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# Effects of High-Intensity Interval Training on Body Composition, Muscle Strength, and Cardiovascular Endurance in Junior Soccer Referees

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## ABSTRACT

This study examined the effects of an 8-week high intensity interval training (HIIT) program on body composition, muscular strength, and cardiovascular endurance in junior soccer referees. Thirty male referees (19.04 ± 2.31 years) were randomly assigned to a HIIT group (n = 15) or a control group (CON; n = 15). The HIIT group trained three times per week for 30 minutes at 60 to 80% of maximum heart rate, while the CON group maintained their usual activities. Body composition (body fat percentage [BF%] and body mass index [BMI]) was assessed using a Bioelectrical Impedance Analysis [BIA] device. Muscle strength was measured by one-repetition maximum (1RM) tests for leg curl. Cardiovascular endurance was assessed using the Yo-Yo Intermittent Recovery Test Level 1 (Yo-Yo IR1), and the results were used to estimate $VO_2$ max. Results showed significant within-group improvements in the HIIT group, including reductions in BF% and BMI and increases in leg curl strength, and $VO_2$ max ($P < 0.05$). In contrast, the CON group showed no significant changes in any variable ($P > 0.05$). Significant between-group differences were observed in all outcomes, favoring the HIIT group ($P < 0.05$). In conclusion, an 8-week HIIT program effectively improves body composition, muscular strength, and cardiovascular endurance in junior soccer referees, whereas no improvements were observed in the control group. HIIT may serve as a practical and time-efficient training strategy for enhancing referee-specific fitness.

**Key Words:** HIIT, Soccer Referees, Body Composition, Muscle Strength, Cardiovascular Endurance

## INTRODUCTION

Soccer refereeing is characterized by complex and intermittent physical demands that require officials to maintain optimal positioning, perceptual accuracy, and decision-making consistency throughout the duration of a match. Time–motion analyses have demonstrated that soccer referees typically cover total distances ranging from 9 to 12 km per match, with frequent bouts of high-intensity running, sprinting, rapid accelerations, decelerations, and changes of direction (1,2,4,5). These physical demands are often performed under conditions of cumulative fatigue, which may negatively influence both physical performance and officiating accuracy if referees are inadequately prepared (6,27,30).

To meet these demands, high levels of cardiovascular endurance, neuromuscular strength, and appropriate body composition are considered essential components of referee-specific fitness (2,4). Previous research has shown that referees with superior aerobic fitness are better able to sustain high-intensity running performance during the latter stages of matches and maintain closer proximity to play, thereby improving decision-making quality (1,3,27). Conversely, insufficient physical conditioning has been associated with increased fatigue-related errors, reduced movement efficiency, and elevated risk of musculoskeletal injury (7,8). Specifically, the intermittent nature of refereeing involves frequent explosive accelerations and rapid decelerations. These actions place immense mechanical stress on the posterior thigh muscles (hamstrings), which are responsible for decelerating the lower leg during the swing phase of sprinting and stabilizing the knee during changes of direction. Consequently, hamstring strength is a critical factor in mitigating injury risk and sustaining high-intensity performance throughout a match.

Despite the well-documented physical requirements of soccer refereeing, junior referees often lack access to structured and systematic physical conditioning programs. Training at this developmental stage is frequently informal, inconsistent, or limited to match participation alone, which may be insufficient to induce meaningful physiological adaptations (8,9). This is of particular concern given that junior referees represent a critical phase in the officiating pathway, during which foundational physical capacities are established, and long-term performance trajectories may be influenced (2,27).

High-intensity interval training (HIIT) has emerged as a time-efficient and effective training modality for improving both aerobic and anaerobic fitness. HIIT typically consists of repeated bouts of high-intensity exercise interspersed with short recovery periods, producing substantial cardiometabolic stress within relatively short training durations (10-12,29). A growing body of evidence indicates that HIIT can elicit significant improvements in maximal oxygen uptake ($VO_2$ max), intermittent running performance, neuromuscular function, and body composition across diverse athletic and physically active populations (13-17).

Importantly, the physiological characteristics of HIIT closely resemble the activity profile of soccer refereeing, which is defined by repeated high-intensity efforts interspersed with brief recovery phases (4,13,26). Studies conducted in soccer players and referees have demonstrated that HIIT-based interventions can improve intermittent running capacity and aerobic fitness more effectively than traditional continuous training methods (3,14,16). However, despite increasing interest in HIIT, empirical evidence examining its effects on junior soccer referees remains limited, particularly with respect to its combined influence on body composition, muscular strength, and cardiovascular endurance.

Therefore, the purpose of the present study was to investigate the effects of an 8-week high-intensity interval training (HIIT) program on body composition, lower-body muscular strength, and cardiovascular endurance in junior soccer referees. It was hypothesized that the HIIT intervention would result in significant improvements in these fitness components compared with a control condition.

## METHODS

### Subjects

Thirty junior male soccer referees (aged 18 to 20 years) registered with the Jieyang City Football Association (China) participated. The participants were randomly assigned to an HIIT Group (n = 15) or a Control Group (CON, n = 15). All were free of cardiovascular or neuromuscular disorders and provided informed consent.

### Procedures

The HIIT Group participated in a supervised training program three times per week for 8 weeks while maintaining their usual daily routines. Exercise intensity was continuously monitored using heart rate monitors and prescribed based on each participant's maximum heart rate (HRmax). Each session lasted approximately 50 minutes, including a 10-minute warm-up, a 30-minute main HIIT phase, and a 10-minute cool-down.

The main session consisted of 6 bodyweight exercises (leg raises, explosive step-ups, alternating side hops, scissor jumps, lateral step-ins, and squat jumps), each performed for 1 minute in an alternating-intensity format. Heart rate was maintained between 120 and 140 bpm (60-70% HRmax) and 140-170 bpm (70-80% HRmax). Completion of the 6 exercises constituted one circuit, and the participants performed 3 circuits per session with 2-minute rest intervals between circuits. This structured protocol ensured controlled intensity (60-80% HRmax), training effectiveness, and participant safety. The CON Group maintained their normal daily activities.

Pre- and post-intervention assessments included body composition (BF% and BMI measured using a Bioelectrical Impedance Analysis [BIA] device), lower-body muscle strength (1RM of leg curl), and cardiovascular endurance (Yo-Yo IR1 test, with $VO_2$ max estimated from performance).

### Statistical Analyses

The data are presented as mean ± SD. Paired *t*-tests were used to assessed within-group changes. Independent *t*-tests compared post-intervention differences between the Groups. The statistical significance was set at $P < 0.05$, and analyses were performed using the Statistical Product and Service Solutions.

## RESULTS

**Table 1. Participants Characteristics in Both Groups (N = 30).**

| Group | HIIT<br>N = 15 | CON<br>N = 15 |
|---|---|---|
| Age (years) | 19.05 ± 2.1 | 19.02 ± 2.5 |
| Weight (kg) | 61.7 ± 2.3 | 61.5 ±1.9 |
| Height (cm) | 172.6 ± 5.4 | 172.4 ± 5.6 |
| BMI (kg/㎡) | 20.71 ± 2.18 | 20.69 ± 2.09 |

Table 1 presents the baseline characteristics of participants in both the experimental and control groups prior to the intervention. The data indicate that the physical attributes of individuals in the 2 Groups are generally comparable with no significant differences observed ($P > 0.05$). This suggests that the random selection process was effective in ensuring group equivalence at the outset of the study.

### Body Composition

The HIIT Group showed significant reductions in BF% and BMI post-intervention ($P < 0.05$), while the CON Group showed no significant changes (Tables 1 and 2). Post-intervention, the HIIT Group had significantly lower BF% than the CON Group ($P < 0.05$).

**Table 2. Body Fat (%) in Both Groups (N = 30).**

| Group | HIIT | CON |
|---|---|---|
| Pre-Test | 21.13 ± 4.34 | 20.93 ± 3.47 |
| Post-Test | 18.00 ± 3.05$^{+*}$ | 21.20 ± 4.06 |

The data are presented as mean ± SD. $^{+}$Significant difference between the Pre-Test and Post-Test ($P < 0.05$). *Significant difference between the HIIT Group and the Control Group ($P < 0.05$).

**Table 3. BMI (kg/㎡) in Both Groups (N = 30).**

| Group | HIIT | CON |
|---|---|---|
| Pre-Test | 20.71 ± 2.18 | 20.69 ± 2.09 |
| Post-Test | 19.27 ± 1.99$^{+*}$ | 20.58 ± 2.04 |

The data are presented as mean ± SD. $^+$Significant difference between the Pre-Test and Post-Test ($P < 0.05$). *Significant difference between the HIIT Group and the Control Group ($P < 0.05$).

**Muscle Strength**

The 1RM for leg curl increased significantly in the HIIT Group ($P < 0.05$) but not in the CON Group (Tables 3 and 4). The post-intervention strength values were significantly higher in the HIIT Group compared to the CON Group ($P < 0.05$).

**Table 4. Leg Curl (kg) in Both Groups (N = 30).**

| Group | HIIT | CON |
|---|---|---|
| Pre-Test | 35.41 ± 7.16 | 35.64 ± 6.46 |
| Post-Test | 39.99 ± 6.56$^{+*}$ | 35.37 ± 6.83 |

The data are presented as mean ± SD. $^+$Significant difference between the Pre-Test and Post-Test ($P < 0.05$). *Significant difference between the HIIT Group and the Control Group ($P < 0.05$).

**Cardiovascular Endurance**

Yo-Yo IR1 performance (time and distance) and estimated $VO_2$ max improved significantly in the HIIT Group ($P < 0.05$) but not in the CON Group (Table 3). Post-intervention endurance metrics were significantly better in the HIIT Group ($P < 0.05$).

**Table 5. $VO_2$Max (ml/kg/min) in Both Groups (N = 30).**

| Group | HIIT | CON |
|---|---|---|
| Pre-Test | 51.50 ± 1.82 | 51.38 ± 1.65 |
| Post-Test | 52.56 ± 0.46$^{+*}$ | 51.25 ± 1.53 |

The data are presented as mean ± SD. $^+$Significant difference between the Pre-Test and Post-Test ($P < 0.05$). *Significant difference between the HIIT Group and the Control Group ($P < 0.05$).

**DISCUSSION**

The primary finding of the present study was that an 8-week HIIT program elicited significant improvements in body composition, lower-body muscular strength, and cardiovascular endurance in junior soccer referees. These findings support the initial hypothesis and are consistent with previous studies demonstrating the effectiveness of HIIT as a time-efficient training strategy for improving multiple components of physical fitness (10,15-17).

### Body Composition Adaptations

The participants in the HIIT Group exhibited significant reductions in body fat percentage and body mass index; whereas, no meaningful changes were observed in the Control Group. These findings align with previous meta-analyses and systematic reviews reporting favorable effects of HIIT on fat loss compared with moderate-intensity continuous training (17,18,28). The repeated exposure to high-intensity exercise likely increased total energy expenditure and excess post-exercise oxygen consumption, thereby enhancing lipid oxidation during the post-exercise recovery period (18).

From a practical perspective, the improvements in body composition may confer important performance benefits for soccer referees. Lower body fat levels are associated with improved running economy and reduced mechanical load during repetitive high-intensity movements (4,19). Given the substantial movement demands imposed on referees during match play, even modest reductions in body fat may contribute to improved movement efficiency and delayed fatigue onset (8,27).

### Muscular Strength and Neuromuscular Adaptations

The HIIT intervention also resulted in significant improvements in lower-body muscular strength, as evidenced by increased one-repetition maximum values in leg curl exercises. The specific enhancement of hamstring strength (as measured by the leg curl) is of particular importance for soccer referees. Biomechanically, the hamstrings play a vital role in hip extension for propulsion and they provide the eccentric braking force required to control limb momentum during high-speed running and sudden stops. Given that fatigue-induced reduction in hamstring strength is a primary precursor to non-contact injuries in soccer officiating, the observed improvements suggest that HIIT may offer a protective benefit against the high incidence of hamstring strains reported in this population. Although HIIT is traditionally associated with aerobic and metabolic adaptations, recent evidence suggests that high-intensity, explosive, and multi-directional movements can induce meaningful neuromuscular adaptations, particularly in individuals with limited resistance training experience (12,20,23).

The inclusion of plyometric and explosive exercises within the present HIIT protocol likely enhanced motor unit recruitment, neuromuscular coordination, and rate of force development. These adaptations are highly relevant to soccer referees, who must frequently accelerate, decelerate, and change direction in response to rapidly evolving match situations (5,21). Enhanced lower-body strength may therefore improve movement efficiency and contribute to injury prevention by increasing joint stability during high-load actions (7,22).

### Cardiovascular Endurance and Intermittent Performance

Significant improvements in the Yo-Yo Intermittent Recovery Test performance and estimated $VO_2$ max were observed following the HIIT intervention. These results are consistent with previous studies demonstrating the superior efficacy of HIIT for improving both maximal aerobic capacity and intermittent exercise performance (14-16,24). The Yo-Yo IR Test has been widely used to assess the ability to repeatedly perform high-intensity efforts with short recovery periods, and it is considered highly relevant to the physiological demands of soccer refereeing (13,25,26).

The observed improvements in cardiovascular endurance may be attributed to both central and peripheral physiological adaptations, including enhanced stroke volume, improved cardiac output, increased mitochondrial density, and greater oxidative enzyme activity within skeletal muscle (10,14,24). Collectively, these adaptations enhance oxygen delivery and utilization that enable referees to sustain higher work rates and resist fatigue during match play.

### Comparison With the Control Group and Practical Implications

The absence of significant changes in the Control Group highlights the limitations of relying solely on match officiating or unstructured physical activity to improve fitness. While match participation may help maintain baseline fitness levels, it appears insufficient to elicit progressive adaptations in aerobic capacity, muscular strength, or body composition (8,9,27).

From an applied perspective, the present findings support the inclusion of HIIT within referee development programs. HIIT offers a practical and time-efficient training approach that can be implemented with minimal equipment and can be adapted to various training environments. Incorporating HIIT sessions 2 to 3 times per week may enhance physical preparedness while accommodating the scheduling constraints commonly faced by junior referees.

### Limitations and Future Directions

Several limitations should be acknowledged. The relatively small sample size and inclusion of male referees only may limit the generalizability of the findings. Additionally, the intervention period was limited to 8 weeks, and no direct measures of match performance or decision-making accuracy were included. Future research should examine longer intervention periods, include female and elite referees, and explore the transfer of physiological adaptations to on-field officiating performance and decision-making quality (2,30).

### Summary

In summary, the present study demonstrates that an 8-week HIIT program effectively improves body composition, lower-body muscular strength, and cardiovascular endurance in junior soccer referees. These findings provide further evidence supporting HIIT as a sport-specific, time-efficient training strategy aligned with the intermittent physical demands of soccer officiating.

## CONCLUSIONS

The 8-week HIIT program induced significant improvements in body composition, cardiovascular endurance, and lower-body muscular strength, specifically targeting the hamstrings, which are a muscle group essential for coping with the repetitive acceleration and deceleration demands of officiating. The findings support HIIT as a sport-specific, time-efficient training method for referees (15,16). From an exercise physiology perspective, this study contributes evidence that HIIT can simultaneously enhance multiple fitness domains body composition, muscular strength, and cardiovascular endurance through integrated neuromuscular, metabolic, and cardiorespiratory adaptations (7-14). This underscores the efficacy of high-intensity, intermittent training paradigms in optimizing performance for athletic populations with sport-specific, intermittent physical demands.

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# Effects of Blood Flow Restriction versus Protein Supplementation on Muscle and Fat Morphology in Trained Males

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**Huang Q, Manimmanakorn A, Manimmanakorn N, Namboonlue C, La-bantao K, Nithisup p. Hamlin M.** The purpose of this study was to compare the effects of blood flow restriction (BFR) and protein supplementation on muscle and fat morphology in trained males. Thirty trained males were randomly assigned to 1 of 3 Groups and completed a 5-week low-load resistance training program (3 sessions per week). Outcome measures were assessed before and after the intervention. Both BFR and protein supplementation significantly increased muscle mass and thickness compared to baseline ($P < 0.05$), with BFR also resulting in a significant reduction in fat mass ($P < 0.05$). In low-load resistance training, blood flow restriction enhances both muscle and fat adaptations; whereas, protein supplementation primarily improves fat-related outcomes.

**Key Words:** Egg White, Fat, Muscle, Vascular Occlusion

## INTRODUCTION

High-load resistance or conventional resistance training (80% 1RM) was recommended for athlete who want muscle mass and strength gain. Muscle mass is a critical component for enhancing athletic performance in men with a history of consistent training (1). However, high-load resistance exercise can cause muscle and tendon injury. Thus, low-load resistance exercise combined with some strategies such as blood flow restriction (BFR) training is introduced as an alternative technique that has been shown to increase muscle strength and hypertrophy (16,18) while reducing joint stress (2,25). BFR induces muscle growth by creating local tissue hypoxia. Research has demonstrated that BFR combined with low-load training can effectively increase muscle mass and strength in trained males while imposing substantially less mechanical strain on joints compared to conventional high-intensity training (19).

Protein supplementation demonstrated increase muscle mass when combined with exercise especially with may exercise regimens. Protein supplement combined with low-load resistance has less studies. Recent research reports that low-load resistance training combined with blood flow restriction improves muscle mass in elderly people with limited mobility. The protein type is one importance key for build-up muscle mass. Consuming high-quality protein (e.g., whey protein) following exercise can enhance nitrogen balance and maximize the net benefit of training-induced protein synthesis (3,7,20). An adequate supply of amino acids is considered one of the most important factors for overcoming the muscle growth plateau, and trained males exhibit a higher muscle protein turnover rate (11). Egg white protein reported high-quality protein, rich in amino acids needed for muscle synthesis in the human body.

However, limited research has examined the effective protocol of both BFR and protein supplementation which combination of low-load training on body composition (muscle mass, fat mass) as most studies have focused exclusively on muscle adaptations.

In general, athletes prefer muscle mass increase while reduce fat mass. Although excessive fat accumulation can negatively affect athletic performance, adipose tissue serves as a necessary energy reserve for sustaining normal physiological functions and training intensity (24). The metabolic stress induced by BFR may exert additional effects on body fat, beyond influencing muscle protein synthesis by increasing post-exercise oxygen consumption or activating lipolysis-related pathways. Protein supplementation may not directly influence fat metabolism since its primary function is to provide substrates for muscle protein synthesis (13). Nevertheless, comparative research examining these two interventions remains scarce.

Therefore, this study employed a 5-week randomized controlled trial to compare the effects of BFR and protein supplementation on body composition, fat thickness, and muscle thickness in trained males. We hypothesized that both BFR and protein supplementation would improve muscle and fat morphology, and that BFR would exert a more comprehensive effect than protein supplementation.

## METHODS

### Participants

Thirty trained males were recruited (age: 21.1 ± 0.6 years; height: 174.2 ± 2.6 cm; body mass: 69.7 ± 4.2 kg; heart rate: 67.6 ± 8 bpm; body mass index: 21.29 ± 1.81 kg/m²). G*Power software was used to estimate the required sample size based on previous similar studies (13). With a statistical power of 0.80, a significance level of α = 0.05, and an effect size of f = 0.40 (indicating a moderate difference between group means), the calculated sample size was 7 participants per Group. To account for an anticipated dropout rate of approximately 30%, the target recruitment was set at 10 participants per Group, resulting in a total of 30 participants across the 3 Groups. The ***Inclusion Criteria*** were: (a) aged between 18 and 22 years; (b) at least 2 years of resistance training experience; and (c) the ability to abstain from alcohol, caffeine, and strenuous exercise for 24 hours prior to testing. The ***Exclusion Criteria*** were: (a) any musculoskeletal injury within the previous 6 months; (b) a history of cardiovascular disease, hypertension, thrombosis, or any other condition that might contraindicate participation; and (c) any other circumstances that could interfere with the study. After receiving a comprehensive explanation of the study's purpose, procedures, and potential risks, all the participants provided written informed consent.

**Procedures**

This study employed a randomized controlled trial design. The participants were randomly assigned to 1 of 3 Groups (Figure 1): (a) low-load resistance training only (LRT, n = 10); (b) low-load resistance training combined with blood flow restriction (BFR, n = 10); and (c) low-load resistance training combined with egg white protein supplementation (EPS n = 10). The study was approved by the Human Ethics Committee of Khon Kaen University (approval No. HE651425) and was conducted in accordance with the Declaration of Helsinki.

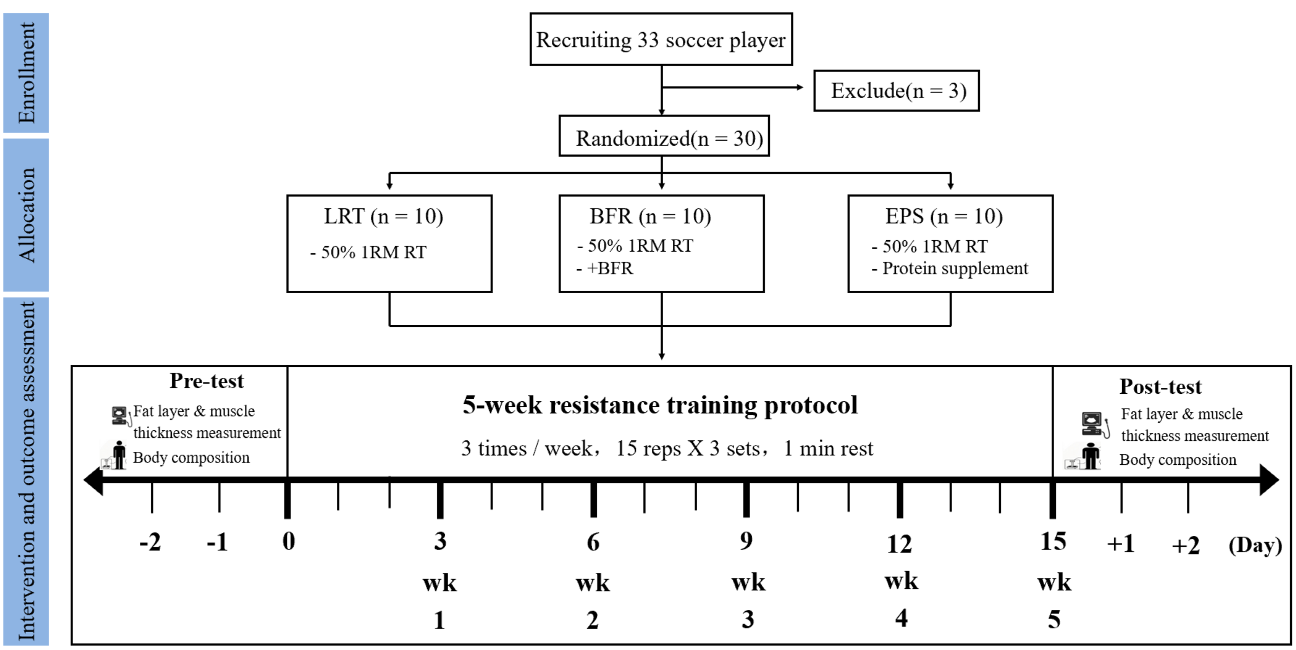



**Figure 1. Experimental Protocol and Training Protocols.**

All the participants performed resistance training at 50% of their one-repetition maximum (1RM) 3 times per week for 5 weeks (Figure 2). The training protocol consisted of knee extension and knee flexion exercises using plate-loaded machines (knee extension: Nautilus One™ S6LE; knee flexion: Nautilus EVO™ S9LCP; Nautilus, Inc., USA). Each session consisted of 3 sets of 15 repetitions with a 60-sec rest between sets. Concentric and eccentric phases were each performed over 2 sec, that was controlled by a metronome. Each training and testing session was preceded by a standardized warm-up that consisted of 5 minutes of active stretching and relaxation exercises.

***Blood Flow Restriction Protocol***
A pneumatic cuff (B-Strong, USA) was applied to the most proximal part of the thigh. Arterial occlusion pressure (AOP) was determined for each participant by placing a Doppler ultrasound probe (LOGIQ e, GE, USA) over the popliteal artery and gradually inflating the cuff until the arterial pulse signal disappeared. During the first 2 weeks of training, cuff pressure was set at 60% AOP, and for the remaining 3 weeks, it was increased to 80% AOP. The participants were instructed to report any discomfort or unusual pain during exercise immediately.

***Protein Supplementation Protocol***
During the intervention period, the participants in the EPS Group consumed egg white protein tablets (KKU 1, KKU Select Pharmaceutical Factory, Khon Kaen, Thailand) daily. The dosage was 2 tablets with each meal (breakfast, lunch, and dinner), totaling 6 tablets per day and providing 21 g of protein (3.5 g/tablet × 6 tablets). Supplementation began on the first day of training and continued daily for 5 weeks. In addition, the participants were instructed to refrain from taking any additional medications or supplements throughout the study period.

***Muscle Thickness and Fat Thickness Assessment***
Ultrasound imaging (LOGIQ e, GE Model Co., Ltd., USA) was used to measure the thickness of the vastus lateralis (VL) muscle and the overlying subcutaneous fat in the non-dominant leg. During measurements, the participants lay supine with their lower limbs relaxed and extended naturally. To avoid excessive pressure on the underlying tissue, the linear probe (5-13 MHz) was positioned perpendicular to the skin surface. The measurement site for the VL was determined as the midpoint between the greater trochanter of the femur and the superior border of the patella, corresponding to the largest portion of the muscle belly. Once a clear image was obtained, the probe was positioned along the longitudinal axis of the muscle, and the image was frozen. The distance between the superficial and deep aponeuroses was measured. The average of 2 independent measurements at each site was used for statistical analysis. Subcutaneous fat thickness was assessed from ultrasound images obtained at the same site, with the fat layer defined as the distance between the epidermal surface and the superficial aponeurosis. To ensure blinding, all measurements were performed by a qualified rehabilitation medicine physician who was unaware of Group allocation.

***Body Composition Assessment***
Body composition was assessed using bioelectrical impedance analysis (Seca mBCA, Hamburg, Germany) to determine pre- and post-intervention body mass, skeletal muscle mass, lean body mass, and body fat percentage. The participants wore light clothing during the assessment and removed all metal objects, shoes, socks, and heavy outerwear. They stood barefoot on the device, ensuring full contact between their feet and the metal electrode plates. Their hands rested naturally at their sides while gripping the metal handles on either side of the device to ensure complete contact between their palms and the electrodes. They remained relaxed and upright, keeping their arms away from their torso to avoid contact during

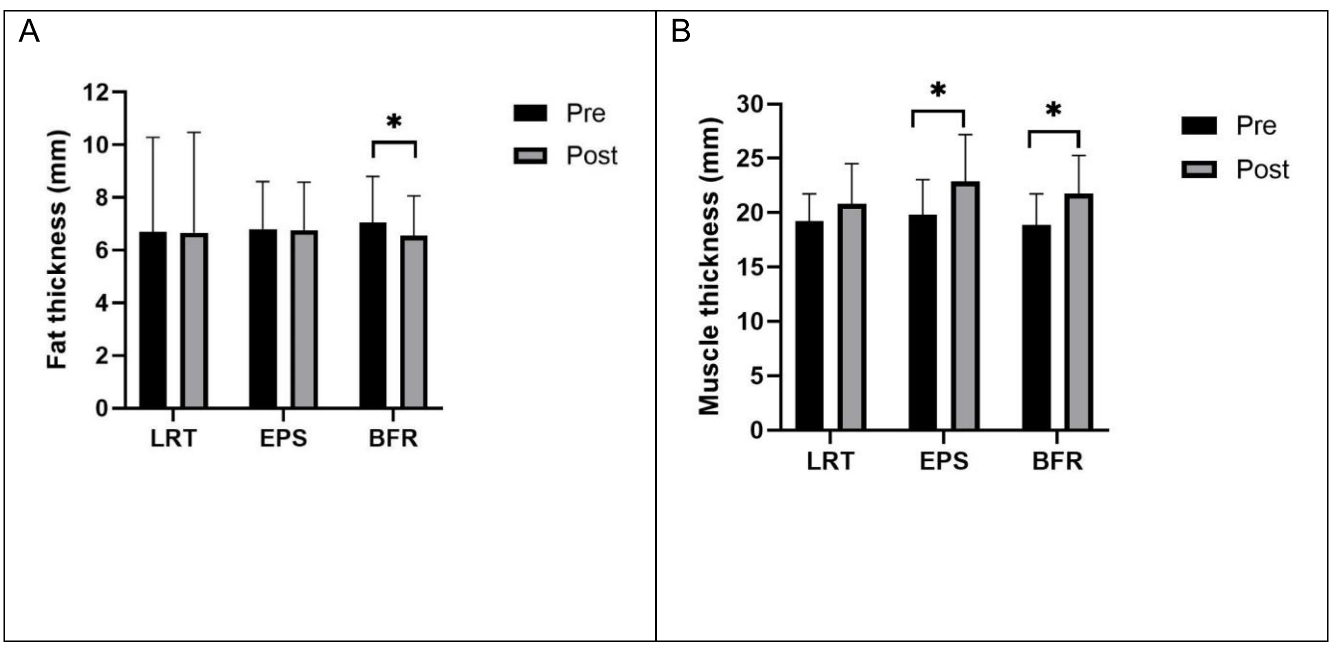
measurement. The same trained technician performed the measurement and recorded the data once the displayed impedance value had stabilized. To ensure accuracy and reproducibility, all the assessments were performed in the morning following a standardized preparation protocol: (a) fasting for at least 10 hours; (b) no strenuous exercise for 48 hours prior to testing; (c) avoidance of alcohol and caffeine for 24 hours prior to testing; (d) no excessive water intake for 4 hours prior to testing; and (e) bladder emptying immediately before measurement.

### Statistical Analysis

Statistical analyses were performed using SPSS (version 26.0, IBM). Descriptive data were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to assess differences between the Groups. *Post hoc* analyses were not conducted since no significant differences were observed between the Groups at baseline. Within-group changes from pre- to post-intervention were analyzed using paired *t*-tests. The significance level for all statistical tests was set at $P < 0.05$.

## RESULTS

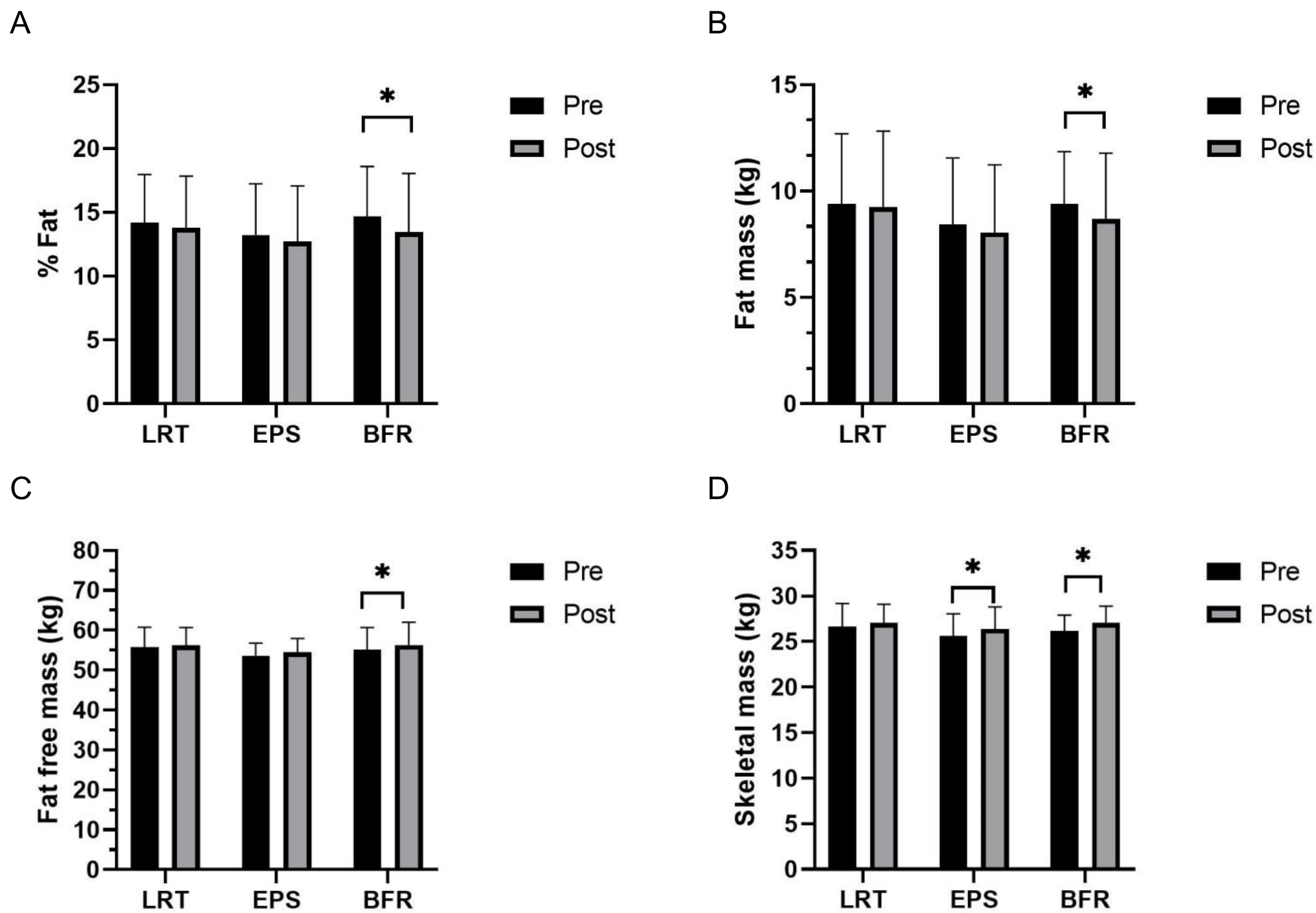
After 5 weeks of training, the BFR Group demonstrated significant changes in both fat thickness and muscle thickness compared to baseline. Fat thickness decreased from 7.04 ± 1.76 mm to 6.55 ± 1.51 mm ($P = 0.057$), while muscle thickness increased from 18.86 ± 2.88 mm to 21.73 ± 3.54 mm ($P = 0.047$). In the EPS Group, muscle thickness increased from 19.81 ± 3.24 mm to 22.86 ± 4.33 mm ($P = 0.008$). One-way ANOVA revealed no significant differences between Groups for either muscle thickness or fat thickness (Figure 2).




Note: * Within-group differences (Pre vs. Post), P < 0 .05.

**Figure 2. Fat Thickness (A) and Muscle Thickness (B) Before and After Exercise Training.**

After 5 weeks of training, the BFR Group demonstrated significant changes in body composition parameters compared to baseline, including percentage body fat, fat mass, fat-free mass, and skeletal muscle mass. Percentage body fat decreased from 14.68 ± 3.92% to 13.45 ± 4.60% ($P = 0.021$), fat mass decreased from 9.40 ± 2.46 kg to 8.70 ± 3.08 kg ($P = 0.047$), fat-free mass increased from 55.11 ± 5.62 kg to 56.31 ± 5.74 kg ($P = 0.008$), and skeletal muscle mass increased from 26.15 ± 1.74 kg to 27.05 ± 1.83 kg ($P = 0.001$). In the EPS Group, skeletal muscle mass increased from 25.61 ± 2.43 kg to 26.38 ± 2.42 kg ($P = 0.008$). One-way ANOVA revealed no significant differences between Groups for percentage body fat, fat mass, fat-free mass, or skeletal muscle mass (Figure 3).




Note： * Within-group differences（Pre vs. Post）, $P < 0.05$.

**Figure 3. Body Components Before and After Exercise Training Include %Fat (A), Fat Mass (B), Fat-Free Mass (C), and Skeletal Mass.**

## DISCUSSION

This study investigated the effects of 5 weeks of low-load resistance training (50% 1RM) combined with either blood flow restriction (BFR) or protein supplementation (EPS) on fat and muscle morphology in trained males. The main findings were that, compared to baseline, BFR significantly reduced percentage body fat and fat mass while increasing lean body mass, skeletal muscle mass, and vastus lateralis thickness. In contrast, protein supplementation significantly increased only skeletal muscle mass and vastus lateralis thickness.

The present study demonstrated that BFR decreased fat mass and increased muscle thickness. These findings are consistent with previous research findings (10,22). By partially restricting venous blood flow, BFR creates a localized hypoxic environment and promotes metabolic accumulation within the muscle tissue. This stimulates the recruitment of type II muscle fibers and enhances the acute secretion of anabolic hormones such as growth hormone that ultimately leads to muscle hypertrophy (9,17). Recent studies have shown that BFR training significantly increases post-exercise excess oxygen consumption (EPOC) and enhances exercise-related energy expenditure compared to training without BFR (4). These responses may be attributed to increased intramuscular acidity induced by exercise (26). Metabolic stress is known to activate type III afferent nerve fibers, which are sensitive to hydrogen ion concentration, leading to increased ventilation and heart rate (12), and consequently elevating energy expenditure during BFR exercise. This additional energy expenditure necessitates the utilization of body fat stores that may explain the reduction in fat mass observed in the BFR Group. Furthermore, fat breakdown may be directly or indirectly triggered by metabolite accumulation induced by BFR training. Studies have indicated that lactate, in addition to being a metabolic byproduct, may function as a signaling molecule that influences adipocyte activity via the G protein-coupled receptor (GPR81), thereby promoting fat mobilization (21).

In contrast to BFR, protein supplementation primarily increased skeletal muscle mass and thickness, while fat-related parameters remained largely unchanged. The observed increases in skeletal muscle mass were consistent with previous findings (5). However, no evidence was found to support a beneficial effect of protein supplementation on fat loss (27). Protein supplementation during resistance training provides ample substrates for muscle protein synthesis, thereby enhancing muscle fiber repair and growth (15). Exogenous amino acid supplements, particularly leucine, are effectively channeled toward muscle protein synthesis pathways, provided that energy balance is maintained (6). During exercise-induced muscle protein breakdown and synthesis, the additional amino acid substrates optimize the net protein balance without being diverted toward fat storage or inducing additional energy expenditure. This comparison indirectly suggests that the fat-reducing effect of BFR does not arise from an overflow of protein metabolism but is more likely attributable to its unique metabolic stress characteristics.

Although BFR demonstrated a more comprehensive effect than protein supplementation within the group, including muscle gain and fat loss, this advantage did not translate into statistically significant differences between the Groups. It is possible that the 5-week intervention period may not have been sufficient for the full effects of either intervention to manifest as statistically significant between-group differences. While both BFR and protein supplementation increase key outcome measures such as skeletal muscle mass and muscle thickness, low-load training alone (such as in the Control Group) may only induce modest adaptations. However, despite their distinct underlying mechanisms, protein supplementation likely acting through substrate optimization and BFR through metabolic stress, their net effects on protein synthesis may converge, resulting in similar magnitudes of adaptation. In addition, inter-individual variability in response may be a key influencing factor. Due to variations in genetic background, training status, and other individual characteristics, trained males exhibit considerable inter-individual variability in response to the same intervention (8). The underlying mean differences between the Groups may be partially masked by this considerable intra-group variability. For example, while some individuals may be more sensitive to the timing or dosage of protein supplementation, others may exhibit a heightened metabolic stress response to BFR. The sample size of this study may not have been sufficient to offset the loss of statistical power resulting from this considerable variability, despite following a standard design.

This study provides several practical implications for training. Protein supplementation is an effective and straightforward strategy when the primary goal is to increase muscle mass without increasing metabolic stress (28). Blood flow restriction training offers unique value due to its pleiotropic effects when the goal is body recomposition, particularly during periods when high-intensity training is not feasible (e.g., during the competitive season, recovery phases, or weight-cutting periods) (23). For individuals requiring long-term body composition management (e.g., athletes in weight-class sports), BFR may serve as a valuable adjunct to regular training, as its fat-loss effects may accumulate over time.

### Limitations in this Study

This study has several limitations. First, the 5-week intervention period may not have been sufficient for the fat-loss effects of BFR to fully manifest and result in statistically significant between-group differences. Second, the scope of mechanistic investigation was limited by the absence of relevant hormone measurements (e.g., growth hormone). Third, although the participants were instructed to maintain similar dietary habits, inter-individual variations in energy intake may have confounded the observed changes in muscle and fat mass.

## CONCLUSIONS

In low-load resistance training, BFR improves muscle thickness, fat thickness, and body composition, while protein supplementation increases muscle thickness and skeletal muscle mass. Therefore, BFR may be a suitable strategy for trained individuals seeking simultaneous fat loss and muscle gain; whereas, protein supplementation may be more appropriate for those primarily aiming to increase muscle mass. The long-term effects of these two interventions warrant further investigation.

## ACKNOWLEDGMENTS

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# Impact of Forward Head Posture on Postural Stability and Neck-Shoulder Muscle Pain Sensitivity in Computer-Based Workers

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**ABSTRACT**

**Thosap S, Hongkan C, Choosongsang S, Kongtong W**. Forward head posture (FHP) has become increasingly prevalent among office workers due to prolonged screen use. The purpose of this study was to investigate differences in static and dynamic postural stability and pressure pain threshold (PPT) between computer-based workers with and without FHP. Thirty-four female computer-based workers 25 to 40 years of age were classified into an FHP Group (craniovertebral angle [CVA] ≤ 48°) or a normal head posture (NHP) Group (CVA > 48°) using digital photogrammetry. Postural stability was assessed using the Biodex Balance System under 12 conditions involving variations in surface stability, visual input, and dynamic difficulty. The PPT of the upper trapezius (UT) and levator scapulae (LS) muscles was measured using a pressure algometer. Group differences were analyzed using independent *t*-tests or Mann–Whitney U Tests, with statistical significance set at $P < 0.05$. Participants with FHP demonstrated significantly greater anterior-posterior stability index (APSI) during foam surface with eyes closed condition under static balance compared with those with NHP ($P < 0.05$). Moreover, the FHP Group showed significantly higher APSI, medial-lateral stability index (MLSI), and overall stability index (OSI) during dynamic balance tasks at stability levels 9 and 5, particularly under reduced sensory conditions, compared with the NHP Group ($P < 0.05$). The PPT values of both the UT and right LS were significantly lower in the FHP Group than in the NHP Group ($P < 0.05$). These findings indicate that FHP adversely affects both static and dynamic postural stability, particularly under conditions requiring effective sensory integration, and is associated with increased neck-shoulder muscle pain sensitivity. Early identification of FHP and implementation of ergonomic interventions may help reduce postural instability and prevent the progression of musculoskeletal discomfort among computer-based workers.

**Key Words:** Computer-Based Worker, Forward Head Posture, Postural Stability, Pressure Pain Threshold

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## INTRODUCTION

Forward head posture (FHP) has become increasingly prevalent in modern work environments due to prolonged digital device usage and extended computer-based tasks. The working-age population, which forms the largest proportion of the national labor force, typically spends more than 6 hours per day in seated positions, often in suboptimal ergonomic conditions. These behavioral patterns contribute significantly to postural deviations, with FHP being among the most common and clinically relevant abnormalities. Biomechanically, FHP is characterized by anterior translation of the head relative to the shoulders, resulting in increased cervical lordosis, thoracic kyphosis, and heightened mechanical load on the cervical spine (2,15).

In 2019, Mahmoud et al. (14) reported that anterior head displacement dramatically increases compressive forces that act on the cervical vertebrae, which lead to chronic muscular overactivity and fatigue. Their findings are further supported by a 2025 study (19) that demonstrated increased stiffness and reduced elasticity of the superficial neck musculature including the upper trapezius (UT) in individuals with FHP. Such alterations in muscle tone and mechanical properties may lead to myofascial tenderness, decreased cervical endurance, and an elevated risk of neck and shoulder muscle pain.

Proper postural control requires effective interaction among visual, vestibular, and somatosensory systems. Balance is achieved when the body maintains its center of gravity (COG) within the base of support (BOS) through coordinated neuromuscular responses. Because the head serves as a primary sensorimotor platform that contains visual and vestibular organs, changes in head position may disrupt sensory integration and influence the ability of the central nervous system in maintaining equilibrium (6). In fact, several studies (3,8) have reported associations between FHP and postural instability.

Kang and colleagues (8) reported that computer-based workers with FHP exhibited increased postural sway during natural standing compared to those with normal cervical alignment. Similarly, individuals with pronounced FHP displayed reduced ankle joint range of motion and impaired static balance (7). Additionally, research has shown that FHP significantly affects dynamic balance performance, particularly when assessed using objective tools such as the Biodex Balance System (3).

However, findings across the literature remain inconsistent. Silva and Johnson (17) reported that induced FHP did not significantly alter postural control in healthy young adults. Their findings suggest that the sensorimotor system may be able to compensate for mild or experimentally induced postural deviations in asymptomatic individuals. This conflicting outcome highlights the need for more rigorous, multi-condition balance assessments to evaluate whether FHP truly compromises postural stability in the at-risk populations, such as computer-based workers.

In addition to balance impairments, FHP is strongly associated with increased neck pain and muscle tenderness. Evidence from systematic reviews indicates that decreased craniovertebral angle (CVA), which is characterized as FHP, correlates with greater neck pain intensity and disability (14). Pressure pain threshold (PPT), a semi-objective indicator of mechanosensitivity has been shown to decrease in individuals with chronic postural strain. Prolonged static postural loading, such as sustained computer work, has been demonstrated to significantly reduce the PPT of superficial cervical muscles, including the UT and levator scapulae (LS), which reflects an increase in mechanosensitivity and muscle tenderness associated with repetitive postural stress (20).

Despite increasing evidence that links FHP with cervical dysfunction and pain, few studies have comprehensively examined both balance performance and muscle pain sensitivity within the same cohort, particularly in asymptomatic individuals with FHP using standardized assessments across multiple sensory and dynamic conditions. Most studies have evaluated either static or dynamic balance in isolation or have primarily focused on symptomatic populations, without addressing early functional changes in individuals with FHP who do not yet report pain. These limitations leave important questions unanswered regarding how FHP influences the integrated relationship between sensorimotor control and musculoskeletal sensitivity in contemporary work environments.

For desk-based workers, who experience prolonged exposure to postural strain, understanding the interaction between FHP, balance impairment, and neck-shoulder muscle tenderness in asymptomatic individuals is essential to shape preventive ergonomic interventions and rehabilitation strategies. Therefore, the purpose of this study was to provide a comprehensive examination of FHP in asymptomatic desk-based workers by assessing its impact on multidimensional postural stability and neck-shoulder muscle pain sensitivity, quantified using PPT, thereby offering a better understanding how modern occupational habits may shape functional balance performance and musculoskeletal health.

## METHODS

### Subjects

A total of 34 sedentary female computer-based workers 25 to 40 years of age were recruited. All the participants had a normal body mass index (BMI) of 18.50 - 22.90 kg/m² based on Asian population standards. The participants reported at least 6 hours per day or more than 30 hours per week of computer screen time as assessed by self-report (8,15). Participants were excluded if they engaged in regularly structured physical activity, defined as ≥ 150 minutes per week of moderate-intensity aerobic activity or ≥ 75 minutes per week of vigorous-intensity aerobic activities, in accordance with the World Health Organization recommendations, or if they participated in sports (11).

In addition, individuals with a history of surgery or fractures, or those presenting with musculoskeletal or neuromuscular disorders, were excluded (8). Participants in the FHP Group had a CVA ≤ 48°; whereas, those with a CVA > 48° were assigned to the normal head posture (NHP) Group (1,16). Written informed consent was obtained from all the participants, and the study was approved by the Ethics Committee of Chulabhorn Royal Academy (EC 088/2565).

The sample size was determined using G*Power version 3.1.9.4 based on a previous study that reported a significant difference in the anterior–posterior stability index (APSI) between individuals with FHP and controls during a dynamic balance task (3). Using an effect size of 0.97, an alpha level of 0.05 and a statistical power of 0.80, the minimum required sample size was 28 participants. To account for an estimated 20% dropout rate and to enhance statistical stability, the final sample size was increased to 34 participants, who were equally allocated to the FHP Group (N = 17) and the NHP Group (N = 17).

**Procedures**

***Craniovertebral Angle (CVA)***

Cervical posture was classified using digital photogrammetry, a widely accepted method for identifying FHP. Non-reflective markers (20-mm diameter) were placed on the tragus of the ear and the C7 spinous process. Lateral images were captured using a Nikon D5100 digital camera positioned at shoulder height and at a standardized distance of 1.5 m from each participant (1,3). The CVA was analyzed using Kinovea motion-analysis software (version 0.9.5) that measured the angle formed between a horizontal reference line through C7 and the line joining C7 to the tragus. Based on previously validated cut-off point, the participants with CVA ≤ 48° were categorized as having FHP, while those with CVA > 48° were classified as having NHP (1,16). This cut-off point has been shown to effectively distinguish individuals with meaningful postural deviation. To ensure measurement reliability, CVA assessment was conducted by a blinded examiner who demonstrated excellent intra-rater reliability, with an intraclass correlation coefficient (ICC) of 0.89, which is consistent with high-quality postural measurement standards.

***Pressure Pain Threshold (PPT)***

The PPT of the UT and LS was evaluated using a pressure algometer (Model 12-0300 MMT) with a 1 $cm^2$ probe. The UT site was located at the midpoint between the 7th cervical spine and acromion, while the LS site was identified approximately 2 cm above the superior medial border of the scapula. The participants were examined while sitting in an upright position with the arm supported to prevent muscle guarding (5,18). Pressure was applied perpendicular to the muscle until the participants indicated pain by either raising their hand or reporting it verbally. Three trials were recorded per site, each separated by 2 minutes of rest, and mean values were used for analysis. This protocol aligns with previous research demonstrating strong reliability for PPT assessment (18). All PPT measurements were performed by a trained examiner who was blinded, with a high intra-rater reliability (ICC = 0.90).

***Postural Stability Assessment***

After the PPT assessment, the postural stability was assessed using the Biodex Balance System SD (Biodex Medical Systems, Shirley, NY, USA), a validated and reliable instrument for quantifying static and dynamic postural control. The system provides 3 stability indices: (a) the overall stability index (OSI), representing the composite variance of platform displacement; (b) the APSI; and (c) the medial-lateral stability index (MLSI), with lower scores indicating superior postural stability (3).

The participants completed 12 balance testing conditions, comprising both static and dynamic tasks. Static balance was evaluated under 4 conditions: firm and foam surfaces with eyes open and eyes closed. Dynamic balance was assessed at platform stability levels 9 and 5, with level 9 representing greater platform stability and level 5 representing lower platform stability, on both firm and foam surfaces, again under eyes-open and eyes-closed conditions. Each condition consisted of 3 trials of 20 seconds, with 10 seconds of rest between trials. To minimize the influence of fatigue and carryover effects, a 60-second rest interval was provided between the testing sessions, including the static, dynamic level 9, and dynamic level 5 conditions (1,3). The order of all testing conditions was randomized to reduce potential learning effects. The participants were tested barefoot and positioned on the platform using a standardized foot placement protocol. They were instructed to maintain the on-screen cursor as close to the center of the target as possible throughout each trial. All assessments were conducted by the same blinded examiner under consistent environmental conditions.

### Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics (version 26.0; IBM, Armonk, NY, USA). Data distribution was assessed for normality using the Shapiro-Wilk Test. Between-group comparisons were conducted using independent *t*-tests for normally distributed variables and Mann-Whitney U Tests for variables that violated normality assumptions (1,3). Specifically, Mann-Whitney U Tests were applied to dynamic balance variables assessed on the foam surface with eyes closed, including OSI and APSI at stability level 9, as well as OSI, APSI, and MLSI at stability level 5. All remaining balance variables were analyzed using independent *t*-tests. Effect sizes were calculated using Cohen's d and interpreted as follows: a value of 0.2 is considered a small effect, 0.5 a medium effect, and 0.8 or above a large effect. The level of statistical significance was set at $P < 0.05$.

## RESULTS

### Participant Characteristics

There were no significant differences in the participant characteristics between the FHP Group and the NHP Group, except for the CVA (Table 1). The CVA was significantly smaller in the FHP Group than in the NHP Group ($P < 0.001$), confirming appropriate group classification.

**Table 1. Characteristics of Participants in the PF and NHP Groups.**

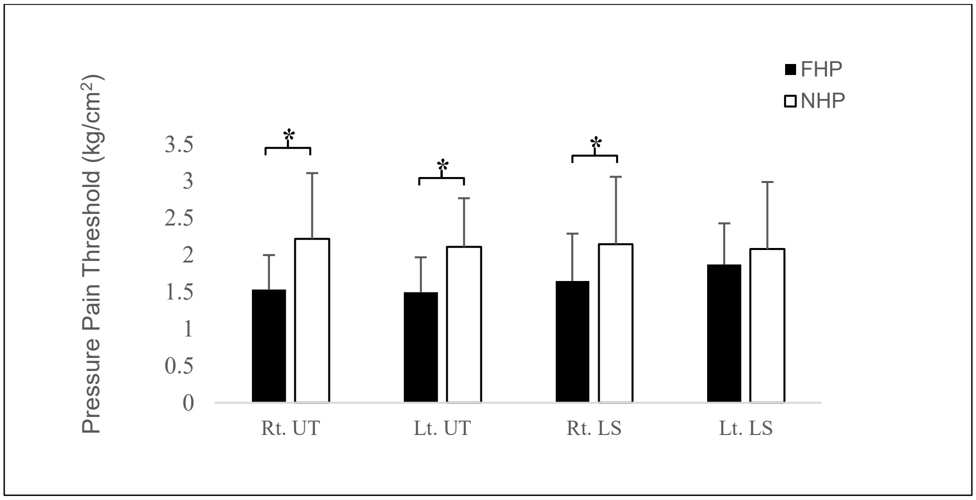
| Characteristics | FHP Group (N = 17) | NHP Group (N = 17) | P-value |
|---|---|---|---|
| **Age** (year) | 31.82 ± 4.69 | 30.94 ± 3.94 | 0.086 |
| **Weight** (kg) | 54.74 ± 5.24 | 53.77 ± 6.20 | 0.432 |
| **Height** (m) | 1.59 ± 0.06 | 1.60 ± 0.07 | 0.692 |
| **BMI** ($kg/m^2$) | 21.60 ± 1.61 | 21.08 ± 1.41 | 0.158 |
| **Daily computer use** (hour/day) | 7.76 ± 1.90 | 8.29 ± 2.26 | 0.684 |
| **Work experience** (year) | 8.99 ± 6.98 | 7.99 ± 5.23 | 0.093 |
| **CVA** (degree) | 45.75 ± 1.54 | 54.71 ± 3.96 | $< 0.001^*$ |

**BMI**, Body Mass Index; **CVA**, Craniovertebral Angle; **FHP**, Forward Head Posture; **NHP**, Normal Head Posture. ***** $P < 0.05$.

### Pressure Pain Threshold (PPT)

The FHP Group demonstrated significantly lower PPT values in the right UT ($P = 0.012$, effect size = 0.380), left UT ($P = 0.044$, effect size = 0.673), and right LS ($P = 0.040$, effect size = 0.207) compared with the NHP Group (Figure 1).

**Figure 1. The Pressure Pain Threshold (PPT) Values in the FHP Group and the NHP Group.**




**Rt**: Right, **Lt**: Left, **UT**: Upper Trapezius, **LS**: Levator Scapulae, *P < 0.05

**Postural Stability**

During static balance testing, the FHP Group demonstrated significantly greater APSI on the foam surface with eyes closed compared with the NHP Group (P = 0.002, effect size = 0.216) (Table 2).

**Table 2. Postural Stability Index during Static Balance Test.**

| Postural Stability Index | FHP Group (N = 17) | NHP Group (N = 17) | P-value | Effect Size |
|---|---|---|---|---|
| **Firm Surface, Eyes Open** | | | | |
| APSI | 2.02 ± 1.48 | 2.37 ± 2.56 | 0.473 | 0.172 |
| MLSI | 1.07 ± 0.73 | 0.86 ± 1.05 | 0.079 | 0.591 |
| OSI | 2.37 ± 1.40 | 2.58 ± 2.61 | 0.593 | 0.112 |
| **Firm Surface, Eyes Closed** | | | | |
| APSI | 2.46 ± 1.94 | 2.30 ± 1.80 | 0.904 | 0.109 |
| MLSI | 0.91 ± 0.45 | 0.73 ± 0.78 | 0.098 | |
| OSI | 3.13 ± 2.05 | 2.56 ± 1.76 | 0.380 | 0.311 |
| **Foam Surface, Eyes Open** | | | | |
| APSI | 2.18 ± 1.06 | 2.05 ± 1.40 | 0.930 | 0.113 |
| MLSI | 1.16 ± 0.63 | 1.02 ± 0.79 | 0.341 | 0.207 |
| OSI | 2.83 ± 1.01 | 2.64 ± 2.61 | 0.103 | 0.162 |
| **Foam Surface, Eyes Closed** | | | | |
| APSI | 3.52 ± 1.85 | 1.97 ± 0.75 | 0.002* | 0.216 |
| MLSI | 1.63 ± 0.57 | 1.37 ± 0.99 | 0.105 | 0.352 |
| OSI | 4.13 ± 1.6 | 2.66 ± 1.45 | 0.211 | 0.67 |

**APSI**, Anterior-Posterior Stability Index; **MLSI**, Medial-Lateral Stability Index; **OSI**, Overall Stability Index; **FHP**, Forward Head Posture; **NHP**, Normal Head Posture. * P < 0.05.

During dynamic balance testing at stability level 9, the FHP Group exhibited significantly higher APSI (P = 0.008, effect size = 0.760), MLSI (P = 0.041, effect size = 0.162), and OSI (P = 0.011, effect size = 0.821) on the foam surface with eyes closed compared with the NHP Group (Table 3).

**Table 3. Postural Stability Index during Dynamic Balance Test at Stability Level 9.**

| Postural Stability Index | FHP Group (N = 17) | NHP Group (N = 17) | P-value | Effect Size |
|---|---|---|---|---|
| **Firm Surface, Eyes Open** | | | | |
| APSI | 2.20 ± 1.71 | 2.02 ± 1.98 | 0.078 | 0.391 |
| MLSI | 1.87 ± 0.99 | 1.98 ± 1.32 | 0.321 | 0.611 |
| OSI | 2.55 ± 2.18 | 2.09 ± 1.79 | 0.095 | 0.237 |
| **Firm Surface, Eyes Closed** | | | | |
| APSI | 3.28 ± 1.77 | 3.01 ± 2.13 | 0.129 | 0.277 |
| MLSI | 3.04 ± 2.12 | 2.78 ±1.55 | 0.078 | 0.517 |
| OSI | 3.67 ± 2.44 | 3.12 ± 2.41 | 0.213 | 0.241 |
| **Foam Surface, Eyes Open** | | | | |
| APSI | 3.89 ± 2.61 | 2.98 ± 2.75 | 0.098 | 0.989 |
| MLSI | 3.23 ± 1.78 | 2.99 ± 2.89 | 0.076 | 0.231 |
| OSI | 4.54 ± 2.76 | 4.12 ± 2.69 | 0.322 | 1.091 |
| **Foam Surface, Eyes Closed** | | | | |
| APSI | 4.99 ± 2.18 | 4.01 ± 2.24 | 0.008* | 0.760 |
| MLSI | 4.89 ± 1.96 | 4.54 ± 2.89 | 0.041* | 0.162 |
| OSI | 5.11 ± 2.77 | 5.36 ± 1.85 | 0.011* | 0.821 |

**APSI**, Anterior-Posterior Stability Index; **MLSI**, Medial-Lateral Stability Index; **OSI**, Overall Stability Index; **FHP**, Forward Head Posture; **NHP**, Normal Head Posture. * P < 0.05.

At stability level 5, the FHP Group demonstrated significantly greater APSI on the firm surface with eyes open (P = 0.021, effect size = 0.836) compared with the NHP Group. Additionally, significantly higher MLSI was observed on the foam surface with eyes open (P = 0.003, effect size = 0.990) compared with the NHP Group. On the foam surface with eyes closed, the FHP Group exhibited significantly greater APSI (P = 0.017, effect size = 0.174), MLSI (P = 0.039, effect size = 0.278), and OSI (P = 0.045, effect size = 0.259) compared with the NHP Group (Table 4).

**Table 4. Postural Stability Index during Dynamic Balance Test at Stability Level 5.**

| Postural Stability Index | FHP Group (N = 17) | NHP Group (N = 17) | P-value | Effect Size |
|---|---|---|---|---|
| **Firm Surface, Eyes Open** | | | | |
| APSI | 4.42 ± 2.98 | 3.77 ± 2.09 | 0.021* | 0.836 |
| MLSI | 4.21 ± 2.52 | 3.67 ± 2.66 | 0.087 | 0.611 |
| OSI | 4.78 ± 2.78 | 4.12 ± 2.51 | 0.132 | 0.373 |
| **Firm Surface, Eyes Closed** | | | | |
| APSI | 5.98 ± 3.12 | 4.99 ± 3.42 | 0.104 | 0.322 |
| MLSI | 5.74 ± 3.05 | 5.10 ± 2.78 | 0.899 | 1.120 |
| OSI | 6.32 ± 3.88 | 5.37 ± 3.91 | 1.005 | 0.434 |
| **Foam Surface, Eyes Open** | | | | |

| | | | | |
|---|---|---|---|---|
| APSI | 6.66 ± 2.10 | 5.94 ± 2.87 | 0.067 | 1.021 |
| MLSI | 6.56 ± 2.39 | 5.12 ± 3.01 | 0.003* | 0.990 |
| OSI | 7.27 ± 3.87 | 6.78 ± 3.79 | 0.069 | 0.992 |
| **Foam Surface, Eyes Closed** | | | | |
| APSI | 8.03 ± 4.29 | 7.22 ± 3.87 | 0.017* | 0.174 |
| MLSI | 6.89 ± 3.12 | 5.96 ± 3.67 | 0.039* | 0.278 |
| OSI | 9.12 ± 3.71 | 8.58 ± 3.99 | 0.045* | 0.259 |

**APSI**, Anterior-Posterior Stability Index; **MLSI**, Medial-Lateral Stability Index; **OSI**, Overall Stability Index; **FHP**, Forward Head Posture; **NHP**, Normal Head Posture. * $P < 0.05$.

## DISCUSSION

The present study investigated the influence of FHP on postural stability and neck-shoulder muscle pain sensitivity in computer-based workers. The primary findings indicate that individuals with FHP exhibit significantly reduced static and dynamic balance, particularly under conditions that challenge sensory integration as well as increased pain sensitivity of the UT and LS muscles compared with individuals without FHP. Although participant characteristics were not matched between the Groups, independent *t*-tests revealed no significant differences in these characteristics, except for the CVA. This suggests that the observed differences in postural stability and muscle pain sensitivity were not influenced by participant characteristics other than the presence of FHP versus NHP.

### Postural Stability

Postural control depends on the integration of visual, vestibular, and somatosensory inputs to maintain the COG within the BOS (4). FHP alters head alignment relative to the trunk, shifting the COG anteriorly and potentially disrupting vestibular signaling and cervical proprioceptive input. Static balance testing on an unstable surface with eyes closed challenges both visual input and the reliability of somatosensory feedback, thereby increasing reliance on vestibular information. Under these conditions, effective sensory reweighting is essential for maintaining stability. The significantly greater anterior-posterior instability observed in the FHP group under this condition suggests impaired multisensory integration when reliance on vestibular and cervical afferents is increased (1,4,8). Altered head posture may influence the orientation of the vestibular apparatus and disrupt afferent signaling from upper cervical mechanoreceptors, both of which contribute to spatial orientation and postural reflex control. Consequently, individuals with FHP may demonstrate a reduced ability to appropriately reweight vestibular input when visual and somatosensory cues are compromised, resulting in increased postural sway.

These findings are consistent with previous studies reporting greater COG sway velocity and total sway displacement in individuals with FHP during static balance tasks performed on unstable surfaces with visual deprivation (12). Prior authors have suggested that structural and neuromuscular alterations associated with FHP may impair sensorimotor integration, thereby reducing postural stability. Collectively, the present results support the concept that FHP may influence balance regulation through altered sensory processing rather than solely through mechanical postural deviation.

The dynamic balance findings further support this interpretation. The increased APSI, MLSI, and OSI observed at stability levels 9 and 5 - under both eyes-open and eyes-closed conditions - indicate impaired postural control in both anterior–posterior and medial–lateral directions in individuals with FHP. Dynamic postural control requires rapid neuromuscular responses, precise trunk–cervical coordination, and continuous sensory recalibration to counteract platform perturbations. In individuals with FHP, anterior displacement of the head may shift the COG forward and alter trunk–cervical coupling, thereby increasing mechanical demand and postural sway during dynamic tasks. As platform instability increases (e.g., higher Biodex challenge levels), effective integration and reweighting of sensory inputs become increasingly critical (1,4). The greater instability observed at these levels suggests that FHP may compromise adaptive postural strategies under heightened mechanical and sensory demands. Importantly, group differences were amplified under eyes-closed conditions, reinforcing the role of impaired sensory integration rather than purely mechanical imbalance. In the absence of visual input, reliance on vestibular and proprioceptive feedback increases substantially. If altered cervical alignment affects afferent signaling or vestibular orientation, compensatory responses may be insufficient, resulting in elevated sway indices (4,8,13). Collectively, these findings suggest that FHP is not merely a static postural deviation but may represent a sensorimotor control alteration that adversely affects dynamic balance regulation, particularly under sensory-challenging conditions.

These findings are consistent with previous studies demonstrating impaired dynamic postural stability in individuals with FHP (1,3). A previous study reported reduced dynamic stability in individuals with FHP compared with controls during Biodex Balance System testing, indicating compromised postural control mechanisms (1). Similarly, another study observed significantly higher OSI, APSI, and MLSI in individuals with FHP under dynamic conditions, indicating reduced dynamic postural stability (3). These impairments have been attributed to anterior displacement of the head, which shifts the COG and increases mechanical and neuromuscular demands on the postural control system. Additionally, altered cervical alignment may impair proprioceptive input from cervical mechanoreceptors, reducing the accuracy of sensory feedback required for rapid postural corrections during dynamic tasks. Importantly, previous research has suggested that increased MLSI may result from impaired coordination between proximal and distal body segments, as effective control of distal segment motion depends on stable proximal alignment (3). In individuals with FHP, disruption of head-neck-trunk alignment may compromise proximal stability and alter postural control strategies, resulting in less efficient coordination between body segments during balance tasks. Consequently, individuals with FHP may rely on less effective stabilization mechanisms when responding to platform perturbations, contributing to increased MLSI. Collectively, these mechanisms provide further support for the present findings and highlight the adverse influence of FHP on dynamic postural stability.

### Neck-Shoulder Muscle Pain Sensitivity

The reduced PPT observed in the UT and LS in the FHP group indicates increased mechanical sensitivity, reflecting heightened muscle pain sensitivity despite the absence of clinical symptoms. In FHP, anterior displacement of the head increases the external moment arm and places greater mechanical demand on the cervical extensor and scapular stabilizing muscles to maintain head and neck alignment. This sustained loading may lead to muscle fatigue, increased intramuscular tension, and mechanical stress on surrounding connective tissues, which can lower the threshold for pain perception (9,10,20). Additionally, prolonged postural stress may alter muscle fiber length and impair normal muscle function, further reducing the tolerance of these muscles to external pressure.

These findings are consistent with previous studies demonstrating an association between FHP and increased neck-shoulder pain and muscle tenderness (9,21). A previous study reported that a decreased CVA, indicating greater severity of FHP, was a significant predictor of cervical pain, as prolonged forward head alignment increases mechanical stress on cervical muscles and connective tissues (9). Similarly, another study reported that sustained forward head and neck posture during computer work significantly reduced the PPT of the UT and LS, indicating increased muscle tenderness due to continuous mechanical loading and postural stress (21). These previous findings support the present results and suggest that FHP contributes to increased muscle tenderness and heightened pain sensitivity in the neck–shoulder muscles despite the absence of clinical pain.

### Limitations in this Study

This study has several limitations that should be considered when interpreting the findings. First, the sample consisted exclusively of female computer-based workers within a specific age range and BMI category, which may limit the generalizability of the findings to males, other age groups, or populations with different occupational or physical characteristics. Second, although major confounding conditions were controlled through strict inclusion and exclusion criteria, other factors such as psychosocial stress, ergonomic workstation variability, and daily postural habits were not objectively quantified and may have influenced postural control and muscle pain sensitivity. Finally, muscle tenderness assessment was limited to the UT and LS, and other muscles involved in cervical and scapular stabilization were not evaluated. Future studies including broader populations and additional neuromuscular assessments are needed to expand understanding of the functional consequences of FHP.

## CONCLUSIONS

This study demonstrated that computer-based workers with FHP exhibit impaired postural stability and greater neck-shoulder muscle pain sensitivity despite the absence of clinical symptoms compared with individuals without FHP. Individuals with FHP showed greater instability during both static and dynamic balance tasks, particularly under sensory-challenging conditions, indicating compromised sensorimotor control. In addition, reduced PPT in the UT and LS suggests increased muscle pain sensitivity associated with altered cervical alignment. These findings highlight the importance of early identification of FHP and the implementation of ergonomic and corrective strategies to improve balance control and prevent the progression of musculoskeletal symptoms in sedentary computer-based workers.

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# Enhancing Muscle Quality and Functional Capacity in Aging: Potential Roles for Beta-Alanine–Derived Carnosine

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## ABSTRACT

Age-related declines in skeletal muscle function contribute to reduced mobility, increased fall risk, and loss of independence in older adults. While preservation of muscle mass has historically been emphasized, emerging evidence indicates that deterioration in muscle quality, including impaired excitation–contraction coupling, reduced intracellular buffering capacity, mitochondrial dysfunction, and increased oxidative stress play a critical role in functional decline. Carnosine (β-alanyl-L-histidine) is an intramuscular dipeptide with roles in intracellular pH buffering, antioxidant defense, carbonyl scavenging, and modulation of calcium sensitivity. Because β-alanine availability is the rate-limiting determinant of carnosine synthesis in skeletal muscle, supplementation provides a targeted strategy to increase intramuscular carnosine concentrations. This narrative review synthesizes mechanistic and applied literature examining carnosine biology and the effects of β-alanine supplementation on skeletal muscle function in aging populations. Literature was identified through structured searches of biomedical and exercise science databases including PubMed/MEDLINE, Scopus, and Google Scholar. Evidence was qualitatively synthesized across domains related to muscle quality, intracellular buffering, oxidative stress, and supplementation outcomes in older adults. The results indicate that β-alanine supplementation reliably increases intramuscular carnosine concentrations in both younger and older adults. Elevated muscle carnosine may enhance fatigue resistance through improved buffering of hydrogen ions, preservation of calcium sensitivity, and attenuation of oxidative and carbonyl stress. In older adults, supplementation has been associated with improvement in physical working capacity and exercise tolerance. Although the effects on muscle mass and maximal strength appear limited. Conclusions: β-alanine–derived carnosine represents a mechanistically relevant target for supporting muscle quality in aging skeletal muscle, when integrated with resistance training and adequate nutrition β-alanine supplementation may contribute to strategies aimed at preserving functional capacity and independence in older adults.

**Key Words:** Aging; β-alanine; Carnosine; Fatigue Resistance; Functional Performance; Intracellular Buffering; Mitochondrial Function; Muscle Quality; Oxidative Stress; Sarcopenia

## INTRODUCTION

Aging is accompanied by a progressive decline in skeletal muscle mass, strength, and functional capacity that collectively increases the risk for disability, falls, hospitalization, and mortality (13). While the loss of muscle mass, traditionally termed sarcopenia, has long been emphasized, accumulating evidence suggests that deterioration in muscle quality often precedes or exceeds reductions in muscle size. Muscle quality encompasses neuromuscular activation, excitation–contraction coupling efficiency, metabolic resilience, mitochondrial integrity, fatigue resistance, and force production relative to cross-sectional area (4,33).

Importantly, age-related declines in physical performance cannot be explained solely by reductions in lean mass. Aging skeletal muscle demonstrates impaired intracellular buffering capacity, reduced calcium sensitivity, mitochondrial dysfunction, increased oxidative stress, and altered redox signaling (4,28,38). These changes contribute to early fatigue during both high-intensity and submaximal activities, limiting functional independence even when muscle mass is partially preserved.

Current nutritional strategies to preserve muscle function have largely focused on optimizing protein intake and supplementing creatine to support muscle protein synthesis and rapid ATP regeneration (8,11). While these approaches improve lean mass and maximal strength, they do not fully address persistent declines in fatigue resistance, intracellular acidosis tolerance, or contractile efficiency (31). Thus, complementary interventions targeting the biochemical determinants of muscle quality are warranted.

Carnosine (β-alanyl-L-histidine) is an intramuscular dipeptide with multifunctional roles in intracellular pH buffering, antioxidant defense, carbonyl scavenging, and potential modulation of calcium sensitivity (1,3,45). Muscle carnosine concentrations decrease with aging (44), potentially contributing to impaired buffering capacity and increased susceptibility to metabolic stress. Because β-alanine availability is the rate-limiting determinant of carnosine synthesis in skeletal muscle (19,25), supplementation with β-alanine provides a targeted strategy to augment intramuscular carnosine stores.

This review synthesizes current evidence regarding the biological relevance of β-alanine–derived carnosine in aging skeletal muscle, evaluates existing supplementation data in older adults, and discusses its potential integration within multimodal strategies aimed at preserving muscle quality and functional capacity.

## METHODS

This manuscript was conducted as a narrative review using structured literature identification and selection procedures. The primary objectives of the review were to examine age-related alterations in skeletal muscle quality with particular emphasis on intracellular buffering capacity, excitation–contraction coupling efficiency, oxidative stress, and mitochondrial dysfunction: (a) to synthesize current knowledge regarding carnosine biology and the mechanistic relevance of β-alanine as the rate-limiting precursor for intramuscular carnosine synthesis; and (b) to evaluate human supplementation studies investigating β-alanine in older adults and clinical or at-risk aging populations with specific attention to functional outcomes, fatigue resistance, safety, and translational application.

A structured search of the literature was performed using electronic databases commonly utilized in biomedical, nutrition, and exercise science research, including PubMed/MEDLINE, Scopus, and Google Scholar. Searches were conducted without strict publication date limitations; however, emphasis was placed on peer-reviewed human trials, mechanistic investigations, systematic reviews, meta-analyses, and consensus statements relevant to aging skeletal muscle and β-alanine supplementation. Foundational mechanistic studies were included when necessary to contextualize biochemical and physiological pathways.

Search terms were used individually and in combination and included aging, sarcopenia, frailty, muscle quality, excitation–contraction coupling, mitochondrial dysfunction, oxidative stress, redox signaling, carnosine, β-alanine, muscle carnosine, CARNS1, carnosine synthase, buffering capacity, hydrogen ions, calcium sensitivity, reactive oxygen species, reactive carbonyl species, advanced glycation end products, lipid peroxidation, 4-hydroxynonenal, supplementation, physical working capacity, fatigue threshold, time-to-exhaustion, resistance training, and oral nutritional supplements. Reference lists of relevant articles and review papers were also screened to identify additional studies not captured in the primary search.

Studies were included if they: (a) provided mechanistic insight into carnosine physiology; (b) examined age-related changes in skeletal muscle function relevant to buffering, mitochondrial integrity, or oxidative stress; or (c) evaluated β-alanine supplementation in human participants with reported outcomes related to muscle carnosine content, functional performance, fatigue resistance, physical working capacity, strength, or safety. Human studies involving older adults or middle-aged populations were prioritized when discussing clinical and translational implications, although data from younger cohorts were included when necessary to establish mechanistic plausibility. Both randomized controlled trials and controlled experimental studies were considered. Systematic reviews and meta-analyses were used to contextualize overall evidence strength when available.

The **Exclusion Criteria** included non-English publications without accessible translation, abstracts lacking sufficient methodological detail, and studies in which β-alanine or carnosine effects could not be isolated from multi-ingredient interventions without appropriate control groups. Animal and in vitro studies were included selectively when necessary to explain underlying mechanisms, but they were interpreted cautiously with regards to translational applicability.

Findings were synthesized qualitatively and organized according to thematic domains aligned with the purpose of the review, including age-related determinants of muscle quality, carnosine biology and synthesis, intracellular buffering and fatigue mechanisms, oxidative and carbonyl stress pathways, supplementation studies in older adults, clinical considerations in frailty and sarcopenia, and practical implications for integration with resistance training and nutritional strategies. Emphasis was placed on mechanistic consistency, clinical relevance, and identification of current gaps in the literature requiring further investigation.

## DISCUSSION

### Current Nutritional Strategies to Preserve Muscle Function in Aging Populations

Adequate protein intake is fundamental for maintaining skeletal muscle mass and function in aging populations. However, the research indicates that many older adults consume less than the recommended protein intake, which contributes to sarcopenia and reduced physical performance (8,16,46). While the World Health Organization recommends a minimum intake of 0.8 $g\cdot kg^{-1}\cdot day^{-1}$ for adults, accumulating evidence and dietary guidance from the European Society for Clinical Nutrition and Metabolism (ESPEN) indicate that older individuals benefit from higher intakes of 1.0-1.2 $g\cdot kg^{-1}\cdot day^{-1}$ (21). ESPEN further recommends 1.2-1.5 $g\cdot kg^{-1}\cdot day^{-1}$ for the at-risk clinical populations that also includes frail, multimorbid, acutely ill, or malnourished individuals (46).

Optimizing protein quantity, quality, and distribution enhances muscle protein synthesis and attenuates muscle loss; however, protein alone does not fully prevent age-related declines in muscle endurance, fatigue resistance, or functional performance (8). Thus, while foundational, protein optimization does not comprehensively address the metabolic determinants of muscle quality.

Creatine supplementation has demonstrated beneficial effects in older adults due to its role in rapid ATP regeneration via the phosphocreatine system (24). Creatine may also stimulate muscle protein synthesis by increasing mechanistic target of rapamycin (mTOR) pathway activation and insulin-like growth factor-1 (IGF-1) expression (11). By enhancing phosphocreatine availability, creatine may increase high-intensity force production and partially mitigate age-related declines in muscle performance (23). Meta-analyses and randomized controlled trials have indicated improvements in lean body mass, maximal strength, chair-rise time, and gait speed in older populations when taking the minimum daily recommended dose of creatine (5 g) (23,42). Creatine appears generally safe in healthy older adults with no consistent evidence of renal impairment when appropriately monitored (23).

However, responsiveness to creatine is variable (10), and its primary physiological effect is the enhancement of short-duration, high-intensity efforts. Creatine does not fully protect against sustained fatigue or prolonged decreases in muscular endurance (31). Therefore, regardless of the improvement in strength and lean mass, functional fatigue resistance often remains compromised. Older adults frequently continue to experience declines in endurance capacity, fatigue resistance, and mobility. These deficits are linked to impaired metabolic buffering, mitochondrial dysfunction, and altered excitation–contraction coupling (31). Current nutritional strategies primarily target hypertrophy and maximal strength, leaving a mechanistic gap in interventions aimed at preserving muscle quality and metabolic resilience.

However, given carnosine's established role as an intramuscular pH buffer and modulator of calcium sensitivity, augmenting muscle carnosine content may represent a targeted strategy to enhance fatigue resistance and delay metabolic stress in aging skeletal muscle (18).

**Carnosine Biology and Age-Related Decline**

Age-related reductions in muscle carnosine content have been reported (44), potentially impairing intracellular buffering capacity and increasing susceptibility to metabolic acidosis during exertion. These reductions may contribute to a decrease in exercise tolerance and functional capacity independent of muscle mass loss.

Carnosine is a multifunctional dipeptide (β-alanyl-L-histidine) enriched in skeletal muscle following β-alanine supplementation, as β-alanine availability is the rate-limiting determinant of carnosine synthesis (25,45). Skeletal muscle expresses high concentrations of carnosine synthase (CARNS1), allowing intracellular synthesis when β-alanine substrate availability increases (19). Elevations in muscle carnosine have been associated with improvements in exercise capacity and high-intensity exercise performance (27,37), enhanced fatigue resistance (18), and potential roles in cellular protection via antioxidant and carbonyl-scavenging mechanisms (9). These effects have been observed across recreationally active individuals (25,26), trained athletes (18), and older adults (15) that suggest the increase in skeletal muscle carnosine may promote physiological benefits across diverse populations.

The most well-established mechanism by which elevated muscle carnosine enhances skeletal muscle function is through its capacity to buffer intracellular hydrogen ions ($H^+$) (27). Under these conditions, ATP resynthesis relies heavily on glycolysis, a process by which the breakdown of glucose is accompanied by increased proton production that contributes to reductions in intracellular pH (i.e., metabolic acidosis). Decreases in muscle pH have been shown to impair contractile performance through multiple fatigue mechanisms, including reduced calcium sensitivity of the contractile apparatus, impaired cross-bridge cycling, and altered excitation-contraction coupling (4,20).

For carnosine to function as an intracellular buffer, it must first be synthesized within skeletal muscle, which is a process governed by β-alanine availability. β-alanine enters muscle fibers primarily via sodium- and chloride-dependent transporters, including the taurine transporter (TauT; SLC6A6) (45). Once inside the myocyte, β-alanine combines with L-histidine via CARNS1, forming carnosine (19). Intracellular histidine concentrations are generally sufficient, such that β-alanine availability controls the rate of carnosine synthesis (25). β-alanine supplementation has been shown to increase muscle carnosine content within 4 to 10 weeks, with greater relative increases observed in type II muscle fibers (18).

Carnosine's buffering capacity arises from the imidazole ring of its histidine residue that has a pKa of approximately 6.83 (1), which closely aligns with the pH range (~6.5-6.8) reached during high-intensity exercise (20,36). Buffers are most effective when pH approximates their pKa, making carnosine particularly well suited to attenuate exercise-induced acidosis (27,45). In its unprotonated form, carnosine accepts protons at the imidazole nitrogen, reducing free intracellular $H^+$ concentration and slowing the decline in muscle pH (1). By mitigating intracellular acidosis, elevated carnosine may indirectly preserve glycolytic enzyme activity and calcium sensitivity of the myofilaments (4,20), thereby supporting the maintenance of force production and delaying fatigue during high-intensity exercise (18,27).

While carnosine is traditionally recognized as an acute ergogenic aid for high-intensity exercise, experimental studies demonstrate that carnosine attenuates protein carbonyl formation and glycation reactions in vitro (3). In addition to its buffering capacity, carnosine has been shown to possess antioxidant and anti-glycation properties, particularly through its ability to quench reactive carbonyl species (RCS) generated during lipid peroxidation and metabolic stress (2,3). Reactive oxygen species (ROS) production is well-established to increase in response to repeated intense muscle contractions, aging, and metabolic dysfunction (4,38). Although exercise acutely enhances endogenous antioxidant defenses, chronic elevations in oxidative stress are associated with mitochondrial dysfunction, protein carbonylation, and impaired metabolic regulation (28,38).

Carnosine has been demonstrated to act as both a reactive carbonyl species scavenger and, to a lesser extent, a direct ROS modulator (2,3). Specifically, carnosine forms stable adducts with α,β-unsaturated aldehydes such as 4-hydroxynonenal (HNE), thereby limiting protein cross-linking and reducing accumulation of lipid peroxidation byproducts that contribute to oxidative damage (3). Furthermore, carnosine reacts with reactive carbonyl intermediates generated during glycoxidation and lipid peroxidation, forming stable adducts that limit advanced glycation end-product (AGE) formation (3). Because carbonyl modification contributes to structural and functional impairment of long-lived proteins (40), elevated intracellular carnosine may help attenuate oxidative modification of myofibrillar and mitochondrial proteins under conditions of metabolic stress.

Thus, beyond its role in buffering exercise-induced acidosis, elevations in intracellular carnosine may contribute to preservation of cellular integrity under metabolic stress. These mechanisms may be particularly relevant in aging and chronic metabolic disease, where oxidative stress, mitochondrial dysfunction, and declines in muscle quality contribute to functional impairment independent of muscle mass loss.

**Evidence for Beta-Alanine Supplementation in Older Adults**

While β-alanine supplementation has traditionally been associated with sports performance, there is growing interest in whether its use may be beneficial for older adults. This is particularly relevant given that muscle carnosine content declines with age (44), potentially due to reduced muscle-loading activity, progressive sarcopenia (and associated reductions in muscle cross-sectional area), and/or lower dietary protein intake (14). Reduced muscle carnosine may impair intracellular buffering capacity and increase susceptibility to metabolic acidosis during exertion, which could contribute to declines in exercise tolerance and functional capacity independent of muscle mass loss. Consequently, age-related reductions in both physical activity and muscle carnosine availability may compromise buffering capacity and the ability to perform functional or anaerobic-demanding activities.

Based on other actions of muscle carnosine including antioxidant (free radical scavenging, metal ion chelation) and anti-inflammatory properties (12,29), increasing intracellular carnosine levels via β-alanine supplementation may be an adjunct nutritional approach to supporting mitochondrial function and fatigue resistance alongside functional attributes. Although limited studies exist, provision of 3.2 g β-alanine per day for 12 weeks using sustained-release dosing has been shown to increase muscle carnosine content in gastrocnemius muscles (+85.4%) (15). This is comparable to previous studies in younger individuals (26), suggesting that the uptake of β-alanine into muscle and corresponding increases in intramuscular carnosine levels are not impeded by the aging process (15).

Only a few studies have been conducted investigating the impact of β-alanine supplementation on functional outcomes that may be pertinent to frailty and risk of falls/injury. As such, this clearly highlights the need for further research in this domain. However, use of a sustained release formula (CarnoSyn™) at 2.4 $g \cdot d^{-1}$ in divided doses for 90 days was shown to significantly increase physical working capacity at the fatigue threshold test (PWCFT) by 28.6% ($P < 0.05$) compared to placebo (43). Such findings are also supported elsewhere (15) when considering submaximal aerobic tasks. Also, a shorter duration β-alanine supplementation (28 days at 2.4 g·d$^{-1}$) has been shown to increase time-to-exhaustion tasks to a similar extent (24%) in older adults (60.5 ± 8.6 yrs) and may positively impact exercise-associated reductions in executive function (22). These findings may have implications for increased functional capacity and/or resistance to fatigue that may impact fatigue-associated incidences.

In the longer duration study (43), Stout and colleagues noted that the response rate was positive at 67%. However, this also indicates that 33% of the participants were potentially non-responders, which should be taken into consideration. Importantly, the findings from this study (43) appeared to be greater than previously observed in younger individuals (who demonstrated a 14.5% increase in PWCFT after 28 days supplementation of β-alanine at 1.6 g·d$^{-1}$) (41). While evidence is limited, these findings suggest that functional capacity benefits from increasing muscle carnosine following β-alanine supplementation may be more prominent in older adults. This emphasizes the need for further research to support these observations.

Considering appetite suppression (32), and the potential for reduced protein intake with age (25), combining β-alanine with protein from an oral nutritional supplement (ONS) may be a beneficial approach for older populations. In one study (34), consumption of 1.6-2.4 g·d$^{-1}$ of β-alanine (in 2 doses) for 12 weeks significantly improved PWCFT in an older, mixed population (age: 70.7 ± 6.2 yrs) compared to just ONS. While non-significant between β-alanine conditions, it is noteworthy that the relative change in PWCFT was greater in the group taking the lower dose (1.6 g·d$^{-1}$; 17.8%) versus those taking the higher dose (2.4 g·d$^{-1}$; 13.6%); possibly indicating a lower threshold dose to elicit functional benefits.

However, in the same study, sit-to-stand (STS) and handgrip strength (relative to muscle quality) were only improved when the higher dose of β-alanine was consumed (34). The potential increase in intramuscular carnosine from β-alanine supplementation may affect calcium ion sensitivity, which impacts muscle force production or delaying fatigue. However, for STS, this may also be partly explained by the provision of additional protein and energy, given that significant changes (10.7%) were also observed with the ONS group (compared with 22.2% for the higher dose of β-alanine). Additionally, other studies (7,15,35) did not find any effect of β-alanine (2.4-3.2 g·d$^{-1}$) on STS, time-up-and-go, 6 min walking or isotonic strength tests compared with placebo in similar aged participants over comparable timeframes. One explanation for this may be that if individuals are already relatively healthy and functionally active or undertaking resistance training, the underlying impact of increased carnosine levels may be limited (7,15) in contrast to those who are frailer.

Overall, it appears that β-alanine supplementation is both safe and well tolerated in older adults when consumed in doses ranging from 1.6 to 3.2 g·d$^{-1}$ (7,14) for 10 to 12 weeks. In some individuals, marginally lower intakes (1.6 g·d$^{-1}$) may also be suitable for supporting functional activities (e.g., daily exercise programs), especially if paresthesia is noted at higher doses. However, adjunct use of β-alanine supplementation does not appear to enhance other functional measures associated with strength gains, despite the increase in intramuscular carnosine.

**Beta-Alanine in Clinical and At-Risk Aging Populations**

Older adults frequently experience declines in muscle function that extend beyond normal aging, which include conditions such as sarcopenia, frailty, and disease-related functional impairment (13). These conditions are associated with reduced physical independence, increased fall risk, hospitalization, and mortality. Importantly, fatigue resistance and neuromuscular efficiency often decline even when muscle mass is partially preserved, highlighting a need for interventions that target muscle quality in addition to hypertrophy or strength alone (42,43). Beta-alanine–derived carnosine may be relevant in these contexts because of its roles in intracellular buffering, calcium handling, and antioxidant activity (45).

Sarcopenia is characterized by the progressive loss of muscle mass, strength, and functional performance, while frailty reflects a broader decline in physiological reserve across multiple systems (13). Mechanistically, the aging muscle demonstrates reduced buffering capacity, impaired excitation–contraction coupling, increased oxidative stress, and mitochondrial dysfunction (33). Muscle carnosine content also appears to decline with age, particularly in type II fibers, which may exacerbate early fatigue and impair physical performance (45). Because beta-alanine availability is rate-limiting for carnosine synthesis, supplementation may partially restore intramuscular carnosine levels and improve tolerance to physical activity in older adults.

Several studies have examined beta-alanine supplementation in older or clinical populations. Stout and colleagues reported improvements in physical working capacity and neuromuscular fatigue threshold following beta-alanine supplementation in older adults, suggesting potential benefits for functional performance (42,43). Other trials have demonstrated increases in muscle carnosine content and modest improvements in endurance-type outcomes without substantial changes in muscle mass (26,39). While the literature remains limited compared with general populations, these findings support the concept that beta-alanine may improve fatigue resistance rather than directly increase strength or hypertrophy.

In clinical or undernourished populations, beta-alanine supplementation must be interpreted within the broader context of nutritional status. Low energy availability, inadequate protein intake, and micronutrient deficiencies remain the primary nutritional drivers of functional decline in aging populations. Beta-alanine alone cannot compensate for malnutrition or insufficient protein intake, and its potential benefits are likely greatest when combined with adequate dietary intake and structured exercise. Multi-ingredient nutritional supplements that include beta-alanine have been studied in older adults, with improvements observed in measures of functional performance such as physical working capacity and sit-to-stand ability. However, evidence remains limited and largely confined to a single randomized trial (34,45), and the studies often involve multi-component interventions, short durations, or surrogate outcomes that emphasize the need for independent replication and longer-term trials.

Beta-alanine may be most useful as part of multimodal strategies targeting sarcopenia and frailty. Resistance training remains the cornerstone intervention for preserving muscle function in aging, while adequate protein intake supports muscle protein synthesis (30). Creatine supplementation has demonstrated benefits for strength and lean mass, and beta-alanine may provide complementary improvements in fatigue resistance and exercise tolerance, potentially enhancing participation in rehabilitation or training programs (6). This integrated approach aligns with current evidence indicating that supplements should support, not replace, nutrition and exercise interventions.

Available data suggest beta-alanine supplementation is generally well tolerated in older adults, with paresthesia as the most common side effect, typically mitigated by divided dosing (5). No consistent evidence indicates adverse renal or hepatic outcomes in healthy individuals, although clinical populations with complex comorbidities or polypharmacy should be monitored under medical supervision.

Overall, evidence supporting beta-alanine use in clinical and at-risk aging populations is promising but still emerging. Beta-alanine is unlikely to meaningfully improve muscle mass in isolation, but it may enhance fatigue resistance and functional capacity when combined with resistance training and adequate nutrition. Future randomized controlled trials in sarcopenic, frail, and clinical populations are needed to clarify optimal dosing, long-term safety, and clinically meaningful outcomes.

## CONCLUSIONS

Age-related declines in skeletal muscle function represent a major contributor to the loss of independence, increased fall risk, and reduced quality of life in older adults. While traditional strategies have focused primarily on preserving muscle mass, it is increasingly recognized that deterioration in muscle quality, including impaired excitation–contraction coupling, reduced buffering capacity, mitochondrial dysfunction, and increased oxidative stress, plays a critical role in functional decline. These physiological changes contribute to early fatigue, reduced exercise tolerance, and diminished physical performance, even in the presence of relatively preserved muscle mass.

Carnosine, an intramuscular dipeptide synthesized from β-alanine and histidine, possesses several biological properties that directly target these underlying determinants of muscle quality. Through its capacity to buffer intracellular hydrogen ions, modulate calcium sensitivity, and act as an antioxidant and reactive carbonyl scavenger, carnosine contributes to maintenance of metabolic stability and contractile function during periods of physiological stress. Because β-alanine availability is the rate-limiting step in carnosine synthesis, supplementation provides an effective strategy to elevate muscle carnosine concentrations and potentially enhance fatigue resistance.

Evidence from human studies indicates that β-alanine supplementation increases intramuscular carnosine levels in older adults and may improve measures of physical working capacity and exercise tolerance. Although current findings suggest that β-alanine is unlikely to directly increase muscle mass or maximal strength, improvements in fatigue resistance and metabolic resilience may support greater participation in physical activity and rehabilitation programs. In this context, β-alanine may serve as a complementary nutritional strategy alongside resistance training, adequate protein intake, and other evidence-based interventions aimed at preserving functional capacity during aging.

Despite these promising findings, the current body of literature examining β-alanine supplementation in older or clinical populations remains relatively limited. Many studies involve small sample sizes, short intervention durations, or surrogate physiological outcomes rather than clinically meaningful endpoints. Future randomized controlled trials are therefore needed to determine optimal dosing strategies, long-term safety, and the extent to which improvements in fatigue resistance translate into meaningful improvements in mobility, independence, and quality of life in aging populations.

In summary, β-alanine–derived carnosine represents a biologically plausible and mechanistically distinct target for supporting muscle quality in aging skeletal muscle. While further research is warranted, current evidence suggests that augmenting intramuscular carnosine may help mitigate fatigue-related declines in functional performance. When integrated within comprehensive approaches that include resistance exercise and appropriate nutritional support, β-alanine supplementation may contribute to strategies aimed at preserving mobility, resilience, and functional independence across the aging lifespan.

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# Yoga as an Integrative System for Health: Philosophical Foundations, Physiological Mechanisms, and Preventive Implications — A Narrative Review

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**ABSTRACT**

**Sivaphongthongchai A.** The purpose of this study was to examine yoga as an integrative system for health by synthesizing classical yoga philosophy with contemporary scientific research. Yoga has increasingly attracted attention within health science as a multidimensional mind–body practice with potential applications in disease prevention and health promotion. Although yoga is widely practiced in modern societies, its conceptual foundations are often interpreted primarily through biomedical perspectives without adequate consideration of its classical philosophical context. This narrative review explores the philosophical foundations of yoga and the eight-limbed framework described in classical texts, emphasizing the role of yogāsana as an embodied practice that integrates posture, breath, and attention. It also reviews current evidence regarding the physiological mechanisms associated with yogāsana practice, including modulation of autonomic nervous system activity, neuroendocrine stress regulation, inflammatory processes, and musculoskeletal function. By integrating the traditional knowledge with the modern biomedical perspectives, this review suggests that yogāsana may function as a multisystem regulatory practice supporting long-term physiological balance and preventive health strategies.

**Key Words:** Autonomic Regulation, Integrative Health, Mind–Body Medicine, Yoga, Yogāsana

## INTRODUCTION

Chronic noncommunicable diseases (NCDs) have become one of the most significant global health challenges of the 21st century. Conditions such as cardiovascular disease, metabolic disorders, and chronic musculoskeletal pain are strongly associated with modifiable lifestyle factors including physical inactivity, chronic stress, and maladaptive behavioral patterns (1). These conditions contribute substantially to global morbidity and mortality and place considerable economic pressure on health systems worldwide. As a result, contemporary health research has increasingly emphasized the importance of preventive strategies that address behavioral and physiological determinants of health rather than relying exclusively on pharmacological treatment after disease onset.

Within this context, mind–body practices have attracted growing attention as potential complementary approaches to health promotion and disease prevention. Among these practices, yoga has become one of the most widely studied mind-body disciplines. Yoga has increasingly been recognized as a holistic health practice that integrates physical movement, breathing regulation, and mental awareness (2,3,6,7). A growing body of clinical and experimental research suggests that yoga-based interventions may influence both physiological and psychological dimensions of health (24,33). Unlike conventional exercise modalities that primarily target cardiovascular fitness or muscular strength, yoga integrates physical movement, breathing regulation, and attentional training into a single practice. This multidimensional approach suggests that yoga may influence several regulatory systems simultaneously.

One of the key pathways through which yoga may influence health involves the regulation of stress physiology. Chronic psychological stress is known to disrupt homeostatic processes within the body by activating neuroendocrine and inflammatory pathways that contribute to disease development (20). Sustained activation of these stress-response systems has been associated with metabolic dysfunction, cardiovascular risk, immune dysregulation, and a variety of stress-related disorders. Consequently, interventions that support adaptive stress regulation have become a central focus within integrative health research.

Recent studies examining yoga-based practices suggest that these interventions may influence physiological stress responses through multiple mechanisms. For example, yoga practice has been associated with improvements in autonomic nervous system regulation, reductions in perceived stress, and changes in physiological markers related to stress reactivity (22,23). These findings suggest that yoga may contribute to improved psychophysiological resilience by supporting regulatory processes across neural, endocrine, and behavioral systems.

In addition to stress regulation, yoga practices may influence physical health through their effects on movement, posture, and musculoskeletal function. Yogic postural practices involve coordinated muscular engagement, controlled breathing patterns, and sustained attentional focus. These elements collectively contribute to improved body awareness and functional movement patterns (33). Unlike many exercise programs that focus primarily on intensity or repetition, yoga practice often emphasizes stability, alignment, and gradual refinement of movement, which may support long-term musculoskeletal health and injury prevention.

Despite the growing body of research examining the clinical effects of yoga interventions, the conceptual foundations of yoga are often interpreted primarily through biomedical frameworks without sufficient attention to their classical philosophical origins. Traditional yoga literature describes yoga not merely as physical exercise but as a comprehensive system of self-regulation encompassing ethical conduct, bodily discipline, breath regulation, and attentional training. Understanding yoga within this broader conceptual framework may therefore provide deeper insight into the mechanisms through which yoga practices influence health and well-being.

The purpose of this narrative review is to examine yoga as an integrated system for health by synthesizing insights from classical yoga philosophy and contemporary scientific research. Specifically, this article explores the philosophical foundations of yoga, the structure of the eight-limbed path, the physiological mechanisms associated with yogāsana practice, and the potential role of yoga as a preventive health strategy. By integrating traditional knowledge with modern scientific evidence, the purpose of this review is to contribute to a more comprehensive understanding of yoga within the emerging fields of integrative health, lifestyle medicine, and rehabilitation science.

**Philosophical Foundations of Yoga**

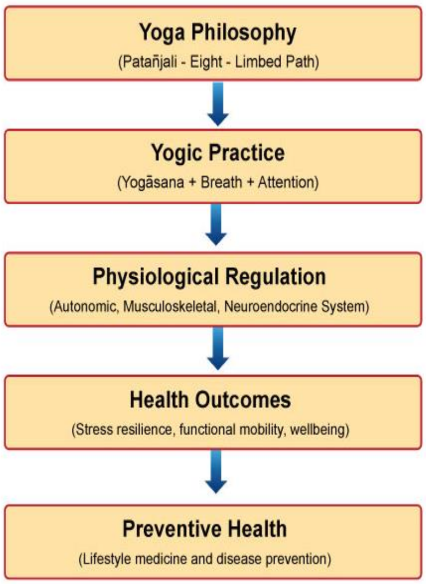
Yoga has traditionally been understood as a comprehensive system for cultivating balance within the body, mind, and consciousness. Classical yoga philosophy describes yoga not merely as a physical discipline but as an integrated framework for regulating mental activity and developing clarity of awareness. The foundational definition of yoga is articulated in the Yoga Sūtra attributed to Patañjali, which describes yoga as the cessation of the fluctuations of the mind (*citta-vṛtti-nirodhaḥ*) (4). Within this philosophical framework, yoga practice aims to stabilize mental processes and cultivate a state of sustained awareness through disciplined practice and self-regulation.

Traditional interpretations of the Yoga Sūtra emphasize that disturbances of the mind arise from habitual patterns of perception, emotional reactivity, and conditioned behavior. Yoga practice is therefore presented as a systematic pathway for transforming these patterns and cultivating psychological stability. Classical scholars describe yoga as a progressive process through which practitioners develop ethical discipline, body stability, and meditative awareness (11). From this perspective, yoga functions as a holistic framework for the regulation of behavior, physiology, and cognition.

Although yoga philosophy originated within ancient contemplative traditions, its conceptual structure can be interpreted in ways that resonate with contemporary perspectives in psychology and neuroscience. The emphasis on attentional regulation, behavioral discipline, and awareness of internal states parallels modern models of self-regulation and cognitive control. These parallels have contributed to increasing scientific interest in yoga as a potential framework for understanding mind–body interactions.

In modern yoga scholarship, the teachings of B.K.S. Iyengar have played a particularly important role in interpreting classical yoga philosophy within the context of contemporary life. Iyengar emphasized that yoga should be understood as a method for integrating physical health, mental clarity, and discipline awareness (16). In his commentary on the Yoga Sūtras, Iyengar described yoga as a process through which the body, breath, and mind become progressively aligned, allowing awareness to function with greater stability and precision. This interpretation highlights the central role of embodied practice in the development of mental steadiness.

**Figure 1. Conceptual Framework Illustrates the Relationship Between Classical Yoga Philosophy and Health-Related Outcomes.**




The model proposes that yoga philosophy, particularly the eight-limbed path described by Patañjali, informs yogic practices such as yogāsana, breathing regulation, and attentional training. These practices influence multiple physiological systems, contributing to improved health outcomes and supporting preventive health strategies.

Within the Iyengar tradition, the body is viewed as an accessible entry point for cultivating awareness and self-regulation. Postural practice (*āsana*) is therefore not understood simply as a form of physical exercise but as a method for refining sensory perception and stabilizing the nervous system. Through careful attention to alignment, muscular engagement, and breathing patterns, practitioners develop increasing sensitivity to the relationship between bodily states and mental processes (17). This emphasis on embodied awareness represents a distinctive feature of Iyengar's interpretation of yoga philosophy.

Recent developments in contemplative neuroscience provide additional perspectives that support this interpretation. Research suggests that practices involving focused attention and awareness of bodily sensations may influence neural systems associated with attention, emotional regulation, and interoceptive processing (30). Interception, the perception of internal bodily signals plays an important role in maintaining physiological balance and supporting adaptive responses to environmental demands. Studies examining mindfulness and contemplative practices have shown that training in interoceptive awareness can alter neural representations associated with bodily perception and emotional processing (9).

These findings suggest that the philosophical principles described in classical yoga traditions may correspond with mechanisms increasingly recognized within contemporary scientific research. Practices that cultivate awareness of bodily states, breathing patterns, and attentional processes may contribute to improved psychophysiological regulation. From this perspective, yoga philosophy provides a conceptual framework through which bodily discipline, mental training, and self-regulatory processes become integrated.

Understanding yoga within this philosophical framework is therefore important for interpreting the potential health effects of yoga-based practices. Rather than viewing yoga solely as a form of physical exercise, classical teachings present it as a comprehensive system of self-regulation that integrates ethical conduct, bodily awareness, and mental discipline. This broader perspective provides an important conceptual foundation for examining how specific components of yoga practice, particularly yogāsana may influence physiological and psychological processes related to health. Contemporary interpretations of classical yoga philosophy have increasingly explored its relevance for modern health sciences and contemplative studies (8,10,14).

**The Eight-Limbed Path as a Model of Self-Regulation**

Classical yoga philosophy presents practice as a structured pathway for the development of self-regulation. This framework is most clearly articulated in the eight-limbed system (*aṣṭāṅga yoga*) described in the Yoga Sūtra attributed to Patañjali [4]. Rather than representing a sequence of isolated practices, the eight limbs are traditionally understood as interconnected dimensions of a comprehensive discipline aimed at stabilizing the body, regulating the mind, and cultivating awareness.

The eight limbs of yoga consist of yama (ethical restraints), niyama (personal observances), āsana (postural practice), prāṇāyāma (regulation of breathing), pratyāhāra (withdrawal of the senses), dhāraṇā (concentration), dhyāna (meditation), and samādhi (integrative awareness) (11). Together, these components describe a progressive system in which ethical behavior, bodily discipline, respiratory regulation, and mental training interact to support psychological stability and expanded awareness.

From a functional perspective, the eight-limbed model can be interpreted as a framework for behavioral, physiological, and cognitive regulation. Ethical disciplines such as *yama* and *niyama* provide behavioral foundations that support psychological stability and reduce interpersonal conflict. These ethical guidelines encourage self-reflection, moderation, and intentional behavior, which may contribute to reduced stress and improved emotional regulation (12,13).

The practices of āsana and prāṇāyāma form the physiological core of the system. Yogic postures cultivate stability, mobility, and bodily awareness, while controlled breathing practices regulate respiratory rhythms and influence autonomic balance (15). In the teachings of B.K.S. Iyengar, these practices are described as essential methods for developing both physical health and mental steadiness (16). Iyengar emphasized that precise attention to posture, breath, and alignment refines sensory awareness and supports the integration of body and mind.

Within this framework, pratyāhāra, dhāraṇā, and dhyāna represent progressively deeper stages of attentional training. Pratyāhāra refers to the regulation of sensory input and the ability to withdraw attention from distracting stimuli. Dhāraṇā involves sustained concentration on a chosen object of awareness, while dhyāna describes a continuous flow of attention without interruption (4). These stages collectively cultivate the capacity for sustained attention and emotional regulation.

The final limb, samādhi, represents a state of integrated awareness in which the distinction between observer and object of attention becomes diminished. While traditional texts describe samādhi in contemplative and spiritual terms, modern interpretations often view it as an advanced state of cognitive integration and attentional stability (11).

Contemporary research in contemplative science has increasingly explored how practices derived from yoga and meditation influence self-regulatory processes. Conceptual models have suggested that yoga may support self-regulation through mechanisms involving attentional control, emotional processing, and body awareness (29). These processes may influence neural networks associated with executive function, interoception, and emotional regulation.

Although modern yoga practice often emphasizes physical postures, the eight-limbed framework suggests that yogāsana represents only one component within a broader system of self-development. Nevertheless, postural practice occupies a unique role because it provides a direct interface between physical experience and mental awareness. Through coordinated movement, controlled breathing, and focused attention, yogāsana may serve as a practical gateway through which practitioners begin to develop the broader capacities described in the classical yoga tradition.

Understanding the eight-limbed path as a model of self-regulation provides an important conceptual foundation for interpreting yoga within contemporary health science. Rather than viewing yoga solely as a form of exercise or relaxation technique, this framework highlights its multidimensional nature as a system integrating behavioral discipline, physiological regulation, and attentional training. This perspective provides a useful conceptual bridge between traditional yoga philosophy and emerging scientific research on mind–body practices.

**Table 1. A Summary of the Classical Eight Limbs of Yoga and Their Functional Interpretation in the Context of Health Science and Self-Regulation.**

| Limb | Classical Description | Functional Interpretation in Health Science |
|---|---|---|
| Yama | Ethical restraints | Behavioral regulation and social stability |
| Niyama | Personal observances | Self-discipline and psychological orientation |
| Āsana | Postural practice | Musculoskeletal stability and body awareness |
| Prāṇāyāma | Regulation of breathing | Autonomic regulation and respiratory control |
| Pratyāhāra | Withdrawal of the senses | Sensory regulation and attentional filtering |
| Dhāraṇā | Concentration | Sustained attention and cognitive control |
| Dhyāna | Meditation | Continuous attentional stability |
| Samādhi | Integrative awareness | Deep integration of cognitive and perceptual processes |

**Yogāsana as an Embodied Practice for Physiological Regulation**

Within the classical framework of yoga, yogāsana represents the embodied dimension of practice through which stability of the body and clarity of awareness are cultivated. In traditional yoga literature, āsana is not merely defined as physical posture but as a condition of steadiness and ease that supports sustained attention and internal awareness. Classical interpretations emphasize that postural practice provides the physical foundation upon which deeper aspects of yoga practice can develop (17).

In modern yoga traditions, particularly within the teachings of B.K.S. Iyengar, yogāsana has been elaborated as a systematic method for cultivating both physical health and mental stability. Iyengar emphasized that the practice of postures should involve the coordinated integration of alignment, breath regulation, and attentive awareness. In this approach, the body becomes a medium through which practitioners develop refined perception of balance, muscular engagement, and internal sensation (16). Through sustained attention to these elements, yogāsana practice may progressively enhance bodily awareness and stability.

A distinguishing feature of yogāsana practice is the emphasis on structural alignment and controlled muscular engagement. Unlike conventional exercise modalities that prioritize repetitive movement or muscular exertion, yoga postures often involve sustained positions in which the practitioner maintains balanced activation of multiple muscle groups. This process encourages postural stability, improved joint positioning, and greater proprioceptive awareness. Over time, these adaptations may contribute to improved functional movement patterns and musculoskeletal balance.

The emphasis on sustained positioning also plays an important role in developing interoceptive awareness, which refers to the perception of internal bodily signals such as breathing patterns, muscular tension, and visceral sensations. Contemporary conceptual models of yoga suggest that enhanced interoception may represent one of the key mechanisms through which yoga influences self-regulation and emotional stability (29). By directing attention toward bodily sensations during posture practice, practitioners may develop increased sensitivity to physiological states and improved capacity for self-regulation.

Another important aspect of yogāsana practice involves the coordination between posture and breathing. Although breathing regulation is formally categorized under prāṇāyāma, breathing awareness is frequently integrated into postural practice. Iyengar described breathing as an essential element that connects bodily structure with mental awareness. The synchronization of breathing with movement and posture may influence respiratory rhythms and contribute to autonomic balance within the body (15). This interaction highlights the multidimensional nature of yoga practice, in which musculoskeletal, respiratory, and attentional processes operate together.

Research examining yoga interventions has increasingly suggested that postural practice may influence multiple dimensions of health. Comparative studies of yoga and conventional exercise have reported improvements in flexibility, functional mobility, and perceived well-being among individuals practicing yoga-based programs (1). In addition, clinical reviews of yoga research have identified beneficial effects on stress regulation, physical functioning, and general health outcomes (33). These findings suggest that yogāsana practice may contribute to health through mechanisms that extend beyond simple physical conditioning.

Importantly, the effects of yogāsana appear to arise not only from mechanical movement but from the integration of movement, breath regulation, and attentional training. This integration reflects a central principle within traditional yoga philosophy: that bodily discipline and mental regulation are mutually reinforcing aspects of practice. Through sustained engagement with posture, breathing, and awareness, yogāsana may therefore function as an embodied method for regulating both physiological and psychological processes.

Understanding yogāsana within this broader conceptual framework provides an important bridge between traditional yoga philosophy and contemporary health science. Rather than functioning solely as a form of exercise, yogāsana can be interpreted as a structured method for cultivating embodied awareness and supporting physiological regulation. This perspective provides a foundation for examining how yoga practice may influence autonomic function, stress physiology, and adaptive health processes explored in the following section (18,19,21).

**Table 2. Functional Components of Yogāsana Practice and Their Physiological Significance Within a Health Science Framework.**

| Component of Practice | Description | Potential Physiological Relevance |
|---|---|---|
| Postural alignment | Balanced positioning of skeletal structures | Joint stability and musculoskeletal efficiency |
| Sustained positioning | Maintaining postures with controlled effort | Proprioception and neuromuscular control |
| Muscular coordination | Balanced activation of stabilizing muscles | Functional movement regulation |
| Breath integration | Coordinated breathing during posture practice | Respiratory rhythm and autonomic balance |
| Attentional awareness | Focus on internal bodily sensations | Interoception and self-regulation |

**Physiological Mechanisms of Yogāsana Practice**

The health effects of yoga practice have increasingly been examined through the lens of modern physiology and integrative medicine. Chronic stress and sedentary lifestyles are recognized contributors to a wide range of non-communicable diseases, including cardiovascular disorders, metabolic dysfunction, and chronic pain conditions that are often associated with dysregulation of multiple physiological systems, including the autonomic nervous system (ANS), neuroendocrine pathways, and inflammatory processes. In this context, yoga has attracted scientific interest as a multimodal intervention capable of influencing several regulatory systems simultaneously (1,2). Emerging research suggests that yoga may influence neurophysiological processes involving autonomic regulation, interoception, and emotional processing (23,25,26,28).

One of the most frequently discussed mechanisms involves modulation of the autonomic nervous system. Chronic psychological stress is associated with increased sympathetic activity and reduced parasympathetic tone, a pattern that contributes to impaired cardiovascular regulation and elevated physiological stress responses. Yoga practice, particularly when combining physical postures with controlled breathing and attentional focus, may promote a shift toward parasympathetic dominance. Reviews examining heart rate variability (HRV), a widely used marker of autonomic balance, have reported that yoga interventions are associated with increased parasympathetic activity and improved autonomic regulation (22).

Beyond autonomic markers, neurophysiological mechanisms have also been proposed. Experimental studies suggest that yoga practice may influence inhibitory neurotransmitter systems in the brain, particularly gamma-aminobutyric acid (GABA), which plays a key role in the regulation of stress, mood, and anxiety. Increased GABA activity has been proposed as one potential mechanism through which yoga exerts calming and stress-reducing effects on the nervous system (29).

Autonomic regulation also interacts closely with neuroendocrine stress pathways, particularly the hypothalamic–pituitary–adrenal (HPA) axis, which is central to the physiological stress response. Dysregulation of the HPA axis is commonly associated with chronic stress and inflammatory disorders. Evidence from mind–body research suggests that yoga practice may influence HPA axis activity and stress hormone regulation, thereby contributing to improved stress resilience and physiological homeostasis (2,21).

Another physiological mechanism involves respiratory–vagal interactions. Slow, controlled breathing patterns commonly integrated within yoga practice may stimulate vagal afferent pathways through mechanical and neural mechanisms associated with respiration. The respiratory vagal stimulation model proposes that breathing patterns can directly influence autonomic regulation through vagal pathways, thereby affecting cardiovascular function and emotional regulation (5).

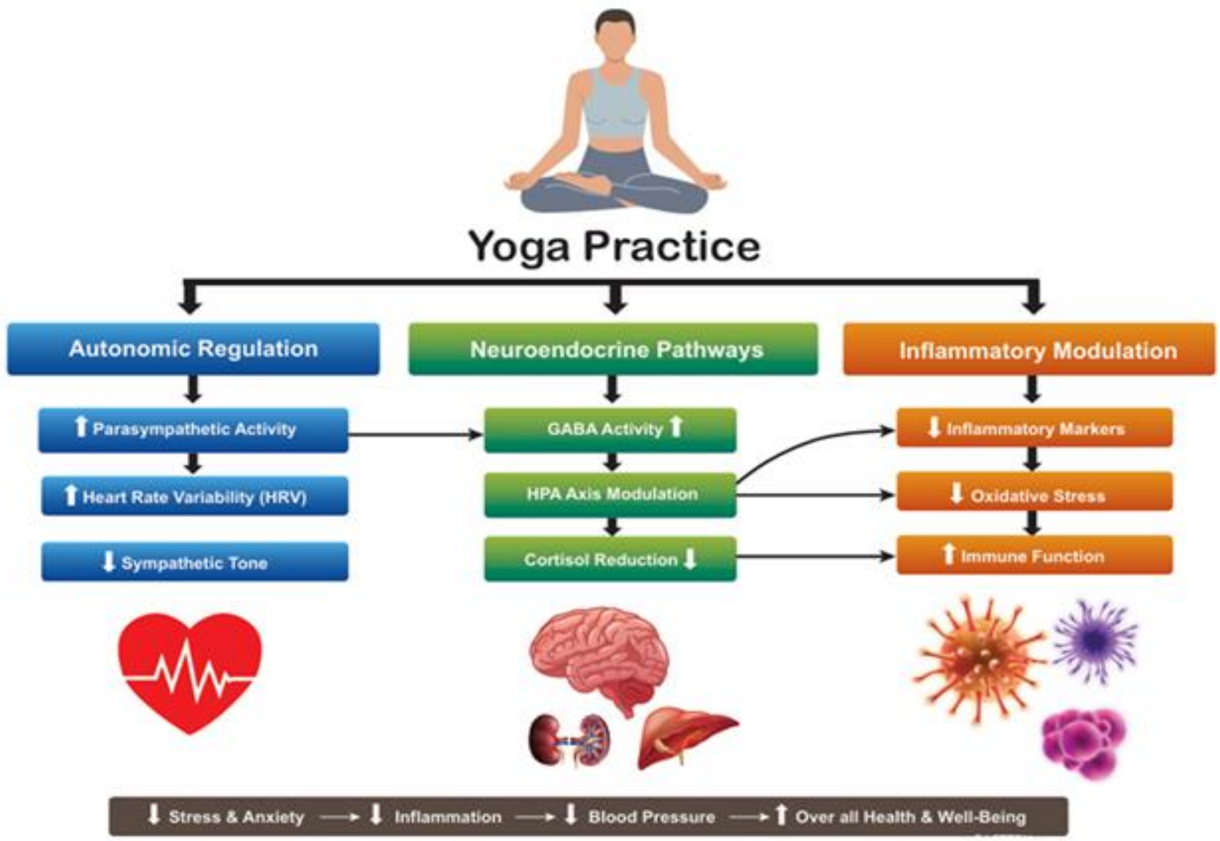
In addition to autonomic and neuroendocrine pathways, yoga practice has been linked to modulation of inflammatory processes. Chronic low-grade inflammation is increasingly recognized as a central mechanism underlying many lifestyle-related diseases. Mind–body interventions, including yoga, have been associated with reductions in inflammatory markers and improved immune regulation in several clinical studies (21,24).

Clinical research examining yoga interventions has also reported beneficial effects on cardiovascular risk factors. Systematic reviews and meta-analyses indicate that yoga practice may contribute to reductions in blood pressure, improvements in lipid profiles, and enhanced cardiovascular regulation. These findings suggest that yoga may influence cardiovascular health through integrated effects on autonomic regulation, stress physiology, and physical activity levels (5,6).

Yoga interventions have also been investigated in musculoskeletal and chronic pain populations. Evidence from systematic reviews suggests that yoga may improve pain outcomes and functional mobility in individuals with chronic low back pain and related musculoskeletal conditions (7,32). These benefits may be explained by a combination of biomechanical factors, enhanced body awareness, and neurophysiological mechanisms related to pain modulation. Emerging evidence also suggests that yoga-based interventions may improve functional capacity and daily activity performance in older adults. Similarly, a pilot study examining an adaptive chair yoga program reported improvements in functional fitness and daily activity outcomes following a structured intervention (27).

Taken together, these findings suggest that yogāsana practice may influence health through multiple interconnected physiological pathways. Rather than acting solely as a form of physical exercise, yoga may function as a multisystem regulatory practice integrating movement, respiration, and attentional processes. This integrated approach may help explain why yoga-based interventions have demonstrated beneficial effects across a wide range of health outcomes in clinical and population studies (33).

**Figure 2. Conceptual Model Illustrating Potential Physiological Pathways.**




Conceptual model illustrating potential physiological pathways through which yoga practice may influence health outcomes. Yoga practice may promote autonomic regulation (increased parasympathetic activity, increased heart rate variability, and reduced sympathetic tone), neuroendocrine modulation (enhanced GABA activity, regulation of the hypothalamic–pituitary–adrenal axis, and reduced cortisol), and inflammatory modulation (reduced inflammatory markers and oxidative stress with improved immune function). These integrated mechanisms may contribute to reduced stress and anxiety, decrease blood pressure and inflammation, and improved overall health and well-being.

## DISCUSSION

The present review synthesizes classical yoga philosophy and contemporary physiological research to examine how yogāsana practice may influence human health through multiple interconnected biological pathways. While yoga is often perceived primarily as a form of physical exercise, the evidence discussed in this review suggests that yogāsana represents a more complex regulatory practice that integrates physical movement, respiratory control, and attentional processes. This multidimensional nature distinguishes yoga from conventional exercise modalities and may explain its broad range of reported health effects.

From a traditional perspective, yogāsana is situated within the broader framework of the eight-limbed path described in the Yoga Sūtras of Patañjali. Classical interpretations emphasize that the purpose of yogāsana is not merely physical flexibility or strength but the cultivation of stability, balance, and attentional clarity within the body and mind (4,11,16). In modern practice, this perspective has been further elaborated in the teachings of B.K.S. Iyengar who emphasized precision of alignment, sustained attention, and coordinated breathing as essential elements of posture practice (16,17). These features may contribute to the distinctive physiological and psychological effects associated with yoga.

One of the most consistent findings in scientific literature concerns the effects of yoga on autonomic nervous system regulation. Chronic stress and many lifestyle-related disorders are associated with increased sympathetic activation and reduced parasympathetic activity. Several reviews and experimental studies indicate that yoga practice may enhance parasympathetic tone and improve autonomic balance, as reflected in measures such as heart rate variability (22). Improved autonomic regulation may in turn contribute to better cardiovascular function and reduced physiological stress responses.

Neurophysiological mechanisms may also play an important role. Research examining the effects of yoga on brain chemistry has suggested that yoga practice may influence inhibitory neurotransmitter systems, particularly gamma-aminobutyric acid (GABA), which is involved in the regulation of anxiety and emotional stability (29). These findings support the hypothesis that yoga may exert calming effects on the nervous system through neurochemical pathways in addition to behavioral and psychological mechanisms.

Another important mechanism involves the interaction between autonomic regulation and neuroendocrine stress pathways. The hypothalamic–pituitary–adrenal (HPA) axis plays a central role in the physiological stress response, and dysregulation of this system has been linked to numerous chronic diseases. Evidence from studies of mind–body interventions suggests that yoga practice may influence HPA axis activity and contribute to reductions in stress-related hormonal responses (2,21). This interaction between autonomic and neuroendocrine regulation may help explain the stress-reducing effects frequently reported in yoga research.

Breathing patterns commonly integrated within yoga practice may further contribute to these regulatory processes. Slow and controlled breathing has been proposed to stimulate vagal pathways through respiratory cardiac interactions, thereby enhancing parasympathetic activity and influencing emotional regulation. The respiratory vagal stimulation model provides a physiological explanation for how breathing practices associated with yoga may influence autonomic balance and cardiovascular regulation (5).

Beyond autonomic and neuroendocrine pathways, yoga may also influence inflammatory processes. Chronic low-grade inflammation is increasingly recognized as a key contributor to cardiovascular disease, metabolic disorders, and other chronic conditions. Evidence from mind–body medicine research suggests that yoga practice may reduce inflammatory activity and improve immune regulation, potentially contributing to improved health outcomes (24).

Clinical studies examining yoga interventions have also reported beneficial effects across a variety of health conditions. Systematic reviews indicate that yoga may improve cardiovascular risk factors such as blood pressure and metabolic indicators (6,28). Yoga-based interventions have also been shown to reduce pain and improve functional mobility in individuals with chronic musculoskeletal disorders, including chronic low back pain (7,32). These findings suggest that yoga may influence both physiological regulation and functional physical capacity.

Taken together, the evidence supports the view that yogāsana practice may act through multiple interacting physiological mechanisms rather than a single pathway. By combining movement, breath regulation, and attentional focus, yoga may simultaneously influence autonomic function, neuroendocrine regulation, inflammatory processes, and musculoskeletal health. This integrative model may help explain why yoga interventions have demonstrated benefits across diverse physical and psychological health outcomes (33). Clinical studies have also suggested that yoga interventions may influence neurochemical and emotional regulatory processes (31,32).

From a broader perspective, the integration of traditional yoga knowledge with contemporary biomedical research may contribute to the development of new approaches in preventive medicine and integrative health. Rather than viewing yoga solely as a complementary therapy, future research may increasingly consider yoga as a lifestyle-based regulatory practice capable of supporting long-term health and resilience.

## CONCLUSION

This review highlights yogāsana as an embodied practice that integrates movement, breathing awareness, and attentional regulation within the broader framework of classical yoga. Synthesizing insights from traditional yoga philosophy and contemporary scientific research suggests that yogāsana may influence health through multiple physiological pathways, including autonomic regulation, neuroendocrine modulation, and improvements in musculoskeletal function. Rather than functioning solely as a form of physical exercise, yogāsana can be understood as a multidimensional practice that supports self-regulation and adaptive physiological balance. Interpreting yogāsana within this integrative framework may help bridge classical yoga traditions with modern health science and provide a conceptual foundation for incorporating yoga-based practices into preventive health and rehabilitation strategies.

Future studies should further clarify the physiological pathways through which yogāsana influences health and well-being. In particular, the research that integrates autonomic regulation, neuroendocrine responses, inflammatory biomarkers, and musculoskeletal adaptations may provide deeper insight into the mechanistic flow linking yogāsana practice with positive health outcomes. Well-designed controlled trials and standardized intervention protocols will also be essential for advancing clinical applications in preventive health and rehabilitation.

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# Acute Effects of a Date Palm (*Phoenix dactylifera* L.) Gel on Anaerobic Performance in Football Players: A Randomized Controlled Crossover Trial

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## ABSTRACT

**Suksaard S, Thanpiam P, Phakaprapaboonnakul P, Ganogpichayagrai A, Pinijsuwan S.** The purpose of this study was to investigate the acute effects of a date palm gel on anaerobic performance and metabolic response in football players. Fourteen healthy male university football players (aged 20.86 ± 1.79 years, $VO_2$ max 51.72 ± 3.12 ml/kg/min) participated in the study. Thirty minutes before the test, the participants were randomly assigned to ingest either date palm gel or maltodextrin control gel, then they performed cycling using the 30S-Wingate Test. The result showed that some differences in anaerobic performance outcomes were observed between conditions with higher peak power, mean power, and total work in the date palm gel condition, while minimum power did not differ. Blood glucose concentration was higher in the maltodextrin control gel at 30 minutes post-ingestion; subsequently, glucose levels declined and remained relatively stable during the post-test recovery period in both conditions. Blood lactate concentration, heart rate, and RPE increased after exercise and peaked at 15 minutes post-test, followed by a gradual decline during recovery, with no significant differences across time. These findings suggest a potential beneficial effect of date palm gel on acute anaerobic performance and may be considered an alternative supplement option for athletes before high-intensity exercise.

**Key Words:** Anaerobic Performance, Date Palm, Energy Gel, Football

## INTRODUCTION

Football is defined as a high-intensity intermittent sport that is characterized by continuous periods of low to moderate-intensity running and short high-speed running and sprints. Although more than 90% of total energy expenditure is derived from the aerobic energy system during soccer matches, the anaerobic system remains crucial during rapid movements, including sprinting, acceleration, and direction changes (5,11). These actions emphasize the importance of anaerobic power for successful performance because it requires the rapid development of force and power over a short period and is more effective in critical situations that require maximum or nearly maximum effort to be repeated in gameplay (1,16).

In aerobic metabolism, the oxidation of carbohydrates and fats is used to support prolonged and repeated activity. In contrast, anaerobic energy metabolism depends predominantly on carbohydrate substrates, particularly intramuscular glycogen to rapidly generate ATP during short-duration, high-intensity efforts. Therefore, carbohydrate availability is a critical determinant of performance in high-intensity intermittent sports, such as football. Although intramuscular glycogen is the primary substrate for anaerobic energy production, acute carbohydrate ingestion before exercise may indirectly influence performance by elevating blood glucose availability and supporting metabolic responses during and after high-intensity efforts (7,14,17).

Energy gel is an ergogenic supplement that has attracted attention as a potential functional food that has a lot of carbohydrates and additional electrolytes, and it is also simple to use, lightweight, and portable. Athletes can take energy gels before and during training or competition to boost or keep their blood sugar levels, slow down muscle fatigue, and improve their exercise performance (8,18,23). Energy gels have been shown to improve intermittent high-intensity exercise capacity (21). Differences in carbohydrate composition, particularly glucose–fructose combinations, may partly explain the variability in performance outcomes observed across studies (9,14,15,22).

Date palm (*Phoenix dactylifera* L.) is a natural carbohydrate source with approximately 70% of its carbohydrate content consisting of glucose and fructose and high levels of micronutrients, such as copper, selenium, potassium, and magnesium, and it is also rich in bioactive phytochemicals, particularly polyphenols and flavonoids that have significant antioxidant properties (2,12,13). Acute ingestion of date extract before high-intensity exercise has been shown to increase pre-exercise blood glucose levels, delay the onset of fatigue, and improve exercise tolerance, suggesting its potential role as a natural ergogenic aid (10). In addition, a randomized, double-blind, placebo-controlled trial reported that short-term date seed (*Phoenix dactylifera* L.) supplementation was associated with reductions in exercise-induced oxidative stress and favorable changes in selected anaerobic performance parameters, including increased peak power and reduced fatigue index following high-intensity interval training (19).

However, existing evidence has primarily focused on exercise tolerance and fatigue-related outcomes, while the acute pre-exercise effects of date palm gel on anaerobic performance have not been well characterized. Therefore, the purpose of this study was to investigate the acute pre – exercise effects of date palm gel on anaerobic performance during a 30-second Wingate Test in football players. Additionally, post-exercise blood glucose and blood lactate levels were measured at multiple time points to gain further understanding of metabolic response (blood glucose and blood lactate) following high-intensity exercise.

## METHODS

### Subjects

Fourteen healthy male university-level football players (aged 18-23 years) were recruited. The **Inclusion Criteria** required the participants to have a maximum oxygen consumption ($VO_2$ max) that exceeded 45 ml/kg/min (3), at least 5 years of competitive football experience, and a cleared Physical Activity Readiness Questionnaire (PAR-Q). The **Exclusion Criteria** were any acute illness or health condition, or a history of health problems or food allergies, especially Date palm, or ingredients in gel, or missing data collection days. The participants who met the Inclusion Criteria signed an informed consent form following the Declaration of Helsinki, agreeing to participate in the study voluntarily. The study was approved by the Mae Fah Luang University Human Research Ethics Committee (approval number: 22135-18).

### Procedures

#### *Study Design*

A randomized, double-blind, placebo-controlled, crossover design was employed. Participants visited the laboratory on 3 separate occasions, with a 7-day washout period between the experimental sessions. The first session was dedicated to inclusion criteria and baseline assessments. During the subsequent sessions, the participants completed 2 experimental exercise trials. To standardize the physiological conditions, the participants were instructed to reduce their training volume and record all dietary intake for 72 hours before each trial. Additionally, an 8-hour overnight fast was required. On each performance test day, trials were conducted at the same time of day.

#### *Preliminary Testing*

The participants completed orientation and performed a $VO_2$ max test using a graded exercise protocol to exhaustion on a bicycle ergometer (Wattbike Pro, UK). Heart rate (HR) was continuously monitored using a heart rate monitor (Polar H10, USA). The ramp protocol commenced at an initial workload of 50 W with increments of 25 W every 2 minutes, maintaining a constant cadence of 60 RPM until volitional exhaustion. $VO_2$ max was considered valid when at least two of the following criteria were met: (1) a maximum HR within 10 beats·min$^{-1}$ of the age-predicted maximum (220 - age), (2) a respiratory exchange ratio (RER) > 1.10, and (3) a rating of perceived exertion (RPE) ≥ 17 on the Borg scale.

#### *Dietary Control*

The nutritional intervention consisted of 2 iso-caloric gels (50 mL, 70 kcal each). The positive control (CON) was a maltodextrin gel. The experimental intervention (DAT) was a proprietary date palm gel formulated from *Phoenix dactylifera* L. fruit. The nutritional profile of the DAT gel was determined using standardized food composition databases (13) and was matched to the CON gel for total carbohydrate-derived energy. Both gels included 0.5 g of carrageenan as a thickening agent to ensure a similar texture. To maintain a double-blind design, the gels were presented in unlabelled packaging and were consumed followed by 200 mL of water.

#### *Experimental Sessions*

The participants arrived at the laboratory at 7:30 AM for each experimental session. In a randomized crossover design, the participants were randomly assigned to receive either one packet of DAT or CON, which was consumed within 5 minutes followed by the ingestion of 250 mL of water. A 30-minute rest period was observed before commencing the exercise protocol.

The session began with a standardized warm-up that consisted of 5 minutes of low-intensity cycling. Subsequently, the participants performed a 30-second Wingate Anaerobic Test, pedalling at maximum speed against a constant resistance of (set at 0.075 kg per kg of body weight) (6). All the trials were conducted in a climate-controlled environment at 23°C. Following the test, the participants were allowed ad libitum water consumption.

### *Measurements*

#### *Anaerobic Performance*

Anaerobic performance variables (i.e., peak power, mean power, minimum power, fatigue index, and total work) were recorded immediately upon completion of the Wingate Anaerobic Test. Heart rate (HR) was monitored continuously and recorded at baseline (pre-ingestion), 30 minutes post-ingestion, and at 15 minutes, 30 minutes, and 45 minutes post-exercise using a heart rate monitor (Polar H10, Finland).

#### *Physiological Responses*

Capillary blood samples were collected via finger prick to determine blood glucose and lactate concentrations at baseline, at 30 minutes post-ingestion, and at 15 minutes, 30 minutes, and 45 minutes post-exercise. Blood glucose was analyzed using a portable glucose analyzer (Accutrend Performa, Roche Diagnostics, Germany), and blood lactate was measured using a lactate analyzer (Accutrend Pus, Roche Diagnostics, Germany). Both devices were calibrated according to the manufacturer's guidelines before each testing session.

#### *Subjective Measurement*

Subjective ratings were assessed at the same time points as the physiological measurements. Gastrointestinal (GI) symptoms (i.e., nausea, vomiting, fullness, abdominal pain, heartburn, and bloating) were evaluated using a 10-point Likert scale that ranged from 1 (no symptoms) to 10 (extreme severity). Similarly, subjective appetite and thirst (hunger and thirst) were recorded. The Rating of Perceived Exertion (RPE) was measured using the Borg 1-10 scale to quantify the intensity of physical effort, where 0 represented 'nothing at all' and 10 represented 'maximal effort'.

#### *Statistical Analyses*

The data were expressed as mean ± SD. The sample size was chosen based on a power calculation (version 3.1.9.2; G*Power, Düsseldorf, Germany) with an effect size of 0.5 and a power of 0.8. Statistical testing was conducted using the SPSS version 29.0, and an alpha level of $P < 0.05$ for all statistical analyses. Data normality was assessed using the Shapiro-Wilk Test. Anaerobic performance and RPE were compared using Paired Samples *t*-tests. Physiological variables over time were analyzed using the Two-way Repeated Measures ANOVA. For GI symptoms and appetite scores, which violated normality assumptions, the Wilcoxon Signed-Rank Test was employed to compare differences between trials at each time point.

## RESULTS

Table 1 presents the baseline characteristics of the participants. The sample showed characteristics typical of trained football players.

**Table 1. The Participants Characteristics.**

| Characteristics | Mean ± SD |
|---|---|
| **Age** (years) | 20.86 ± 1.79 |
| **Body Weight** (kg) | 69.07 ± 4.36 |
| **Height** (cm) | 173.14 ± 5.41 |
| **Body Mass Index** (BMI, $kg/m^2$) | 22.95 ± 2.03 |
| **Experience in Football** (years) | 7.29 ± 2.57 |
| **$VO_2$ Max** (ml/kg/min) | 51.72 ± 3.12 |

Table 2 summarizes anaerobic performance outcomes following DAT and CON ingestion. Significant differences between conditions were observed for several performance variables, whereas minimum power did not differ between gels.

**Table 2. Anaerobic Performance.**

| Anaerobic Performance Data | Type of Gel | | P-value |
|---|---|---|---|
| | **DAT** Mean ± SD | **CON** Mean ± SD | |
| **Peak power** (W) | 843.53 ± 110.47 | 774.78 ± 126.02 | .002* |
| **Peak power** (W/kg) | 13.15 ± 1.37 | 12.08 ± 1.45 | .001* |
| **Minimum Power** (W) | 287.46 ± 83.89 | 320.66 ± 90.71 | .244 |
| **Mean Power** (W) | 577.95 ± 87.83 | 546.07 ± 85.38 | <.001* |
| **Mean Power** (W/kg) | 8.88 ± 0.69 | 8.42 ± 0.76 | .004* |
| **Fatigue Index** (FI) | 65.60 ± 10.53 | 58.63 ± 9.35 | .04* |
| **Total Work** (kJ) | 16.85 ± 26.01 | 15.95 ± 24.63 | .001* |

*Significant level at ($P < 0.05$)

Figures 1 A-D present the following comparisons of (A) blood glucose, (B) blood lactate, (C) heart rate, and (D) RPE between DAT and CON trials. Values are mean ± SD. *Significant difference between trials ($P < 0.05$).

Figure 1A shows the changes in blood glucose concentration across time following ingestion of DAT and CON are shown in Figure A. Blood glucose increased after gel ingestion with a significantly higher concentration observed in the CON condition at 30 minutes post-ingestion (DAT: 113.43 ± 6.44 and CON: 124.71 ± 10.05 mg/dL). Thereafter, glucose levels declined and remained relatively stable during the post-test recovery period in both conditions.

(A)

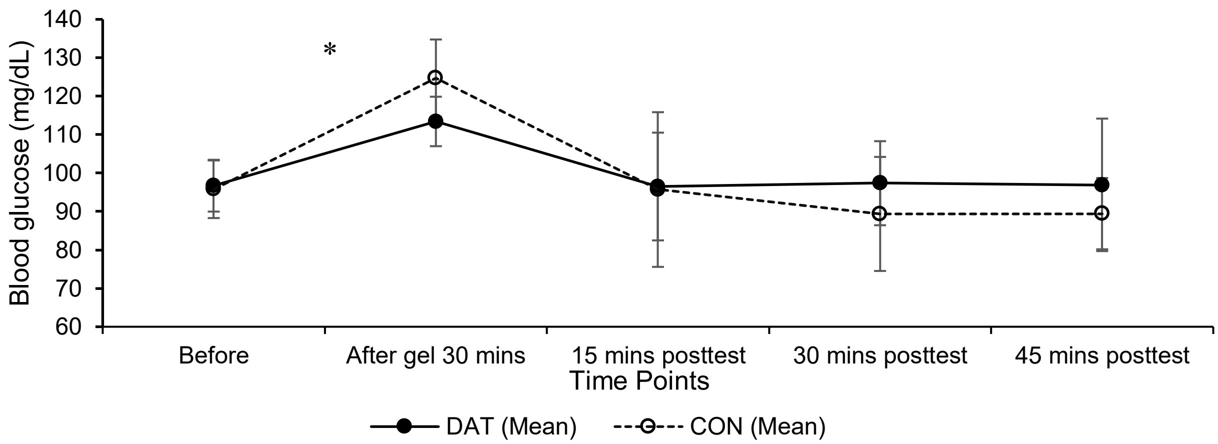



Figure 1B shows changes in blood lactate concentration across time following ingestion of DAT and CON. Blood lactate increased after exercise in both conditions, reached a peak at 15 minutes post-test, and gradually declined during the recovery period. No significant differences between conditions were observed across the measured time points.

(B)

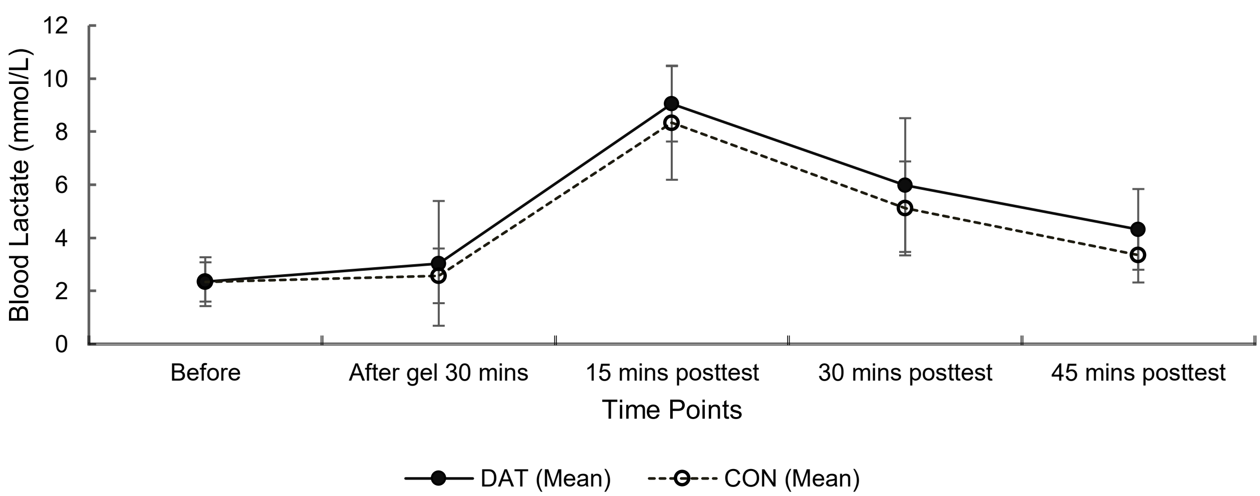



**Figure 1C** shows the changes in heart rate across time following ingestion of DAT and CON. Heart rate increased after gel ingestion and reached its highest value at 15 minutes post-test in both conditions (DAT:9.05 ± 1.42 and CON:8.34 ± 2.15 mmol/L). Thereafter, heart rate gradually declined during the post-test recovery period with similar patterns observed between DAT and CON across all time points.

(C)

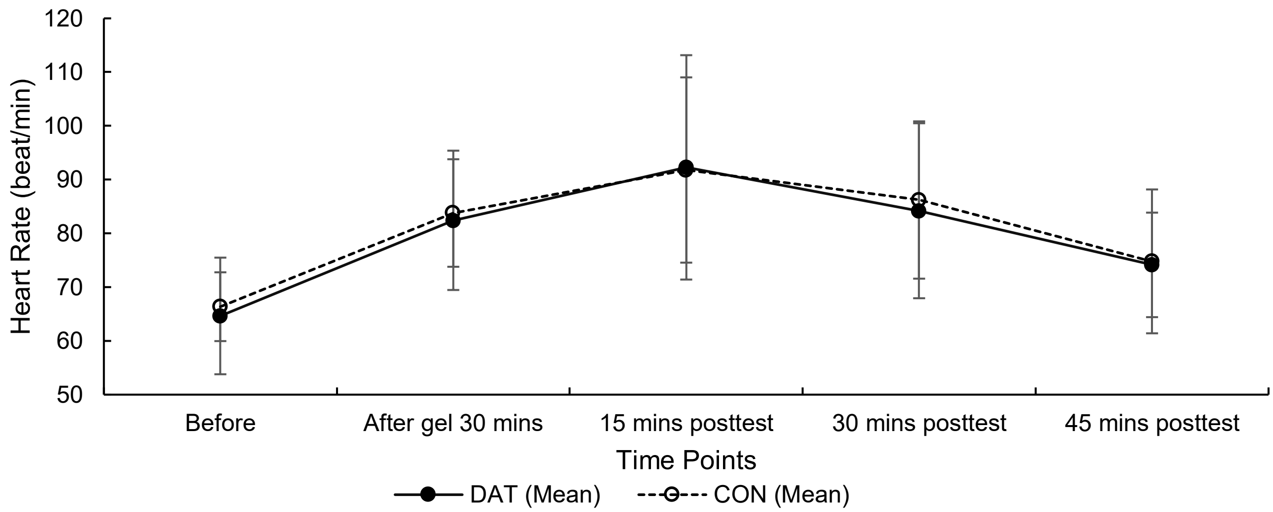
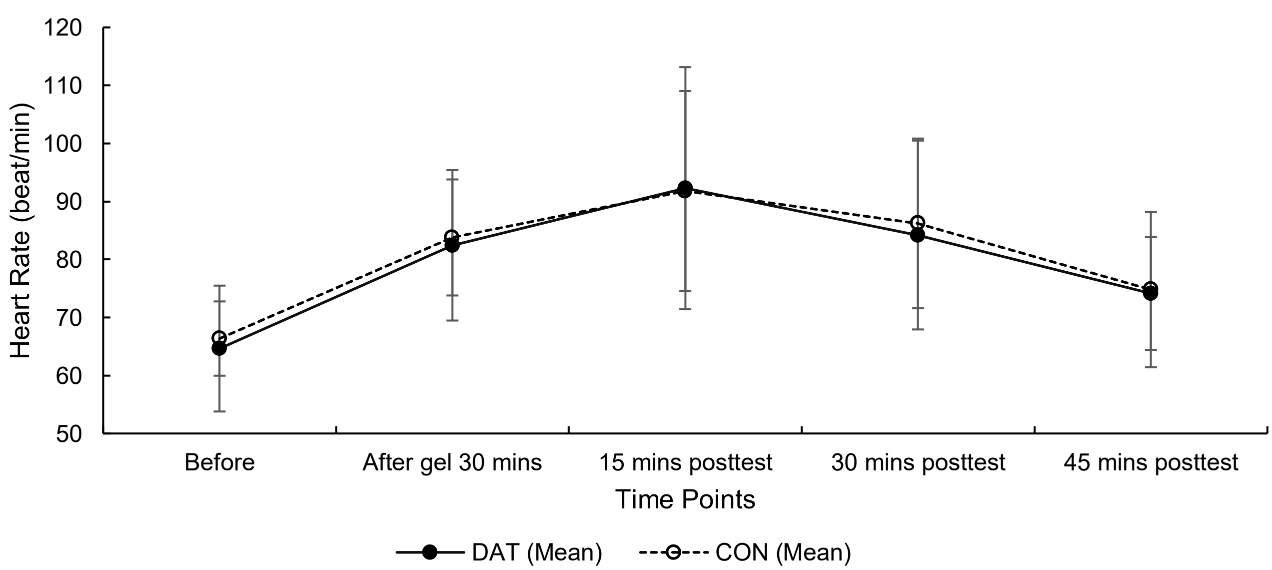



**Figure 1D** shows the changes in RPE across time following ingestion of DAT and CON. RPE increased after exercise, reaching the highest value at 15 minutes post-test in both conditions. Thereafter, RPE gradually declined during the post-test recovery period. Similar patterns were observed between DAT and CON across all time points.

(D)



## DISCUSSION

The present study investigated the acute effects of a date palm gel (DAT) compared with a maltodextrin-based control gel (CON) on anaerobic performance in football players. The main finding was that DAT ingestion resulted in significantly higher peak power (PP), mean power (MP), and total work. Specifically, peak power increased by 9% and mean power by 6% compared with the maltodextrin control. While the fatigue index (FI) was approximately 12% higher in the DAT, the elevated FI likely reflects a concomitant response to higher peak power production and may indicate a shift toward a more power-dominant performance profile rather than a clear impairment of anaerobic capacity. The 30-second Wingate Test, although performed in a laboratory setting, accurately represents performance attributes relevant to football, including short maximal sprints and power that is explosive. The observed enhancements in PP and MP following the consumption of DAT may have practical relevance for specific sprint-related activities in football, particularly actions requiring rapid force production, such as short sprints and explosive accelerations.

Because the gels were iso-caloric, the observed performance divergence likely reflects differences in the carbohydrate matrix of the DAT. Unlike maltodextrin, a glucose polymer, date palm provides a natural blend of glucose and fructose in an approximately 1:1 ratio. Consistent with this, blood glucose was transiently higher in the CON condition at 30 minutes post-ingestion that reflects the rapid absorption characteristics of maltodextrin. It should be acknowledged that evidence supporting an ergogenic effect of carbohydrate ingestion on short-duration, maximal exercise remains inconsistent. This inconsistency has been highlighted in previous reviews, which suggest that the ergogenic effects of acute carbohydrate supplementation during intermittent or maximal exercise are highly dependent on factors such as carbohydrate type, dosage, and timing rather than total caloric intake (4).

In this context, co-ingestion of glucose and fructose may influence carbohydrate availability by engaging both SGLT1 and GLUT5 transporters that potentially affect metabolic signalling or carbohydrate handling rather than acting solely through substrate delivery. Although such mechanisms have been more consistently demonstrated during prolonged exercise, several studies have shown that glucose–fructose combinations increase total carbohydrate oxidation rates (9,20). Accordingly, date palm extract ingestion has been shown to increase pre-exercise blood glucose availability without exacerbating metabolic stress markers such as blood lactate or heart rate during high-intensity exercise (10).

Furthermore, the DAT demonstrated superior gastrointestinal (GI) tolerance compared with the CON. The participants consuming the DAT reported lower ratings of stomach fullness and abdominal pain with a non-significant trend towards reduced nausea. Consistent with these perceptual responses, the incidence of GI symptoms was lower in the DAT Group than in the CON Group across all reported categories (stomach fullness, abdominal pain, and nausea: $n = 1$ for DAT vs. $n = 2$ for CON). Improved GI tolerance may represent a practical consideration in football-specific contexts, where repeated high-intensity actions can increase susceptibility to GI discomfort.

## CONCLUSION

The findings suggest a potential beneficial effect of pre-exercise date palm gel ingestion on anaerobic performance as indicated by higher peak power, mean power, and total work compared with the maltodextrin control gel, while minimum power remained unchanged. Metabolic and perceptual responses, including blood glucose, blood lactate, heart rate, and RPE, demonstrated similar post-exercise patterns between the conditions. Therefore, date palm gel may be considered a potential alternative supplement for athletes before high-intensity exercise.

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# Muscle Adaptations to Hypoxic Resistance Training Occur Independent of Hematological Shifts

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## ABSTRACT

**Zheng M, Manimmanakorn A, Huang Q, La-bantao K, Choocheepwatana P, Chotipinit T, Manimmanakorn N, Labantao K, Yang G.** This study evaluated the effects of low-load resistance training (LL-RT) under hypoxic conditions on muscle strength, body composition, and hematological variables. While high-load training is the gold standard for hypertrophy, low-load resistance training serves as a vital alternative for athletes during rehabilitation or dense competitive schedules. However, LL-RT often yields sub-optimal adaptations under normal oxygen levels (normoxia). Hypoxic exposure during exercise potentiates metabolic stress, which may enhance anabolic signaling and neuromuscular adaptation. However, the combined effects of hypoxia and LL-RT on body composition, strength, and hematological responses in team-sport athletes remain underexplored. Twenty male athletes were randomly assigned to either normoxic (RT, $FiO_2$ = 21%) or hypoxic (RTH, $FiO_2$ = 13.6%, ~3420m simulated altitude) Groups. All athletes completed 5 weeks of lower-body LL-RT (50%1RM, 3 sets × 15 reps, 3 sessions/week). Body composition (via bioelectrical impedance), dynamic strength (1RM for knee extension and flexion), and hematological variables were assessed pre- and post-intervention. Both Groups exhibited significant increases in knee extension and flexion strength relative to baseline ($P < 0.05$). Notably, the RTH Group achieved significantly greater improvement in knee extension 1RM compared to the RT Group (33.11 ± 15.81 vs.18.38 ± 7.26 kg, $P < 0.05$). Furthermore, the RTH Group demonstrated significant within-group increases in fat free mass, total skeletal muscle mass, and leg muscle mass ($P < 0.05$), whereas no such changes were observed in the RT Group. Neither Group showed significant alterations in hematological markers (RBC, Hb, Hct, WBC). These results suggest that LL-RT in a normobaric hypoxic environment augments lower-body strength and lean mass adaptations more effectively than normoxic training, without influencing hematological parameters. Consequently, hypoxia serves as a potent adjunct for athletes when high-load training is contraindicated or unfeasible.

**Key Words:** Body Composition; Hypoxia; Low-load Resistance Training; Muscle Strength

## INTRODUCTION

Resistance training (RT) is widely recognized for its ability to enhance muscular strength, power, and hypertrophy, which are critical determinants of sports performance (31). Traditional RT programming often emphasizes high mechanical loads (≥70%of one-repetition maximum, 1RM) to optimize neuromuscular adaptations (1). However, high-load training is not always feasible or safe for certain athletic populations, such as athletes during in-season periods, those undergoing rehabilitation from injury, or individuals with orthopedic limitations (16). Research indicates that low-load resistance training (LL-RT, ≤50%1RM) alone may provide a sub-optimal hypertrophic stimulus compared to high-load training (28). In contrast, LL-RT combined with hypoxia has emerged as a viable alternative to effectively promote muscular adaptations (4,15).

For athletes, who require a combination of strength, power, and endurance, identifying time efficient training strategies that optimize multiple performance domains is of high practical relevance. While previous studies have demonstrated the efficacy of LL-RT combined with hypoxia in improving muscle function (5,15,18), few have systematically investigated whether such short term interventions also influence hematological parameters. Moreover, existing evidence suggests that athletes' adaptive responses to hypoxic RT may be modulated by many factors such as duration of training, training load, training volume, inter-set rest interval and hypoxia dose (3,25).

A robust body of evidence has examined the physiological and performance-related effects of hypoxic training. Studies have demonstrated that resistance exercise performed under hypoxic conditions can enhance muscular strength and hypertrophy to a greater extent than equivalent normoxic training, even when the mechanical load is matched (9,18). For instance, Manimmanakorn et al. (2013) reported that after 5-weeks of knee flexor and extensor training (20%1RM, 3 sessions/week) under normobaric hypoxia ($SpO_2$≈ 80%), female netball athletes showed a 15.0%increase in maximal voluntary contraction and a 6.1% increase in quadriceps cross-sectional area, both significantly greater than those observed under normoxia (18). Similarly, Martínez-Guardado et al. (9) found that 7-weeks of full-body resistance training to failure (65-80%1RM, 3 sessions/week) under normobaric hypoxia ($FiO_2$ = 13%) led to significant gains in muscle mass and reductions in fat mass in untrained males, with elevated hematocrit levels observed after a detraining period. In addition, repeated sprint training (4-weeks) in hypoxia has been shown to improve sprint velocity (6,8) and power output (17). Most studies reporting improvements in anaerobic performance have employed training durations ranging from 4 to 8 weeks (2,24,25). Furthermore, prolonged hypoxic exposure has been associated with enhanced aerobic capacity via hematological adaptations. For example, Fernández-Lázaro et al. (2022) and Wonnabussapawich et al. (2017) reported significant increases in red blood cell count and hemoglobin concentration following 8 weeks of living and training at moderate altitude combined with intermittent hypoxic exposure (7,33). Meta-analyses by Huang et al. (2023) and Westmacott et al. (2022) further support these findings, both concluding that hypoxic training is more effective than normoxic training in enhancing maximal oxygen uptake ($VO_2$max) (11,32).

Despite the widespread application of hypoxic training, no consensus has been reached regarding the minimal intervention duration required to induce significant changes in hematological markers such as hemoglobin (Hb), hematocrit (Hct), red blood cell count (RBC), and erythropoietin (EPO). Some studies suggest that short-term hypoxic exposure (2–4 weeks) is sufficient to stimulate EPO secretion and subsequently increase red blood cell production (10,13), whereas other studies indicate that low-dose or short-duration exposure (e.g., 3-5 weeks, 3 h/week) fails to elicit significant changes in hematocrit or hemoglobin levels (23,26). There is a lack of clarity regarding whether a shorter, 5-week intermittent hypoxic resistance training (RT) program can concurrently drive adaptations in both anaerobic performance (linked to muscle mass) and the hematological markers essential for oxygen transport. Therefore, it is necessary to examine whether a 5-week hypoxic LL-RT protocol can induce meaningful changes in both muscular and hematological outcomes in athletic populations.

The purpose of this study was to investigate the effects of a short period hypoxic program (5-week LL-RT, 50%1RM) performed under normobaric hypoxic conditions ($FiO_2$ = 13.6%, simulated altitude 3,420m) on body composition, maximal dynamic strength, and hematological parameters in healthy male athletes. We hypothesized that low-load resistance training in a hypoxic environment would outperform identical training in normoxia in terms of lower-body strength and lean mass, with altering hematological markers. Our results offer a framework for tailoring exercise programs in sports settings where high-load training is impractical.

## METHODS

### Participants

Twenty healthy male athletes (age: 19–24 years) from university soccer, basketball, and volleyball teams volunteered for this randomized controlled trial. All participants were engaged in regular sport-specific training but had not performed systematic resistance training for at least three months prior to the study. Additional inclusion criteria required no exposure to altitudes above 1000 m in the preceding six months and the absence of any known cardiovascular, respiratory, or musculoskeletal disorders. All procedures were explained in detail, and written informed consent was obtained before participation. The study protocol received ethical approval from the Institutional Ethics Committee of Khon Kaen University (Approval No. HE651425).

Participants were stratified by primary sport and randomly assigned to either a normoxic training Group (RT, n = 10) or a hypoxic training Group (RTH, n = 10). This stratification ensured balanced representation of sports backgrounds across Groups, minimizing potential bias from differential training histories.

### Procedures

Both Groups completed a 5-week lower-body resistance training program (3 sessions/week on Monday, Wednesday, and Friday) consisting of seated knee extension and prone knee flexion exercises performed on fixed-resistance machines (Nautilus One™ S6LE and Nautilus EVO™ S9LCP, USA). Training intensity was set at 50%of each participant's one-repetition maximum (1RM), with three sets of 15 repetitions per exercise and 60-second rest intervals between sets. A metronome paced each movement to ensure 2-second concentric and eccentric phases. Range of motion was standardized from 90° flexion to full extension (0°) for knee extension, and from full extension to 90° flexion for knee flexion. Before each session, participants completed a 10-minute warm-up comprising 7 minutes of stationary cycling and 3 minutes of dynamic lower-limb stretching.

The RT Group trained under normoxic conditions (ambient air, $FiO_2$ ≈ 21%). The RTH Group breathed a hypoxic gas mixture ($FiO_2$ = 13.6%, simulating ≈ 3,420 m altitude) via a facemask connected to a normobaric hypoxic generator (Everest Summit II, Hypoxico, USA) throughout each session. This $FiO_2$ was selected based on Namboonlue et al. (2020), who demonstrated the efficacy of this moderate simulated altitude for enhancing training adaptations (22).

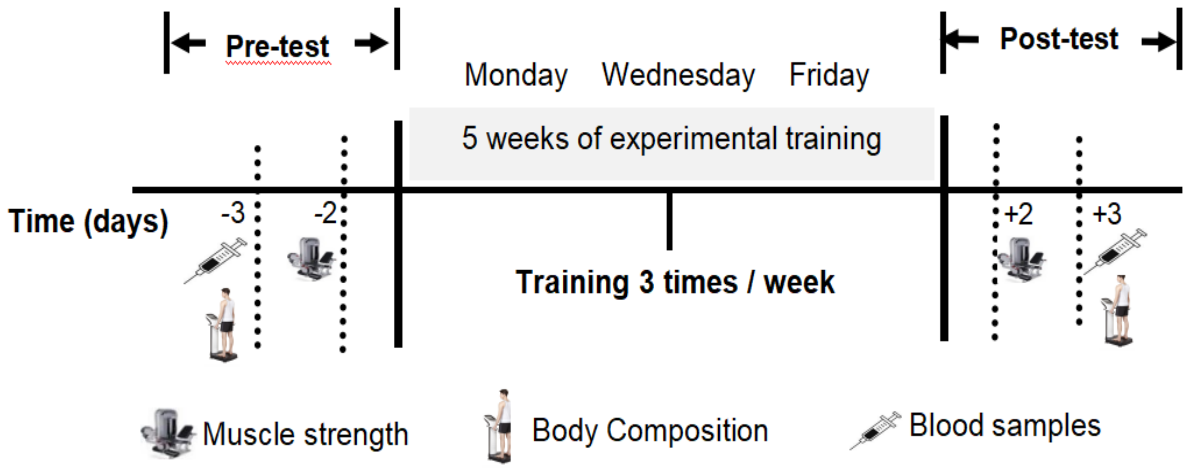


**Figure 1. Outline of Training and Testing Schedule.**

**Measurements**
All measurements were conducted 1-3 days prior to and following the 5-week intervention (Figure 1). A double-blind design was employed, with the researcher masked to Group assignments to mitigate potential observer bias during testing.

**Body Composition Assessment**
Body composition was evaluated using a medically approved, multi-frequency bioelectrical impedance analysis (BIA) device (SECA mBCA, Seca GmbH & Co. KG., Hamburg, Germany). Following standardized procedures, the participants were assessed in the morning after an overnight fast and having emptied their bladder. They stood barefoot on the device's electrode platform wearing light indoor clothing. The measured parameters included fat mass, fat-free mass, body fat percentage, total muscle mass, trunk muscle mass, arm muscle mass, and leg muscle mass. This BIA system has demonstrated good validity and reliability in athletic populations, thus allowing for the precise detection of subtle changes in body composition.

**Muscle Strength Measurement**
Maximal dynamic strength of the knee extensors and flexors was assessed using a one-repetition maximum (1RM) test based on a 10-repetition maximum (10RM) protocol. Following a standardized warm-up, participants performed sets with progressively increasing loads until they were unable to complete 10 full repetitions with proper form. The highest successfully lifted load for 10 repetitions was recorded, and the corresponding 1RM was estimated using the Brzycki equation (4): 1RM = weight lifted / [1.0278 – (0.0278 × number of repetitions)]. This method provided a safe and reliable estimation of maximal strength for both movements.

**Haematological Variables Measurement**
Fasting venous blood samples (10 mL) were collected from the antecubital vein by qualified medical personnel in the morning. The samples were placed in vacuum blood collection tubes containing anticoagulant, stored under cool conditions, and transported to the cooperating hospital laboratory within 2 hours for analysis. A fully automated hematology analyzer was used

to determine the following parameters: red blood cell (RBC) count, hemoglobin(Hb) concentration, hematocrit (Hct), white blood cell (WBC) count, platelet count, neutrophils, lymphocytes, eosinophils, and basophils. These hematological indices were measured to evaluate the effects of the training intervention on fundamental blood physiological status.

### Statistical Analyses

The data were analyzed using SPSS software (Version 28.0; IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD). The normality of data distribution was initially assessed using the Shapiro-Wilk Test. For data that met the assumption of normality: paired-sample *t*-tests were used to evaluate within-group differences (pre-test vs. post-test); independent-sample *t*-tests were used to compare the mean change scores (Δ = post-test value – pre-test value) between the RT and RTH Groups. For data not conforming to a normal distribution, equivalent non-parametric tests were applied (Wilcoxon Signed-Rank Test for within-group comparisons and Mann-Whitney U Test for between-group comparisons of Δ scores). Statistical significance was set at $P < 0.05$.

## RESULTS

### Participant Characteristics

Twenty athletes completed the intervention (RT Group, n = 10; RTH Group, n = 10). As shown in Table 1, there were no significant baseline differences between the 2 Groups in terms of age, height, weight, BMI, resting heart rate, and resting blood pressure ($P > 0.05$).

**Table 1. The Baseline Characteristics of the RT and RTH Groups.**

| Parameters | RT (n = 10) | RTH (n = 10) | P-value |
|---|---|---|---|
| **Age** (year) | 21.60 ± 0.90 | 21.80 ± 1.20 | 0.912 |
| **Height** (cm) | 176.30 ± 5.70 | 175.00 ± 6.13 | 0.629 |
| **Weight** (kg) | 72.60 ± 18.08 | 71.63 ± 15.94 | 0.900 |
| **BMI** (kg·m$^{-2}$) | 23.18 ± 4.63 | 23.27 ± 4.27 | 0.965 |
| **Resting Heart Rate** (bpm) | 72.40 ± 12.37 | 71.20 ± 13.40 | 0.837 |
| **SBP** (mmHg) | 119.00 ± 12.09 | 114.50 ± 9.36 | 0.364 |
| **DBP** (mmHg) | 71.10 ± 6.76 | 71.80 ± 7.38 | 0.827 |

Values are means ± SD. RT, low-load (50%1 RM) resistance training ($FiO_2$ = 21%); RTH, low-load (50%1 RM) resistance training combined with hypoxia ($FiO_2$ = 13.6%); **BMI =** Body Mass Index; **SBP =** Systolic Blood Pressure; **DBP =** Diastolic Blood Pressure.

### Changes in Body Composition

Following the 5-week intervention, body composition changes demonstrated distinct patterns between the 2 Groups (Table 2). The RTH Group showed significant positive adaptations in several metrics: compared to pre-intervention, post-intervention values for fat-free mass, total skeletal muscle mass, and leg skeletal muscle mass increased significantly ($P < 0.05$). In contrast, none of the body composition metrics in the RT Group exhibited statistically significant changes from pre-intervention to post-intervention ($P > 0.05$). Further between-group comparison of the change magnitudes (Δ scores) revealed that the RTH Group exhibited larger positive changes across all metrics compared to the RT Group; however, none of these between-group differences reached statistical significance ($P > 0.05$).

**Table 2. Comparison of Body Composition Changes Before and After Intervention Between RT and RTH Groups.**

| Parameters | RT (n = 10) | | | RTH (n = 10) | | | Between-Group Δ (P-value) |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Δ | Pre | Post | Δ | |
| **Fat mass** (kg) | 12.54 ± 11.41 | 12.42 ± 11.37 | -0.13 ± 1.98 | 11.75 ± 9.47 | 10.69 ± 8.35 | -1.06 ± 2.27 | 0.344 |
| **Fat Free Mass** (kg) | 60.05 ± 8.53 | 60.81 ± 8.50 | 0.76 ± 2.19 | 59.89 ± 8.63 | 60.77 ± 9.14 * | 0.89 ± 1.14 | 0.873 |
| **Body Fat Percentage** (%) | 15.31 ± 9.51 | 14.92 ± 9.67 | -0.39 ± 2.05 | 15.05 ± 8.46 | 13.75 ± 8.01 | -1.30 ± 2.3 | 0.355 |
| **Total Muscle Mass** (kg) | 28.78 ± 4.64 | 29.32 ± 4.57 | 0.54 ± 0.92 | 29.00 ± 4.83 | 29.70 ± 4.78 * | 0.70 ± 0.72 | 0.669 |
| **Trunk Muscle Mass** (kg) | 12.44 ± 1.97 | 12.66 ± 1.82 | 0.21 ± 0.31 | 12.73 ± 2.32 | 12.96 ± 2.10 | 0.23 ± 0.36 | 0.903 |
| **Leg Muscle Mass** (kg) | 12.74 ± 2.09 | 12.87 ± 2.05 | 0.13 ± 0.48 | 12.93 ± 2.02 | 13.24 ± 2.26 * | 0.30 ± 0.37 | 0.385 |
| **Arm Muscle Mass** (kg) | 3.41 ± 0.59 | 3.48 ± 0.57 | 0.07 ± 0.10 | 3.29 ± 0.71 | 3.38 ± 0.67 | 0.10 ± 0.12 | 0.577 |

Data are presented as mean ± standard deviation (SD). Δ (Delta change) represents the mean change score (post-test minus pre-test). * Significant $P < 0.05$ (pre vs. post).

### Changes in Muscle Strength

Post-intervention, maximal dynamic strength (1RM) for both knee extension and knee flexion significantly increased from baseline in both Groups (RT: $P < 0.001$; RTH: $P < 0.001$), demonstrating that low-load resistance training effectively enhanced strength under both environmental conditions (Figure 1). Furthermore, between-group comparisons revealed that the magnitude of improvement in knee extension 1RM was significantly greater in the RTH Group (33.11 ± 15.81 kg) than in the RT Group (18.38 ± 7.26 kg; $P = 0.03$). Although the increase in knee flexion 1RM was numerically larger in the RTH Group (16.50 ± 10.90 kg) than in the RT Group (11.55 ± 5.90 kg), the difference did not reach statistical significance ($P = 0.223$).

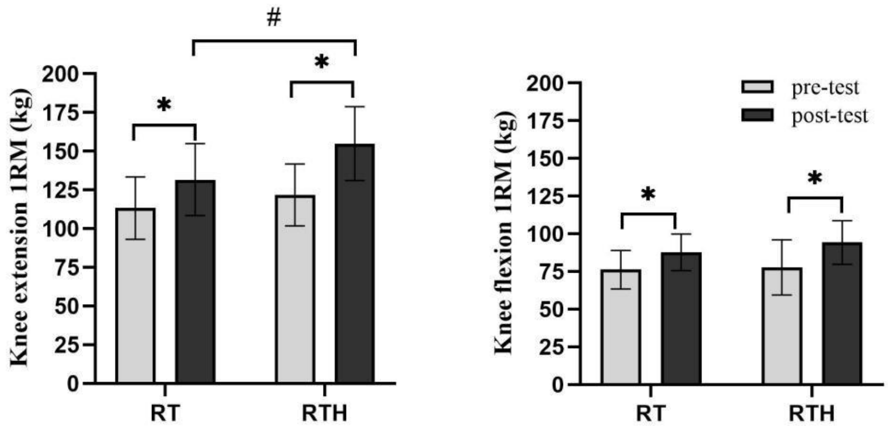


**Figure 1. Comparison of Muscle Strength Before and After the Intervention in RT and RTH Groups.** *Significant within-group effect ($P < 0.05$, pre vs. post). #Significant between-group difference ($P < 0.05$) for delta change (Δ) values.

**Changes in Hematological Variables**
As shown in Table 3, none of the monitored routine hematological variables showed significant changes in either Group following the 5-week intervention (P > 0.05). Specifically, parameters reflecting oxygen-carrying capacity, including red blood cell count (RBC), hemoglobin concentration, and hematocrit, as well as those related to inflammation and immune status, including white blood cell count (WBC) and its differential components (neutrophils, lymphocytes, eosinophils, basophils, and platelet count), which reflects coagulation function, all showed no statistically significant differences in within-group pre-post comparisons or in between-group comparisons of change scores.

**Table 3. Comparison of Hematological Variables Changes Before and After Intervention Between RT and RTH Groups.**

| Parameters | RT (n = 10) | | | RTH (n = 10) | | | Between Group Δ (P-value) |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Δ | Pre | Post | Δ | |
| **RBC** ($\times 10^{12}$/L) | 5.47 ± 0.44 | 5.41 ± 0.59 | -0.06 ± 0.26 | 5.52 ± 0.65 | 5.46 ± 0.62 | -0.06 ± 0.22 | 0.942 |
| **Hb** (g/dL) | 14.05 ± 0.60 | 14.05 ± 0.60 | -0.19 ± 0.71 | 14.52 ± 1.25 | 14.39 ± 1.30 | -0.13 ± 0.57 | 0.837 |
| **Hct** (%) | 42.70 ± 2.75 | 42.00 ± 1.83 | -0.70 ± 2.26 | 43.50 ± 3.10 | 43.60 ± 3.34 | 0.10 ± 1.73 | 0.386 |
| **WBC** ($\times 10^9$/L) | 7.01 ± 1.75 | 6.52 ± 1.57 | -0.49 ± 1.23 | 6.88 ± 1.36 | 6.79 ± 1.15 | -0.09 ± 1.15 | 0.463 |
| **Platelet Count** ($\times 10^9$/L) | 247.90 ± 57.92 | 245.00 ± 59.40 | -2.90 ± 29.11 | 260.00 ± 41.94 | 251.50 ± 35.00 | -8.50 ± 25.53 | 0.653 |
| **Neutrophils** (%) | 59.70 ± 6.83 | 57.10 ± 6.70 | -2.60 ± 3.95 | 58.80 ± 8.32 | 59.80 ± 7.28 | 1.00 ± 5.01 | 0.091 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Lymphocytes (%)** | 30.90 ± 7.06 | 32.50 ± 6.95 | 1.60 ± 4.38 | 31.20 ± 7.89 | 31.20 ± 7.89 | -1.20 ± 4.32 | 0.167 |
| **Eosinophils (%)** | 3.10 ± 3.14 | 3.30 ± 2.11 | 0.20 ± 1.69 | 3.20 ± 1.69 | 3.80 ± 2.04 | 0.60 ± 0.84 | 0.511 |
| **Basophils (%)** | 0.30 ± 0.48 | 0.60 ± 0.52 | 0.30 ± 0.67 | 0.30 ± 0.48 | 0.10 ± 0.32 | -0.20 ± 0.42 | 0.062 |

Data are presented as mean ± standard deviation (SD). Δ (Delta change) represents the mean change score (post-test minus pre-test). Abbreviations: **RBC** = red blood cell count, **Hb** = hemoglobin, **Hct =** hematocrit, **WBC** = white blood cell count.

## DISCUSSION

This randomized controlled trial systematically compared the effects of a 5-week low-load resistance training (LL-RT) program conducted under normoxic versus hypoxic ($FiO_2$ = 13.6%) conditions on body composition, muscle strength, and blood parameters in athletes. The results demonstrated that while both training environments significantly increased maximal knee extension strength, the hypoxic condition appeared to confer additional benefits by promoting more favorable trends in lean body mass, total skeletal muscle mass, and leg muscle mass without inducing in hematological adaptation.

The enhanced muscular hypertrophy and strength gains observed following LL-RT in hypoxia align with the contemporary hypothesis of "synergistic metabolic stress potentiation." The hypoxic environment itself constitutes a physiological stressor that, by reducing arterial oxygen saturation, exacerbates local tissue hypoxia within the exercising muscles. This amplifies the inherent metabolic stress of LL-RT (29). Previous studies indicate that exercise under hypoxia leads to a more pronounced decrease in muscle tissue oxygen saturation, precipitating and intensifying anaerobic glycolysis, thereby accelerating the accumulation of metabolites such as lactate and hydrogen ions (30). This potentiated metabolic milieu is theorized to promote protein synthesis through various molecular pathways, including activation of the mechanistic target of rapamycin (mTOR) signaling cascade, induction of muscle cell swelling stimulating mechanosensitive channels, and upregulation of local growth factors like Insulin-like Growth Factor 1 (IGF-1) (27). The more pronounced gains in muscle mass observed in the RTH Group are consistent with findings by Kon et al. (14) and Manimmanakorn et al (19). Both studies reported that LL-RT under acute hypoxia elicited a greater growth hormone release after acute bout training, which may represent a key endocrine mechanism underlying hypoxia-augmented muscular adaptation.

Furthermore, hypoxia may enhance strength performance by influencing neuromuscular adaptations. Although LL-RT employs relatively low mechanical loads, the increased metabolic strain induced by hypoxia could optimize neural drive by elevating motor unit recruitment thresholds and enhancing synchronization, thereby improving the efficiency of force production at the neural level (19). The significantly greater increase in knee extension 1RM observed in the RTH Group compared to the RT Group not only reflects potential increases in muscle cross-sectional area (18) but may also imply superior neural adaptations (19,20,21). This warrants further investigation using techniques such as surface electromyography or neurophysiological assessments.

The present study found that after 5 weeks of intervention, the RTH Group demonstrated significant increases in fat-free mass, total muscle mass, and leg muscle mass relative to baseline, whereas no such changes were observed in the RT Group. Concurrently, both Groups showed significant improvements in lower-body maximal strength (knee extension and flexion 1RM) from baseline, with the RTH Group exhibiting a significantly greater increase in knee extension strength compared to the RT Group. These findings suggest that low-load resistance training in a hypoxic environment can effectively promote concurrent gains in muscle mass and strength. Regarding body composition, although the RTH Group showed significant within-group improvements in several metrics, the between-group differences in the magnitude of change did not reach statistical significance. This may be partly attributed to the relatively small sample size (n = 10 per Group), which limited statistical power. Alternatively, it may suggest that the potentiating effect of hypoxia on muscle mass accrual requires a longer intervention period or more sensitive measurement techniques to become fully apparent.

Importantly, the increases in fat-free mass, total muscle mass, and leg muscle mass observed in only the RTH Group were positively associated with improvements in knee extension and flexion function. This finding carries meaningful physiological implications: increases in muscle mass, particularly in the lower limb musculature directly involved in knee extension strength, provide the structural foundation for strength gains. Similar findings were reported by Manimmanakorn et al. (18) in female netball athletes, who demonstrated significant increases in muscle strength following 5 weeks of low-load hypoxic training, with effects superior to the normoxic Group . This aligns with the trend observed for lower-limb muscle mass in our study. However, other research (2,9) has noted that the impact of hypoxia on muscle hypertrophy may be modulated by various factors, including pre-training status, hypoxia exposure modality (intermittent vs. continuous), and training program design.

Another significant finding of this study is that 5 weeks of hypoxic LL-RT did not induce significant alterations in key hematological variables, including red blood cell count, hemoglobin concentration, or white blood cell count. This indicates that the intensity and duration of the hypoxic stimulus in this protocol were insufficient to trigger the classic "altitude-induced polycythemia" adaptation, and no marked signs of inflammatory or immune stress were observed. This result carries positive practical implications that suggests performing LL-RT for 5 weeks, 3 times per week, in hypoxia simulating approximately 3,420 m altitude is a safe intervention for healthy athletes that does not perturb basal hematological homeostasis. This finding is consistent with Roels et al. (26), who reported that 7 weeks of intermittent hypoxic interval training (approximately 114 min per week) did not elicit significant improvements in performance beyond normoxic training, nor did it induce any hematological modifications in well-trained cyclists and triathletes. However, other research has indicated that more prolonged or intense hypoxic exposure may elicit adaptive changes in the hematopoietic system. For instance, Fernández-Lázaro et al. (7) demonstrated that 8 weeks of living and training at 1,065 m combined with intermittent hypoxic exposure ($FiO_2$ = 10-13%, 90 min/day) significantly increased hemoglobin concentration, red blood cell count, and erythropoietin levels in elite athletes. Similarly, Kasperska et al. (12) reported that 10 days of intermittent hypoxic exposure (1 hour/day) combined with wrestling training elevated erythropoietin levels and reticulocyte counts. Moreover, Wonnabussapawich et al. (33) found that after 8 weeks of living and training at 825 m supplemented with intermittent hypoxic training at 3,000 m, the altitude training Group exhibited significantly higher red blood cell counts and hemoglobin concentrations compared to the sea-level Group at day 14 post-intervention.

The findings of this study indicate that a 5-week LL-RT protocol combined with hypoxic exposure ($FiO_2$ = 13.6%) is sufficient to reach the threshold for gains in muscle strength and mass, but insufficient to induce hematological changes, which appear to require longer hypoxic exposure. This dissociation between muscular and hematological adaptations highlights the distinct time courses and underlying mechanisms of these two physiological systems. The future research studies should explore the dose-response relationship of different hypoxic regimens (i.e., concentration, duration, and frequency) on hematological outcomes.

**Limitations in this Study**

Several limitations of this study must be acknowledged. First, the sample size was relatively small. Second, the study did not directly measure intramuscular metabolic byproducts (such as blood lactate and/or intramuscular pH) or molecular markers (e.g., mTOR phosphorylation levels), therefore, the evidence for the "augmented metabolic stress" mechanism remains indirect.

**Practical Implications and Future Research Directions**

This study demonstrates that 5 weeks of hypoxic LL-RT effectively enhances muscle strength and lean mass without altering hematological markers. For athletes during competitive seasons or rehabilitation, this strategy offers a safe alternative when high-load training is not feasible. Coaches may consider incorporating portable hypoxic devices into training programs to optimize neuromuscular adaptations while minimizing mechanical stress. Future research should investigate longer intervention periods (≥8 weeks) to determine the threshold for hematological adaptations and explore dose-response relationships across different hypoxic concentrations and training protocols.

## CONCLUSIONS

Five weeks of low-load resistance training in a hypoxic environment ($FiO_2$ = 13.6%) enhances lower-body strength and promotes lean mass gains more effectively than equivalent normoxic training in the athletes, without disturbing hematological homeostasis. These findings support hypoxic LL-RT as a viable alternative when high-load training is impractical, offering a safe and effective strategy for optimizing muscular adaptations during periods of load management.

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# Effects of Khon Kaen Qigong on Metabolic Flexibility in Older Adults with Mild Cognitive Impairment: A Randomized Control Trial

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## ABSTRACT

**Hao Gao, Narisara Premsri, Guang Yang, Orathai Tunkamnerdthai, Apiwan Manimanakorn, Terdthai Tong-Un, Rujira Nonsa-ard, Naruemon Leelayuwat.** This study explored the acute and training effects of Khon Kaen Qigong (KKQ), which combines Qigong (Baduanjin and Wuqinxi) and Muay Thai (Wai-Khru session) on metabolic flexibility via expired-air variables in older adults with mild cognitive impairment. Correlation of all responses with cognition was also investigated. This is pre-test and post-test parallel study that consisted of 2 Groups: Exercise Group and the Control Group. Both Groups randomly performed 2 assessments before (acute effect) and after (training effect) 12 weeks. At each assessment the subjects performed a single session of 30-minute KKQ (Exercise Group) or reading (Control Group). On both days, expired air was collected before, during, and after KKQ/reading to measure rates of oxygen uptake and carbon dioxide production. Both were used to calculate carbohydrate and fat oxidation rates, respiratory exchange ratio, and energy expenditure. The training consisted of 60-minute KKQ/day, 3 days/week, while those in the Control Group continued to live a sedentary lifestyle. Almost all absolute metabolic responses from expired-air values acutely increased during KKQ compared with reading and returned to baseline at the end of recovery (all $P < 0.01$). Compared to the Control, training increased the fat-burning proportion and energy expenditure while compromised the carbohydrate utilization proportion. All metabolic responses via the expired-air variables after the training were correlated to cognition. Together with the lack of injury and high adherence, KKQ training could be recommended as an alternative intervention for improving respiratory function, metabolism, and cognition for older adults with cognitive function impairments.

**Key Words:** Aging, Cognitive Function, Exercise, Metabolic Rate, Substrate Utilization

## INTRODUCTION

This study is composed of many experiments exploring the acute and chronic effects of Khon Kaen Qigong (KKQ), which combined Qigong (Baduanjin and Wuqinxi) with Muay Thai (Wai Khru session) on different body systems that included metabolic, cardiorespiratory, neurological, and musculoskeletal responses in sedentary older adults with mild cognitive impairment (MCI). Baduanjin, as one of the most common forms of Chinese Qigong, is an aerobic exercise combined with breathing and deep relaxation (8). Wuqinxi, another style of Qigong exercise, is based on the principles of yin and yang, incorporating movements (2). The Wai Khru session provided warming up purpose for Muay Thai that is Thai martial art. Two recent articles in our research group found that KKQ training improved cognitive function, neck and thoracic abnormalities (29), and inflammation (30) in older adults with MCI. This study emphasized metabolic flexibility in fuel selection responses to KKQ in these adults.

The proportion of elderly individuals in the global population is projected to increase from 12% in 2015 to 22% by 2050 (27). Aging is associated with metabolic inflexibility that causes metabolic diseases related to MCI (3,4,25), which is a state between normal aging and dementia (11). Accordingly, the prevalence of age-related metabolic disorders and MCI increased around the world that is a major health problem. In this study, metabolic flexibility was determined by whole body fat and carbohydrate oxidation rates calculated by expired gas. In fact, aging with (18,19) or without (9) metabolic inflexibility had decreased fat oxidation leading to impaired lipid and energy metabolism. Additionally, the elevated carbohydrate reliance, as shown by the increase in respiratory exchange ratio (RER) values is also observed in aging individuals (5).

Markers of respiratory function, such as expired-air oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) were used to calculate substrate oxidation rate, energy expenditure, and RER (7,10). These markers are related to cognition, for example, the impaired cerebral blood flow contributes to cognitive decline through hypoperfusion and reduced oxygen consumption in brain tissues (31). Hence, intervention that alters these markers could influence not only metabolic flexibility, but also respiratory and cognitive functions.

Recently, our research group found potential benefits of KKQ training on improved cognition in participants with MCI (29). Interestingly, we also found increasing oxygen consumption and ventilation in older adults with normal cognition (31), which implies improving brain blood flow. This may be the case with MCI. However, no previous study has explored this specific phenomenon. Furthermore, our previous publication demonstrated that KKQ is a very-low-intensity exercise (32). This exercise intensity yielded the greatest fat oxidation rate compared with other exercise intensities in a sedentary young Thai population (6). As such, KKQ training may also contribute to enhanced fat utilization and energy consumption in participants with MCI. Exploring the effects of KKQ gives the growing remedy of MCI among aging populations. However, no previous evidence of KKQ on metabolic flexibility from expired-air markers has been reported in this population.

This study mainly investigated the acute and training effects of KKQ on fat oxidation rate in older individuals with MCI. The subsequent aims were to explore both effects on carbohydrate oxidation rate, RER, energy expenditure, and expired air variables. Additionally, a correlation between all metabolic variables and cognitive function was determined. We hypothesized that both effects of KKQ would have beneficial changes on fat utilization and other variables in the participants. Furthermore, we expected the training-improved metabolic flexibility would be linked to their improved cognitive function.

## METHODS

### Research Design

This research is a randomized controlled, pre-test and post-test parallel study. Randomization was performed by a computer-generated random allocation sequence using Research Randomizer. The CONSORT reporting guidelines were followed to report on parallel group randomized trials (15).

### Subjects

From June to December 2021, the participants were recruited from Khon Kaen province. Their age range was 60 to 75 years. The participants were screened by physical examination, health questionnaires (Supplement 2), and Physical Activity Readiness Questions (PAR-Q) (25) to assess readiness to exercise. The participants were included if they: (a) had a Montreal Cognitive Assessment (MoCA) score less than 26 (20); (b) were living a healthy sedentary lifestyle with no underlying diseases that might affect the exercise activity; (c) had a mild abnormal blood pressure, blood glucose, and lipid profile maintained by their medications; and (d) had no long-term (>2 years) experience with regular practice of exercise, meditation, yoga, Muay Thai, or any type of exercise. Ethical approval was obtained from the Ethics Committee of Khon Kaen University following the Declaration of Helsinki (HE641163). The study was registered under TCTR NO:20211228001 on 27 December 2021. All participants received oral and written explanations about the study before signing the consent form. The recruitment methods included advertisements on Khon Kaen University platforms, Facebook.com, and through words of mouth within the university and local community. Details of the information to include when reporting a randomized trial are shown in Supplement 1, Table 1. CONSORT flowchart containing details of the enrollment and progress through the RCT was shown in our previous study (29). All the experiments were performed at the Nutrition and Exercise Laboratory, Faculty of Medicine, Khon Kaen University, Thailand.

The sample size was calculated based on the improvement in fat oxidation rate by using G*Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), which is consistent with a study by Tunkamnerdthai et al. (22). Power was set at 80%, alpha at 5%, and Cohen’s effect size at 0.50. The sample size for each Group was 25 participants, including a 20% dropout rate.

This study was a simple, random allocation sequence of 1:1 using the Research Randomizer. The participants were assigned either to the Control Group (CG) or the Exercise Group (KKG) using their unsorted identification codes by a researcher at the Exercise and Sport Sciences Development and Research Group. The codes were in the same color and size in opaque envelopes. All adjudicators, data collectors, data analysts, and participants were blinded to the experiment codes until the study was completed.

## Procedures

After passing the screening, the participants were randomly divided into 2 Groups of 25 each: (a) the KKG; and (b) the CG (Figure 1). All assessments were performed in the morning at 8:00 a.m. after 8 hours of fasting without alcohol consumption or smoking at the Nutrition and Exercise Laboratory, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. At the first assessment, all the participants received daily dietary (Supplement 1, Table 2) and physical activity (Supplement 1, Table 3) record for 3 days (2 weekdays and 1 weekend day) and returned to the researcher after the first and the last assessment. The room temperature and humidity were kept comfortable and quiet throughout the experiment.

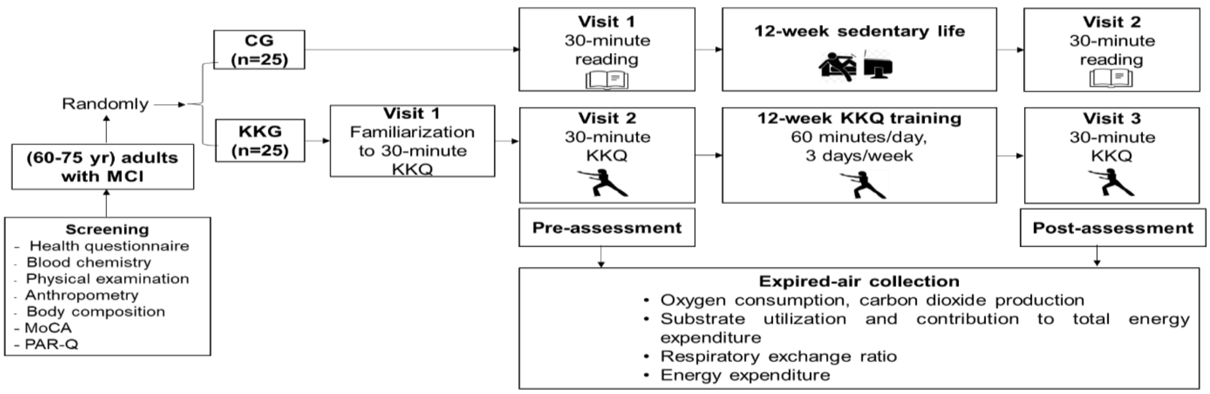


**Figure 1. Protocol of the Study.** MoCA = Montreal Cognitive Assessment, PAR-Q = Physical Activity Readiness Questionnaire, MCI = Mild Cognitive Impairment, CG = Control Group, KKG = Khon Kaen Qigong Group, KKQ = Khon Kaen Qigong.

### *KKG*

The participants visited the laboratory 3 times (Figure 1). On their first visit, the participants performed a 30-minute KKQ (2 bouts of 12 movements, which are described in a previous article (29) to familiarize themselves with the exercise. On the second (pre-assessment) and the third visits (post-assessment), they arrived at the laboratory and rested for 15 minutes. Then their blood pressure, heart rate, oxygen saturation, and body temperature (Automatic sphygmomanometer (UA-767 Plus, OX, UK) were recorded. Next, they performed a 5-minute warm-up by stretching the upper and lower limbs and trunk, followed by a 30-minute KKQ and a 30-minute recovery. Throughout both assessments, expired gas was collected to measure $VO_2$ (L/min) and $VCO_2$ (L/min) using a gas analyzer. The exercise training was performed in group in the participants' community hall. Twelve weeks between the first visit and the second visits, they trained by practicing 60-minute KKQ/day, 3 days/week. After completion, they performed the last assessment, which was the same as the second.

### *Control and Safety Monitoring of KKQ*

All the participants wore comfortable flat shoes and sportswear. For 12 weeks, all the participants received records to monitor their exercise training and injuries (29). They returned the records at the last assessment. They were told that they could stop their participation at any time. During the exercise assessment and training, a certified KKQ leader guided and corrected their movements. In addition, they were phoned by a researcher every week to remind them to attend the practice and maintain their dietary intake, and not to do any other exercises except KKQ. The participants were asked to concentrate on every movement with pursed lip breathing during the KKQ practice.

**Control Group**

The subjects in the CG participated in both assessments like those of the KKG, except that they read Buddhist books instead of undergoing KKQ at the first assessment and maintained a sedentary lifestyle during the 12 weeks (Figure 1).

**Outcome Measurements**

All the anthropometry and body composition measurements and the physiological characteristics were shown in our previous study (29).

***Expired-Air Collection***

The gas analyzer (Oxycon Mobile V-707270 SBx, Hoechberg, Germany) was calibrated before the data collection using a three-liter syringe. The calibration used surrounding air (20.93%$VO_2$ and 16.00%$VCO_2$) and calibrated gas (5.0% $O_2$ and 0.04% $CO_2$). After arriving at the laboratory, the participants sat quietly for 15 minutes before wearing the testing equipment, including a respiratory mask. Then, they laid down while remaining awake throughout the process until they reached a stable resting state. Then, expired air collection was done throughout 15 minutes before, during 30-minute KKQ/reading, and during the 30-minute recovery. The $VO_2$ (L/min) and $VCO_2$ (L/min) measurements from the gas analyzer were used to determine rates of fat and carbohydrate oxidation (11), RER (11), and energy expenditure (7).

**Statistical Analyses**

All the data were tested by using SPSS (version 21.0; SPSS Inc., Chicago, IL, USA). Distribution was analyzed by the Kolmogorov-Smirnov Test. The normal distribution data were expressed as mean ± SD and analyzed using repeated measures ANOVA, with *Post Hoc* Bonferroni Test for sub-groups differences. The data which were not normally distributed were presented as median (IQR) and compared for within-group differences using the Wilcoxon Signed Rank Test and for between-group differences using Mann Whitney U Test was used. The intervention analysis was based on a modified intention-to-treat principle. The significance level was less than 0.05.

**RESULTS**

The participants in the KKG (n = 22, 21 females and 1 male) and the CG (n = 23, 22 females and 1 male) completed the experiment and were analyzed for the study outcomes (29). No participant changed their nutritional and physical activity habits (30). The compliance with the exercise training was 93%. There were no injuries throughout the experiments in the Exercise Group.

**Characteristics of the Participants**

The baseline characteristics of the 2 Groups of participants were similar but there was a significantly lower MoCA score in the CG than the KKG ($P < 0.05$) (29). In the CG, 14 people were overweight, and 1 was obese, all of them were women. In the KKG, 16 people were overweight (15 females, one male) and one was obese (one female). Overweight/obesity participants had a BMI between 23 and 29.9 kg/$m^2$ (based on the Asia-Pacific criteria) (World Health Organization. Regional Office for the Western Pacific, 2000) (28). The rest of the indicators, including heart rate, blood pressure, and oxygen saturation were within the normal standard range. However, there were seven and two participants with diabetes mellitus type 2 in CG and KKG, respectively.

Whereas there were nine and eight participants with hypertension in CG and KKG, respectively. All of them had medications but could maintain them throughout the experiments. No injuries during and after the exercise throughout all experiments.

**Main Outcomes**

***Fat Oxidation Rate***

*Acute Effect (Pre-assessment)*

At pre-assessment, KKQ increased fat oxidation rates over baseline until 10 minutes into KKQ with greater values than CG and returned to baseline during recovery (all P < 0.01) (Figure 3A）. In KKG, fat oxidation rates during recovery were lower than during KKQ. There were no significant differences over time in all fat oxidation rates of CG (Figure 3A).

*Training Effect (Post-Pre-assessment)*

Absolute Data

After the 12-week training, KKG shows more changes in all fat oxidation rates. All rates significantly increased over baseline almost throughout KKQ and returned to baseline during recovery (all P < 0.01). These oxidation rates were lower during recovery than during KKQ (all P < 0.01). No significant differences over time in all fat oxidation rates of CG (Figure 3A).

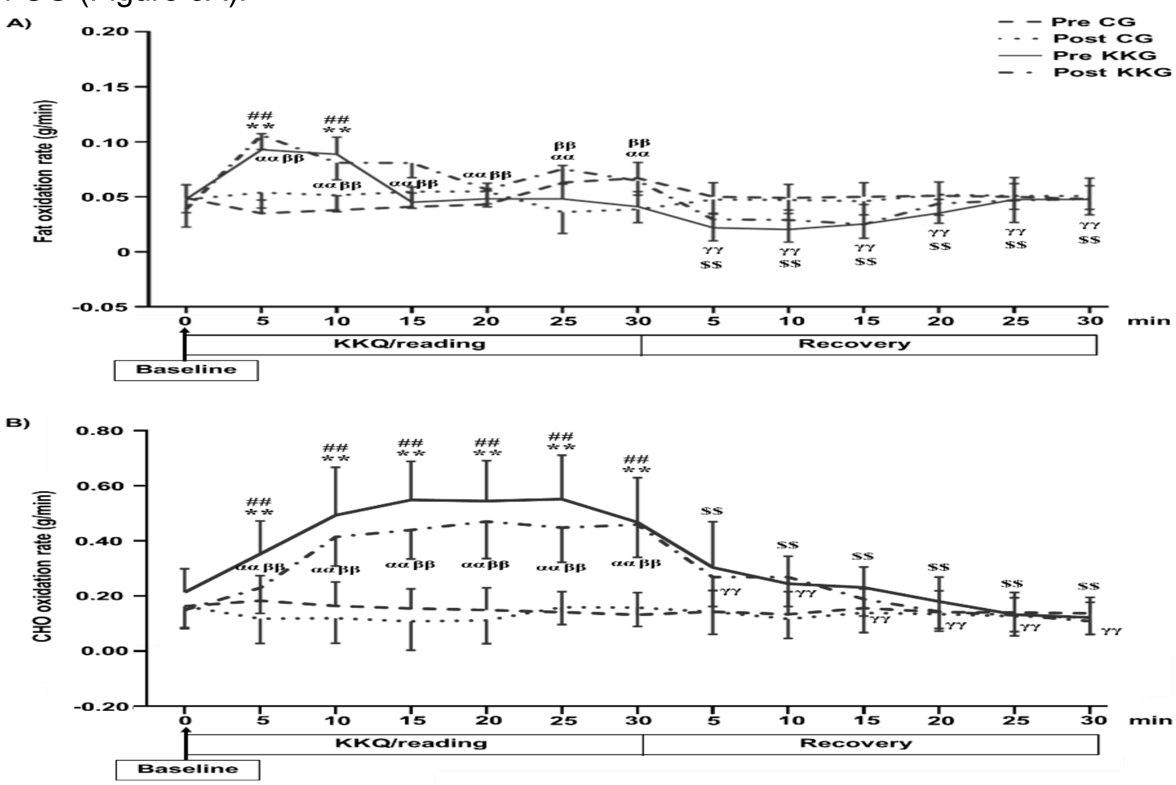


**Figure 3. Absolute Data of Fat (g/min) (A) and CHO Oxidation Rates (g/min) (B) at Baseline, during KKQ/Reading and Recovery in KKG (n = 22, 21 females and 1 male) and CG (n = 23, 22 females and 1 male).** All data are expressed as mean ± SD. **difference from baseline within the Group at pre-assessment (P < 0.01), $^{\$\$}$difference from during KKQ in KKG at pre-assessment (P < 0.01), $^{\#\#}$difference from CG at the same time point and at pre-assessment (P < 0.01), $^{\alpha\alpha}$difference from baseline within group at post-assessment (P < 0.05, 0.01), $^{\gamma\gamma}$difference from during KKQ and at post-assessment (P < 0.01), $^{\beta\beta}$difference from CG at the same time point and at post-assessment (P < 0.01). CG = Control Group, KKG = Khon Kaen Qigong group, KKQ = Khon Kaen Qigong.

Changes Data
KKG had significant increases in changes of fat oxidation rate compared to the baseline compared with pre-training. The changes were higher than those in CG in all fat oxidation rates during the last five minutes during KKQ. No significant differences in other time points in changes of fat oxidation rates of KKG and all time points of CG (Figure 4A）.

**Secondary Outcomes**
***Carbohydrate Oxidation Rate***
*Acute Effect (Pre-assessment)*

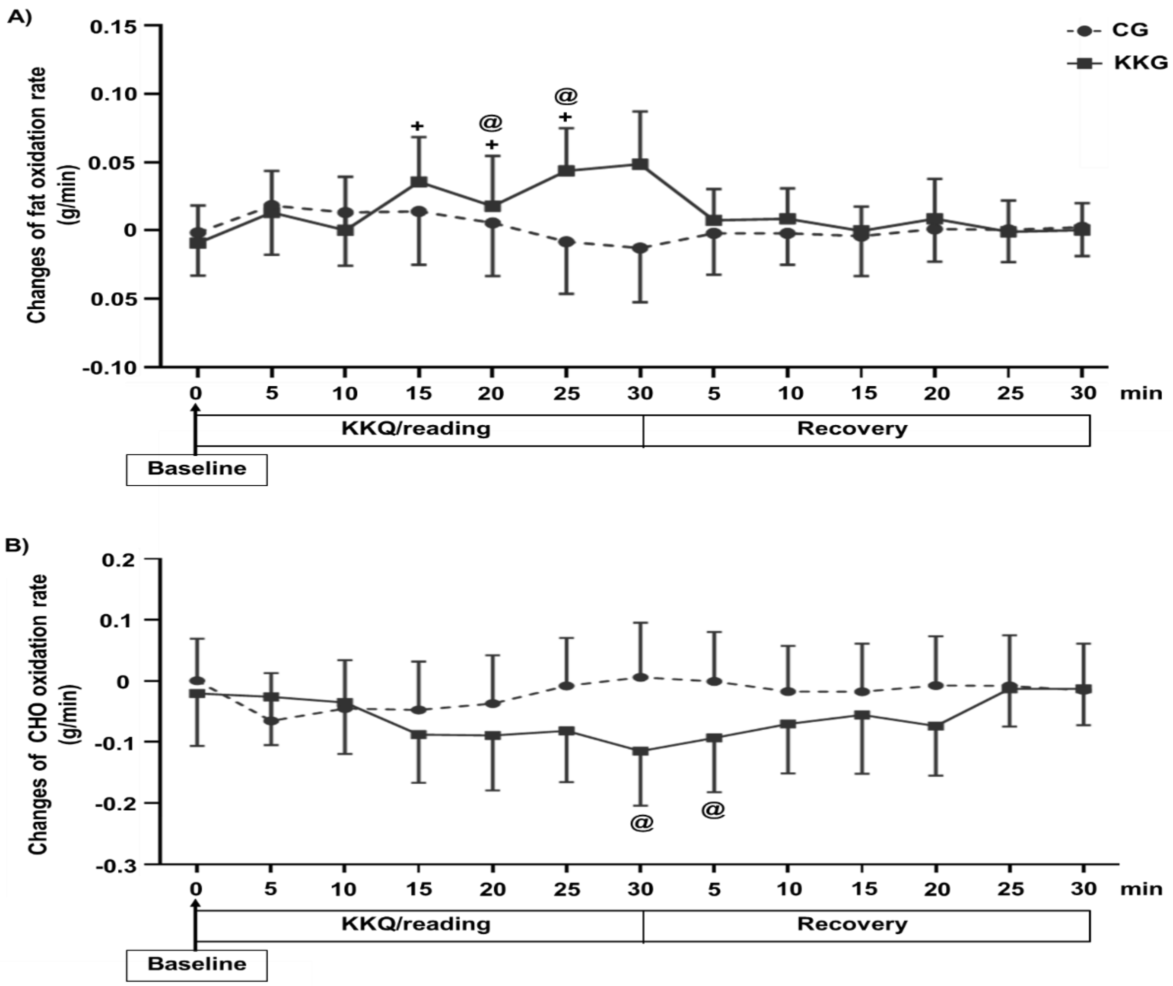


**Figure 4. Changes (post-pre-assessment) of Fat (g/min) (A) and Carbohydrate Oxidation Rates (g/min) (B) at Baseline, during KKQ/reading, and Recovery in KKG (n = 22, 21 females and 1 male) and CG (n = 23, 22 females and 1 male).** All data are expressed as mean ± SD. Repeated measured ANOVA is used to reveal significant differences within and between groups. Bonferroni is a *Post Hoc* Test. $^{+}$difference from the change at the baseline in KKG (P < 0.05), $^{@}$difference from the change in CG at the same time point (P < 0.05). CG = Control Group, KKG = Khon Kaen Qigong group, KKQ = Khon Kaen Qigong.

At pre-training, KKG increased all rates of carbohydrate oxidation over baseline with greater values than CG throughout KKQ and returned to baseline during recovery (all P < 0.01, Figure 3B). KKG had lower rates of carbohydrate during recovery than during KKQ. There were no significant differences over time in all carbohydrate oxidation rates of CG (all P < 0.01, Figure 3B).

*Training Effect (Post-Pre-assessment)*

Absolute Data

After 12 weeks of training, KKG had the same changes in carbohydrate oxidation rates over baseline, with higher values than CG throughout KKQ and returned to baseline during recovery (all P < 0.01, Figure 3B). In KKG, all rates of carbohydrate oxidation during recovery were lower than during KKQ. There were no significant differences over time in all rates of carbohydrate oxidation of CG (all P < 0.01, Figure 3B.

Changes Data

KKG had significant decreases compared to the baseline and was lower than CG during the end of KKQ and 5 minutes of recovery for carbohydrate oxidation rate, during the last 10 minutes (all P < 0.01, Figure 4B). There were no significant differences in other time points in changes in all rates of KKG and all time points of CG.

***Contribution to Total Energy Expenditure***

*Acute Effect (Pre-assessment)*

In KKG, the percentage of carbohydrate utilization increased over fat from 10 minutes of KKQ to 5 minutes and during the last 15 minutes of recovery (all P < 0.01, Figure 5). The percentage of fat oxidation rate did not change throughout the experiment.

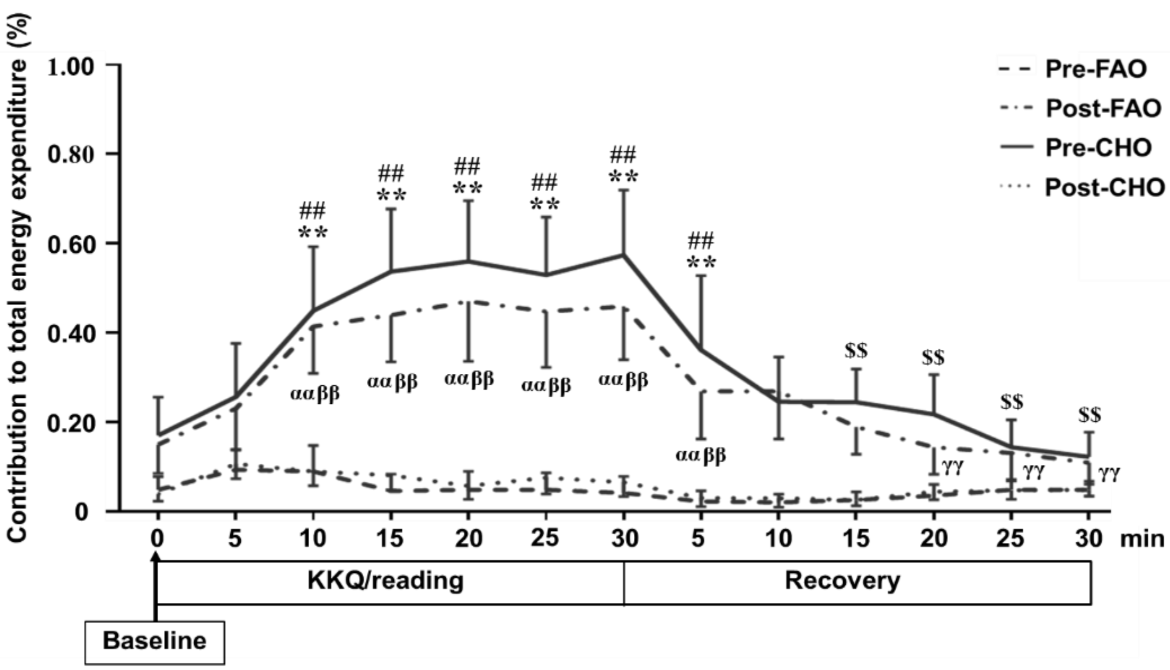


**Figure 5. Contribution to Total Energy Expenditure (%) at Baseline, during KKQ exercise and Recovery in KKG (n = 22, 21 females, one male).** All data are expressed as mean ± SD. **difference from baseline within group at pre-assessment (P < 0.01), $^{\$\$}$difference from during KKQ at pre-assessment (P < 0.01), $^{\#\#}$difference from fat at the same time point and at pre-assessment (P < 0.01), $^{\alpha\alpha}$difference from baseline within group at post-assessment (P < 0.01), $^{\gamma\gamma}$difference from during KKQ at post-assessment (P < 0.01), $^{\beta\beta}$difference from fat at the same time point and at post-assessment (P < 0.01). CG = Control Group; KKG, Khon Kaen Qigong Group, Pre-FAT = fat contribution at pre-assessment, Post-FAT = fat contribution at post-assessment, Pre-CHO, carbohydrate contribution at pre-assessment, Post-CHO = carbohydrate contribution at post-assessment.

*Training Effect (Post-Pre-assessment)*
Absolute Data
Like pre-training, in KKG after 12 weeks, the percentage of carbohydrate utilization increased over fat from 10 minutes of KKQ until 5 minutes and during the last 10 minutes of recovery (all P < 0.01, Figure 5). Fat contribution did not change throughout the experiment.

Changes Data
After 12 weeks of training, the change in fat contribution was positively greater than baseline at 15 minutes (P < 0.05). The change of fat contribution was greater than carbohydrate contribution from 15 minutes of KKQ to 5 minutes of recovery (all P < 0.05, Figure 6). Carbohydrate contribution did not change throughout the experiment.

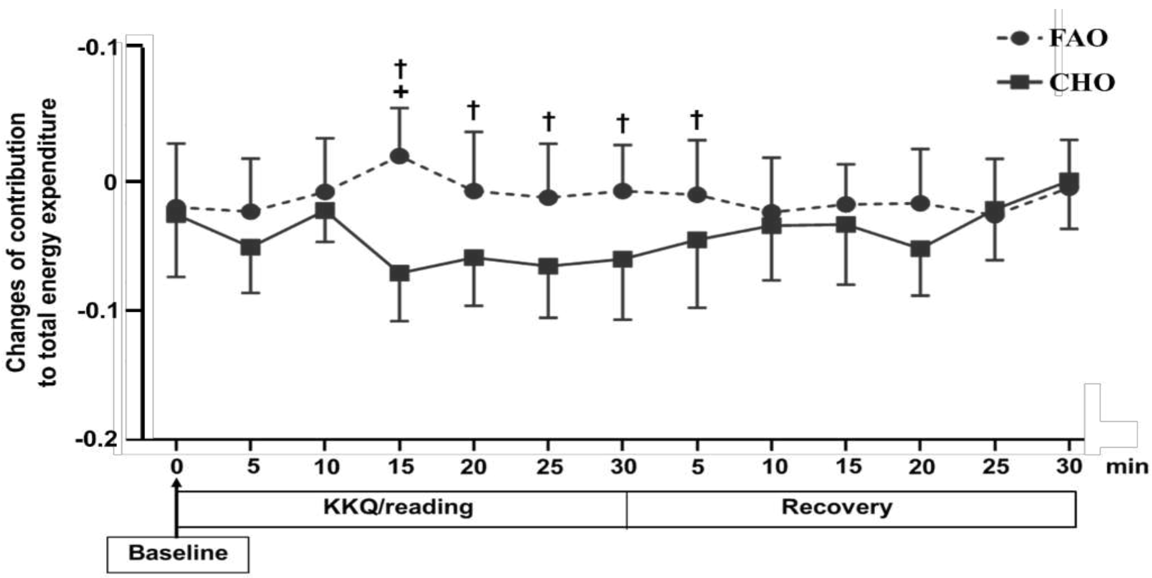


**Figure 6. Changes (Post-Pre-assessment) of Contribution to Total Energy Expenditure, at Baseline, during KKQ, and Recovery in KKG (n = 22, 21 females and 1 male).** All data are expressed as mean ± SD. Repeated measured ANOVA is used to reveal significant differences within and between groups. Bonferroni is *Post Hoc* Test. +difference from the change at the baseline (P < 0.05), †difference from the change in CHO at the same time point (P < 0.05). CG = Control Group, KKG = Khon Kaen Qigong group; KKQ, Khon Kaen Qigong, CHO = Carbohydrate.

## ***Respiratory Exchange Ratio***
*Acute Effect (Pre-assessment)*
The RER in the KKG increased compared to baseline and was higher than in the CG from 15 minutes of KKQ until 20 minutes of recovery and then returned to baseline afterward (all P < 0.01 (Figure 7A). Respiratory Exchange Ratio during the last 10 minutes of recovery was lower than during KKQ.

*Training Effect (Post-Pre-assessment)*
Absolute Data
After 12 weeks of training, the RER in the KKG increased compared to baseline and was higher than in the CG from five to 15 minutes of recovery (all P > 0.05, Figure 7A). There was no significant change in the CG at any time point before and after the

intervention. The RER during the last 10 minutes of recovery was lower than during KKQ.

Changes data

The decreases in the RER during 15 to 30 minutes of KKQ compared to those at baseline were greater than those in the CG. The CG showed no significant changes at any time point (all $P < 0.05$, Figure 8A).

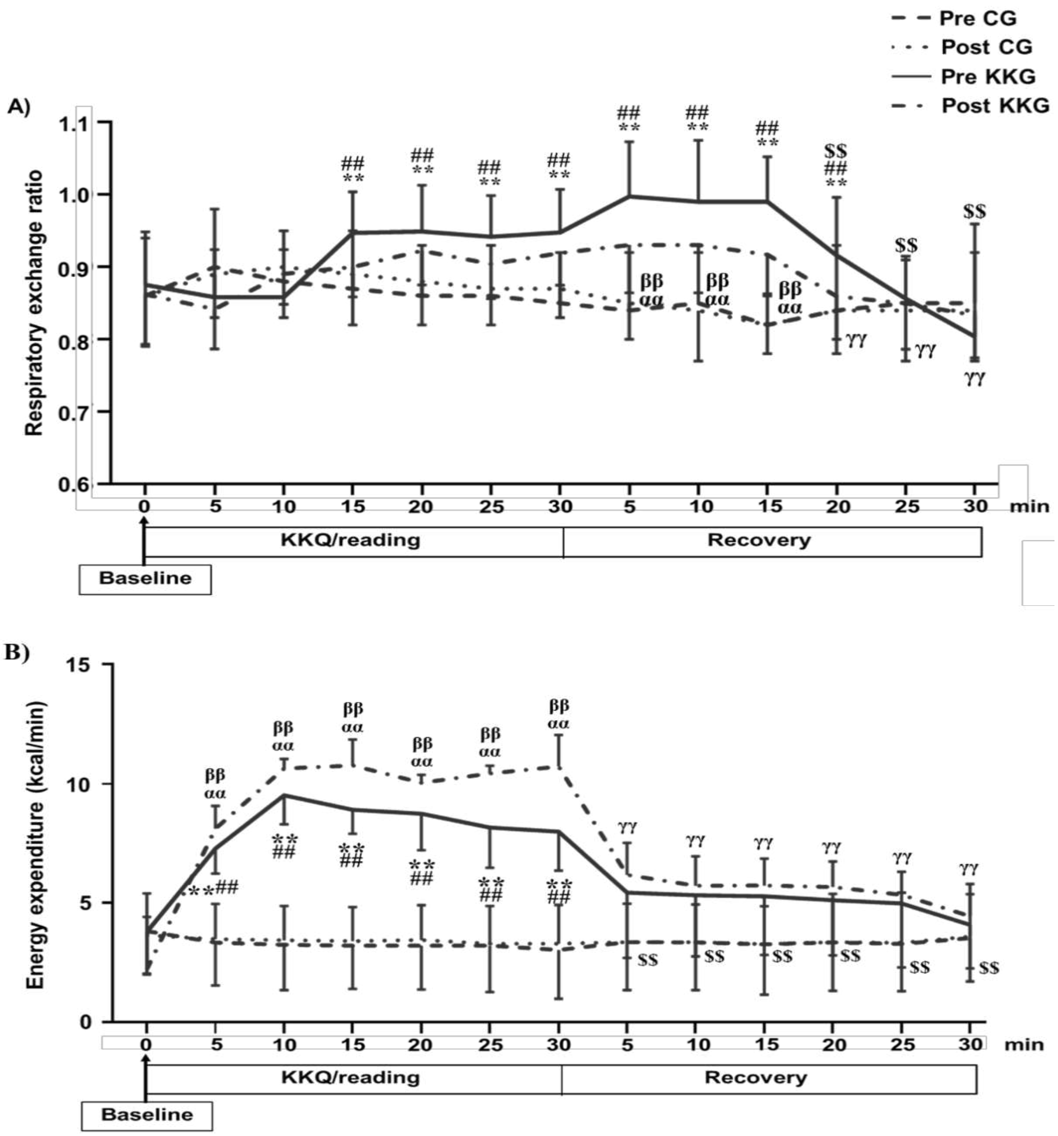


**Figure 7. Absolute Data of Respiratory Exchange Ratio (A) and Energy Expenditure (kcal/min) (B) at Baseline, during KKQ/reading, and Recovery of KKG (n = 22, 21 females and 1 male) and CG (n = 23, 22 females and 1 male).** All data are expressed as mean ± SD. $^{**}$difference from baseline within group at pre-assessment ($P < 0.01$), $^{\$\$}$difference from during KKQ at pre-assessment ($P < 0.01$), $^{\#\#}$difference from CG at the same time point and at pre-assessment ($P < 0.01$), $^{\alpha\alpha}$difference from baseline within group at post-assessment ($P < 0.01$), $^{\gamma\gamma}$difference from during KKQ at post-assessment ($P < 0.01$), $^{\beta\beta}$difference from CG at the same time point and at post-assessment ($P < 0.01$). CG = Control Group, KKG = Khon Kaen Qigong Group.

***Energy Expenditure***

*Acute effect (Pre-assessment)*

In the KKG group, energy expenditure continuously increased throughout the KKQ process and returned to baseline levels during the recovery period. During the KKQ, energy expenditure in the KKG was significantly higher than baseline (all P < 0.01) and significantly higher than those in the CG (all P < 0.01). Additionally, during the recovery period, energy expenditure at all time points were lower than during the KKQ period (all P < 0.01, Figure 7B).

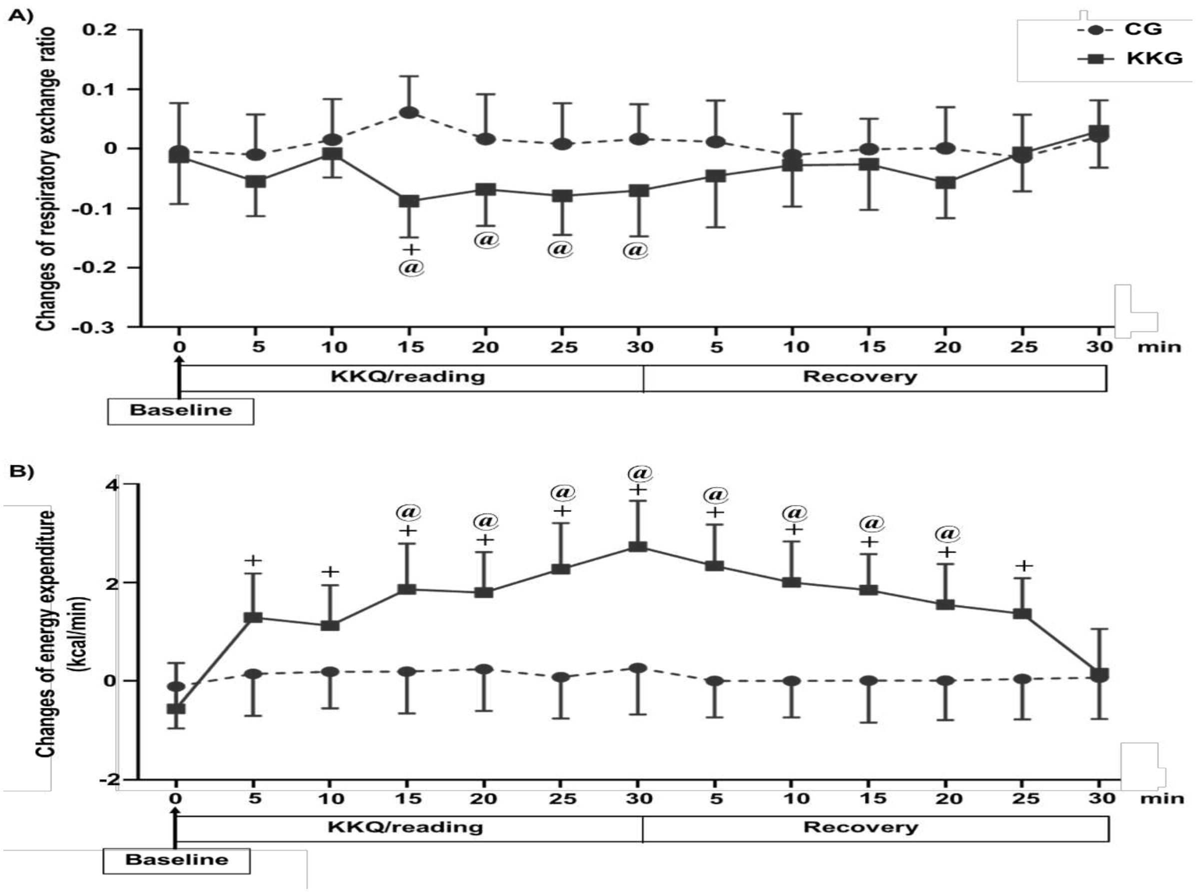


**Figure 8. Changes (Post-Pre-assessment) of Respiratory Exchange Ratio (A) and Energy Expenditure (kcal/min) (B) at Baseline, during KKQ/reading, and Recovery in KKG (n = 22, 21 females, one male) and CG (n = 23, 22 females, one male).** All data are expressed as mean ± SD. Repeated measured ANOVA is used to reveal significant differences within and between groups. Bonferroni is *Post Hoc* Test. +difference from the change at the baseline in KKG (p < 0.05), @difference from the change in CG at the same time point (P < 0.05). CG = Control Group, KKG= Khon Kaen Qigong Group, KKQ = Khon Kaen Qigong.

*Training Effect (Post-Pre-assessment)*

*Absolute Data*

After the intervention, the KKG group remained elevated during the recovery period, significantly higher than baseline (all P < 0.01) and significantly higher than the recovery values of the CG (all P < 0.01). The differences disappeared by the 30 minutes of the recovery period, approached baseline levels, and were lower than during the KKQ period (P < 0.01). The CG showed no significant changes at any time point before or after the intervention (Figure 7B）.

*Changes Data*

Compared to baseline, the change in the KKG showed a significant increase starting from the five minutes of KKQ and lasting until the 25 minutes of the recovery period ($P < 0.05$). Compared to the CG, it was significantly higher from the 15 minutes of KKQ until the 25 minutes of the recovery period ($P < 0.05$, Figure 8B).

***Expired-air Variables***

*Acute Effect (Pre-assessment)*

$VO_2$ (Figure 9A) and $VCO_2$ (Figure 9B) in the KKG increased over baseline and reading throughout the KKQ and returned to baseline levels during recovery. At all-time points during recovery in KKG, both values were significantly lower than during KKQ (all $P < 0.01$).

*Training Effect (Post-Pre-assessment)*

Absolute Data

Like pre-training, after 12 weeks of intervention, $VO_2$ (all $P < 0.01$, Figure 9A) and $VCO_2$ (all $P < 0.01$, Figure 9B) in the KKG increased over baseline and reading throughout the KKQ and returned to baseline levels during recovery. At all-time points during recovery in KKG, both values were significantly lower than during KKQ (all $P < 0.01$, Figure 9A and 9B).

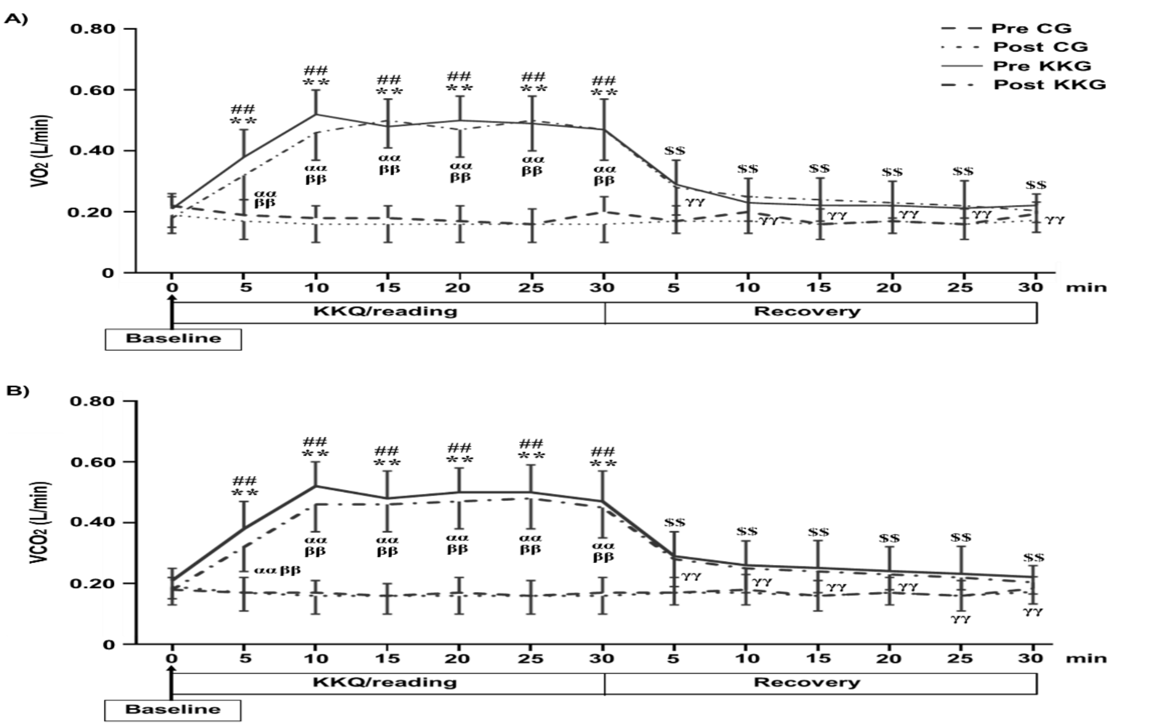


**Figure 9. Absolute Data of $VO_2$ (L/min) (A) and $VCO_2$ (L/min) (B) at Baseline, during KKQ/reading, and Recovery in KKG (n = 22, 21 females and 1 male) and CG (n = 23, 22 females and 1 male).** All data are expressed as mean ± SD. **difference from baseline within group at pre-assessment ($P < 0.01$), $^{\$\$}$difference from during KKQ in KKG at pre-assessment ($P < 0.01$), $^{\#\#}$difference from CG at the same time point and at pre-assessment ($P < 0.01$), $^{\alpha\alpha}$difference from baseline within group at post-assessment ($P < 0.01$), $^{\gamma\gamma}$difference from during KKQ in KKG and at post-assessment ($P < 0.01$), $^{\beta\beta}$difference from CG at the same time point and at post-assessment ($P < 0.01$). CG = Control Group, KKG = Khon Kaen Qigong Group, $VO_2$ = oxygen consumption rate, $VCO_2$ = carbon dioxide production rate.

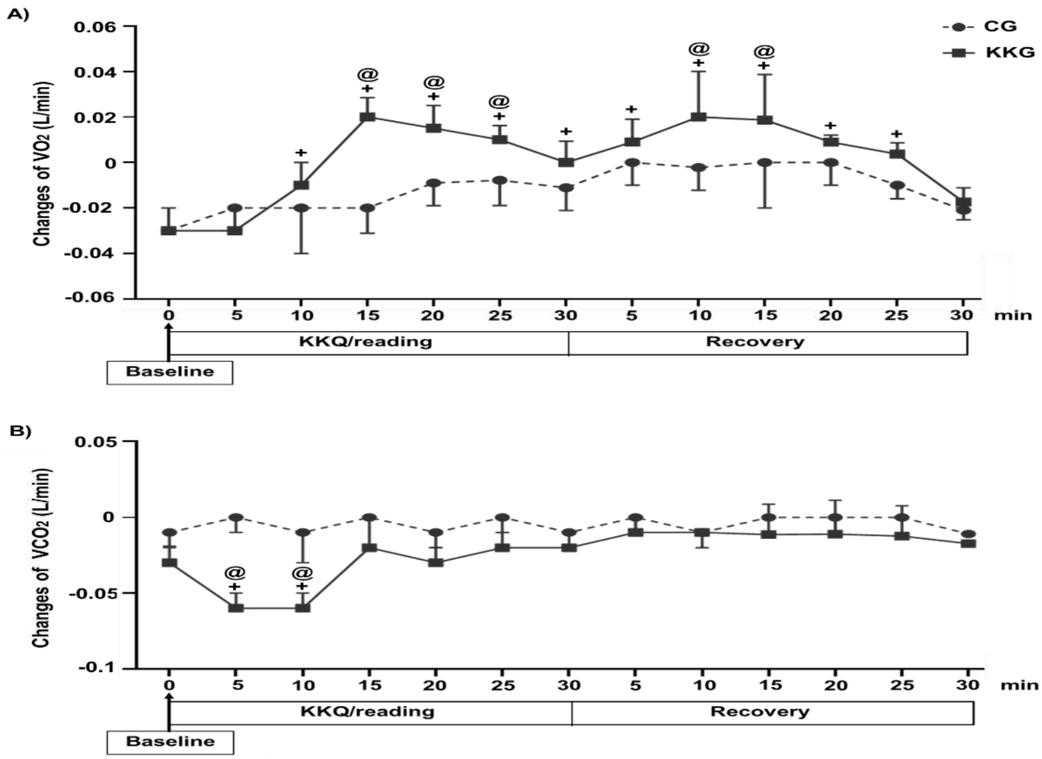


**Figure 10. Changes (Post-Pre-assessment) of $VO_2$ (A) and $VCO_2$ (B) at Baseline, during KKQ/reading, and Recovery in KKG (n = 22, 21 females and 1 male) and CG (n = 23, 22 females and 1 male).** All data are expressed as mean ± SD. Repeated measured ANOVA is used to reveal significant differences within and between groups. Bonferroni is Post hoc test. [+]difference from the change at the baseline in KKG (P < 0.05), [@]difference from the change in CG at the same time point (P < 0.05). CG= Control Group, KKG= Khon Kaen Qigong Group, KKQ = Khon Kaen Qigong, $VO_2$ = oxygen consumption rate, $VCO_2$ = carbon dioxide production rate.

*Changes Data*

Compared to changes at baseline, those of $VO_2$ in KKG were greater from five minutes of KKQ/reading to 25 minutes of recovery. Furthermore, changes in $VO_2$ in KKG were greater than in CG for 15-25 minutes and 10-15 minutes of recovery (all are P < 0.05, Figure 10A). Unlike $VO_2$, $VCO_2$ had fewer significant changes compared to those at baseline and CG. It decreased and was lower than in CG during 5-10 minutes of KKQ (all P < 0.05, Figure 10B).

***Resting Metabolic Rate***

There were no significant differences between time and group difference in resting metabolic rate (kcal/day) (Table 1).

***Correlation between MoCA Scores and Metabolic Variables***

At pre-assessment, neither group showed a correlation between MoCA scores and metabolic variables including fat and carbohydrate oxidation rates, contribution to total energy expenditure, RER, energy expenditure, $VO_2$, and $VCO_2$ (Table 2). At post-assessment, KKG showed significant correlation between MoCA scores and all metabolic variables (P<0.01). No significant correlation were found in the CG.

**Table 1. Resting Metabolic Rate (kcal/day) at Pre- and Post-assessment in Both Groups.**

| | CG | | | KKG | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | P-Value[a] | Pre | Post | P-Value[b] | MD [95% CI] | P-Value[c] | P-Value[d] |
| **Resting Metabolic Rate (kcal/day)** | 1155.3±182.3 | 1188.6±249.7 | 0.521 | 1238.8 (1092.8-1399.6) | 1285.2±233.5 | 0.254 | 13.16 [-697.77, 724.09] | 0.224 | 0.541 |

**The data are expressed as mean ± SD, median (interquartile range), mean difference [95% CI], and P value of CG (n = 23, 22 females and 1 male) and KKG (n = 22, 21 females and 1 male).** The intervention analysis was based on a modified intention-to-treat principle. The normal distribution data were expressed as mean ± SE and analyzed using repeated measures ANOVA, with *Post Hoc* Bonferroni Test for sub-groups differences. The data which were not normally distributed were presented as median (IQR) and compared for within-group differences using the Wilcoxon Signed Rank Test and for between-group differences using Mann Whitney U Test. P-value[a], duration effects (within CG), P-value[b], duration effects (within KKG), P-value[c], between groups at baseline, P-value[d], between groups after intervention. CG = Control Group. KKG = Khon Kaen Qigong Group, CI = confidence intervals.

**Table 2. Correlation Between MoCA Scores and Metabolic Variables at Pre- and Post-assessment in Both Groups.**

| | Pre-assessment | | | | Post-assessment | | | |
|---|---|---|---|---|---|---|---|---|
| | CG | | KKG | | CG | | KKG | |
| | r | P-value | r | P- value | r | P-value | r | P- value |
| **Fat Oxidation Rate** (g/min) | 0.133 | 0.387 | 0.088 | 0.438 | 0.103 | 0.429 | 0.525 | **0.002** |
| **Carbohydrate Oxidation Rate** (g/min) | -0.110 | 0.451 | -0.069 | 0.439 | -0.110 | 0.401 | -0.441 | **0.005** |
| **Contribution of Fat to Total Energy Expenditure** (%) | 0.135 | 0.365 | 0.090 | 0.445 | 0.015 | 0.430 | 0.505 | **0.002** |
| **Contribution of Carbohydrate to Total Energy Expenditure** (%) | -0.140 | 0.415 | -0.095 | 0.460 | -0.092 | 0.450 | -0.478 | **0.002** |
| **Respiratory Exchange Ratio** | -0.160 | 0.300 | -0.105 | 0.415 | -0.132 | 0.330 | -0.472 | **0.001** |
| **Energy Expenditure** (kcal/min) | 0.135 | 0.326 | 0.121 | 0.372 | 0.125 | 0.326 | 0.521 | **0.0005** |
| **Oxygen Consumption Rate** (L/min) | 0.181 | 0.252 | 0.128 | 0.355 | 0.159 | 0.258 | 0.551 | **0.001** |
| **Carbon Dioxide Production Rate** (L/min) | -0.162 | 0.306 | -0.103 | 0.404 | -0.126 | 0.331 | -0.489 | **0.001** |

**Pearson correlation analysis (normally distributed data) and Spearman correlation analysis (non-normally distributed data) were used to assess correlation among indicators of CG (n = 23, 22 females and 1 male) and KKG (n = 22, 21 females and 1 male).** The data presents r and P value. CG = Control Group, KKG = Khon Kaen Qigong Group.

**DISCUSSION**

This is the first study to investigate the acute and training effects of KKQ on metabolic flexibility determined by whole body substrate oxidation rate via expired air variables in individuals with MCI. Correlation between these variables and cognition was also determined. We found that KKQ acutely increased carbohydrate and fat utilization by relying more on carbohydrate. Furthermore, KKQ increased RER, energy expenditure, and expired gas concentration during exercise. However, after the training, although carbohydrate was still the main energy source during the exercise, fat contribution was increased. Furthermore, the training increased oxygen consumption and energy expenditure during exercise and recovery. In addition, all changes in the metabolic flexibility according to the training were positively correlated to cognitive function.

Our main hypothesis, which is expected to find the beneficial effect of KKQ on fat utilization was supported. Although our results showed reliance on carbohydrate more than fat as fuel utilization during a single bout of exercise, fat utilization increased and carbohydrate utilization decreased during the exercise after the training. This is comparable with the inverse changes of RER during exercise regarding the training compared to the Control Group. This showed that the training lowered RER during the exercise, which means more fat was used (11). This is confirmed by the greater fat contribution to total energy expenditure than carbohydrate, which is shown by this study.

In summary, it reflects that the KKQ training increased fat burning during the exercise reflecting its potential in weight reduction. The greater reliance on carbohydrate than fat during KKQ in this study is consistent with our previous research in exercise with the same intensity in sedentary young untrained participants (6) and dyslipidemic women (22). In contrast, other studies found that during this exercise intensity, fat is equally utilized in dyslipidemic men (26) or more utilized in trained men (24) and obese participants (12). A discrepancy may be due to many factors influencing the energy source, such as fitness status, nutritional status, sex, and age (1,14,21).

In addition, the impact of both acute and training KKQ on expired air variables leading to changes in other metabolic responses was also as expected. That is, this study showed that KKQ acutely increased oxygen uptake and carbon dioxide production. This has been supported by a previous study that investigated a single bout of KKQ (32), and the increased oxygen uptake has been shown in Tai Chi Easy (13,17) in populations with normal cognition. This study also found increased energy expenditure, which is produced by the increased oxidation process. This phenomenon is consistent with a previous study presenting increases in whole body oxygenation and energy responses to Tai Chi Easy, which is a low-intensity exercise (17).

Furthermore, these metabolic results provide potential effects of KKQ training on improving respiratory response, fat oxidation, and energy cost in individuals with MCI. Hence, this study found that the metabolic responses to KKQ training were correlated with cognitive function. This is consistent with previous studies that reported metabolic diseases are related to MCI (4,23). Together with the lack of injury, KKQ is an alternative remedy for improving health for people with MCI and metabolic diseases.

The strength of this research could be the training in group activity within the participants' community. They performed the exercise with their friends and within their own environment. This made them familiar with the exercise, which led to a high adherence to the training. Additionally, the exercise leader was the same person throughout the training. In addition, the information regarding diet intake and energy expense, which are necessary for research in metabolic field were recorded. The records showed similar diet intake and energy use before the training (30). The results show no bias of both factors. Furthermore, all the exercise protocol and collection were very well controlled according to the CONSORT protocol (15).

**Limitations in this Study**

This study has several limitations to address when interpreting the findings. First, possible molecular mechanisms clarifying the beneficial effects of KKQ training were not performed, for example, glucose and lipid kinetics during the KKQ. Secondly, most of the participants were females who have different metabolism from males (16). Therefore, the results of this study cannot be applied to the male individuals. Lastly, the participants of KKG had a higher MoCA scores at pre-assessment than those of the CG. Although this may be a bias of baseline cognition for the participants, the results were analyzed by a standard statistical tool adjusting the baseline.

Future research should examine circulating markers of cognitive impairment, such as plasma Aβ42/40 ratio and plasma brain-derived neurotrophic factor. Regarding a sex bias on metabolism, there should be an exploration of the effects of KKQ on the metabolic responses in male older adults with mild cognitive impairment and other diseases, such as cardiopulmonary and metabolic diseases. Lastly, further studies might examine the impact of this novel combined exercise training on quality of life and other physiological markers of cardiovascular and neuromuscular parameters to understand their broader benefits.

## CONCLUSIONS

Acute KKQ had the potential to increase substrate utilization, energy expenditure, and respiratory variables of older adults with MCI. Although carbohydrate was the main energy source during KKQ, the exercise training can increase the fat-burning proportion, oxygenation, and energy expenditure. All metabolic responses to KKQ training were correlated with cognitive function. Together with the lack of injury and high adherence, KKQ training should be recommended as an alternative intervention for older individuals with respiratory, metabolic, and cognitive impairments.

## ACKNOWLEDGMENTS

We thank the Research Administration and Innovation Division of Khon Kaen University for the research grant. We thank Natnaree Chaipiphat for her research assistance. We also thank all Village Health Volunteers and all staff of the Public Health Units and the Ministry of Public Health for their cooperation in recruiting the participants. We appreciate all the participants for their willingness to participate in this study.

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## Supplementary Documents

**Supplement 1, Table 1. Consort Checklist of This Study.**
**Supplement 1, Table 2. Dietary Intake Record.**
**Supplement 1, Table 3. Physical Activity Record.**

**Supplement 2, Participant's Characteristics and Health Questionnaire Form.**

**Supplement 1, Table 1. Consort Checklist of This Study.**

## CONSORT 2010 Checklist of Information to Include When Reporting a Randomised Trial*

| Section/Topic | Item No | Checklist Item | Reported on Page No |
|---|---|---|---|
| **Title and Abstract** | | | |
| | 1a | Identification as a randomized trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 1 |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 2 |
| | 2b | Specific objectives or hypotheses | 3 |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 3 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | N/A |
| Participants | 4a | Eligibility criteria for participants | 3 |
| | 4b | Settings and locations where the data were collected | 3 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were administered | 4-5 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 5 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | N/A |
| Sample size | 7a | How sample size was determined | 4 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | 4 |
| Randomization: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 4 |
| | 8b | Type of randomization; details of any restriction (such as blocking and block size) | 4 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 4 |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 4 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how | 4 |
| | 11b | If relevant, description of the similarity of interventions | 5 |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 6 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | 6 |
| **Results** | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome | 4 |
| | 13b | For each group, losses and exclusions after randomization, together with reasons | 4 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 3 |

| Section/Topic | Item No | Checklist Item | Reported on Page No |
|---|---|---|---|
| | 14b | Why the trial ended or was stopped | Because of the completion of this research. |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 6-16 |
| Numbers analyzed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | 6-16 |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 6-16 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | N/A |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | 14 |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 6 |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 17 |
| Generalizability | 21 | Generalizability (external validity, applicability) of the trial findings | 17-18 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 17-18 |
| **Other Information** | | | |
| Registration | 23 | Registration number and name of trial registry | 3 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | 3 |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 18 |

Citation: Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials. BMC Medicine. 2010;(8):18.  *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist, see www.consort-statement.org.

**Supplement 1, Table 2. Dietary Intake Record.**

Dietary Intake Record

"Effects of Qigong combined with Muay Thai on metabolic responses via expired gas analysis in older adults with mild cognitive impairment: a randomized control trial"

Participants ID …………………………………………. Date ……………………………

| Time | Food | Amount |
|---|---|---|
| | | |
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**Supplement 1, Table 3. Physical Activity Record.**

Physical Activity Record

"Effects of Qigong combined with Muay Thai on metabolic responses via expired gas analysis in older adults with mild cognitive impairment: A randomized control trial"

Participants ID …………………………………………. Date …………………………….

| Physical Activity | Duration (min) |
|---|---|
| | |
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| | |

**Supplement 2, Subject's Characteristics and Health Questionnaire Form.**

"Effects of Qigong combined with Muay Thai on metabolic responses via expired gas analysis in older adults with mild cognitive impairment: A randomized control trial"

Please fill in your information. They will be kept secret and used only for this research.

Research ID code ...................

Date...........Month..........................Year............

1. Name-surname.........................................................................................................................
2. Sex ( ) male ( ) female
3. Career .........................................................................
4. Birth date……..... Month ………..…… Year .............
5. Age .....................Year ............................ Month ............................
6. Address ........................................................................................................................
7. Body weight ...................................... kg. Body height .......................... cm.
8. Marital status ( ) Single ( ) Married ( ) divorce Number of children ..................
9. Underlying diseases
   - ( ) Cardiovascular disease Please specify…………………………………………...
   - ( ) Neurological disease Please specify…………………………………………………...
   - ( ) Diabetes type 2
   - ( ) Musculoskeletal disease Please specify…………………………………………...
   - ( ) Respiratory disease Please specify ………………………………………………..
   - ( ) Surgery..........................................................................……..........Date............................
   - ( ) Others....................................................................................................................................
   - ( ) No
10. Medication (name, number, and frequency/day) ..............................................………..
11. Underlying diseases of your family
    - ( ) No ( ) Yes Please specify ……………………………………………..……….…………...
12. Smoking ( ) Don't smoke ( ) Smoke

    Please specify the number and frequency/day for how long ...................................………..
13. Drink ( ) Don't drink ( )

    Please specify the quantity and frequency/day for which it will last .....................................

15. Drug addict ( ) Don't take ( ) Take

Please specify the quantity and frequency/day for which it will last .....................................…………...

16. History of exercise

16.1. In the past 4 months, how often per week did you regularly exercise..........…………...

16.2. What type of exercise? …………………………………………………..………..…….

Please define whether you are a sports player ………………………..………….......

16.3. What intensity of the exercise

( ) low (can speak and sing) ( ) moderate (cannot speak but can sing) ( ) high (cannot talk and cannot sing)

16.4. Have you ever had an injury from the exercise

( ) No ( ) Yes Please specify ..……………………………………………………........

Signature...........................................................

(..........................................................)

Date..........Month........................Year................

We thank you for your report.

# Impact of Resistance Exercise Training on Renal Morphofunctional Characteristics: A Sex-Based Comparison in Wistar Rats

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## ABSTRACT

**Emily R Cordeiro, Isadora G Almeida, Nathalia B Silva, Isabela BM Silveira, Letícia M Monteiro, Debora CK Lichtenecker, Rogério Argeri, Guiomar N Gomes.** This study evaluated the effects of resistance training on renal morphofunctional parameters in male and female Wistar rats. Two-month-old rats were allocated into 4 Groups: Male Sedentary (MS Group), Male Exercise (ME Group), Female Sedentary (FS Group), and Female Exercise (FE Group). The exercise Groups performed progressive resistance training on vertical climbing a ladder 5 times per week for 6 weeks. At protocol completion, blood pressure (BP) and renal function were evaluated. Urine and blood samples were collected to determine creatinine clearance (Clcr) and for biochemical analysis. The kidneys were harvested for morphological evaluation, including glomerular tubularization, interlobular artery structure (media-to-lumen ratio), and expression of α-smooth muscle actin (α-SMA) and CD68-positive cells by immunohistochemistry (CEUA/UNIFESP: 9009241022). The data are expressed as mean ± SEM; two-way ANOVA, $P \leq 0.05$. Resistance training did not significantly alter BP, but Clcr was reduced in the FE Group. Glomerular tubularization was higher in males than in females and was not affected by exercise. The media-to-lumen ratio of interlobular arteries was higher in females and further increased in the FE Group, possibly related to greater sympathetic activation. No significant changes were observed in α-SMA expression, while CD68-positive cells were reduced in the FE Group. In conclusion, 6 weeks of resistance exercise did not affect BP but induced functional and vascular alterations in females without increasing renal injury markers. Further studies are needed to better understand sex-specific renal responses to exercise.

**Keywords**: Creatinine Clearance, Renal Function, Resistance Training, Sex Differences

## INTRODUCTION

Physical activity, such as aerobic exercise is widely claimed to be beneficial to health (23). Recently, resistance training has also been pointed out as a strategy efficient in health promotion (8,16,18), and it is especially recommended to individuals with type 2 diabetes, obesity, and cardiovascular diseases (6,8,16,18,23).

Although there are many studies in this area, few focus on the effects of physical training on the renal system. During rest, the kidneys receive about 20% of the cardiac output. However, during exercise, this blood flow is reduced due to the activation of renal sympathetic nerves and the release of hormones from the renin-angiotensin-aldosterone system, among other mediators (25). This response results in a transitory renal hypoperfusion (12,25). In this situation, it is important to consider whether the physiological adaptation may affect kidney morphology and/or function, especially in relation to chronic physical training.

Chronic renal disease is a globally growing health problem with serious economic and social implications (10). Given that physical activity is an effective tool for preventing and treating several clinical conditions, it is relevant to understand the undesirable effects and limitations of this procedure. Thus, the purpose of the present study is to deepen knowledge of the effects of resistance training on renal function by using an experimental model with rats of both sexes to evaluate the potential impact of this intervention and to investigate the possible differences related to biological sex.

## METHODS

### Experimental Design

Wistar rats (male and female, 2 months old) were obtained from the Center for the Development of Experimental Models for Medicine and Biology (CEDEME/UNIFESP). The experimental protocol used was analyzed and approved by the University Ethics Committee (protocol 9009241022). The rats were housed in ventilated cages in a room maintained at a constant temperature of 22°C under a 12-hour light/dark cycle (lights on at 7 am). They had free access to standard laboratory chow and water throughout the experimental protocol.

There were 4 Experimental Groups: Male Sedentary (MS Group), Male Exercise (ME Group), Female Sedentary (FS Group), and Female Exercise (FE Group). The animals in the ME and FE Groups underwent a progressive resistance training program. The others stayed in the same room in the housing cage.

### The Protocol of Resistance Exercise Training

Initially, the animals were habituated to the climbing apparatus, a 110 cm-high × 18 cm-wide 80° inclined vertical ladder (2 cm grid steps). A housing chamber (L×W×H=20×20×20 cm) was positioned at the top of the ladder and used as a refuge during the resting period. Habituation occurred over 3 days, with 3 attempts per day. At first, the animal was placed in the refuge for 60 seconds, and then the trials were conducted [modified from Cassilhas et al. (5)]. The rats underwent progressive resistance exercise, 5 sessions a week for 6 weeks. Each training session has 8 rounds of ladder climbing with a progressive load attached to the animal's tail (fixed with a Coast lock Snap Swivel ring and Scotch 23-Scotch 3M tape). In the first 2 sets, the load was of 50% of the maximum load set for each animal. Following this, the load was increased according to the series, with the third and fourth at 75%, the fifth and sixth at 90%, and the seventh and eighth at 100% of the maximum load. A 60-second rest interval is provided between each set. Each animal's training session lasts between 20 and 30 minutes. Every week (on Mondays), a new maximum-load test was performed to determine the new load for each animal's sessions. At the end of 6 weeks of exercise, the maximum load carried by the animals was 815.5 ± 22.9 g (223.4 ± 9.65% of body weight) for the ME Group and 639.1 ± 27.7 g (255.1 ± 11.03% of body weight) for the FE Group.

**Assessment of Systolic Blood Pressure**

At 4 months of age, blood pressure was measured using the indirect tail plethysmography technique as described previously (11). Before the evaluation, the rats underwent an adaptation period to the measurement protocol. Following this, 5 measurements were taken in sequence for each rat, and the caudal pressure value was obtained by the average of these measurements and expressed in mmHg.

**Renal Function Parameters**

After the blood pressure evaluation, the rats were placed in metabolic cages for 24 hours to collect urine, followed by blood sample collection to determine creatinine, sodium, and potassium concentrations. Creatinine levels in plasma and urine were measured using the Jaffé method, and the creatinine clearance was determined to estimate the glomerular filtration rate. Sodium and potassium concentrations were measured by flame photometry (Analyser 910). Plasma urea was determined using an enzymatic method (Labtest Diagnóstica). Arterial pH, HCO3, pCO2, and pO2 were determined in a whole blood sample with an i-STAT Clinical Analyzer (Abbott Point of Care Inc.).

**Morphological Analysis**

The kidneys were weighed, fixed in Bouin's fluid, and embedded in paraffin for morphological assessment. Histological sections (5μm thick) were stained with hematoxylin and eosin. Images were acquired (200× magnification) on a microscope (Nikon H550L) connected to a microcomputer by a video camera (Sony-CCD-IRIS). The analysis of glomerular tubularization was carried out, as established by Simon et al. (22), which consists of evaluating approximately 100 glomeruli from each animal and recording how many of them have, within the Bowman's capsule, proximal convoluted tubule characteristic cells.

The immunohistochemistry assays were used to identify the CD68-positive cells (macrophages/monocytes) and α-SMA (Alpha-Smooth Muscle Actin). For this purpose, the tissue sections were incubated for 12 hours at 4°C with monoclonal anti-CD68 and anti-αSMA antibodies (1:500, Serotec; 1:1000, Dako). The reaction product was determined using an immunoperoxidase polymer (Histofine, Nichirei Biosciences Inc.). After the reaction, the slides were dehydrated and mounted. For quantitative analysis, the percentage of the area or positive cell number was assessed in 20 consecutive fields for each sample (×200 magnification); each field had an area of 275,000 $\mu m^2$.

**Statistical Analysis**

All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc). The normality assumption was based on Shapiro-Wilk's Test. The results were expressed as mean ± standard error (SEM) and analyzed using a two-way ANOVA followed by Tukey's *post hoc* test. The level of significance was set at 5% ($P \leq 0.05$).

## RESULTS

### *Changes in Anthropometric and Functional Parameters*

The anthropometric parameters of the studied Groups are shown in Figure 1. The results confirm the dimorphism of the studied species, as evidenced by differences in corporal parameters (body weight-bw and naso-anal length). One interesting finding was that although the females were lighter than the males, the maximum load carried by the females, expressed as a % of body weight (bw), was larger than that observed in the males, reaching values near 250% of bw. The maximum load in the males was 200% of bw. This result shows that females perform better in this resistance exercise. Oyadanich et al. (17) also observed that the female mice subjected to treadmill training had better performance compared to the male mice. The effect observed in the mice was suggested to be due to differences in the distribution of myosin heavy chains, among others (17). There were no significant differences in blood pressure after the training; only sex differences, with females presenting lower values.

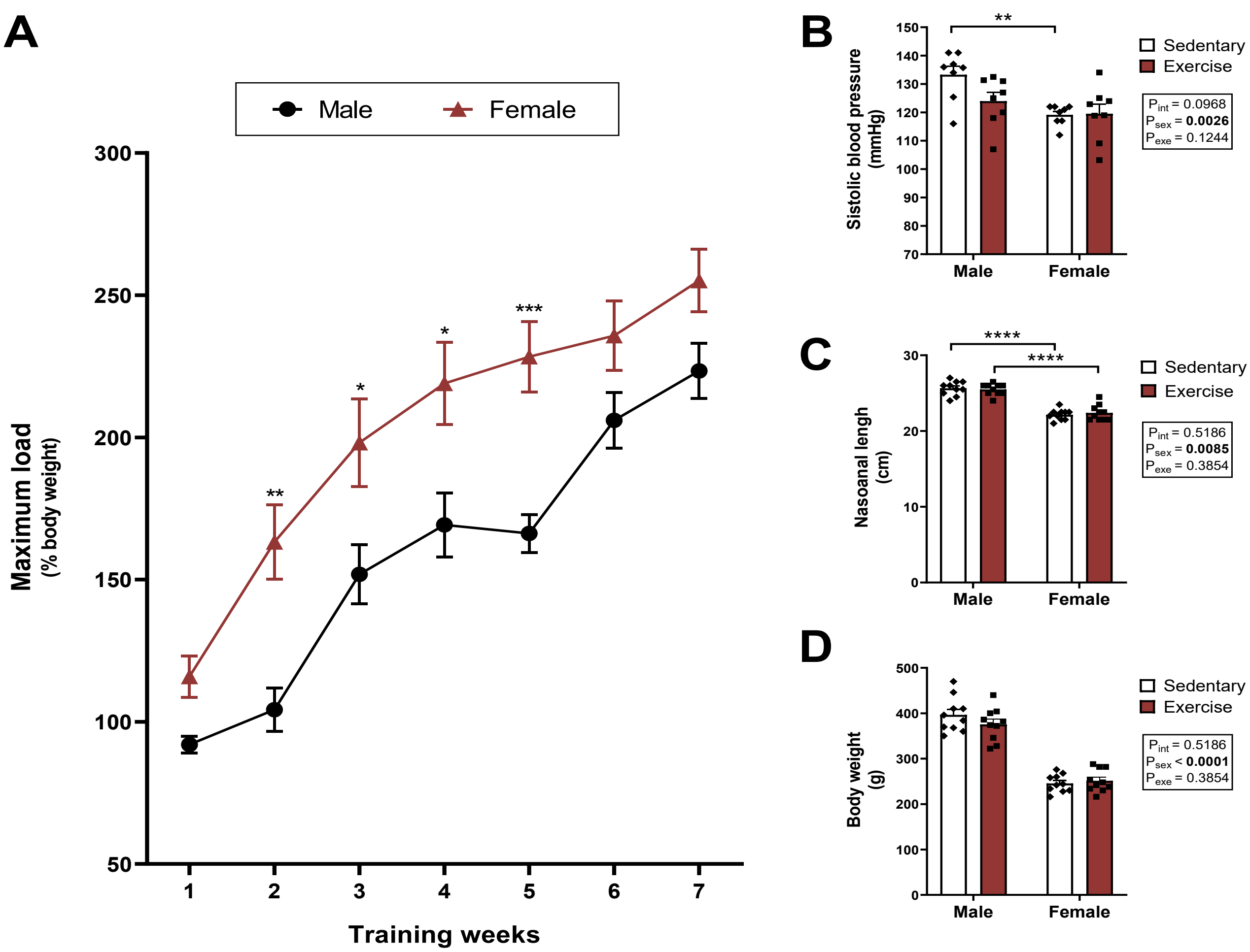


**Figure 1. Maximum Load Carried by Animals Over Weeks of Resistance Training (A), Systolic Blood Pressure (B), Nasoanal Length (C), and Body Weight (D) Observed in 4-Month-Old Rats Submitted to a Resistance Exercise Training Protocol.** Differences were considered significant when P $\leq$ 0.05 (bold); Tukey post-test after two-way-ANOVA. Values are means ± standard error.

Regarding renal function parameters, we observed that females had higher urinary flow than males, without alterations in response to physical exercise (Table 1). Plasma urea concentration was lower in the females; physical activity increased this parameter in both experimental Groups. Regarding plasma creatinine concentration, we observed an increase in the females with exercise. However, there was no increase in the excreted load of this solute, resulting in a decrease in creatinine clearance in the FE Group. Blood acid-base balance parameters were within normal range and showed no significant differences between Groups.

**Table 1. Creatinine Clearance, Urinary Flow, and Blood Parameters Observed in 4-Month-Old Rats Submitted to a Resistance Exercise Training Protocol.**

| Parameter | MALE | | FEMALE | | Two-way ANOVA |
|---|---|---|---|---|---|
| | Sedentary | Exercise | Sedentary | Exercise | |
| **Creatinine Clearance** (mL/min/kg) | 7.41 ± 0.44 | 6.97 ± 0.37 | 6.38 ± 0.36 | 5.36 ± 0.27$ | $P_i$ = 0.4303<br>**$P_s$ = 0.0010**<br>$P_e$ = 0.0561 |
| **Urinary flow** (mL/min/kg) | 0.024 ± 0.001 | 0.023 ± 0.001 | 0.032 ± 0.002$ | 0.033 ± 0.002$ | $P_i$ = 0.5226<br>$P_s$ < 0.0001<br>$P_e$ = 0.8875 |
| **Blood pH** | 7.36 ± 0.017 | 7.35 ± 0.010 | 7.36 ± 0.012 | 7.33 ± 0.015 | $P_i$ = 0.5626<br>$P_s$ = 0.6353<br>$P_e$ = 0.1681 |
| **$pCO_2$** (mmHg) | 49.9 ± 3.0 | 53.6 ± 1.6 | 46.2 ± 1.3 | 53.5 ± 3.2 | $P_i$ = 0.4321<br>$P_s$ = 0.4136<br>**$P_e$ = 0.0229** |
| **$HCO_3^-$** (mmol/L) | 28.0 ± 0.93 | 29.4 ±0.55 | 26.1 ± 0.90 | 28.2 ± 1.18 | $P_i$ = 0.7088<br>$P_s$ = 0.0964<br>$P_e$ = 0.0660 |
| **Hematocrit** (%PCV) | 43.1 ± 0.88 | 44.7 ± 0.69 | 41.1 ± 1.78 | 40.3 ± 0.94 | $P_i$ = 0.3460<br>**$P_s$ = 0.0142**<br>$P_e$ = 0.7778 |
| **Creatinine** (mg/dL) | 0.33 ± 0.019 | 0.33 ± 0.015 | 0.37 ± 0.021 | 0.47 ± 0.026*$ | **$P_i$ = 0.0145**<br>**$P_s$ < 0.0001**<br>**$P_e$ = 0.0198** |
| **Urea** (mg/dL) | 40.7 ± 1.6 | 46.8 ± 1.0* | 35.4 ± 1.7 | 39.1 ± 1.6$ | $P_i$ = 0.4517<br>**$P_s$ = 0.0002**<br>**$P_e$ = 0.0038** |
| **$[Na^+]_p$** (mmol/L) | 148.1 ± 1.4 | 148.6 ± 1.4 | 148.2 ± 1.2 | 145.5 ± 1.7 | $P_i$ = 0.2673<br>$P_s$ = 0.2920<br>$P_e$ = 0.4533 |
| **$[K^+]_p$** (mmol/L) | 4.36 ± 0.10 | 4.38 ± 0.12 | 3.86 ± 0.08$ | 3.67 ± 0.13$ | $P_i$ = 0.3401<br>**$P_s$ < 0.001**<br>$P_e$ = 0.4568 |

Differences were considered significant when $P \leq 0.05$ (bold); vs. Sedentary* or Male$. Tukey post-test after two-way-ANOVA: interaction ($P_i$), sex ($P_s$) and exercise ($P_e$) effects were analyzed. Values are means ± standard error. Number of measurements: 6-8.

Regarding histological analyses (Figure 2), glomerular tubularization was more frequent in the males than in the females, and that physical activity did not modify this distribution. Given the substantial variability in the diameters of interlobular arteries in the renal cortex, we evaluated the relationship between the width and the diameter of each artery. A positive correlation was observed in both Groups: males and females (Pearson, MS: r = 0.7891, ME: r = 0.6819, FS: r = 0.6851 and FE: r = 0.7780). The analyses of the interlobular arteries showed that the females had higher mean/lumen ratios than the males, and that physical activity further increased this parameter in the females. The expression of alpha SM actin, a known marker of renal injury, was similar in males and females, which indicated no significant renal damage. The expression of CD68, a macrophage marker, was higher in the FS than in the MS, but physical activity reduced macrophage infiltration in the FE Group.

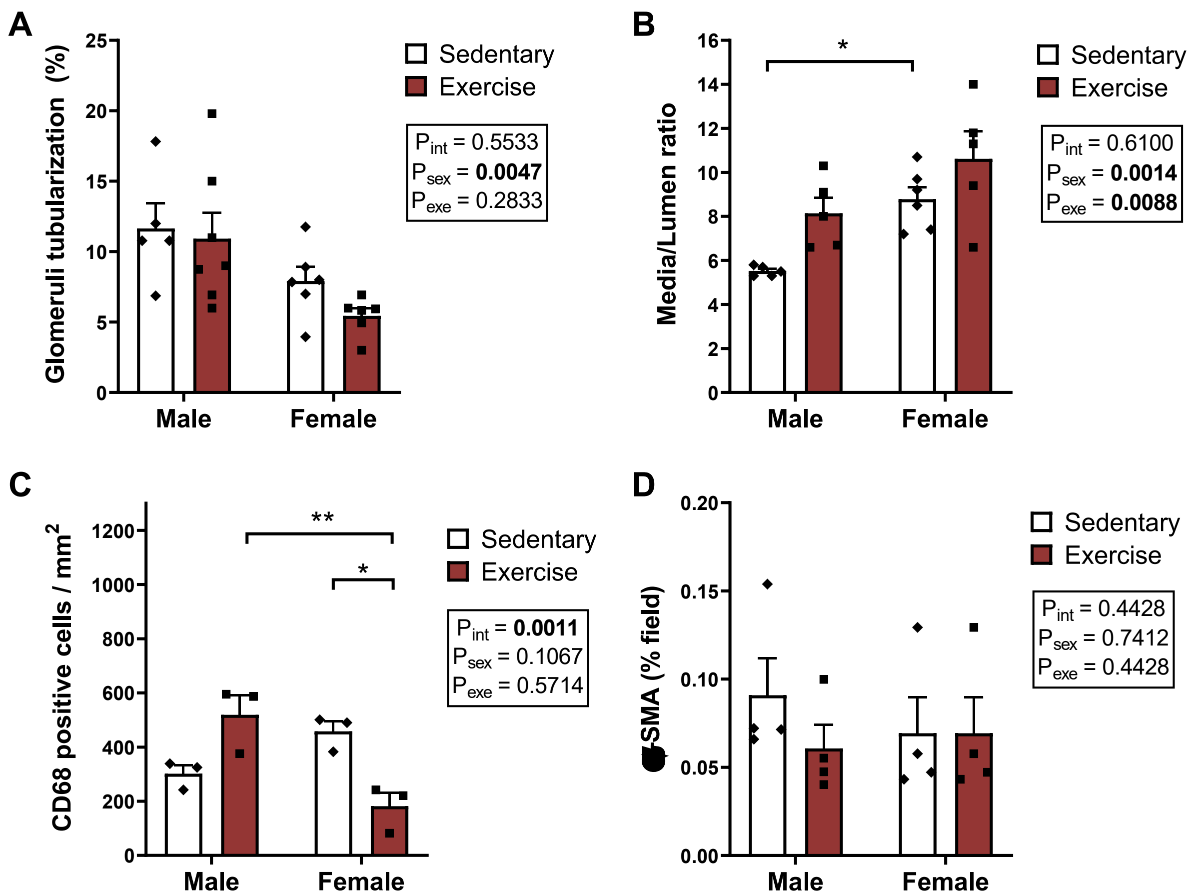


**Figure 2. Glomeruli Tubularization (A), Media/lumen Ratio (B), CD68 Positive Cells (C) and α-Smooth Muscle Actin (α-SMA) (D) Observed in 4-Month-Old Rats Submitted to a Resistance Exercise Training Protocol.** Differences were considered significant when P $\leq$ 0.05 (bold); Tukey post-test after two-way-ANOVA. Values are means ± standard error.

## DISCUSSION

The purpose of this study was to evaluate the effects of resistance exercise training on renal morphofunctional parameters and to compare the effects in male and female Wistar rats. The findings confirmed corporal sexual dimorphism, with the male rats showing higher values of corporal parameters. Regarding renal function parameters, the female rats had higher plasma creatinine levels than the male rats, and exercise significantly increased blood levels of this metabolite. Besides, the female rats had lower creatinine clearance than the males, and exercise significantly decreased this parameter in the females. Urinary flow was higher in the females than in the males, and exercise had no influence.

**Effect of Exercise on Creatinine Clearance**

The increase in serum creatinine observed in the FE Group may be related to the increase in muscle mass induced by exercise (1). In addition, the higher muscle activity favors the production and release of creatinine into the bloodstream (3). Furthermore, in females, the creatine content in muscle fibers is relatively higher than in males, as observed by Forsberg et al. (9), which is a difference that may contribute to this effect. In another way, during physical exercise, the activity of the renal sympathetic nervous system (rSNA) may increase. The rSNA participates in the redistribution of blood flow, privileging the active muscles (14,19), and the increase in the rSNA, depending on the degree, may reduce the glomerular filtration rate (7) due to the reduced renal blood flow induced by vasoconstriction (19).

The resistance exercise protocol applied in this study may have been of high intensity for the females, and the induced vasoconstriction may have persisted longer in the FE Group, impacting the glomerular filtration rate, evaluated by creatinine clearance. In another study, Koçer et al. (14), using a swimming 5 times per week protocol observed that renal resistance arteries from trained female rats showed an increased constrictor response to norepinephrine compared with the Sedentary Group, which confirmed the hypothesis that renal blood flow may reduce significantly during exercise (14). However, further studies are needed to confirm the observed changes in glomerular filtration rate and to clarify the mechanisms involved in this effect.

**Effect of Exercise on Urea Blood Concentration**

In contrast with the results for creatinine plasma concentration, lower blood urea values were observed in the female Groups. However, physical training increased this parameter in both sexes, possibly due to an increase in protein catabolism, which augments urea production (1). The difference in blood urea levels observed between the sedentary Groups may be related to the differences in urinary flow. Females (FS) have higher urinary volume than males (MS). The reduced urinary flow in males seems to be related to the vasopressin action that is 3 times superior in this sex (due to testosterone action) (24). Besides, the vasopressin action extends beyond urinary concentration. It also increases urea reabsorption in the renal collecting ducts (20), thereby increasing blood urea concentration in males.

**Effect of Exercise on Renal Morphology**

Despite the functional alterations, morphological analyses did not reveal significant damage. Glomerular tubularization was greater in the males than in the females, and physical exercise did not change this profile. This histological marker may be associated with the increase in glomerular filtration as well as with the degree of proteinuria (21). However, this last parameter was not evaluated in the present study.

Regarding the media-to-lumen ratio, the females exhibited higher values than the males. The media-to-lumen ratio is a well-established index of small resistance artery structure and is largely independent of vessel size (2). In line with our findings, Bruno et al. (2) reported that women present higher media-to-lumen ratios with aging, a difference potentially linked to greater sympathetic nervous system activity. In the present study, resistance exercise significantly influenced this parameter, suggesting a vascular adaptation associated with physiological changes induced by physical activity. The vascular alterations observed in the FE Group, together with the reduction in glomerular filtration rate, may indicate at least a transient impairment in renal function. Given that chronic kidney disease is more prevalent in women (4,15), these findings highlight the need for further studies to better elucidate the effects of exercise on females' sex and to enhance knowledge and safety regarding intensity and type of physical activity.

The expression of the known marker of renal injury, the alpha SM actin, was similar in males and females, not indicating significant renal damage. The expression of the macrophage marker CD68 was higher in the FS than in the MS. However, physical activity reduced macrophage infiltration in the FE Group. This effect is consistent with evidence suggesting that chronic exercise decreases macrophage infiltration, possibly by modulating the expression of pro-inflammatory cytokines (13).

## CONCLUSIONS

In the present study, the 6-week resistance exercise program did not have a significant impact on blood pressure within the same sex. However, training in the female Group induced changes in renal function and interlobular artery morphology, specifically reducing creatinine clearance and increasing the median-lumen ratio of the arteries without resulting in increased expression of markers of renal injury (α-SMA and CD68). Further studies are needed to clarify the factors underlying the greater impact of this resistance exercise program observed in female rats.

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# Training for the NFL Combine: Effects on Muscle Mass, Strength, and Body Composition

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## ABSTRACT

**Lia Jiannine, Kevin Leung, and Jose Antonio.** This study examined the effects of a 6-week training program for the National Football League (NFL) Combine on body composition and muscular strength in collegiate football players. Thirty-eight participants completed a structured program of resistance training, position-specific drills, and conditioning. Body composition was measured pre- and post-intervention using bioelectrical impedance analysis (InBody 770), and muscular strength was assessed with dominant-hand grip dynamometry. Paired sample *t*-tests compared pre- and post-training values. Skeletal muscle mass increased (51.87 ± 5.98 kg to 52.40 ± 5.66 kg; P = 0.050), and grip strength improved significantly (56.24 ± 9.97 kg to 61.08 ± 11.42 kg; P = 0.006). Lean body mass demonstrated a non-significant trend toward improvement (88.85 ± 10.00 kg to 89.69 ± 9.44 kg; P = 0.061). No significant changes were observed in body fat mass or body fat percentage. These findings suggest that short-term, structured Combine training can increase muscle tissue (specifically, strength in collegiate football players), but meaningful reductions in fat mass may require longer training interventions or additional nutritional strategies.

**Keywords:** Body Composition, Collegiate Athletes, NFL Combine, Strength, Training

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## INTRODUCTION

The NFL Combine is a nationally recognized week-long showcase where select college football players are invited to participate in a series of physical, mental, and medical evaluations to showcase their abilities and enhance their draft stock ahead of the NFL Draft. This invite-only event features 300 to 330 participants annually (1). NFL coaches, general managers, and scouts use the Combine as a critical assessment tool to evaluate each player's performance and draft readiness. Preparation for the Combine typically involves a combination of weight room and on-field training, including speed and agility drills, skill development, and position-specific exercises.

Many athletes strive to optimize their body composition during training to meet the physical demands of their sport. Elite-level collegiate football players can make favorable changes in body composition, even late in their careers, which may improve performance and reduce injury risk (22). Studies on Division I football players also reported in-season changes, including a reduction in body weight by 1.3 kg, an increase in body fat by 0.5%, and a loss of 1.4 kg in lean mass (3,22). However, post-offseason data showed that players rebounded from these changes with lean mass increasing by 2.2 kg and fat mass decreasing by 1.4 kg (23). These findings suggest that in-season body composition declines can be reversed and even improved during the off-season through targeted training and diet (3).

Although body composition does not directly predict performance, studies (1,5,19) suggest that athletes with a leaner, more muscular body composition can perform better in power-endurance sports. One investigation found a correlation between draft status and body composition, showing that drafted players had a significantly lower body fat percentage in 6 of the 8 position groups compared to undrafted players (except quarterbacks and running backs) (16).

Townsend et al. (22) evaluated body composition changes following Combine preparation and found significant improvements in body mass, total body water, and lean mass over a short training period. This program consisted of 4 resistance training sessions per week, 6 days of position-specific training, and Combine-specific drill work. The participants also received dietary counseling from a licensed nutritionist. Of the 9 athletes studied, 3 were drafted and placed on active NFL rosters, 1 was drafted and signed to a practice squad, and 5 others secured contracts as undrafted free agents (22). These results highlight the impact of a structured training and nutrition plan on both physical development and professional success. The purpose of this study is to examine the effectiveness of structured training programs on optimizing body composition and isometric muscular strength in American football players preparing for the National Football League (NFL) Combine.

## METHODS

### Subjects

Thirty-eight collegiate football players (age 22.6 ± 1.8 years, height 187 ± 6.7cm, mass 107.1 ± 20 kg) who just completed the collegiate football season joined an intense, off-campus training program. All 38 players who were enrolled in this combine training program participated in this study. At baseline, body fat mass was 14.81 ± 9.42 kg, body fat percentage was 13.53 ± 5.97%, and skeletal muscle mass was 51.87 ± 5.98 kg.

**Procedures**
A pre-post experimental design was employed over a 6-week training period to examine changes in body composition and isometric grip strength. Both the pre-data and post-data were collected over 2 days, 48 hours apart, with all subjects attending a single session. Body composition was measured at baseline using bioelectrical impedance analysis (BIA) with an InBody 770. Measurements included height, weight, body fat percentage, lean muscle mass, and skeletal muscle mass. All assessments were conducted under standardized conditions. Grip strength was measured with a Harpen dynamometer. The highest value was recorded.

**Training Program Overview**

The athletes completed a 6-week macrocycle consisting of 3 distinct microcycles. The overall training structure is outlined below:

**Drill-Specific Training:**
• 40-yard dash training
• 60-yard Shuttle Agility
• Pro-Shuttle Agility Drill
• 3-Cone "L" Agility Drill

**Position-Specific Training:**
• Position-specific drills and conditioning to prepare for All-Star Football Games
• Training tailored to the NFL Combine and/or College Pro-Day testing components

**Each Week Followed a Standardized Structure:**
• Two days focused on linear speed development (emphasizing the 40-yard dash)
• Two days dedicated to agility and position-specific training
• One day devoted to recovery-based movement and power training (typically conducted in a pool)

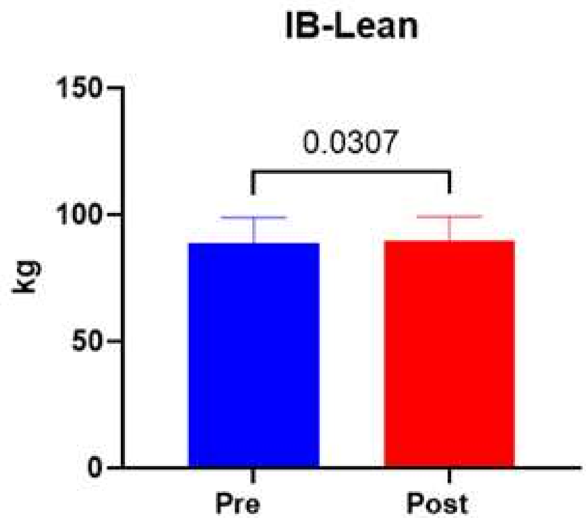
**Resistance Training Focus:**
The strength-training component emphasized event-specific performance, particularly the vertical and broad jumps. The 6-week weight room program included the following progression:
• Weeks 1-2: General preparatory phase
• Weeks 3-4: Emphasis on speed-strength development
• Week 5: Strength/power split phase to maximize power output
• Week 6: Taper phase to optimize performance readiness

**Statistical Analyses**

A paired sample *t*-test was conducted to compare the pre- and post-values for both body composition and grip strength. Values are expressed as the mean ± SD.

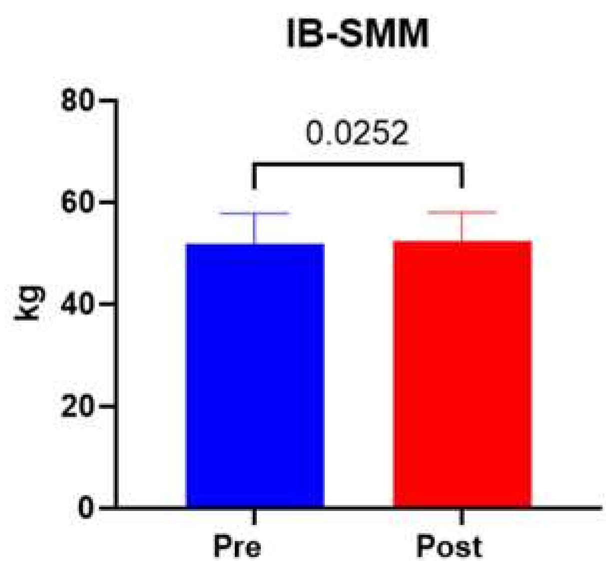
**RESULTS**

Significant improvements were observed following the 6-week program. Lean mass increased from 88.85 ± 10.00 kg to 89.69 ± 9.44 kg, while skeletal muscle mass rose from 51.87 ± 5.98 kg to 52.40 ± 5.66 kg ($P < 0.05$). In addition, handgrip strength improved from 56.24 ± 9.97 kg to 61.08 ± 11.42 kg ($P < 0.01$).




| | Pre | Post |
|---|---|---|
| Mean | 88.85 | 89.69 |
| Std. Deviation | 10.00 | 9.438 |

**Figure 1. Pre- and Post-Training Lean Body Mass (kg) Measured Using the InBody 770. Lean Mass Increased Following the 6-Week NFL Combine Training Program, Indicating a Trend Toward Significance (P = 0.061).**




| | Pre | Post |
|---|---|---|
| Mean | 51.87 | 52.40 |
| Std. Deviation | 5.983 | 5.657 |

**Figure 2. Pre- and Post-Training Skeletal Muscle Mass (kg) Measured Using the InBody 770. Skeletal Muscle Mass Increased Significantly Following the 6-Week NFL Combine Training Program (P = 0.050).**

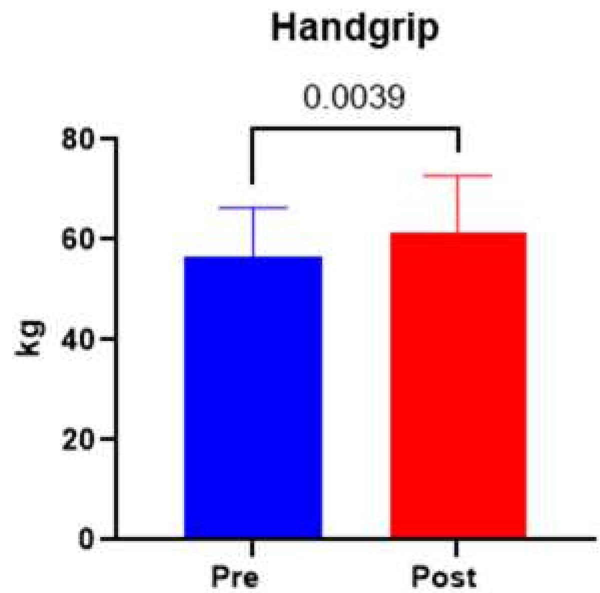



**Figure 3. Pre- and Post-Training Handgrip Strength (kg). Handgrip strength increased Significantly Following the 6-Week NFL Combine Training Program (P = 0.006).**

## DISCUSSION

Our findings align with prior research showing improvements in body composition with structured training within a relatively short training period. Prior investigations (22) have demonstrated that Combine-related performance metrics are not only reliable indicators of athletic readiness but also predictive of draft status across multiple positions (13). In particular, studies have identified body composition as a critical factor with drafted players typically exhibiting lower body fat percentages compared to their undrafted counterparts (16).

However, the present study differs from prior research, which indicates that Combine-specific training reduces fat mass (22). While significant gains were documented in lean muscle tissue, including lean body mass and skeletal muscle mass, reductions in fat mass and body fat percentage did not reach statistical significance. This discrepancy highlights an important nuance. Muscle hypertrophy and neuromuscular adaptations appear to respond rapidly to targeted, high-intensity training within a 6-week window; whereas, meaningful reductions in fat mass are more complex and may require extended training periods, increased caloric expenditure, and/or concurrent dietary adjustments. Thus, our findings suggest that while Combine-specific training is an effective short-term strategy for augmenting muscle-related outcomes, players who are aiming for comprehensive body composition changes should integrate nutritional interventions and potentially longer-term training plans.

### *Limitations in this Study*

This study has several limitations. The relatively small sample size (n = 38) and the absence of a control group make it challenging to attribute all observed changes solely to the training program. Additionally, body composition was assessed using the InBody 770, which relies on bioelectrical impedance analysis (BIA). While convenient and accessible, BIA is more susceptible to error than more precise tools like DEXA, particularly when hydration levels vary.

Another limitation was the use of grip strength as the sole measure of muscular strength. Although grip strength is a reliable indicator of upper-body strength, it does not fully represent the strength and power demands of Combine-specific assessments. Finally, although grip strength improved significantly in the present study, it does not directly replicate Combine-specific strength measures such as the 225-lb bench press. Prior research emphasized the predictive value of Combine-standardized drills for draft status (13,17), suggesting that future studies should examine whether improvements in grip strength transfer to performance on Combine-specific strength assessments.

Also, our findings do not show statistically significant reductions in body fat that suggest short-term training alone is insufficient without dietary monitoring or longer intervention periods. Finally, the participant diets were not monitored, which may have influenced changes in body composition. Future studies should incorporate a larger sample, Combine-specific strength and performance measures, and controlled dietary variables to better evaluate the effectiveness of Combine-specific training programs.

## CONCLUSIONS

A 6-week structured NFL Combine training program can elicit beneficial improvements in body composition and muscular strength. While reductions in body fat mass and body fat percentage did not reach statistical significance, significant improvements were observed in handgrip strength, lean body mass, and skeletal muscle mass. These findings suggest that a short-term Combine-specific training protocol is effective in enhancing muscle-related outcomes, but changes in fat mass may require additional strategies, such as nutrition interventions or an extended training duration.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# Physical Activity and Physical Literacy among Older Adults in Bangkok: A Preliminary Study

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## ABSTRACT

**Ponlasen S, Pongkanpai B**. Population aging is increasing rapidly worldwide, including in Thailand, raising concerns about health and quality of life among older adults. Physical activity (PA) is a key factor for healthy aging, while physical literacy (PL) has emerged as an important multidimensional construct supporting lifelong engagement in physical activity. The purpose of this study is to examine the levels of physical activity and physical literacy among older adults in Bangkok. A cross-sectional study was conducted among 105 adults aged ≥60 years using convenience sampling. The data were collected using structured questionnaires, including the Perceived Physical Literacy for Chinese Elderly Questionnaire (PPLCE) and the International Physical Activity Questionnaire (IPAQ), and analyzed using descriptive statistics. The mean age was 66.23 years, and most of the participants were female (63.81%). The mean physical activity level was 1885.68 MET-min/week (range: 544.5–5468.5), with the participants classified as high (50.48%), moderate (21.90%), and low (27.62%). The mean physical literacy score was 150.29 (min–max: 68–235), with most participants categorized at a moderate level (70.48%), followed by high (20.00%) and low levels (9.52%). These findings indicate that older adults in Bangkok generally demonstrate moderate physical literacy and relatively high physical activity levels. This preliminary evidence may support the development of interventions to promote active and healthy aging in urban Thailand.

**Key Words:** Physical Activity, Physical Literacy, Older Adults, Urban Population

## INTRODUCTION

Population aging is a major global phenomenon that is driven by declining fertility rates and increasing life expectancy. Thailand has already entered an aging society and it is rapidly transitioning toward a super-aged society, with more than 20% of the population aged 60 years and older (National Statistical Office, 2021). This demographic shift presents significant challenges to healthcare systems, economic sustainability, and social structures, particularly due to the increasing burden of chronic diseases and functional limitations among older adults.

Aging is associated with progressive declines in physiological function, including reductions in cardiovascular capacity, muscle strength, balance, and neuromuscular coordination that may lead to frailty and disability (9). Physical inactivity has been identified as a key modifiable risk factor for non-communicable diseases (NCDs), poor mental health, and reduced quality of life. Evidence suggests that a substantial proportion of older adults do not meet recommended levels of physical activity, particularly in low- and middle-income countries (6). Insufficient physical activity has been linked to increased risks of conditions such as diabetes, hypertension, cardiovascular diseases, and depression (7,12).

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure and can be categorized into occupational, household, transportation, and leisure-time activities (3). The World Health Organization (WHO) recommends that older adults engage in at least 150 to 300 minutes of moderate-intensity aerobic activity per week or 75 to 150 minutes of vigorous-intensity activity, in addition to muscle-strengthening and balance-enhancing activities (8). Regular participation in physical activity contributes to improved physical and mental health, reduced risk of chronic diseases, and enhanced functional independence among older adults.

In recent years, the concept of physical literacy has gained increasing attention as a multidimensional construct that supports lifelong engagement in physical activity. Physical literacy is defined as the motivation, confidence, physical competence, knowledge, and understanding to value and take responsibility for engagement in physical activities throughout life (14,15). It comprises 4 domains: (a) affective; (b) physical; (c) cognitive; and (d) behavioral (10). Higher levels of physical literacy are associated with greater participation in physical activity and improved overall health and well-being (5).

Despite the recognized importance of both physical activity and physical literacy, limited evidence exists regarding their levels among older adults in Thailand, particularly in urban settings such as Bangkok. Most previous studies have focused on physical activity alone, while the concept of physical literacy remains underexplored in this population. Therefore, understanding the levels of physical activity and physical literacy among older adults is essential to inform the development of appropriate health promotion strategies.

Accordingly, the purpose of this study is to examine the levels of physical activity and physical literacy among older adults in Bangkok as a preliminary study to provide baseline data for future research and intervention development.

## METHODS

### Subjects

A total of 105 older adults aged 60 years and above were recruited from community settings in Bangkok, Thailand. The participants were selected based on predefined inclusion criteria: (a) individuals aged 60 years or older who were in good health and able to perform activities of daily living independently; (b) had resided in Bangkok for at least 6 months; (c) were able to listen, speak, read, and understand the Thai language; and (d) were willing to participate in the study limitations, or serious medical conditions that could affect participation were excluded. All participants voluntarily participated in the study.

**Research Instruments**

Three instruments were used in this study. First, a structured questionnaire developed by the researchers was used to collect general and health-related information, including sex, age, marital status, educational level, current occupation, monthly income, and body mass index (BMI).

Physical literacy was assessed using the Perceived Physical Literacy for Chinese Elderly Questionnaire (PPLCE) developed by Wang and King (2019). The questionnaire was translated into Thai and back-translated into English following Brislin's (1970) translation procedure, as conducted by Chaichompoo et al. The instrument consists of 47 items across 6 domains: (a) motivation (10 items); (b) physical competence (7 items); (c) environmental interaction (11 items); (d) self-perception (6 items); (e) social interaction (6 items); and (f) knowledge and understanding of physical activity (7 items). Responses were rated on a 5-point Likert scale ranging from 1 (very low) to 5 (very high). The total score ranged from 47 to 235, with higher scores indicating higher levels of physical literacy. Physical literacy levels were categorized into 3 groups: low (47–109), moderate (109.01–172), and high (172.01–235) (2,11).

Physical activity was measured using the International Physical Activity Questionnaire (IPAQ), which assesses physical activity over the past 7 days (4). The questionnaire consists of 27 items covering 4 domains: (a) moderate-intensity activities; (b) vigorous-intensity activities; (c) sitting time; and (d) transportation-related activities. Responses were used to calculate total energy expenditure expressed in MET-minutes per week (MET-min/week). Based on the IPAQ scoring protocol, physical activity levels were classified as low, moderate (≥600 MET-min/week), and high (≥1,500 MET-min/week for vigorous activity or ≥3,000 MET-min/week for total physical activity) (4).

**Procedures**

The study was approved by the Institutional Review Board of Chulalongkorn University (IRB No. 680178), and permission to use the Perceived Physical Literacy for Chinese Elderly Questionnaire (PPLCE) was granted by the original developers.

The data were collected using a cross-sectional survey approach. Data collection was supervised with assistance from 3 trained graduate students from the Faculty of Sports Science. All research assistants received training prior to data collection to ensure consistency in questionnaire administration and participant instruction.

The participants were recruited using convenience sampling from community settings in Bangkok, Thailand, including temples, public parks, local markets, and residential communities. Potential participants were approached and provided with information about the study, including its objectives. Eligibility screening was conducted based on predefined inclusion criteria, and informed consent was obtained prior to participation.

The participants completed the questionnaires that required approximately 15 minutes. Assistance was provided as needed; for participants with reading difficulties, the questions were read aloud, and responses were recorded by the research team. Upon completion, the participants were thanked and received a small token of appreciation. All the data were then prepared for statistical analysis.

**Statistical Analyses**

Descriptive statistics were used to summarize the data. Continuous variables are presented as percentages. All analyses were performed using SPSS (version 27; IBM Corp., Armonk, NY, USA).

## RESULTS

### 1. Participant Characteristics

A total of 105 participants were included in this study, with a mean age of 66.23 years. The majority were female (n = 67, 63.81%), while males accounted for 38 participants (36.19%). Regarding marital status, most participants were married (n = 66, 62.86%), followed by single individuals (n = 26, 24.76%). Smaller proportions were widowed (n = 6, 5.71%), separated (n = 4, 3.81%), and divorced (n = 3, 2.86%).

In terms of educational attainment, the largest group held a bachelor's degree (n = 41, 39.05%), followed by those with higher than a bachelor's degree (n = 17, 16.19%). Participants with primary education accounted for 20 individuals (19.05%), while 13 participants (12.38%) reported no formal education. Smaller proportions had a diploma (n = 8, 7.62%), upper secondary education (n = 4, 3.81%), and lower secondary education (n = 2, 1.90%).

Regarding health status, 67 participants (63.81%) reported no underlying disease; whereas, 38 participants (36.19%) reported having at least one underlying condition. The reported diseases included diabetes mellitus, hypertension, dyslipidemia, and other related chronic conditions.

### 2. Physical Activity and Physical Literacy

The mean physical activity level was 1885.68 MET-min/week, with a minimum of 544.5 and a maximum of 5468.5. The participants were categorized as having high (n = 53, 50.48%), moderate (n = 23, 21.90%), and low physical activity levels (n = 29, 27.62%).

For physical literacy, the mean score was 150.29, with a minimum of 68 and a maximum of 235. The participants were classified as having high (n = 21, 20.00%), moderate (n = 74, 70.48%), and low levels of physical literacy (n = 10, 9.52%) (Table 1).

| Variables | Range Score | Mean | Level (%) | | |
|---|---|---|---|---|---|
| | | | Low | Moderate | High |
| **Physical Activity** | 544.5-5468.5 | 1885.68 | 27.62 | 21.9 | 50.48 |
| **Physical Literacy** | 68-235 | 150.29 | 9.52 | 70.48 | 20 |

## DISCUSSION

This study examined the levels of physical activity and physical literacy among older adults in Bangkok. The findings showed that a substantial proportion of participants had high levels of physical activity, while most demonstrated moderate levels of physical literacy.

### Physical Activity in Elderly in Bangkok

The present study found that more than half of the participants were classified as having high levels of physical activity (50.48%), with a mean physical activity level of 1885.68 MET-min/week. This level exceeds the minimum recommendations of the World Health Organization (WHO), which recommend at least 150 to 300 minutes of moderate-intensity physical activity per week for older adults (8). These findings may be explained by the lifestyle patterns of urban-dwelling older adults in Thailand, where light-to-moderate intensity daily activities, such as walking, household tasks, and participation in community or religious activities contribute substantially to overall physical activity levels.

Consistent with previous research, community-dwelling older adults may accumulate sufficient physical activity through non-exercise activities of daily living rather than structured exercise programs (1). Nevertheless, despite the relatively high proportion of participants classified at a high level, approximately one-third were categorized as having low or moderate levels of physical activity. These findings are consistent with global evidence indicating that insufficient physical activity remains a major public health concern among older adults, particularly in urban and rapidly developing settings (6). Therefore, continued efforts to promote physical activity are essential to ensure that all older adults achieve recommended levels.

**Physical Literacy in Elderly in Bangkok**

Most participants demonstrated a moderate level of physical literacy (70.48%); whereas, fewer exhibited high levels (20.00%). This finding suggests that although older adults may engage in physical activity, their underlying motivation, confidence, knowledge, and understanding related to physical activity may remain underdeveloped.

Physical literacy is widely recognized as a key determinant of lifelong engagement in physical activity, encompassing affective, physical, cognitive, and behavioral domains (10,13). Previous studies have demonstrated that individuals with higher levels of physical literacy are more likely to maintain regular physical activity and adopt healthier lifestyles (5). Therefore, the predominance of moderate-level physical literacy observed in this study highlights the need for targeted interventions to enhance not only physical competence but also motivation, confidence, and knowledge among older adults.

Furthermore, the concept of physical literacy is relatively new in many Asian contexts, including Thailand, and may not yet be fully integrated into public health strategies or community programs. This may partly explain why a large proportion of participants did not reach high levels of physical literacy. Promoting physical literacy through community-based programs, education, and age-appropriate interventions may contribute to improving long-term engagement in physical activity and overall well-being among older adults.

## CONCLUSION

This study demonstrated that older adults in Bangkok generally exhibited relatively high levels of physical activity and predominantly moderate levels of physical literacy. Although most participants achieved sufficient levels of physical activity, physical literacy remained at a moderate level, suggesting potential gaps in motivation, confidence, knowledge, and understanding related to physical activity. These findings highlight the importance of integrating physical literacy into health promotion strategies for older adults. Future interventions should focus not only on increasing physical activity levels, but also on enhancing key components of physical literacy to support sustainable engagement in active lifestyles and promote healthy aging in urban populations.

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# Addictive Effects of Creatine and Caffeine Supplementations in Glucose-Electrolyte-Based Sports Drinks on Soccer Performance in U21 League Players

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## ABSTRACT

**Masodsai K, Chaunchaiyakul R, Pongkanpai B, Chen PN.** Addictive Effects of Creatine and Caffeine Supplementations in Glucose-Electrolyte-Based Sports Drinks on Soccer Performance in U21 League Players. The purpose of this study was to investigate the acute effects of 3 different beverage formulations on agility performance, short-distance sprinting, and fatigue-related variables in trained soccer players. Sixteen male soccer players (age: 20.06 ± 0.57 years; height: 176.19 ± 1.48 cm) participated in a randomized crossover design. The participants consumed 3 different drinks: Drink 1 (control), Drink 2 (containing 25 g glucose and 5 g creatine monophosphate), and Drink 3 (containing 25 g glucose, 5 g creatine monophosphate, and 35 mg caffeine). Agility performance was assessed using the Agility Test (AAT), including response time and directional movement (left and right). Sprint performance was evaluated at 5, 10, 15, and 20 m. Heart rate, blood pressure, and fatigue index were also recorded. The data were analyzed using repeated-measures ANOVA. Drink 3 significantly improved response time ($P = 0.027$) and agility performance in both left ($P = 0.040$) and right ($P = 0.005$) directions compared with Drink 1. Additionally, the 5-m sprint time was significantly reduced following Drink 3 compared with Drink 1 ($P = 0.042$), while no significant differences were observed at longer sprint distances. No significant differences were found among drinks for average power or fatigue index, although a trend toward improvement was observed in Drink 2 and Drink 3. Baseline characteristics and resting physiological variables were not significantly different across conditions. The combined ingestion of glucose, creatine, and caffeine (Drink 3) enhanced agility and short-distance sprint performance in soccer players, particularly in tasks requiring rapid reaction and directional changes. These findings suggest that multi-ingredient ergogenic beverages may be effective for improving high-intensity, neuromuscular performance in intermittent sports.

**Key Words:** Caffeine, Creatine, Isocaloric Supplement, Soccer Agility, Sports Drink

## INTRODUCTION

With the advent of sports beverages, it has become crucial to maintain hydration levels and performance during physical activities, as well as address post-exercise recovery requirements (19). These drinks are formulated by considering the depletion of energy substrates and electrolytes through sweat loss. As a result, sports drinks typically contain specific quantities of carbohydrates and electrolytes such as sodium, potassium, calcium, and magnesium. Caffeine, the most widely consumed psychoactive substance is not only prevalent among sedentary individuals, but also commonly used by athletes due to its known acute ergogenic effects on performance (7). Research has shown that a significant number of athletes, as high as 3 out of 4, consume caffeine before or during sports competitions (13). This popularity of caffeine among athletes was further highlighted in a 7-year study, which revealed a 21% higher concentration of caffeine in urine samples from athletes who consumed caffeine compared to the non-athlete control group (2). Athletes from various sports rely on caffeine to enhance their strength and endurance, with caffeine-containing beverages being popularly consumed before or during sports competitions (13). Various forms of caffeine-containing products, including drinks, chewing gum, gels, and bars are readily available for active individuals (13). Among young, physically active individuals, caffeine-containing energy drinks are particularly popular. For athletes, the effects of caffeine-containing beverages on performance have been found to strongly correlate not only with endurance and strength, but also with cognitive functions (7). However, it is important to note that the ergogenic effects of caffeine can be influenced by several factors, including dosage, training intensity, timing of ingestion, time of day when caffeine is consumed, habitual caffeine consumption, and the type of exercise being performed (6,20). Several reports have highlighted the need to consider these factors when assessing the performance-enhancing effects of caffeine (16).

Creatine, a non-protein amino acid that is naturally found in red meat (18) is a popular nutritional ergogenic aid used by athletes to enhance strength and power (13). Majority of creatine in the body, known as the creatine pool consist of phosphocreatine (PCr) and creatine (Cr), is stored in skeletal muscles and limited to approximately 120 $mmol \cdot kg^{-1}$ of dry muscle mass for an individual weighing 70 kg. During short and high-intensity exercise, intramuscular creatine is degraded into creatinine, a metabolic byproduct, and excreted in the urine (13). Therefore, to maintain normal creatine levels without supplementation, the body needs to replenish about 1 to 3 g of creatine per day, which becomes particularly crucial for power-based athletes (13). Vegetarian athletes, who may not consume meat in their regular diet, may face a greater challenge in maintaining adequate creatine levels and may benefit from creatine supplementation (4). By aiding in the resynthesis of adenosine triphosphate (ATP) through the hydrolysis of PCr into Cr and inorganic phosphate (Pi), creatine can help maintain ATP availability, especially during maximal effort anaerobic sprint-type exercises (1).

In a normal regular diet, the intake of creatine is typically only around 1-2 $g \cdot day^{-1}$, which may not be sufficient to support intense physical activity. Therefore, dietary supplementation of creatine is often necessary (12). Creatine monohydrate is the most commonly used form of creatine supplementation, and its absorption occurs in the blood followed by uptake into the target tissues (9). After ingestion, plasma creatine levels peak and are sustained for approximately 60 minutes. One of the reasons for the widespread popularity of creatine is that it is not banned by any sports organization, making it a common choice among athletes and military personnel (12). Supplementing with creatine has been shown to increase intramuscular phosphocreatine concentrations, which can explain the observed improvements in high-intensity exercise performance. This can result in higher acute anaerobic power and greater training adaptations, particularly in soccer players (5,14). In a study with elite female soccer players, acute improvements in performance of repeated sprint and agility tasks simulating soccer match play were observed after consuming 5 g of creatine 4 times per day for 6 days (5). The most effective way to increase muscle phosphocreatine storage is through ingestion of creatine monohydrate at a dosage of 0.3 to 0.8 $g·kg^{-1}·day^{-1}$ (12). In another study, consuming a total of 20 $g·day^{-1}$ of creatine for 5 to 7 days typically increased total creatine content by 10 to 30% and phosphocreatine stores by 10 to 40% (11). For safety reasons, it is recommended that the individuals consume 3 $g·day^{-1}$ of creatine throughout their lifespan to promote general health (12).

Although creatine and caffeine have been individually studied for their ergogenic properties among athletes, the acute additive effect of combining them in sports drinks is still not well understood. While sports drinks containing carbohydrates and electrolytes have been extensively researched, there is a need to examine the changes in alterations in sports performance when creatine and caffeine are combined in such drinks especially in professional soccer players. Thus, the purpose of this study was to investigate the additive effects of creatine and caffeine supplementations in sports drinks on soccer performance in U21 league players.

## METHODS

### Subjects

The study recruited 16 male U21 league players who met the inclusion criteria, including being between 18 and 25 years old, regularly participating in competitive soccer games, being free from recent musculoskeletal injuries, having no cardiorespiratory problems, and not taking any medications or supplements that may affect the cardiorespiratory system, metabolism, or exercise performance. The participants underwent physical examinations and completed the Physical Activity Readiness Questionnaire (PARQ) by a registered physical therapist and sports scientist. They were instructed to maintain their regular diets, drink water freely, refrain from vigorous physical activity, avoid non-sports drinks such as tea, coffee, alcoholic beverages, and to sleep for at least 8 hours for 2 days before the scheduled test date. The study was conducted during the off-season period and followed the Declaration of Helsinki and regulations of the Institutional Review Board of Chulalongkorn University, Thailand (COA No. 650071).

Prior to inclusion, the participants were provided with comprehensive testing outlines and an explanation of the study, and written informed consent was obtained before conducting the physical tests. All the participants completed 3 trials, with each trial being separated by at least 7 days. They were provided with manufactured isocaloric drinks that were electrolyte-based, with each drink having different compositions. Drink 1 (D1) was a placebo containing 25 g of glucose. Drink 2 (D2) contained 25 g of glucose and 5 g of creatine monophosphate. Drink 3 (D3) contained 25 g of glucose, 5 g of creatine monophosphate, and 35 mg of caffeine. The drinks had identical taste, color, and flavor. The drinks were randomly provided 30-minutes before the agility testing. The participants were asked to record their food and fluid intakes, including the portion sizes of all foods consumed and the volume of all fluids consumed on the day prior to each trial to estimate their normal nutritional status.

## Procedures

On the test day, various measurements were carefully taken. Standing height, body mass, and body composition were measured using a free-standing adjustable stadiometer and using a bioelectrical impedance analyzer (BIA HBF-375, Omron, Japan). Resting blood pressure was determined using an automated sphygmomanometer (HEM-7156A, Omron, Japan). Hydration status was indirectly monitored using urine-specific gravity measured with a refractometer (Atago Master-URC/NM, Japan). Blood samples from fingertips were collected using sterile techniques for baseline glucose (Accu-check guide, Germany) and lactate (Lactate plus, USA) levels. Telemetry heart rates (Polar H10, Poland) were continuously recorded throughout the study.

The Arrowhead Agility Test (AAT) was utilized to measure change of direction (COD) capacity in soccer players conducting on a natural turf outdoor soccer field (17). The AAT time was measured using timing gates (ReactionX, China) positioned at the start line, as described previously (17). The participants sprinted and changed direction maximally throughout the test, stepping around and not over the markers; otherwise, the trial was stopped and reattempted after full recovery (~4 minutes). Four trials in total were completed; 2 with movement initiation to the left side, and 2 with movement initiation to the right side. The order was randomized among the participants to avoid any effect of fatigue on the other side. The participants started when ready, thus eliminating reaction time and completed 2 trials separated by at least 3 minutes of rest. The best of 2 trials for each side was retained for all analysis. Moreover, the 20-m speed test in soccer, a fundamental assessment of acceleration, speed, and quickness, measuring a player's ability to reach maximum velocity over a short distance, was used for all testing occasions (8). All tests were conducted on the same field and zone of the soccer club, with the natural turf being well-maintained to ensure consistent results. The participants were instructed to use the same shoes and sportswear for all testing.

## Statistical Analyses

All the data were presented as the mean and standard error of mean (SEM). When the homogeneity assumption of the variance was met (Leven's Test, $P > 0.05$), the data were carefully analysed using a repeated-measure ANOVA with an LSD *post hoc* test by the SPSS statistical package (version 23; SPSS, USA). Statistical significance was accepted when $P < 0.05$.

# RESULTS

## General Characteristics

The general characteristics of the participants are shown in Table 1. The mean age and body height of all players is 20.06 ± 0.57 years old and 176.19 ± 1.48 cm, respectively. There were no significant differences of all body composition and resting vital signs among the 3 testing occasions ($P > 0.05$).

## Agility Performance

Figure 1 represents the agility performance during 3-randomized drink interventions with the AAT without the unexpected occurrence. The results found that Drink 3 (D3) contained 25 g of glucose, 5 g of creatine monophosphate, and 35 mg of caffeine revealed the significant improvement in the response time ($P = 0.027$, effect size 0.156) and lowering agility time in left

(P = 0.04, effect size 0.097) and right (P = 0.005, effect size 0.187) directions compared with Drink 1. Moreover, when compared with D2, agility time in right direction was significantly lower after consumption D3 (P = 0.022, effect size 0.187).

The speed time was also monitor in 5, 10, 15, and 20 meters, and although it was found significantly lower in the 5-m speed time after D3 when compared with D1 (P = 0.042, effect size 0.062), it was not significantly different among drinks during the later distance. Moreover, there were no significant differences in the average power and fatigue index among the drinks, even though it showed the lower trend in D2 and D3.

**Table 1. Descriptive Data of the Subjects.**

| Variables | Drink 1 (n = 16) | Drink 2 (n = 16) | Drink 3 (n = 16) |
|---|---|---|---|
| **Body Mass** (kg) | 69.46 ± 2.21 | 70.78 ± 2.02 | 69.94 ± 2.18 |
| **BMI** (km·m$^{-2}$) | 22.61 ± 0.54 | 22.89 ± 0.54 | 22.48 ± 0.50 |
| **Body Fat** (%) | 15.19 ± 1.24 | 15.86 ± 1.10 | 14.32 ± 1.08 |
| **Body Muscle** (%) | 36.96 ± 0.28 | 36.42 ± 0.33 | 36.39 ± 0.39 |
| **HR** (beats.min$^{-1}$) | 68.23 ± 1.63 | 72.08 ± 1.40 | 71.44 ± 2.32 |
| **SBP** (mmHg) | 120.23 ± 8.51 | 122.47 ± 8.63 | 120.57 ± 8.26 |
| **DBP** (mmHg) | 72.96 ± 9.82 | 76.28 ± 10.07 | 71.38 ± 10.21 |

Values are mean ± SEM Abbreviations: **BMI**; Body Mass Index, **HR**; Heart Rate, **SBP**; Systolic Blood Pressure, **DBP**; Diastolic Blood Pressure.

## DISCUSSION

The present study investigated the acute effects of 3 beverage formulations on agility and short-distance speed performance in trained soccer players. The primary finding was that Drink 3 (D3), containing glucose (25 g), creatine monophosphate (5 g), and caffeine (35 mg), significantly improved response time and agility performance, particularly in both left and right directional movements, compared with Drink 1 that contained only 25 g glucose. Additionally, D3 enhanced 5-m sprint performance, while no significant differences were observed in longer sprint distances, average power, or fatigue index. These findings indicate that the combined ingestion of carbohydrate, creatine, and caffeine exert ergogenic effects in high-intensity, short-duration, and neuromuscular demanding tasks.

One of the key mechanisms underlying the observed improvements is likely related to caffeine's role in central nervous system stimulation. Caffeine has been shown to enhance alertness, reaction time, and motor unit recruitment via antagonism of adenosine receptors, thereby improving cognitive processing speed and neuromuscular coordination (7). This may explain the significant improvements in response time and agility performance, particularly in tasks requiring rapid decision-making and directional changes, such as the agility test used in this study. The relatively low dose of caffeine (~0.5 mg·kg$^{-1}$, depending on body mass) is noteworthy since low-to-moderate doses have been reported to improve cognitive and skill-based performance without inducing adverse effects (21).

The inclusion of creatine monophosphate in D3 may have contributed to improvements in short-duration, high-intensity performance, such as the 5-m sprint. Creatine supplementation enhances phosphocreatine (PCr) availability, facilitating rapid ATP resynthesis during explosive efforts (12). Although acute creatine ingestion is typically considered less effective than chronic loading, emerging evidence suggests that even short-term or co-ingestion strategies may influence performance when combined with other ergogenic nutrients (12). The improvement observed specifically at 5 m but not at longer distances supports the role of the ATP-PCr energy system, which predominates in the initial seconds of sprinting.

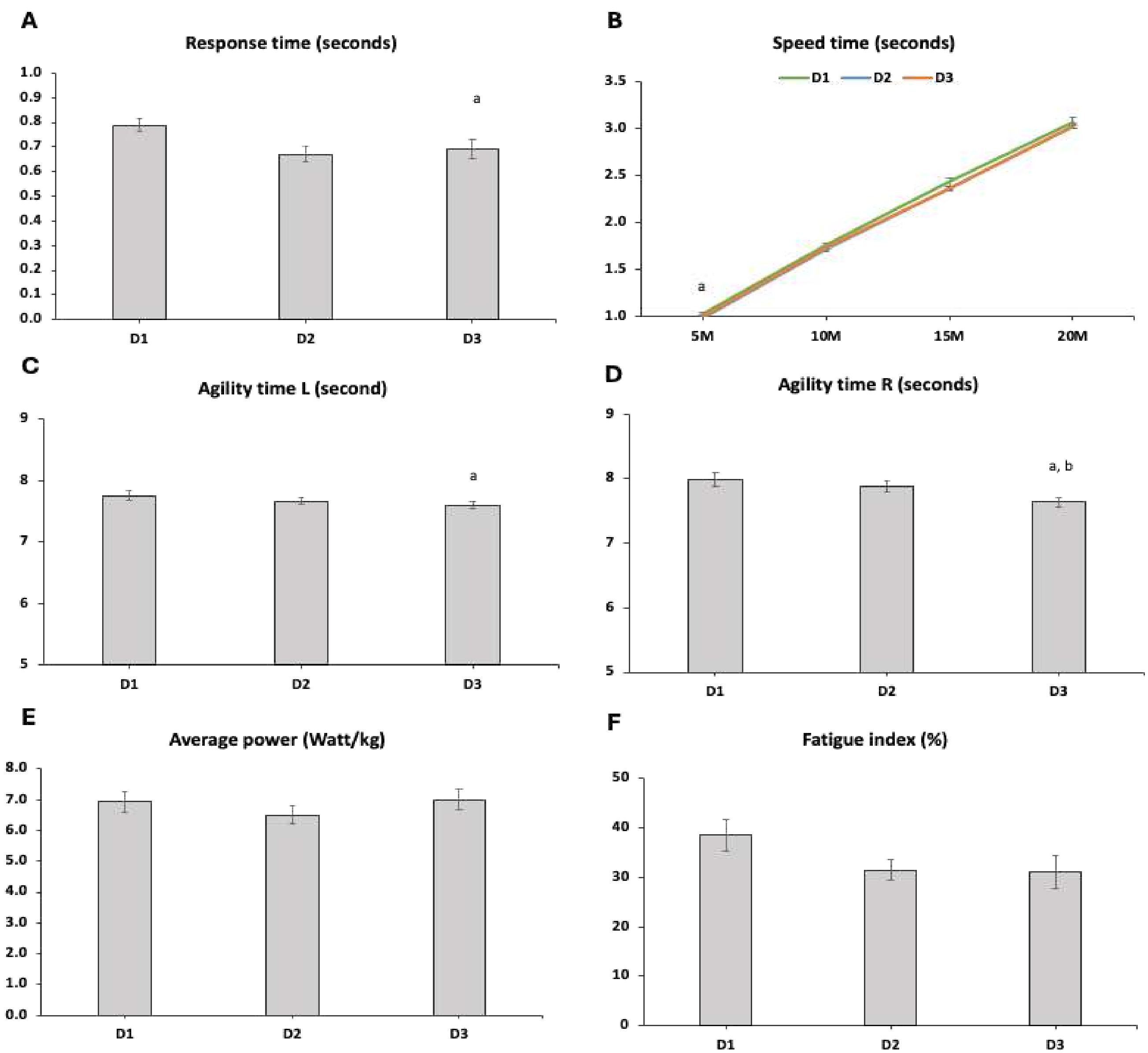


**Figure 1. The Soccer Performance After Drink 1, 2, and 3 (D1, D2, and D3) In This Study.**
Values are mean ± SEM Abbreviations: **a**; significant between D1 and D3, **b**; significant between D2 and D3.

Carbohydrate ingestion (25 g glucose) likely played a complementary role by maintaining blood glucose availability and supporting central drive, particularly in repeated or cognitively demanding tasks (10). Although the exercise duration in this study was relatively short, carbohydrate availability has been shown to influence motor skill performance and perceived effort, even in intermittent high-intensity exercise. The synergistic effect of carbohydrate with caffeine may further enhance performance via both metabolic and central mechanisms (1,3).

Interestingly, no significant differences were observed in average power or fatigue index, although a trend toward improvement was noted in D2 and D3. This may indicate that the intervention primarily influenced neuromuscular coordination and reaction-based performance, rather than sustained anaerobic capacity. Similarly, the absence of differences in 10-20 m sprint performance suggests that the ergogenic effects were most pronounced in initial acceleration phases, rather than maximal sprint velocity, which relies more heavily on biomechanical and muscle-tendon properties.

The lack of differences in baseline characteristics across trials confirms that the randomized crossover design was effective in minimizing confounding variables, strengthening the internal validity of the findings. However, several limitations should be acknowledged. First, the sample size (n = 16) may limit statistical power for detecting small effects in secondary outcomes. Second, the study did not include biochemical markers (e.g., plasma caffeine, creatine uptake, or blood glucose), which could provide mechanistic insights. Third, the absence of a placebo-controlled double-blind design may introduce potential bias. Finally, the acute nature of supplementation limits the ability to generalize findings to chronic use.

From a practical perspective, these findings suggest that a multi-ingredient ergogenic drink combining carbohydrate, creatine, and caffeine may be beneficial for sports requiring agility, and short sprint bursts, such as soccer. Coaches and sports nutritionists may consider such formulations for pre-competition or pre-training strategies, particularly when the goal is to enhance decision-making speed and explosive movement.

## CONCLUSIONS

The present study demonstrates that acute ingestion of a multi-ingredient beverage containing glucose, creatine, and caffeine significantly enhances agility performance and short-distance sprint ability in trained soccer players. The improvements were most evident in response time, directional agility, and 5-m sprint performance, which suggest a primary effect on rapid neuromuscular activation and initial acceleration rather than sustained anaerobic performance.

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