 2], this study found that CLP produced significant improvements across a broad range of well-being indicators. Specifically, CLP was associated with significant reductions in state anxiety (STAI) and negative emotion, as well as significant increases in positive emotion, engagement, accomplishment, meaning, relationship, overall well-being, and a

reduction in loneliness. These results indicate that watching a live comedy performance by comedians with sustained popularity across diverse age groups can rapidly elicit positive psychological changes, likely through sustained audience engagement and shared laughter during the performance. Approximately 90% of participants reported enjoying the performance greatly and laughing frequently, supporting the view that the immediate hedonic experience of laughter serves as a powerful catalyst for well-being enhancement[18, 19].

In contrast, although TNKP produced a somewhat narrower pattern of significant improvements, the findings remain noteworthy. Significant improvements were observed in positive emotion, accomplishment, and negative emotion in the TNKP group, suggesting that traditional Japanese performing arts, which incorporate elements of humor and comic expression within a culturally rich context, also have the capacity to enhance psychological well-being[15]. This is particularly meaningful given that Noh and Kyogen are often perceived as formal and inaccessible art forms; these results indicate that even a single viewing experience may be sufficient to elicit measurable positive psychological effects[20].

Taken together, while CLP demonstrated more rapid and wide-ranging effects on well-being indicators—with significant improvements across most PERMA domains, including positive emotion, engagement, accomplishment, meaning, and relationship, as well as reductions in loneliness and negative emotion—TNKP also produced meaningful changes in affective and motivational domains. These findings suggest that both forms of performing arts may serve as viable cultural interventions for the promotion of psychological well-being.

With respect to the biological indicators of stress, salivary cortisol—a well-established biomarker of physiological stress and an index of hypothalamic–pituitary–adrenal (HPA) axis activity[16, 21]—was measured in both groups. Between-group comparisons revealed that the TNKP group exhibited higher cortisol levels than the CLP group at both pre- and post-performance time points. Nevertheless, both groups demonstrated significant reductions in cortisol following the performance. Given that cortisol is subject to circadian variation[16], pre-performance cortisol concentrations were compared between morning and afternoon sessions within the TNKP group; no significant difference was found (Figure.2), confirming that the observed post-performance reductions in cortisol were not attributable to diurnal fluctuation. These findings suggest that both comedy and traditional performing arts can effectively attenuate physiological stress responses, as reflected by reduced cortisol secretion. The decrease in cortisol suggests that theater viewing has a relaxing effect, which may be attributed to attentional distraction and emotional engagement within a safe context, together suppressing HPA axis activity and reducing cortisol levels[22, 23].

Regarding DHEA concentrations, the TNKP group tended to show higher pre-performance levels compared to the CLP group, although this difference did not reach statistical significance. Importantly, post-performance DHEA concentrations were significantly higher in the TNKP group than in the CLP group (Figure.3). As with cortisol, DHEA is known to exhibit circadian variation[17]; however, no

significant difference in pre-performance concentrations was observed between morning and afternoon sessions within the TNKP group (Figure.4), ruling out diurnal effects as a confounding factor.

The cortisol-to-DHEA (C/D) ratio, which serves as an integrated index of neuroendocrine balance between stress load and recovery capacity, as well as a marker of stress resilience and aging[12, 13], was significantly higher in the TNKP group than in the CLP group at both pre- and post-performance time points. Importantly, within the TNKP group, the C/D ratio decreased significantly from pre- to post-performance (Figure.5), indicating a favorable shift in the neuroendocrine stress profile. DHEA is known to exert anti-glucocorticoid and neuroprotective effects and has been associated with stress resilience and recovery[12, 13, 24, 25].

This reduction reflects not merely a decline in cortisol but also the maintenance of stable DHEA levels, indicating that stress was attenuated without suppressing the body's recovery systems. In contrast, the CLP group exhibited decreases in both cortisol and DHEA, with no significant change in the C/D ratio. This pattern suggests a rapid, release-based form of stress reduction in which both the stress hormone (cortisol) and the protective hormone (DHEA) declined concurrently—consistent with a transient relaxation response rather than a sustained shift in neuroendocrine balance. This interpretation aligns with earlier evidence that short-term positive emotions, such as laughter, can temporarily attenuate stress hormone activity and reduce cortisol levels[25, 26].

From a mechanistic perspective, these findings suggest that different types of performing arts may engage distinct modes of stress regulation. The reduction in the C/D ratio observed in the TNKP group may reflect a more integrative form of stress processing, in which cognitive and emotional engagement—potentially involving reflective processes and meaning-making—facilitates adaptive regulation of the hypothalamic–pituitary–adrenal (HPA) axis. This integrative processing may promote a sense of coherence, defined as a global orientation in which experiences are perceived as comprehensible, manageable, and meaningful, thereby supporting adaptive stress regulation[9, 27]. In contrast, the absence of change in the C/D ratio in the CLP group may reflect a more transient, affective release-based response. Although both performances were experienced in a non-threatening context, the observed differences may ultimately indicate distinct modes of emotional processing: rapid affective release in the case of comedy, versus cognitively mediated integration in the case of traditional performing arts. This distinction is particularly relevant in the context of healthy aging, as a lower C/D ratio has been associated with greater stress resilience and more favorable aging-related outcomes[12, 13].

Limitations

Several limitations of the present study should be acknowledged. First, the non-randomized pre–post comparative design precludes causal inference, and the possibility of selection bias cannot be excluded. Participants self-selected into performance groups based on personal preference, which may have introduced systematic differences in baseline characteristics, personality traits, or cultural familiarity

that could have influenced the observed outcomes. Second, the sample sizes differed considerably between groups (TNKP: $n = 44$; CLP: $n = 119$), and the demographic profiles were not fully comparable. The TNKP group was disproportionately composed of participants in their 20s and students, whereas the CLP group was predominantly composed of participants in their 40s and 50s and company employees. Given that both cortisol and DHEA are known to vary with age[14, 28] and occupational stress[29–31], these differences may have confounded the biomarker results and limit the generalizability of the findings. Third, the study did not include an active control condition, such as a rest period or attendance at a non-arts event, making it difficult to determine whether the observed changes were due to the specific content of each performance or to general factors common to any live event, such as social atmosphere or the natural relaxation that occurs over time. Fourth, as all measurements were taken immediately before and after the performances, the durability of the observed effects remains unknown. Fifth, salivary biomarker levels may have been influenced by factors such as food intake or physical activity prior to sampling, despite instructions to refrain from these beforehand. Finally, the study was conducted in a specific cultural and geographic context in Japan, which may limit the transferability of findings to other populations with different levels of cultural familiarity with Noh, Kyogen, or live comedy.

Implications and Future Directions

The present findings collectively suggest that cultural performing arts—whether contemporary comedy or classical Japanese theater—may serve as accessible and enjoyable interventions for promoting psychological well-being and modulating stress-related biomarkers. Across both groups, reductions in salivary cortisol and improvements in positive emotion within the PERMA framework were observed, underscoring the shared health-promoting potential of diverse cultural experiences. The more pronounced and rapid effects observed in the CLP group across subjective well-being measures may reflect the immediacy of laughter-induced positive affect, whereas the distinct neuroendocrine profile observed in the TNKP group—particularly the significant reduction in the C/D ratio—may point to unique pathways through which traditional performing arts influence physiological stress regulation. If replicated in future studies, these findings may position traditional cultural performing arts such as Noh and Kyogen as novel, culturally grounded strategies for healthy aging promotion, complementing rather than competing with the well-documented benefits of laughter-based interventions. Future studies with larger and more diverse samples, more closely matched in age and occupational background, and including active control conditions and longitudinal follow-up assessments, are warranted to further elucidate the mechanisms underlying these effects—particularly the roles of aesthetic contemplation, rhythmic engagement, cultural familiarity, and meaning-making in modulating neuroendocrine responses. Mechanistic studies incorporating neuroimaging or autonomic nervous system monitoring may also help clarify the distinct physiological pathways engaged by different forms of cultural participation. Ultimately, a deeper understanding of these processes may contribute to the development of evidence-based cultural health promotion strategies that are both sustainable and broadly accessible across diverse populations.

Conclusion

This study investigated the differential effects of live comedy performance (CLP) and traditional Japanese Noh and Kyogen performance (TNKP) on psychological well-being and stress-related biomarkers in a general adult population. Both groups showed significant reductions in salivary cortisol and improvements in positive emotion, accomplishment, and negative emotion, indicating that cultural performing arts—regardless of genre—can effectively attenuate physiological stress responses and enhance affective well-being.

Nevertheless, important differences were observed between the two performance types. CLP produced more rapid and wide-ranging improvements across most PERMA domains and significantly reduced state anxiety and loneliness, consistent with the well-documented immediate benefits of laughter-induced positive effects. In contrast, TNKP was uniquely associated with a significant reduction in the cortisol-to-DHEA (C/D) ratio, driven by cortisol reduction in the context of stable DHEA levels, suggesting a more integrative mode of stress regulation with implications for healthy aging.

In conclusion, this study demonstrated that even among populations with no difference in daily laughter frequency, the physiological effects obtained varied depending on the nature of the performance viewed. While viewing comedy contributes to stable stress relief and the maintenance of well-being, viewing Noh and Kyogen appears to possess a unique interventional potential—inducing a state of deep tranquility following a period of moderate sympathetic nervous system activation, thereby resetting endocrine balance and restoring biological youthfulness. These findings suggest that comedy and traditional performing arts are complementary cultural health resources, and that incorporating diverse performing arts into public health frameworks may represent a sustainable and meaningful approach to well-being promotion across the lifespan.

Methods

Participants

The participants were recruited from people aged 18–64 years who attended a CLP in Hirosaki, Aomori, Japan, in July 2023. The exclusion criterion was inability to understand Japanese.

Procedure

Study design and Settings.

This study employed a non-randomized, quasi-experimental pre–post design among volunteer attendees of cultural performances. Traditional Noh and Kyogen Performance (TNKP) was observed at Hirosaki city hall on November 3, 2024, and Comedy Live Performance (CLP) was also observed at same hall on February 21, 2025. Both participants were informed of the study purpose, methods, risks, and benefits, and provided written informed consent. Participants were asked to arrive early at the venue to complete

a pre- questionnaire. They also completed a post- questionnaire. Each participant received an honorarium after all pre- and post-research data had been collected.

Intervention

1. Traditional Noh and Kyogen Performance(TNKP)

Noh is a form of musical drama performed in coordination with utai (chanting, including song and dialogue) and hayashi (instrumental accompaniment). A distinctive feature of many Noh performances is the use of masks (noh-men) worn by the actors. The characters portrayed are not limited to humans but also include deities, demons, spirits, and other supernatural beings. Emotions are expressed through highly stylized and minimalistic movements, through which the narrative unfolds.

Kyogen, in contrast, is a dialogue-centered theatrical form. It does not employ elaborate stage settings; instead, all elements are conveyed through language and gesture. A defining characteristic of Kyogen is its emphasis on humor. Drawing on the everyday lives and folktales of common people in the medieval period, it sharply depicts human nature and behavior, transforming them into broad humor and comic expression.

In this study, participants attended a combined performance integrating both Noh and Kyogen, with a total duration of approximately two hours.

2 Comedy Live Performance(CLP)

Four famous Japanese comedians performed their routines at a two-hour CLP that was organized by the Division of Cultural Promotion in Hirosaki City with the aim of supporting citizens' health through comedy entertainment as a form of culture. The comedians were Sisonnu, Chocolate Planet, Ginshari and Panther.

Salivary sample collection

Saliva samples were collected before and after each performance. The TNKP consisted of two sessions (morning and afternoon). Saliva samples were collected at 9:00 (pre-TNKP-AM) and 12:00 (post-TNKP-AM) for the morning session, and at 13:00 (pre-TNKP-PM) and 16:00 (post-TNKP-PM) for the afternoon session. For the CLP, samples were obtained at 16:00 (pre-CLP) and 19:00 (post-CLP). The participants were asked not to drink or eat 1 hour before the saliva collections. Saliva collections were used the passive drool method with the Saliva Collection Aid, an assisting tool for collecting the whole saliva. There were using the Saliva Collection Aid (Salimetrics, LLC, State College, PA, USA) and immediately stored on ice. And then stored at -20°C until analysis.

Measurement of salivary cortisol (sCort)

sCort levels were measured using a salivary cortisol enzyme immunoassay kit (Salimetrics) according to the manufacturer's instructions. On the day of the assay, saliva samples were completely thawed, vortexed, and centrifuged at 1,500×g for 15 min; the resulting supernatants were collected for analysis. Briefly, 25µL of each sample and 200µL of enzyme conjugate were added to the microplate wells. The plate was shaken at 500 rpm for 5 min and then incubated at room temperature for 1 h. After washing the wells four times with wash buffer, 200µL of TMB (3,3',5,5'-Tetramethyl benzidine) substrate solution was added, followed by shaking at 500 rpm for 5 min and incubation in the dark for 25 min. The reaction was terminated by adding 50µL of stop solution and shaking at 500 rpm for 3 min. The absorbance at 450 nm was measured using a multimode microplate reader, Infinite 200 PRO F-Plex (Tecan Group Ltd., Männedorf, Switzerland). Cortisol concentrations were determined using a four-parameter logistic (4PL) curve fit via the 4PL Curve Calculator (AAT Bioquest, Inc., Pleasanton, CA, USA).

Measurement of salivary dehydroepiandrosterone (DHEA)

DHEA levels were measured using a salivary DHEA enzyme immunoassay kit (Salimetrics) according to the manufacturer's instructions. On the day of the assay, saliva samples were completely thawed, vortexed, and centrifuged at 1,500×g for 15 min; the resulting supernatants were collected for analysis. Briefly, 50µL of each sample and 150µL of enzyme conjugate were added to the microplate wells. The plate was shaken at 500 rpm for 5 min and then incubated at room temperature for 3 h. After washing the wells four times with wash buffer, 200µL of TMB substrate solution was added, followed by shaking at 500 rpm for 5 min and incubation in the dark for 25 min. The reaction was terminated by adding 50µL of stop solution and shaking at 500 rpm for 3 min. The absorbance at 450 nm was measured using a multimode microplate reader, Infinite 200 PRO F-Plex (Tecan Group Ltd.). DHEA concentrations were determined using a 4PL curve fit via the 4PL Curve Calculator (AAT Bioquest, Inc.).

Japanese State-Trait Anxiety Inventory (STAI)

The STAI is a scale developed by Shimizu et al. that measures anxiety state using a 4-point scale ranging from 4: completely true to 1: completely false. Pre- and post-measures were taken[32].

PERMA- Profiler Japanese version

Wellbeing was assessed using the Japanese version of the PERMA-Profiler[33, 34], a 23-item self-report measure developed to assess flourishing across multiple psychosocial domains. The original measure was constructed based on Seligman's PERMA theory of wellbeing[35], which conceptualizes flourishing as arising from five distinct but interrelated pillars: Positive Emotion (P), Engagement (E), Relationships (R), Meaning (M), and Accomplishment (A).

The PERMA-Profiler comprises 15 core items (three per PERMA domain) and eight additional filler items that assess overall wellbeing (one item), negative emotion (three items: anxiety, anger, and sadness), loneliness (one item), and self-perceived physical health (three items). All items are rated on an 11-point Likert scale ranging from 0 to 10, with endpoint anchors varying by item (e.g., 0 = never to 10 = always; 0 = not at all to 10 = completely; 0 = terrible to 10 = excellent). Domain scores for each of the five PERMA

subscales, negative emotion, and physical health are calculated as the mean of the respective three items (range: 0–10). The overall wellbeing score is computed as the mean of the 15 core PERMA items and the single overall happiness item (range: 0–10). Loneliness is treated as a single-item score[33, 34].

The present study utilized the Japanese version of the PERMA-Profilier (PERMA-Profilier KIT version), the reliability and validity of which have been reported by Shiotani et al.³ Permission to use the Japanese version was obtained from the Institute of Psychological Science, Kanazawa Institute of Technology (approval number: 20240706). The measure yielded nine scores in total: five PERMA domain scores (P, E, R, M, A), negative emotion, physical health, loneliness, and overall wellbeing.

Additional questionnaire items included questions regarding sex, age, and occupation.

Data were collected as variables, including Socio-demographic characteristics (age, sex, occupations, preference of TNKP and CLP, how many times laughter in a day, et al.), how fun of TNKP and CLP, satisfaction of TNKP and CLP, except scores of STAI and PERMA- Profiler Japanese version.

Study size.

Repeated measures analysis of variance was used to compare knowledge and comprehension pre and post the TNKP and CLP groups. Using G*Power 3.1.9.7 with “F tests” and “ANOVA” statistics, an effect size of “0.25” (effect size: medium) and power of “0.8” from previous studies, the number of groups of “2” and the number of repetitions of “2”, a total of 34 cases, at least 17 in each group, were calculated. The α level was set at .05 for statistical significance.

Statistical Analysis

All data were analyzed by the Statistical Package for the Social Sciences (SPSS) for Windows, version 28.0 (SPSS Inc., Armonk, NY, USA). Chai-square test was used to analyze the variation in the background of the subjects in each group. Two-way repeated measures ANOVA was conducted to examine the effects of time, group, and their interaction on understanding, anxiety, and psychological well-being. Comparison of understanding, anxiety, and psychological well-being regarding observation of participants pre- and post-data and between groups was performed using Bonferroni adjustment. Since the interaction effects did not consistently correspond with the pairwise comparisons, the pre- and post-intervention between-group and within-group comparisons were considered primary outcomes for interpretation.

Declarations

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions Statement

T.T., N.I. and N.N. conceived and designed the study, including the development of the study methodology. T.T., N.I. and N.N. analyzed data and drafted the manuscript. K.M., N.I. and K.S. conducted data collection and participant recruitment. S.T. performed data curation and managed the study dataset. T.T., N.I. and N.N. created the figures and tables. T.T., N.N. and K.H. provided critical revision of the manuscript for important intellectual content. All authors had full access to all data in the study, take responsibility for the integrity of the data and the accuracy of the data analysis, and critically reviewed and approved the final version of this manuscript for publication.

Declaration of Competing Interests

The authors declare that there are no conflicts of interest.

Content of Publication

Not applicable, as no identifiable information is included in this manuscript.

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki. The study was approved by the Institutional Review Board of Hirosaki University School of Health Sciences (approval No. 2024-012). Participants were advised orally and in written form of the purpose and outline of the study, that the survey information would not be used for any purpose other than this study, that participation was voluntary with no disadvantage incurred depending on whether or not to cooperate. All participants provided written informed consent prior to participation in the study. Personal information was anonymized by replacing the name information with a number.

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Figures

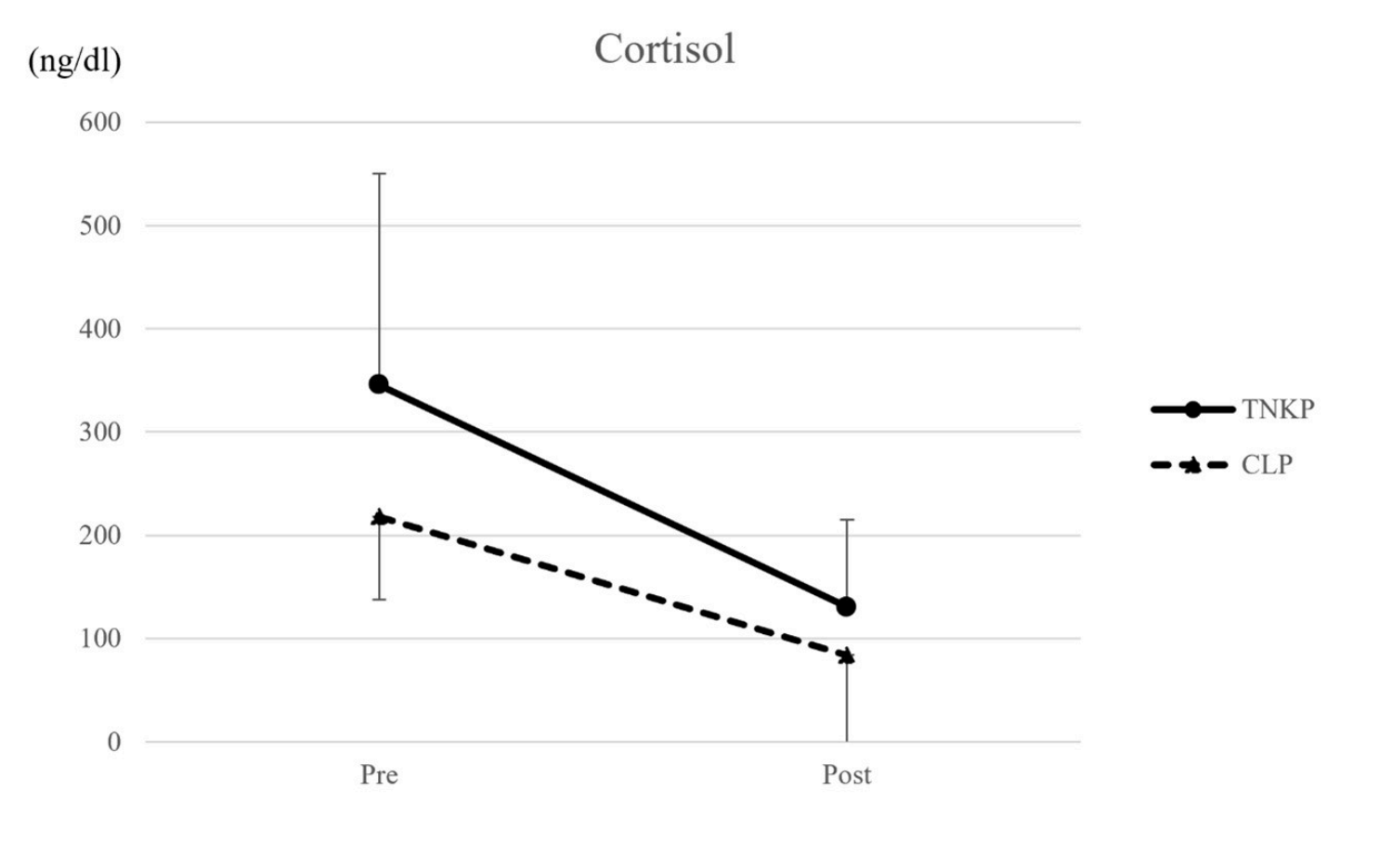


Figure 1

Comparison of salivary cortisol concentrations between the TNKP and CLP groups pre- and post-performance.

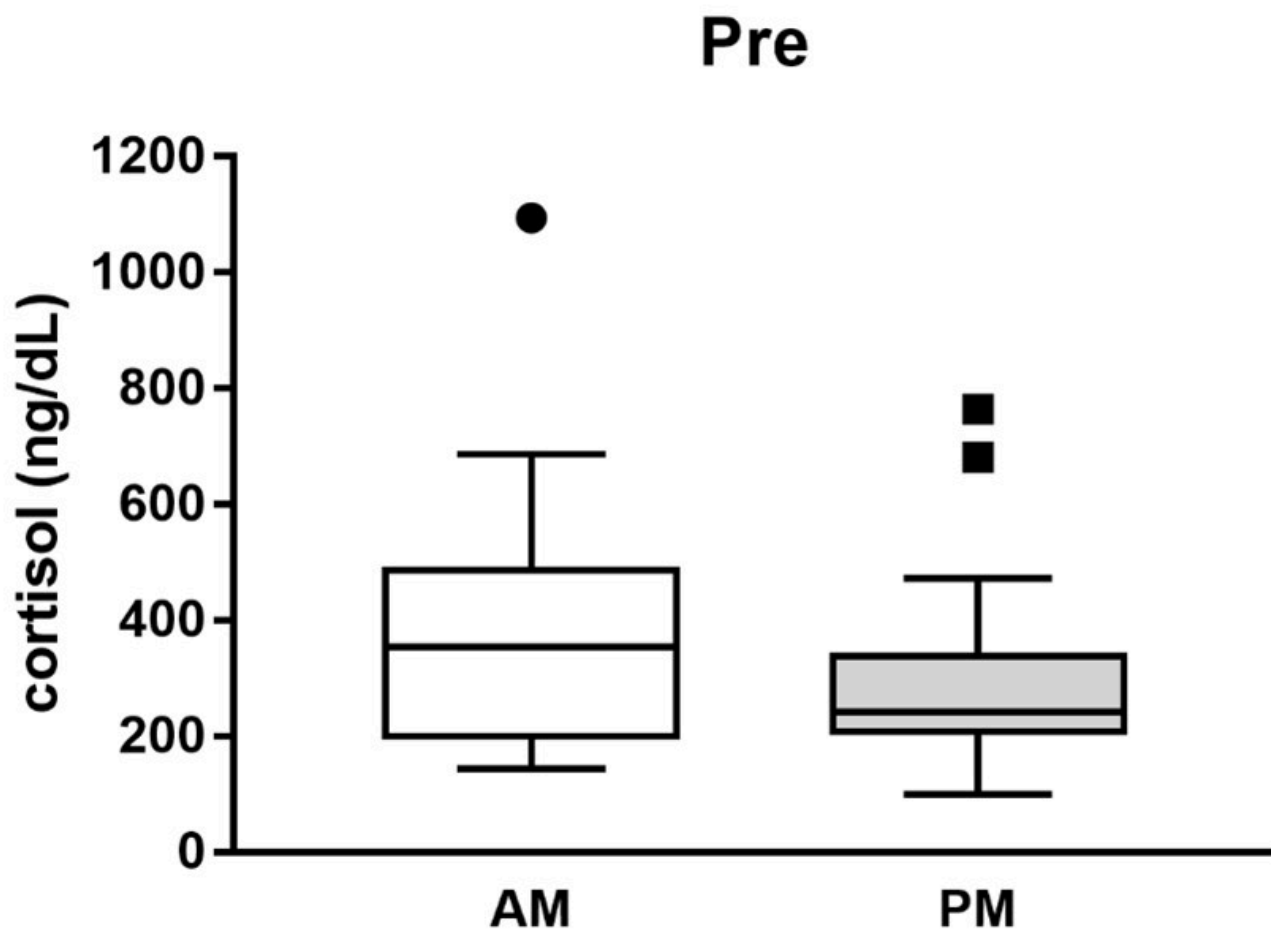


Figure 2

Evaluation of diurnal variation in cortisol levels within the TNKP group (AM vs PM sessions).

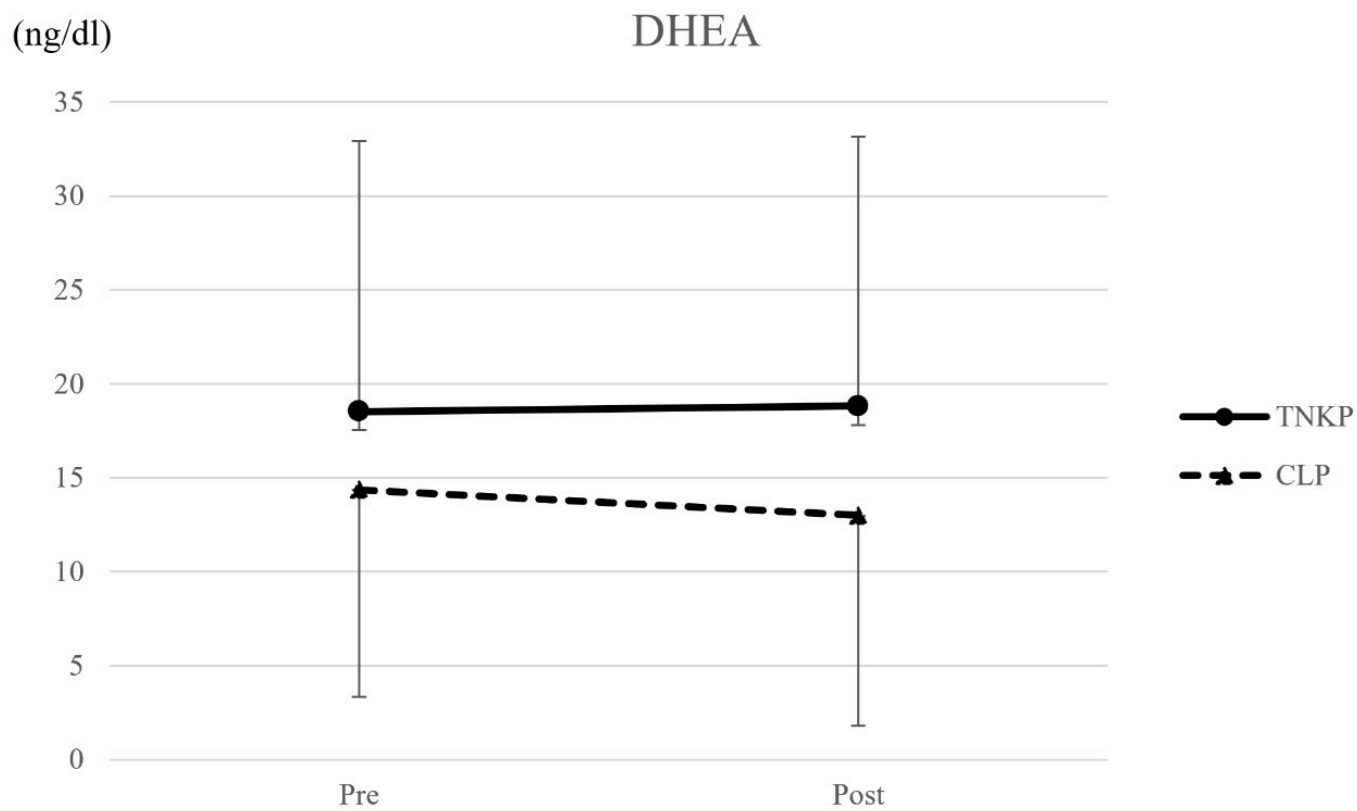


Figure 3

Comparison of salivary DHEA concentrations between the TNKP and CLP groups pre- and post-performance.

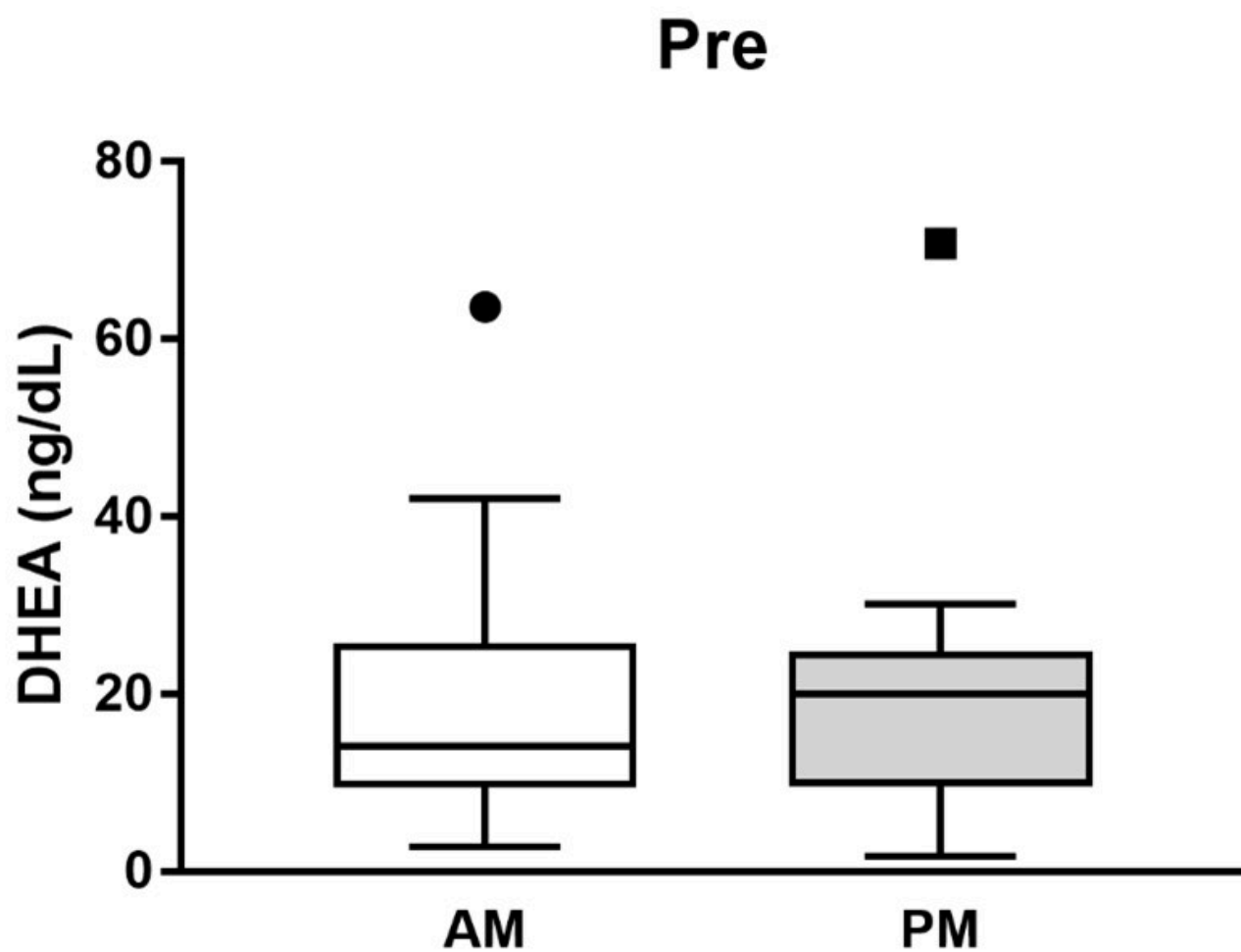


Figure 4

Evaluation of diurnal variation in DHEA levels within the TNKP group (AM vs PM sessions).

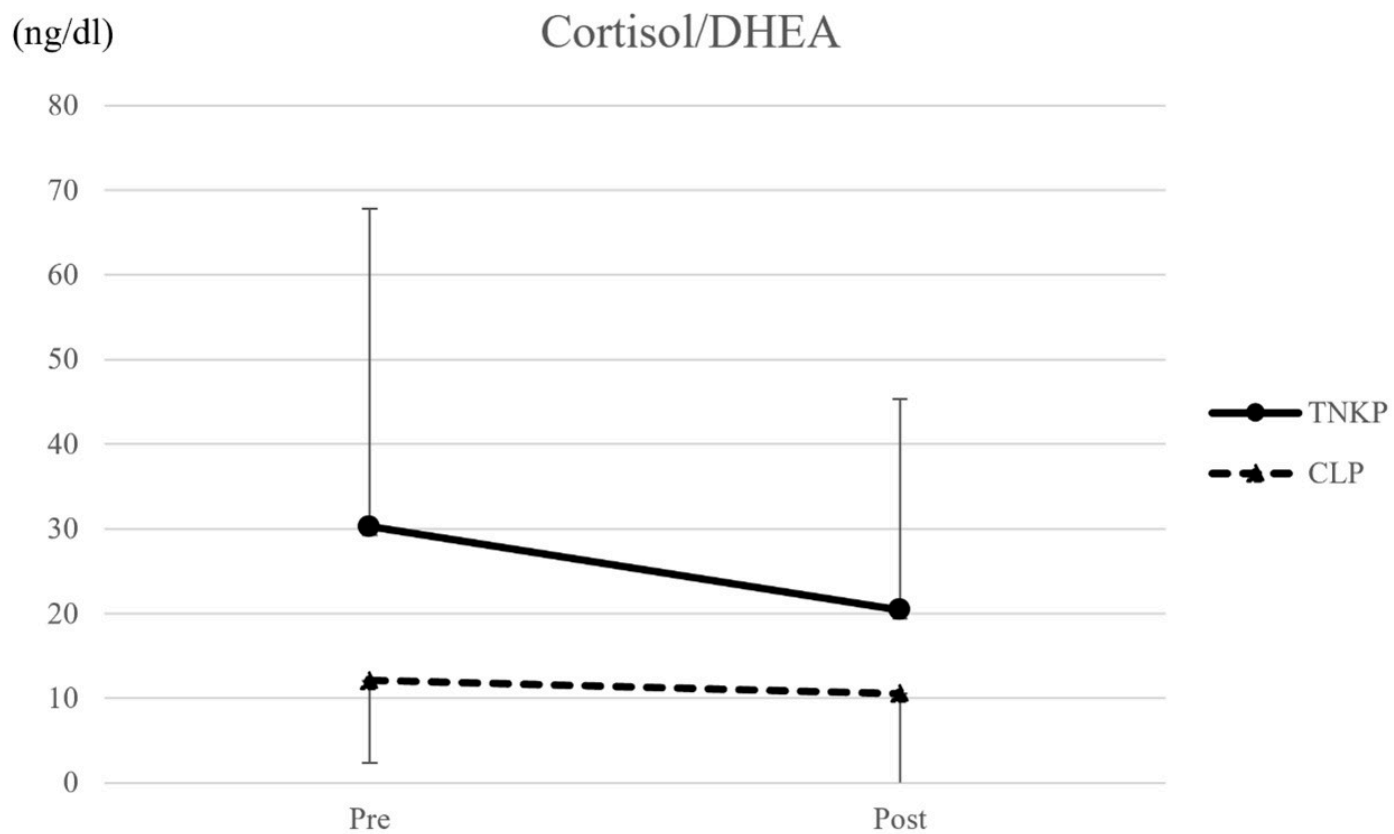


Figure 5

Changes in the cortisol/DHEA (C/D) ratio pre- and post-performance in both groups.