Project Title: The Impact of Fruit and Vegetable Enzyme Supplementation on Aerobic Capacity, and Blood Biochemical Values Following Very Low Volume High-Intensity Interval Exercise - Feasibility Study of Exercise Design Using Motion-Sensing Video Games

Study Protocol

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Introduction

Exercise represents a paradoxical element in health management, offering substantial benefits yet posing potential risks if not properly moderated [1, 2]. High-intensity exercise, while efficacious in improving various health parameters, can lead to oxidative stress, muscle damage, and inflammation [3, 4]. The oxidative stress primarily arises from increased reactive oxygen species production during intensive physical activities [5]. Moreover, exercise-induced fatigue serves as a protective mechanism against overexertion and consequent injuries[6, 7]. In contemporary fitness regimes, HIIE, particularly the Tabata training method, has gained prominence for its effectiveness in enhancing aerobic power, fat oxidation, and muscular endurance [8-10]. These attributes are especially crucial for the elderly population, a demographic that significantly benefits from regular physical activity [11-13] .

Exergaming, an innovative blend of physical exercise and interactive gaming, has emerged as a transformative approach to fitness, especially in engaging diverse age groups in regular physical activity. Its efficacy in enhancing key fitness parameters like aerobic capacity, agility, and coordination, coupled with its ability to make exercise more enjoyable, has been well documented [14-16]. This fusion of technology and exercise not only caters to the digital age but also opens avenues for personalized fitness experiences, adaptable to various demographic needs [17, 18]. While exergaming has been effective across a range of ages, its application in elderly populations presents unique opportunities and challenges. As the elderly population seeks safe, engaging, and effective exercise methods, exergaming could offer a solution that aligns with these requirements. However, integrating HIIE concepts into exergaming for the elderly remains a relatively uncharted territory. HIIE, known for its efficiency in improving cardiovascular health and metabolic function, could significantly benefit older adults, particularly in terms of enhancing functional capacity and overall quality of life [12, 19].

The potential of HIIE within exergaming for the elderly hinges on the balance between intensity and safety. While HIIE is beneficial, it is crucial to adapt its intensity to suit the physiological capabilities and limitations of older individuals. Research indicates that tailored HIIE programs can be both feasible and beneficial for older adults, leading to improvements in cardiovascular health, muscle strength, and metabolic function [20, 21]. Integrating these concepts into exergaming could further enhance adherence and enjoyment, crucial factors in maintaining regular exercise habits in this demographic. Furthermore, the interactive and immersive nature of exergaming can address common barriers to exercise among the elderly, such as lack of motivation or fear of injury. By providing a safe, controlled environment for engaging in HIIE, exergaming can potentially transform the perception and experience of high-intensity workouts for older adults. This is particularly pertinent given the increasing need for innovative exercise interventions that cater to the aging global population [11].

Nutritional supplementation, especially with natural fruit and vegetable enzymes, presents a promising avenue in augmenting exercise performance through their antioxidant, anti-inflammatory, and metabolic benefits [21-28] Such supplementation could potentially optimize lactate metabolism and enhance muscle function during exercise. Recent advancements in nutritional science have highlighted the significant role of natural fruit and vegetable enzymes in enhancing exercise performance. These enzymes are increasingly recognized for their multifaceted health benefits, including their antioxidant, anti-inflammatory, and metabolic-enhancing properties [21, 22]. Notably, their potential impact on exercise physiology, particularly in the context of high-intensity workouts, offers a new perspective on improving athletic performance and recovery.

One of the critical areas where these enzymes show promise is in the modulation of lactate metabolism. Lactate, often produced in higher quantities during intense physical activity, can lead to fatigue and decreased muscle efficiency. The traditional view of lactate as merely a byproduct of anaerobic metabolism has evolved, with current research acknowledging its role as a valuable energy source during prolonged exercise [23]. This shift in understanding opens up new avenues for utilizing enzyme supplementation to optimize lactate utilization. Enzymes such as bromelain and papain, found in pineapples and papayas respectively, have been studied for their potential in improving lactate metabolism. These enzymes are known to facilitate faster clearance of lactate from the bloodstream, thereby enhancing recovery and reducing fatigue [26, 28]. Furthermore, the antioxidant properties of these enzymes play a crucial role in combating oxidative stress, which is often elevated during intense exercise regimes [24, 25]. This reduction in oxidative stress is not only beneficial for immediate recovery but also contributes to long-term muscle health and function. Moreover, the anti-inflammatory actions of these natural enzymes can mitigate the inflammatory response often triggered by high-intensity exercise [27]. By reducing inflammation, these enzymes may enhance muscle recovery and function, thus allowing for more efficient and prolonged exercise performance. This aspect is particularly relevant in training regimens where recovery is as crucial as the exercise itself.

The primary aim of this feasibility study is to examine the effects of fruit and vegetable enzyme supplementation on aerobic capacity and blood lactate response in elderly individuals engaged in HIIE through an exergaming framework. This study is dual-faceted, focusing firstly on the physiological responses and feasibility of an exergaming-HIIE regimen tailored for the elderly, and secondly, on the impact of enzyme supplementation on enhancing these exercise outcomes.

Methods

Participants And Experimental Design

All participants reported a regular exercise habit (3 times per week within the past year). They also completed the Physical Activity Readiness Questionnaire and confirmed no history of upper limb skeletal muscle injury or major injury. Participants were instructed to avoid strenuous activities and the intake of caffeine or muscle-enhancing supplements for 24 hours prior to the experiment. Before the study commenced, all participants provided personal information, completed health questionnaires, disclosed personal medical history, and signed informed consent forms.

The 16 participants underwent the Exergaming HIIE test as an initial assessment (pre-test). Participants engaged in a 5-minute warm-up on a stationary bike, followed by HIIE using the Nintendo Switch Ring Fit Adventure design. The training method was adapted from the research of [8, 9] and consisted of 8 sets of 20 seconds of maximum effort exercise with 30 seconds of complete rest between each set, resulting in a total exercise time of 370 seconds. The HIIE design incorporated training modes targeting the deltoid, pectoralis major, latissimus dorsi, and quadriceps muscles in the Nintendo Switch Ring Fit Adventure. Blood lactate levels, heart rate, and ratings of perceived exertion were recorded before, during, and after exercise, and training load was quantified using TRIMP. Participants were matched and divided into two groups, the enzyme group, and the placebo group, based on their blood lactate levels during HIIE. Each group comprised 8 individuals. Supplementation with vegetable and fruit enzymes or maltodextrin commenced three days after the pre-test and lasted for a total of 14 days. On the 14th day, following the completion of supplementation, the participants underwent the Exergaming HIIE test as a post-test.

Ethical Considerations

The human research ethics committee of the local university approved this study, which was also approved by the human research ethics committee of the National Cheng Kung University, Taiwan. (Approval No. NCKU HREC-E-112-419-2). Users volunteered for this study and agreed to participate by signing an informed consent form.

Supplementation Protocol

After the pre-test, the enzyme group consumed 30 mL of vegetable and fruit enzymes (The contents include needle-leaf cherries, cherries, apples, cranberries, blackberries, black currants, blueberries, beets, broccoli, cabbage, carrots, Concord grapes, cranberries, elderberries, kale, oranges, peaches, papayas, parsley, pineapples, raspberries, red currants, spinach, and tomatoes, etc.)(Enzyme Village, Chiayi, Taiwan) mixed with 150 mL of water twice a day (at breakfast and dinner) for 14 consecutive days. The placebo group followed the same protocol but consumed malt syrup (Amazon, USA) instead until the end of the study. Participants returned to the laboratory each morning to receive the daily supplement, which was administered on-site. Following supplementation, participants reported their dinner intake to the researchers, ensuring compliance with the prescribed supplementation regimen.

Exergaming HIIE Test-Combination Of Exergaming And HIIE

Participants in this experiment engaged in HIIE using the Nintendo Switch Ring-Con within a laboratory environment. All participants completed pre-test and post-test assessments on the same day. The exergame employed in this study was 'Nintendo Switch Fitness Adventure,' which ingeniously blends exercise with an adventure narrative to deliver both physical workouts and gaming enjoyment concurrently. This game is noted for its intuitive, user-friendly interface that accommodates players of all ages. It incorporates a specialized fitness ring -a smart accessory that connects to the Nintendo Switch console. The sensor system utilized two Nintendo controllers: one mounted on the exercise ring and the other secured to the participant’s thigh to enhance gameplay interaction. Through the Ring-Con, participants engaged in diverse physical activities such as weightlifting, yoga, and aerobic exercises. The fitness ring sensor accurately captures and integrates players' movements into the game. The gameplay involves unlocking levels and engaging in fitness challenges that are achieved through actual physical activities. It offers a wide range of exercise routines targeting various muscle groups and provides engaging gaming challenges. The exercise protocol included eight sets of 20-second high-effort exercises, interspersed with 30-second rest intervals, totaling 370 seconds of active exercise time. Specifically, the fitness game mode used was the Adventure Mode in 'Ring Fit Adventure,' comprising exercises targeting the pectoralis major, latissimus dorsi, deltoids, and quadriceps muscles (Figures 2).

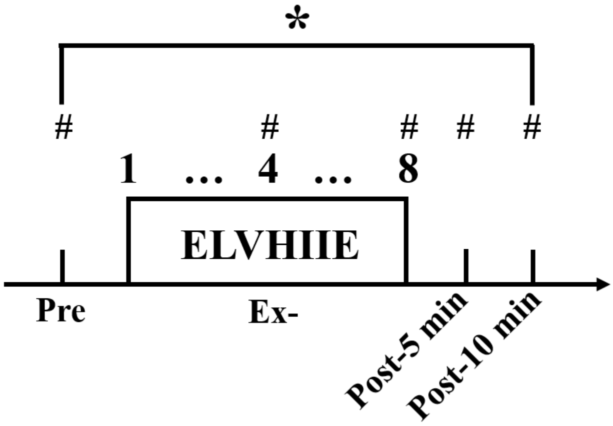

Figure 2. Experimental flowchart. Ex-: bout of HIIE test; Post-5 min: after 5 minutes of HIIE test; Post-10 min: after 10 minutes of HIIE test. Asterisk (*) indicates lactate test. Asterisk (#) indicates test heart rate and RPE.

Blood Lactate Test

The blood lactate was measured at five time points: before exercise, after the fourth and eighth bouts of exercise, and at 5 and 10 minutes after exercise. The blood lactate were analyzed using a Biosen Cline blood analysis system (EKF-diagnostic, Germany). Capillary blood samples of 10 μL were collected and added to red blood cell lysis reagent and stored at low temperature until analyzed. Prior to analysis, instrument standardization and test calibration were performed, and the coefficient of variation was determined to be ≤1.5%. The detection range for blood lactate was 0.5-40 mM [31].

Training Impulse

In this study, the exercise load was represented by the TRIMP [32], which is calculated as the product of exercise intensity and duration. To accommodate the convenience of the experiment, two different TRIMP calculation methods were used, including % HRmax (objective) and RPE (subjective). At the end of each exercise bout (8 bouts in total) and during the recovery period before the next bout (7 bouts in total), participants were asked to report their RPE, and their heart rate was recorded. This process was repeated 8 times.

% HRmax Calculation Method

During the entire HIIE, the participant's HR was recorded every 5 seconds using a heart rate monitor (iHeart Polar, Taiwan) to calculate % HRmax. The block TRIMP method developed by S. Edwards [33] was used, which divides the exercise intensity into 5 blocks with corresponding weighting factors (Table 1). The weighted score of each block was multiplied by the exercise time (min) and then summed to obtain the exercise load (arbitrary unit [AU]). The calculation formula is as follows: Exercise load = (Z1 exercise time × 1) + (Z2 exercise time × 2) + (Z3 exercise time × 3) + (Z4 exercise time × 4) + (Z5 exercise time × 5)

Table 1 The Edwards' block TRIMP calculation method

Zone	Intensity	Weighted score
Z1	50%-60% HRmax	1
Z2	60%-70% HRmax	2
Z3	70%-80% HRmax	3
Z4	80%-90% HRmax	4
Z5	90%-100% HRmax	5

RPE Calculation Method

The TRIMP calculation method of Foster, Hector [34] and Foster, Florhaug [35] was used to calculate the exercise load by multiplying the RPE value of each exercise segment by the exercise time and summing them up. The RPE scale used in this method was the CR-10 version modified by Foster, Florhaug [35] based on Borg, Ljunggren and Ceci [36] (Table 2). The calculation formula is as follows: Exercise load (AU) = Borg's CR-10 RPE score × exercise time (min)

Table 2. Borg's CR-10 RPE

0	Rest
1	Very, very easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	
7	Very Hard
8	
9	
10	Maximal

Statistical Analysis

All the data were analyzed by SPSS for Windows 20.0. Data are expressed as the mean ± standard deviation (SD) and 95% confidence interval (95% CI). A mixed design two-way analysis of variance (ANOVA) (group × time) was used to compare the variables of lactate, heart rate and training impulse between two groups before and after the 14-days of supplementation. Graphs were generated using GraphPad Prism 8.0 (GraphPad Software, San Diego, California, USA). Cohen's conventions for ES (Cohen d) were calculated by the G*Power 3.1 software program (Heinrich-Heine-Universität, Düsseldorf, Germany), where the ESs of 0.2, 0.5, and 0.8 are considered small, medium, and large, respectively. Statistical significance was set as $p < 0.05$.

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