RESEARCH ARTICLE

Alpha-Actinin-3 (*ACTN3*) R577X Polymorphism on Brain-derived Neurotrophic Factor (BDNF) Levels of Pre- and Post-Eccentric Exercised Male Subjects

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Abstract

BACKGROUND: Eccentric exercise, characterized by muscle lengthening underload, elicits physiological responses, including alterations in brain-derived neurotrophic factor (BDNF) levels, crucial for neuroplasticity and exercise adaptations. The Alpha-Actinin-3 (*ACTN3*) gene encodes α-actinin-3, a protein in fast-twitch muscle fibers associated with explosive performance. The R577X polymorphism in *ACTN3* is associated with athletic performance, particularly in power-based activities. However, its influence on the BDNF response to eccentric exercise remains unclear. This study investigated whether the *ACTN3* R577X polymorphism modulates BDNF levels post-exercise.

METHODS: Male subjects aged 18-30 years old, who were not involved in structured physical activity, and abstaining from alcohol and protein supplements within specified periods, were involved in this study. Subjects' genotypes were identified using polymerase chain reaction (PCR) and classified into different *ACTN3* genotypes (RR, RX, XX). All subjects underwent an eccentric exercise protocol. BDNF levels were measured pre-exercise, post-exercise, and 72 hours post-exercise using sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

RESULTS: Most of subjects had RX genotype (52.2%), followed by XX (39.1%) and RR genotypes (8.7%), respectively. BDNF levels decreased significantly across all time points. The RR genotype showed a decrease from approximately 270 pg/mL to 230 pg/mL, while RX and XX genotypes showed similar patterns of reduction. No significant differences in BDNF levels were observed between genotypes at any time point.

CONCLUSION: Eccentric exercise leads to a consistent decrease in BDNF levels, with no significant modulation by *ACTN3* genotype. These findings suggest a uniform response to exercise-induced stress across genotypes.

KEYWORDS: *ACTN3*, BDNF, eccentric exercises, genotype, adaptation

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Introduction

Eccentric exercise, characterized by muscle lengthening under tension, is an essential component of strength training and rehabilitation due to its effectiveness in enhancing muscle strength, stability, and adaptability. During eccentric contractions, muscles experience high tension, resulting in microtrauma and the subsequent release of biomarkers indicative of muscle damage, such as creatine kinase (CK), lactate dehydrogenase (LDH), and brain-derived neurotrophic factor (BDNF).(1) Among these biomarkers, BDNF plays a pivotal role in the repair and adaptation of skeletal muscle tissue.(2,3)

BDNF, a neurotrophin, is crucial for neuroplasticity and has been implicated in muscle repair processes by

promoting satellite cell activation, muscle innervation, strengthening neuromuscular junctions, and regulating insulin-glucose uptake.(3-6) Moreover, BDNF facilitates communication between the nervous system and skeletal muscles, making it a critical marker for assessing the adaptability of muscle tissues to physiological stress induced by eccentric exercise.(7) However, BDNF levels can be influenced by various physiological factors, including hormonal fluctuations, gender differences, and aging, which can affect muscle repair and neuroplasticity.(8-10)

Meanwhile, the Alpha-Actinin-3 (*ACTN3*) gene, expressed in fast-twitch muscle fibers, influences muscle performance and recovery.(11) The R577X polymorphism in the *ACTN3* gene results in three genotypes: RR, RX, and XX, with varying effects on muscle function.(12) While the RR and RX genotypes are associated with better muscle performance, the XX genotype is linked to reduced fast-twitch muscle fiber function and prolonged inflammatory responses to exercise-induced damage.(11-13) Additionally, the absence of α-actinin-3 in XX individuals has been correlated with lower muscle hypertrophy signaling.(14)

The link between *ACTN3* and BDNF lies in their roles in muscle damage and recovery. *ACTN3* genotypes may influence the efficiency of muscle adaptation following exercise-induced damage.(12) Exercise-induced changes in BDNF expression are also influenced by exercise intensity and type. Acute eccentric exercise induces transient reductions in BDNF, with variations across *ACTN3* genotypes.(15) These findings underscore the dynamic interplay between muscle damage, inflammation, and neuroplasticity, highlighting the importance of genetic factors in tailoring exercise and rehabilitation protocols. To minimize the influence of physiological variability, this study focused on male subjects aged 18-30 years, as hormonal and age-related changes can impact BDNF levels and muscle function.(8-9)

Recent studies further explore these relationships. For instance, *ACTN3* genotypes influence the stiffness response of type IIa fibers after eccentric exercise-induced muscle damage, with the RR genotype showing more pronounced stiffness adaptation.(16) Similarly, susceptibility to muscle damage after downhill running is higher in RR individuals compared to X allele carriers.(17) Moreover, variations in *ACTN3* expression under endoplasmic reticulum stress further demonstrate its regulatory complexity.(18)

The BDNF pathway activation is primarily influenced by exercise intensity rather than contraction type, further emphasizing its central role in adaptive responses.(19) Furthermore, hematological responses to exercise, including changes in iron metabolism and inflammation, differ based on *ACTN3* genotypes, with XX individuals exhibiting reduced susceptibility to exercise-induced changes.(20)

While prior studies have explored *ACTN3*'s impact on athletic performance, its influence on BDNF modulation in response to eccentric exercise remains unclear. This study was conducted to investigate the relationship between *ACTN3* R577X polymorphism and BDNF levels prior to and following eccentric exercise, hypothesizing that genetic differences modulate the BDNF response to muscle damage and recovery.

Methods

Study Design and Subject Recruitment

This was an analytical observational study with pre- and post-design which analyzed BDNF values on *ACTN3* polymorphism (rs1815739) at three different times. This study was conducted at the Biomolecular Laboratory, Faculty of Medicine, Universitas Trisakti, Jakarta, from August to November 2023. A total sample size of 46 was determined using the G*Power application version 3.1.9.7 (HHU, Düsseldorf, Germany) to ensure an adequate power of 0.80 and a confidence level of 95%.(21) The Consecutive Non-Random Sampling method was employed to include all eligible participants until the desired sample size was reached, balancing statistical rigor with practical constraints such as time and resources.

Subjects were limited to males aged 18–30 years. This age group was chosen to minimize the influence of age-related physiological changes on muscle repair and BDNF expression, as well as to ensure relatively uniform skeletal muscle function. Male subjects were selected to avoid confounding factors related to hormonal differences, which can affect muscle repair processes and BDNF levels. Subjects were excluded if they engaged in structured physical activity (*e.g.*, resistance or aerobic training) performed for at least 30 minutes per session, 3–4 times per week within the last 6 months, consumed alcohol within 12 hours prior to sampling, and consumed protein powder 3–4 times per week within the last 3 months prior to the study. The protocol of this study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti (No. 89//KER-FK/VII/2023).

Eccentric Exercise Protocol

Subjects that met the inclusion and exclusion criteria were informed and provided with written informed consent.

The exercise protocol involved performing 3 sets of 15 repetitions of bicep curls using a 5 kg dumbbell, with a rest period of 1 minute between sets. This protocol was chosen to induce muscle damage and assess the BDNF response. Blood samples were collected for further analysis at three time points: baseline/pre-exercise (Pre-ex), immediately after exercise (Post-ex), and 72 hours post-exercise (72hPost-ex). The 72hPost-ex (3 days) time point was selected to capture the recovery phase of muscle repair, as previous studies suggest that muscle recovery processes, including BDNF metabolism, can extend over several days.(1,22)

BDNF Measurement

Plasma BDNF levels were measured using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalogue No. E-EL-H0010; Elabscience, Texas, USA) and ELISA Reader MP96 (Safas, Monaco, Monaco). To collect plasma, blood samples from subjects were centrifuged immediately at 2-8°C at 1000×g to ensure sample integrity, as per kit instructions. Plasma samples were stored at -80°C until the targeted number of samples was reached. Prior to testing, plasma was diluted 1:4000 in diluent solution. Briefly, 100 µL of sample was added to the wells and incubated at 37°C, followed by the addition of Biotinylated Detection Antibody and HRP conjugate solutions with intermittent washing. The substrate reagent was then added, incubated, and followed by stop solution. The plate was read at 450 nm, with results calculated using readings corrected at 570 nm.

DNA Isolation

DNA isolation was done using the Quick DNA Miniprep Plus Kit (Catalogue No. D4069; Zymo Research, California, USA). The DNA concentration and purity were measured using a nanophotometer. Samples with the concentrations above 15 ng/µL and A260/280 ratio value between 1.8–2.0 were used for the amplification process. Polymerase chain reaction (PCR) screening of *ACTN3* gene polymorphisms was performed, and the results were analyzed using electrophoresis.

Primer Preparation and PCR Amplification for Identifying *ACTN3* Polymorphism

For the amplification of the *ACTN3* R577X polymorphism, a primer mixture was prepared by combining 4 µL of hACTN-3f primer, 4 µL of hACTN-3r primer, 1 µL of hACTN-3Tif primer, and 2 µL of hACTN-3Cir primer (Table 1).(23) A total of 5 µL of this primer mixture was added to 10 µL of My Taq HS Red Mix and 5 µL of DNA sample to create the reaction mixture. The PCR process was conducted in a thermocycler with the following cycling conditions of 95°C for 2 minutes, followed by 35 cycles of 95°C for 10 seconds, 68°C for 10 seconds, and 72°C for 45 seconds. A final extension at 72°C for 2 minutes was performed at the end of the cycle. The resulting PCR products were analyzed using gel electrophoresis. The samples were run on a 4% agarose gel prepared with 1X TAE buffer. The electrophoresis was performed at 100 volts for 30 minutes. Visualization of the DNA bands was carried out using Fluoresafe DNA staining. The X and R allele showed bands of 318 bp and 413 bp, respectively. Band of 690 bp was considered as PCR control (Figure 1).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 10 software (GraphPad Software, San Diego, CA, USA). The Friedman test was used to evaluate differences in BDNF levels across the three time points and across *ACTN3* genotypes. Post-hoc comparisons were conducted using the Dunn test to identify significant differences between specific time points and genotypes.

Results

Demographic Characteristic of Subjects

The demographic data of the study subjects was described in Table 2. A total of 46 subjects were included in the final analysis. Most subjects were aged 21-25 years (54.3%). The study also included subjects aged 18-20 years (30.4%) and 26-30 years (15.2%). The *ACTN3* genotype distribution was RR (8.7%), RX (52.2%), and XX (39.1%).

Table 1. Primer pairs used for identifying *ACTN3* polymorphism.

Genes	Primers (5' → 3')	PCR Product
hACTN3f	CGCCCTTCAACAACTGGCTGGA	690bp with hACTN3r
hACTN3r	GATGAGCCCGAGACAGGCAAGG	690bp with hACTN3f
hACTN3Tif	CAACACTGCCCGAGGCTGACTG	318bp with hACTN3r
hACTN3Cir	CATGATGGCACCTCGCTCTCGG	413bp with hACTN3f

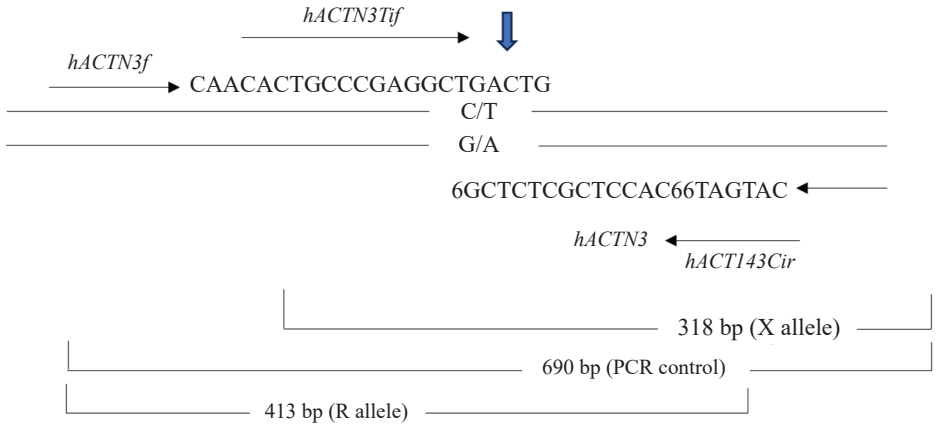

Figure 1. Primer position in *ACTN3* gene specifically for the *ACTN3* polymorphism analysis.(15)

Changes in BDNF Levels Across Exercise Time Points

Figure 2 presented the changes in BDNF levels at three different time points: Pre-ex, Post-ex, and 72hPost-ex. This study results showed that in the initial BDNF levels Pre-ex, an average concentration of approximately 300 pg/mL. While Post-ex, BDNF levels decreased to around 250 pg/mL, and this reduction was statistically significant. At 72hPost-ex, BDNF levels remained around 250 pg/mL, indicating that the decrease in BDNF levels persisted for 72 hours. BDNF levels were significantly lower immediately after exercise compared to before exercise with the p=0.0048, indicating an acute decrease following eccentric exercise. Additionally, levels at 72hPost-ex remain significantly reduced compared to Pre-ex (p=0.0399), but no significant difference was observed between Post-ex and 72hPost-ex.

Genotype-specific Variations in BDNF Levels

The differences of BDNF level in each genotype of *ACTN3* was also analyzed. PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) was initially used to identify the polymorphism of *ACTN3* gene. The RR and XX genotype variations have two DNA bands with lengths of 690 bp and 413 bp, and 690 bp and 318 bp, respectively, while the RX genotype has three DNA bands that were a combination of the RR and RX genotypes (Figure 3). Figure 4 depicted the changes in BDNF levels at three time points across three genotypes of the *ACTN3* gene.

In the subjects with RR genotype, the Pre-ex BDNF levels for individuals with the RR genotype were approximately 270 pg/mL, then decreased to be around 240 pg/mL Post-ex. After 72hPost-ex, the BDNF levels further decreased to be around 230 pg/mL. Meanwhile, the Pre-ex BDNF levels for individuals with the RX genotype were approximately 320 pg/mL, decreased to be around 280 pg/mL Post-ex. BDNF levels remained steady at around 280 pg/mL after 72hPost-ex. Meanwhile, participants with XX genotype showed Pre-ex BDNF levels for individuals with the XX genotype were approximately 320 pg/mL. Then, the BDNF levels decreased to be around 270 pg/mL Post-ex and remained relatively steady at around 270 pg/mL after 72hPost-ex. However, the Friedman test for each genotype of *ACTN3* did not show any significant differences between the time points (p=0.408).

Table 2. Demographic characteristics of study subjects.

Criteria	n (%)
Age	
18-20 years old	14 (30.4)
21-25 years old	25 (54.3)
26-30 years old	7 (15.2)
Genotype	
RR	4 (8.7)
RX	24 (52.2)
XX	18 (39.1)

Discussion

In this study, the association of the *ACTN3* gene and changes in BDNF levels in the blood was analyzed. BDNF acts as a muscle damage marker and can determine the type of exercise suitable for genotype.(24) The age of the subjects can influence changes in muscle age. The results of this study indicate that each person had the *ACTN3* gene at the age of 18-30 years with different genotypes: RR (8.7%), RX (52.2%), and XX (39.1%). The wild type inferred in this research is the R-allele.

The observed decrease in BDNF levels immediately after eccentric exercise and the sustained reduction of

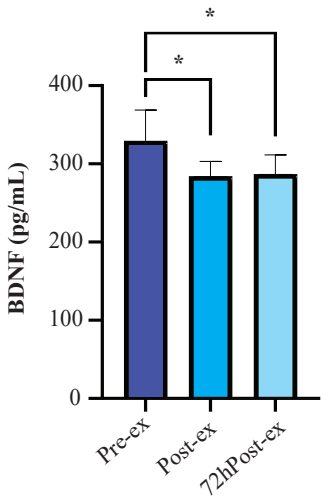

Figure 2. Different levels of BDNF in pre-, post-, and 72 hours post-exercise. Data was presented in mean rank±95% CI. *Significant if $p<0.05$, Friedman test was used to analyze the data.

72 hours post-exercise. This acute reduction of BDNF immediately after exercise can be attributed to physiological stress induced by eccentric contractions, which involve muscle microtrauma and an inflammatory response.(1) Eccentric exercise often induces significant muscle damage and inflammation compared to other types of exercise. (25) The acute stress response to such physical activity can involve the release of stress hormones like cortisol, which has been shown to negatively regulate BDNF expression. (26,27) Furthermore, eccentric exercise triggers a robust inflammatory response as part of the muscle repair process. (22) Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are released in response to muscle damage. These cytokines can inhibit BDNF production or promote its degradation. Additionally, intense exercise increases the metabolic demands on the body, leading to a temporary shift in cellular priorities where resources are allocated to immediate energy production and repair processes rather than the synthesis of neurotrophic factors like BDNF.(28)

The selection of the 72-hour time point was based on previous research indicating that muscle recovery processes, including BDNF metabolism, often extend beyond the immediate post-exercise period and can continue for several days.(1,22,29,30) This timeframe allows for the observation of longer-term adaptations and changes in BDNF levels as the body progresses through different stages of recovery. (1,22) Studies have shown that BDNF levels can fluctuate during this extended recovery phase, providing insights into the dynamics of neuroplasticity and muscle repair. (29,30) This study results show that BDNF levels remain reduced at 72 hours post-exercise compared to pre-exercise levels, suggesting that the body is still in a recovery phase. Since BDNF is crucial for synaptic plasticity and neuronal repair, the sustained reduction in BDNF levels could reflect increased utilization and uptake of BDNF by neurons and muscle cells to facilitate repair and adaptation, rather than a simple reduction in production.(31,32)

Exercise influences neuroendocrine systems, including the hypothalamic-pituitary-adrenal (HPA) axis. The activation of the HPA axis and the subsequent release of glucocorticoids can downregulate BDNF expression. (31) Prolonged activation of this axis following strenuous exercise might contribute to the sustained reduction in BDNF levels observed by 72 hours post-exercise, suggesting that

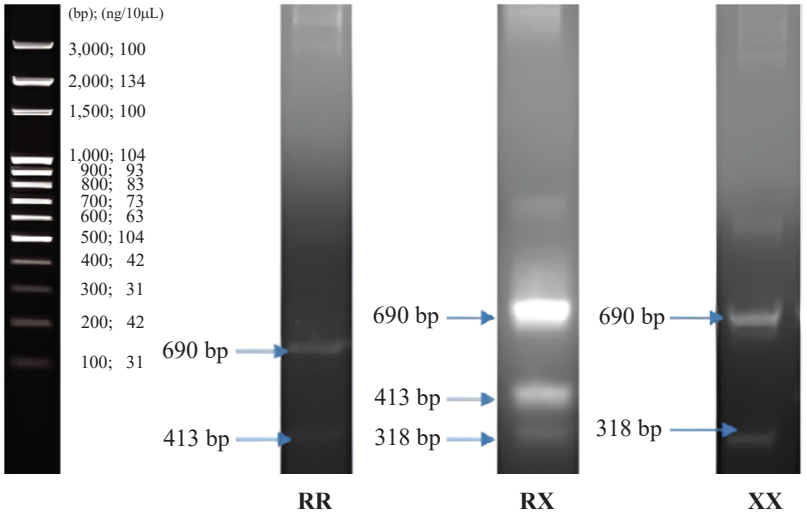

Figure 3. The length of each DNA band represented the RR, RX and XX genotypes of *ACTN3*.

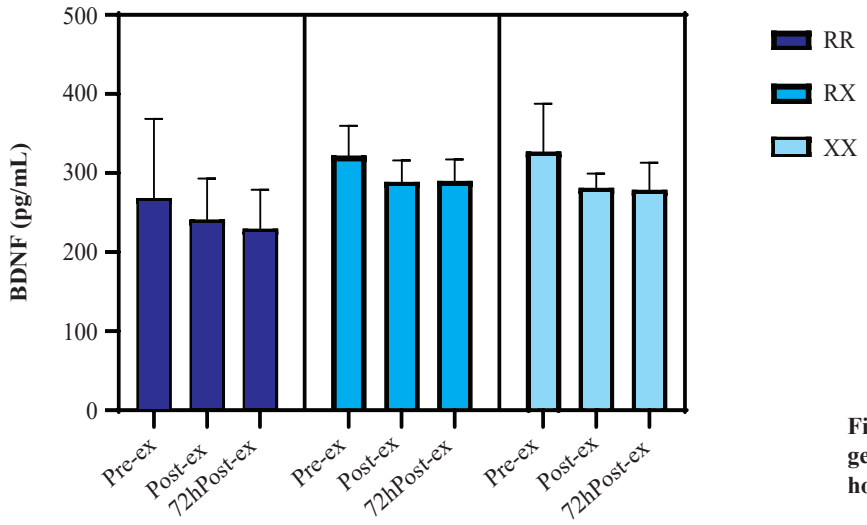

Figure 4. Differential levels of BDNF in three genotypes of *ACTN3* in pre-, post-, and 72 hours post-exercise.

the body is still in a recovery and adaptation phase, where the priority is on repairing damaged tissues and adapting to the new stressor, temporarily diverting resources away from BDNF synthesis.

In this study, the observed differential responses in BDNF levels to eccentric exercise across the *ACTN3* genotypes can be attributed to several key physiological and molecular mechanisms. For RR genotype, the consistent reduction of BDNF levels post-exercise suggests a uniform acute exercise-induced stress response and efficient adaptation mechanisms. These individuals most likely experience balanced inflammatory responses and effective metabolic adaptability, supporting neuronal and muscle repair.(12,29-33)

In contrast, the RX genotype exhibited initial higher BDNF levels, which then decreased post-exercise and remained stable over the course of 72 hours post-exercise. This genotype may have a slightly different regulatory mechanism, potentially due to the presence of both R and X alleles, which could influence muscle fiber composition and recovery efficiency.(17,33,34)

Similarly, the XX genotype exhibits a sustained decrease in BDNF levels post- exercise. This genotype may have a prolonged inflammatory response and inefficient muscle repair processes, resulting in continued suppression of BDNF production. The absence of α-actinin-3 in the XX genotype leads to altered muscle fiber composition and reduced capacity to handle physical stress, resulting in prolonged inflammation.(17,34) Studies suggest that higher oxidative stress levels and altered inflammatory pathways in XX individuals can hinder efficient muscle recovery and prolong inflammation.(35) Additionally, higher oxidative stress and prolonged activation of the HPA axis in XX individuals could further inhibit BDNF expression.(29,31) These factors, combined with reduced fast-twitch muscle fiber function, can lead to a more extended recovery period and lower BDNF levels even after 72 hours of relaxation.(36,37) These findings underscore the importance of considering genetic variations in designing personalized exercise programs and recovery strategies to optimize neurotrophic support and muscle health.

This study had a limited and non-diverse sample size which affects generalizability, a focus on short-term rather than long-term effects, and a lack of control over confounding variables such as diet and stress. It also lacks detailed exploration of the biological mechanisms underlying the observed responses, physiological metrics such as body mass index and waist circumference that could provide insights into metabolic variability, only examines eccentric exercise without comparing other modalities, and uses assessment methods that may not fully capture localized BDNF changes in brain and muscle tissues.

To address limitations and improve the study design, a case-control approach is recommended for future studies. This design would allow for more definitive and comparable clustering/grouping across *ACTN3* genotypes and time points, providing clearer insights into the relationship between genetic variations and BDNF responses. Future studies should also investigate the long-term effects of eccentric exercise on BDNF levels across *ACTN3* genotypes using extended training protocols instead of single exercise sessions. Additionally, incorporating physiological metrics, such as BMI and waist circumference and exploring the influence of other genetic factors or gene-environment

interactions, such as the impact of diet, sleep, and stress, could provide a more comprehensive understanding of neurotrophic responses. Incorporating neuroimaging techniques, such as functional MRI, could further elucidate the relationship between peripheral BDNF levels and central nervous system neuroplasticity in individuals with different *ACTN3* genotypes.

Conclusion

This study found that eccentric exercise leads to a decrease in BDNF levels, with no significant differences in response across *ACTN3* genotypes (RR, RX, XX). The consistent reduction in BDNF levels suggests a uniform response to exercise-induced stress and recovery processes across genotypes. These findings indicate that while *ACTN3* genotypes may not significantly influence BDNF response, understanding the broader genetic and physiological factors involved in exercise recovery remains important.

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Authors Contribution

ASK, AA, ADA, and MDH participated in the research conceptualization and planning. ASK, AA, and ADA handled data acquisition and collection. The experimental data was calculated and analyzed by ASK, AA, ADA, and MDH. ASK and AELS were responsible for drafting the manuscript and creating the figures, while MDH finalized and provided critical revisions to the manuscript.

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