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How Does Acute Blood Flow Restriction Resistance Training Influence Free Fatty Acids in Obese Individuals?

Haodong Tian [1,2], Qiu Xiang [3], Li Huang [1,2], Haowei Liu [1,2], Hanglin Yu [1,2], Jinlong Wu [1,2], Hansen Li [4], Jujiao Kuang [5,6], Xu Yan [5,6,7], Li Peng [1,2]

1 College of Physical Education, Southwest University, Chongqing, China

2 Key Laboratory of Physical Fitness Evaluation and Sports Function Monitoring of General Administration of Sport of China.

3 Chongqing Sports Science Research Institute

4 Sichuan Agricultural University

5 Institute for Health and Sport, Victoria University, Melbourne, Australia

6 Australia Institute for Musculoskeletal Sciences, Melbourne, Australia

7 Department of Medicine-Western Health, The University of Melbourne, Melbourne, Australia

Corresponding Author: Li Peng, Professor in Southwest University. Affiliations: Key Laboratory of Physical Fitness Evaluation and Sports Function Monitoring of General Administration of sport of China; College of Physical Education, Southwest University, Chongqing, China. Email: 804455169@qq.com

Abstract

Objective To investigate the effect of blood flow restriction resistance exercise (BFR-RE) on free fatty acids (FFAs) in obese individuals. **Methods** A two-arm randomized controlled design was employed. A total of 22 eligible subjects were randomly divided into blood flow restriction resistance exercise intervention group (BFR-RE, n=11) and traditional resistance exercise intervention group (RE, n=11). Each participant underwent an acute moderate-intensity exercise intervention. Venous blood samples were collected at Pre, Post 0h, Post 1h, and Post 24h. FFAs, ANG-II, NO, HIF-1α, and VEGF-A were measured. **Results** Significant group effects were observed in FFAs, ANG-II, VEGF-A, and NO; significant time effects were observed in FFAs and NO; significant interactions of group*time were observed in HIF-1α and NO. In BFR-RE group, FFAs significantly decreased at Post 1h and Post 24h; HIF-1α increased significantly at Post 0h, Post 1h, and Post 24h; VEGF-A significantly increased at Post 0h and then decreased until Post 24h. In RE group, FFAs also significantly decreased at Post 1h and Post 24h; HIF-1α significantly decreased at Post 24h; NO significantly decreased at Post 0h, then increased until Post 24h. **Conclusions** BFR-RE showed advantages in reducing the plasma FFAs of obese individuals compared to RE. The vasodilation and angiogenic responses induced by BFR-RE may be the reason for this difference, which supported BFR-RE as a hypoxic training modality to improve obesity.

Keywords: free fatty acids; blood flow restriction resistance exercise; obesity; hypoxia; vascular; angiogenesis

Introduction

Free fatty acids (FFAs) exists in a free, unbound form in the body, serving as vital energy substrates and forming key components of cellular membranes [1]. Obesity is the primary

contributor of abnormal plasma FFAs. The excessive expansion of adipose tissue leads to enhanced lipolysis under various physiological stresses, which promotes the extensive release of plasma FFAs [2]. Consequently, plasma FFAs levels typically remain at a relatively high levels among obese individuals, and acute elevations in plasma FFAs levels are also commonly observed [3]. These acute increases in plasma FFAs have been shown to reduce whole-body insulin-stimulated glucose uptake, causing sustained insulin resistance [4]. Furthermore, evidence also indicated that the acute lowering of the plasma FFAs can effectively improve insulin sensitivity among obese individuals [5]. Therefore, monitoring and real-time regulation of plasma FFAs levels is critically important for the obese population.

Currently, pharmacological interventions to effectively regulate plasma FFAs are highly limited [6], whereas exercise demonstrates some advantages [7,8]. However, existing researches have largely focused on the regulatory effects of aerobic exercise on plasma FFAs levels [9–11], with limited exploration of the role of resistance exercise (RE). There are evidence showing that for individuals with dyslipidemia, skeletal muscle tends to utilize plasma FFAs as an energy source [12]. This suggests that resistance exercise, which involves high engagements of skeletal muscle, may be particularly beneficial for improving FFAs metabolism. Nevertheless, given the positive relationship between exercise intensity and FFAs utilization [13], traditional RE may require relatively high loads to achieve ideal effects. However, due to the high body weight and limited exercise capacity of obese individuals, an ideal intensity for traditional resistance exercise may pose non-negligible risks. Therefore, exercise modalities more suited for obese individuals deserve further exploration.

Blood flow restriction-resistance exercise (BFR-RE), which involves using a specialized tourniquet or cuff to temporarily restrict blood flow to the working muscles during resistance exercises [14], seems to be a potential alternative to traditional RE, and its two features highlight its suitability for obese individuals. Initially, the blood flow restriction has been shown to increase the muscle recruitment and engagement during exercise, allowing for higher intensity at lower loads [15]. Additionally, the restricted blood flow associated with BFR may create a state of localized hypoxia [16], which is proved conductive to decrease plasma FFAs during exercise [17,18].

This research is designed to investigate the effects of acute BFR-RE on plasma FFAs compared to traditional RE among obese individuals. However, since the direct evidence supporting BFR-RE as a form of hypoxic training remains limited, we introduce HIF-1α

(hypoxia-inducible factor-1α) to evaluate the hypoxia in the circulating blood and propose our first hypothesis: BFR-RE can functioned as a hypoxic exercise. HIF-1α is a key factor directly regulated by hypoxic conditions, and it increase sensitively as oxygen decrease [19]. More importantly, we expect to reveal part of the mechanisms of the BFR-RE's impact on FFAs. Given the localized limb compression, we focus on vascular factors that can significantly impact blood flow. Correspondingly, we introduce Angiopoietin-II (ANG-II) to reflect vasoconstriction [20], and nitric oxide (NO) to reflect vasodilation [21]. Besides, because angiogenesis serves as a critical modulator in hypoxia, we involved vascular endothelial growth factor-A (VEGF-A), which functions as the most remarkable factor stimulating angiogenesis in a strictly dose-dependent manner. The investigation would contribute to verify our second hypothesis: BFR-RE's regulation on vascular factors is one of the physiological mechanisms influencing FFAs.

Methods

Participants

Fifty-five male volunteers with obesity were initially recruited for this study. Following a rigorous screening process by two researchers, 25 eligible subjects were finally included. The criteria for inclusion were as follows: (1) being male with a body fat percentage (BF%) greater than 25% [22], (2) having no physical activity limitations, (3) not consuming medications or supplements that significantly affect metabolism, and (4) no regular exercise in the past three months, exercising no more than once per week.

Study design

A two-arm randomized controlled design is employed to determine the effects of BFR-RE and RE on obese individuals. All participants provided informed consent before taking part in the study and underwent a screening for exercise-related risks. Subsequently, the participants were divided into two groups using a random number table method: the blood flow restricted resistance exercise intervention group (BFR-RE, n=13) and traditional resistance exercise intervention group (RE, n=12). Basic information of participants included is presented in table 1.

Intervention

All participants underwent the same intervention procedure (Figure 1) under identical conditions (room temperature of 20 ~ 25℃ and air humidity between 40% ~ 50%). Prior to the intervention, there was a 1-week familiarization period during which the participants received

exercise test to determine the exercise intensity. Additionally, 3 days before the exercise, participants were provided a standardized diet prepared by a professional nutritionist. The diets plan contains approximate ratio of 5:3:2 for carbohydrates, proteins, and fats, while excluding oily and spicy foods. During the intervention, venous blood samples were collected at four time points, including pre-intervention (Pre), immediately post-intervention (Post 0h), 1 hour post-intervention (Post 1h), and 24 hours post-intervention (Post 24h) [23,24]. During the intervention, one participant withdrew from the BFR-RE group voluntarily, and two blood samples (one from each group) did not meet the criteria. This left 11 eligible samples per group.

Resistance Training

RE protocols of the two groups were the same, which targeted at the major muscle groups. The RE sequence comprised of a combination of lower body exercises, including lunges, squats, and standing calf raises; upper body exercises, including biceps curls, lateral raises, and overhead arm extensions; and core training exercises (without any external load), including abdominal crunches, back raises, and planks. Visual demonstrations of the exercises are shown in figure 2. During each exercise session, participants performed the plank exercise for a duration of 30 seconds. For the remaining exercises, 15 repetitions were executed per set. Both interventions involved two sets for each exercise, with a 30-second rest period between sets.

The determination of intensity was achieved during the familiarization period. A talk test based on BOK et al. [25] was employed, in which the participants engaged in a conversation with the experimenters at a stable pace, enabling clear expression of immediate sensations. For obese participants with substantial body weight and joint pressure, we employed lightweight dumbbells for intensity test. Dumbbells weighing 5kg, 7.5kg, and 10kg were prepared for the test. Starting with the 5kg dumbbell, participants completed a full training session while responding to inquiries about their rated perceived exertion (RPE). For RPE evaluation, we employed the Borg Category-Ratio-10 Scale (CR10), which was considered suitable for RE as it exhibit clear liner correlations with some primary fatigue indicators [26]. The experimenters assessed the appropriateness of the load based on the participants' responses during the conversation and their external performance, with targeted RPE set at CR10: 4~5. If the load was deemed appropriate, the test was concluded. If not, the subjects rested for 5 minutes after completing a set of exercises before proceeding to the next weight for testing. This process was repeated until an appropriate load was determined. Consequently, we observed minimal

differences in strength levels among the participants. Ultimately, a pair of 5kg dumbbell was chosen for BFR-RE and 7.5kg dumbbell for RE.

Blood Flow Restriction

To restrict blood flow, two pairs of pneumatic cuffs (manufactured by Bstrong corporation, Park City, USA) were used. Cuffs with a length range of 12~17.5 inches were employed for the upper limbs, and 17.5~57.5-inche cuffs for the lower limbs. Each participant's arterial occlusive pressure (AOP) was calculated based on his thigh dimension [14,27]. For the lower limbs, 80% of the AOP was employed as the pressure, while for upper limbs, the pressure was set 100mmHg lower than lower limbs. The pressure scheme for participants in the BFR-RE group is outlined in Table 2.

Outcome measures

ELISA (Enzyme-Linked Immunosorbent Assay, manufactured by HengYuan Biological Technology corporation, Shanghai, China) was utilized to measure FFAs, hypoxia inducible HIF-1α, ANG-II, NO, and VEGF-A. Blood samples were collected and processed by professional medical personnel at pre-defined time points following the outlined procedures: (1) Venous blood was drawn from the elbow using a vacuum blood collection tube with no additives. The collection tube was then placed in a rack and allowed to clot naturally at 20-24°C for at least 30 minutes. (2) Using a pipette, the upper layer of serum from the blood collection tube was transferred to a centrifuge tube, followed by centrifugation at 4°C and 3000 rpm for 5 minutes. (3) After centrifugation, the supernatant (clear, pale-yellow fluid) was carefully transferred to two new centrifuge tubes, with each sample containing no less than 150μL, and accurately recorded and labeled. (4) All collected samples were placed in a dry ice box before concluding each batch of sample collection and rapidly transferred to a -80°C freezer for preservation. (5) After the completion of the experiment, all frozen samples were transported in a dry ice shipping box during analysis phase.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 and GraphPad Prism 8.0 software. The Shapiro-Wilk method was used to test whether the data were normally distributed. For parametric data following a normal distribution, values were presented as mean ± standard deviation (SD), while for non-normally distributed data, the median ± interquartile range (IQR) was used. In dependent samples t-test was employed to analyze the intergroup differences in normal baseline, and Mann-Whitney U-test was employed to analyzed the intergroup

differences in non-normal baseline. Generalized estimating equations (GEE) were used to test the main effect of time, group, and group*time interaction, and simple effect of time and group would be measured if the interaction of them was significant.

Results

Baseline Comparison

There were significant baseline differences in HIF-1a ($p < 0.01$), and VEGF-A ($p < 0.01$) levels between the two groups, as shown in Table 3. To reduce the interference caused by baseline differences, we included the baseline of HIF-1α and VEGF-A as covariate in the generalized estimating equations (GEE).

Effects of Different Interventions on FFAs and hypoxia

Table 4 demonstrated the results of GEE conducted on the levels of FFAs and HIF-1α in the two groups. For FFAs, significant effects of group ($p < 0.05$) and time ($p < 0.001$) were observed. While for HIF-1α, only significant effect of group*time ($p < 0.001$) was observed.

As is shown in GEE that no interaction of time and group was significant in FFAs, Bonferroni multiple comparisons were employed to analyze the specific variations of FFAs. Figure 3 indicated that both BFR-RE and RE resulted an extremely decrease in FFAs at Post 1h ($p < 0.001$). While FFAs exhibited a more significant decrease in BFR-RE group ($p < 0.001$) at Post 24h than RE group ($p < 0.05$).

Due to the significant time*group interaction observed in HIF-1α, the simple effect analysis was further conducted. Time's simple effects were found significant both in BFR-RE group (Wald $\chi^2 = 21.61$, $p = 0.00$) and RE group (Wald $\chi^2 = 8.99$, $p = 0.03$). Figure 3 demonstrated the differences between time points. In BFR-RE group, HIF-1α increased significantly from Pre to Post 0h ($p < 0.01$). Its levels at Post 1h ($p < 0.05$) and Post 24h ($p < 0.001$) are both significantly higher than that at Pre. While in RE group, HIF-1α decreased significantly from Pre to Post 24h ($p < 0.05$). Groups' simple effects indicated significant differences between groups at Pre (Wald $\chi^2 = 16.43$, $p = 0.00$) and Post 24h (Wald $\chi^2 = 8.85$, $p = 0.00$).

Effects of Different Interventions on vascular factors

Table 5 demonstrated the results of generalized estimating equations conducted on the levels of ANG-II, VEGF-A, and NO. For ANG-II (Wald $\chi^2 = 13.50$, $p = 0.00$) and VEGF-A (Wald $\chi^2 = 25.57$, $p = 0.00$), only significant effect of group was observed. While for NO,

significant effects of group (Wald $\chi^2 = 106.86$, $p = 0.00$), time (Wald $\chi^2 = 15.72$, $p = 0.00$), group*time (Wald $\chi^2 = 69.00$, $p = 0.00$) were observed.

As is shown in GEE that no interaction of time and group was significant in ANG-II and VEGF-A, Bonferroni multiple comparisons was employed to analyze the specific variations of them. As demonstrated by figure 3, no significant variation of ANG-II over time was observed in both BFR-RE group and RE group. While at Post 24h, ANG-II level in BFR-RE group was significantly higher than that of RE group ($p < 0.01$). And for VEGF-A, significant variations over time were observed only in BFR-RE group. VEGF-A increased significantly at Post 0h, then decreased significantly from Post 0h to 24h ($p < 0.01$). And its levels at Post 0h ($p < 0.001$) and Post 1h ($p < 0.01$) are both significantly higher than that at Pre.

Since significant time*group interaction was observed in NO, the simple effects analysis was conducted. Time's simple effects were found significant both in BFR-RE group (Wald $\chi^2 = 11.82$, $p = 0.01$) and RE group (Wald $\chi^2 = 102.75$, $p = 0.00$). In BFR-RE group, NO increased significantly ($p < 0.01$) at Post 0h, then decreased significantly ($p < 0.05$) from Post 0h to 24h. While in RE group, NO decreased significantly ($p < 0.001$), then increased significantly ($p < 0.001$) from Post 0h to 24h. Groups' simple effects indicated significant differences between groups at Post 0h (Wald $\chi^2 = 233.39$, $p = 0.00$), Post 1h (Wald $\chi^2 = 11.71$, $p = 0.00$), and Post 24h (Wald $\chi^2 = 17.40$, $p = 0.00$).

Discussions

To our knowledge, existing studies that employed RE to regulate plasma FFAs of obese individuals remains limited. Besides, this may be the first study to investigate the impact of BFR-RE on FFAs, which may introduce a new exercise modality for obese individuals. The primary finding of the study was that BFR-RE decreased plasma FFAs more significantly than RE, despite both being effective. Specifically, FFAs in two groups both exhibited extremely significant decrease ($p < 0.001$) at Post 1h, which contribute most to the variance throughout the whole observation. However, FFAs in BFR-RE group continuously decreased from Post 1h to Post 24h, while the RE counterparts increased during this phase. This may explain the significant group effect observed, and the different impacts on FFAs between the two exercises (BFR-RE: $p < 0.001$ vs. RE: $p < 0.05$) indicate the advantages of BFR-RE in FFAs consumption.

Unfortunately, we found limited direct evidence to support the advantage of BFR-RE in improving plasma FFAs levels. Nevertheless, there are reviews that reported the correlation between adipose tissue hypoxia (ATH) and obesity [28], And inhibition of adipogenesis and triglyceride synthesis by hypoxia can result the elevated FFAs in the blood during obesity [29]. Hypoxic training has been shown to enhance the hypoxic ventilatory response, thus improving metabolic dysregulation in obese individuals [30]. These observations implied that the decrease in plasma FFAs may come from the hypoxia response induced by BFR.

To confirm the establishment of hypoxia in BFR-RE, we primarily investigated the HIF-1α that directly regulated by hypoxia. In our study, BFR-RE induced a significant increase in HIF-1α levels throughout the entire observation period, with particularly noticeable effects ($p < 0.01$) during the exercise. Similarly, Muangritdech, et al used intermittent hypoxic breathing to establish a conventional hypoxic training model, which successfully induced an increase in plasma HIF-1α and NO levels in the subjects [31]. Besides, Matthew et al. reported a progressive decrease in tissue saturation index (68% to 58%) with increasing BFR (0% LOP to 80% LOP) [32], and local hypoxia tends to be a primary physiological mechanism underlying the training benefits induced by BFR [33]. These findings supported our first hypothesis that BFR-RE may functioned as hypoxic exercise.

In order to reveal the correlation of vasoconstriction and hypoxia induced by BFR, we investigate the variations in ANG-II. And according to our second hypothesis, we assume that BFR-RE may contribute to the vasoconstriction. While surprisingly, no significant change was observed in both groups. This may imply that BFR-RE may not regulate hypoxia by vasoconstriction. Nevertheless, ANG-II exhibited significantly higher level in BFR-RE group that RE counterpart at Post 24h. The delayed effects of the two exercises appear to promote this difference, and we recommend further studies to replicate the investigation. Except for ANG-II, variations in VEGF-A and NO were observed. In BFR-RE group, both two factors significantly increased (VEGF-A: $p < 0.001$, NO: $p < 0.01$) at Post 0h. And the impact of VEGF on NO may explain their synchronic growth, VEGF can upregulate the NO by activate endothelial NO synthase (eNOS) [34,35] and inhibiting NO synthesis can neutralize proangiogenic effects [36]. Subsequently, both VEGF-A and NO exhibited significant recovery. Given the similar trends of VEGF-A and NO, we additionally found that changes of VEGF-A were more pronounced during the whole observation (upwards, $p_{\text{VEGF-A}}$ vs. p_{NO}: 0.001 vs. 0.01; downwards, $p_{\text{VEGF-A}}$ vs. p_{NO}: 0.01 vs. 0.05). This corroborates the downstream involvement of

NO in VEGF-mediated regulation of angiogenesis [37]. In RE group, no significant change of VEGF-A was observed, which indicate that RE may not impact the angiogenesis. However, NO decreased extremely significant ($p < 0.001$) right after RE, and increased significantly until Post 24h. Consequently, these variations have led to significantly differences between the two groups. This result contradicts a known physiology fact that muscle contraction can promote the generation of NO [38,39]. In addition, Bradley et al. found that NOS (dominate the production of NO) is unaffected by physical exercise [40]. Nonetheless, existing evidences mainly employed aerobic exercises to validate the effect on NO, and the results of NO in our study implicate that RE's effect remains further explore. By integrating the results in ANG-II, VEGF-A, and NO, we found that BFR-RE may impact the hypoxia by regulating angiogenesis and vasodilation rather than vasoconstriction.

Conclusion

Our research showed that both BFR-RE and RE acutely reduced plasma FFAs in obese individuals, but BFR-RE had a stronger FFA-lowering effect. The significant vasodilation and angiogenic responses induced by the localized hypoxia of BFR-RE may be the primary reason for this difference, supporting the use of BFR-RE as a hypoxic training modality to improve obesity.

Author's contribution

Conceptualization, HDT, and LP; methodology, HDT, and QX; software, LH, and HWL; check, HDT, and QX; formal analysis, HDT; investigation, LP; resources, LP; data curation, HLY, JLW, and HSL; writing - rough preparation, HDT; writing - review and editing, JJK, YX, and LP; visualization, HDT, and QX; supervision, LP; project administration, LP; receiving funding, LP All authors have read and agreed with the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by ethics committee of of Southwest University Hospital (Ethics Approval NO. SWU-ETH-2023-07-17-011).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participants to publish this paper.

Data Availability Statement

The data used in this study belongs to the research team, and any requests for access must be approved by all authors.

Conflict of Interest Statement

All authors declared that there no conflicts of interest.

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Table 1. Basic Information of Participants

Group	Age (years)	Weight (kg)	BMI (kg/m^2)	BFP (%)	SMM (kg)
BFR-RE	21.36±2.11	90.06±6.47	28.26±1.66	30.57±4.45	34.99±3.75
RE	20.91±1.51	89.59±8.98	28.72±1.95	30.19±3.78	35.29±4.56
t	0.58	0.14	-0.59	0.22	-0.17
p	0.57	0.89	0.56	0.83	0.87

BMI: body mass index; BFP: body fat percentage; SMM: skeletal muscle mass

Table 2. Pressure Scheme for BFR-RE group

Participants	Thigh Dimension (cm)	AOP（mmHg）	Lower pressure（mmHg）	Upper Pressure（mmHg）
1	64	350	280	180
2	61	350	280	180
3	62	350	280	180
4	54	250	200	100
5	55	250	200	100
6	58	300	240	140
7	57	300	240	140
8	61	350	280	180
9	57	300	240	140

10	52	250	200	100
11	56	300	240	140

Table 3. Baseline comparison

Indicator	BFR-RE	RE	t (z)	p
FFAs (μmol/L)	10.16±0.47	9.73±0.62	1.81	0.09
HIF-1α (ng/L)	80.35±3.53	86.20±3.55	-3.87***	0.00
ANG-II (ng/L)	36.12±1.76	33.31±4.88	1.8	0.09
NO (μg/L)	209.11±7.38	203.67±21.48	0.79	0.44
VEGF-A (pg/ml)	393.18±19.5	334.07±50.25	3.637***	0.00

*$P < 0.05$: significant; **$P < 0.01$: very significant; ***$P < 0.001$: extremely significant

Table 4. Repeated measurements of FFAs and HIF-1α

Indicators		FFAs (μmol/L)		HIF-1α (ng/L)	
Groups		BFR-RE	RE	BFR-RE	RE
Pre		10.16±0.47	9.73±0.62	80.35±3.53	86.20±3.55
Post 0h		9.62±0.43	9.46±0.44	86.08±3.68	83.89±5.2
Post 1h		9.37±0.47	8.97±0.59	85.18±2.72	82.13±5.73
Post 24h		10.03±0.48	8.69±1.18	87.62±3.83	81.91±5.37
group	Wald c^2	4.26*		2.42	
	p	0.04		0.12	
time	Wald c^2	180.59***		3.11	
	p	0.00		0.38	
time*group	Wald c^2	4.14		29.30***	
	p	0.25		0.00	

*P < 0.05: significant; **P < 0.01: very significant; ***P < 0.001: extremely significant

Table 5. Repeated measurements of vascular factors

Indicators		ANG-II (ng/L)		VEGF-A (pg/ml)		NO (μg/L)	
Groups		BFR-RE	RE	BFR-RE	RE	BFR-RE	RE
Pre		36.12±0.51	33.31±1.40	393.18±5.61	333.16±14.67	209.11±2.12	203.67±6.18
Post 0h		34.79±0.47	33.38±1.76	441.96±3.97	354.61±22.12	221.06±2.89	174.87±1.53
Post 1h		35.33±0.41	35.36±1.76	426.64±5.78	328.26±21.12	217.25±3.31	187.67±7.50
Post 24h		36.78±0.39	31.89±1.06	413.47±5.44	346.99±23.61	210.86±2.16	191.36±4.00
group	Wald χ^2	13.50***		25.57***		106.86***	
	p	0.00		0.00		0.00	
time	Wald χ^2	1.18		5.91		15.72**	
	p	0.76		0.12		0.00	
time*group	Wald χ^2	4.30		2.38		69.00***	

p	0.231	0.50	0.00

*P < 0.05: significant; **P < 0.01: very significant; ***P < 0.001: extremely significant

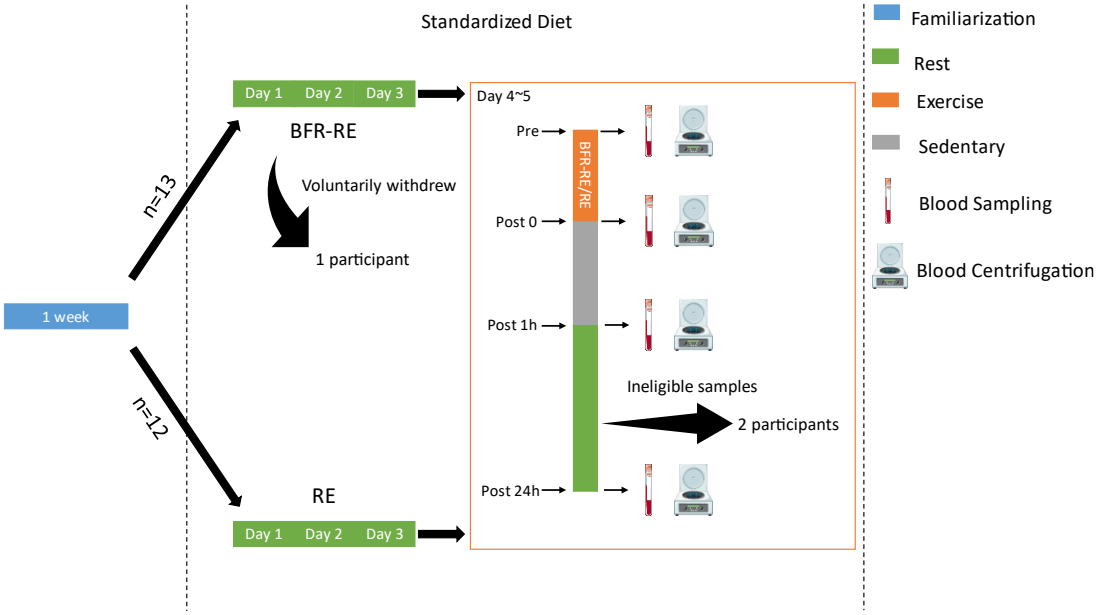

Figure 1. Experimental Procedure

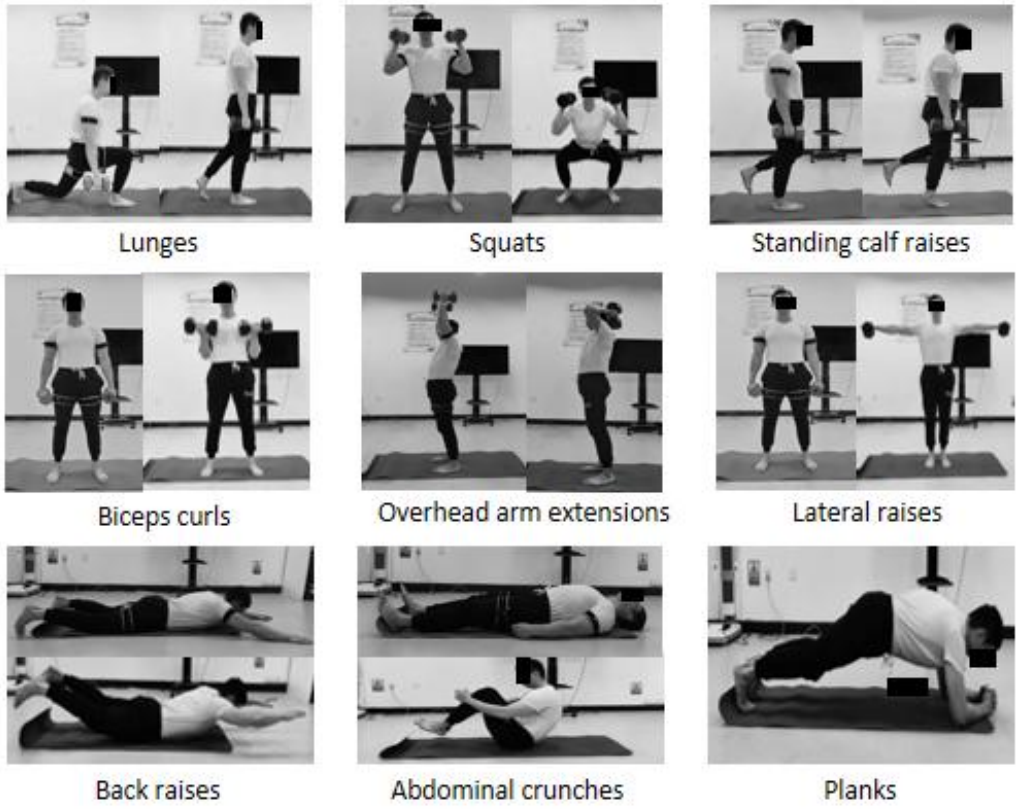

Figure 2. Exercise Demonstrations

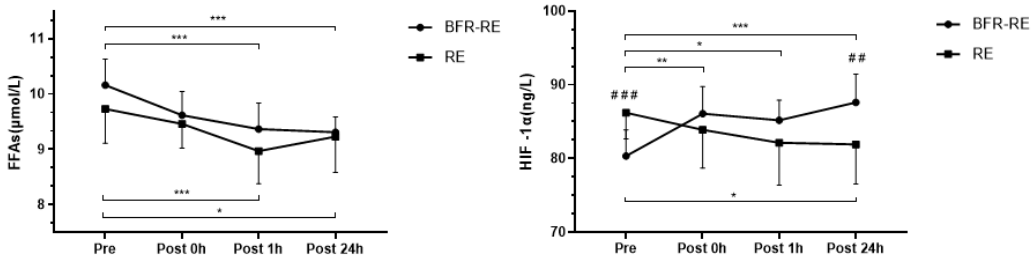

Figure 3. The variations in FFAs and HIF-1α

"*": significant difference between time points; "#": significant difference between groups. "*": $p < 0.05$, significant; "**": $p < 0.01$, very significant; "***": $p < 0.001$, extremely significant. "#": $p < 0.05$, significant; "##": $p < 0.01$, very significant; "###": $p < 0.001$, extremely significant.

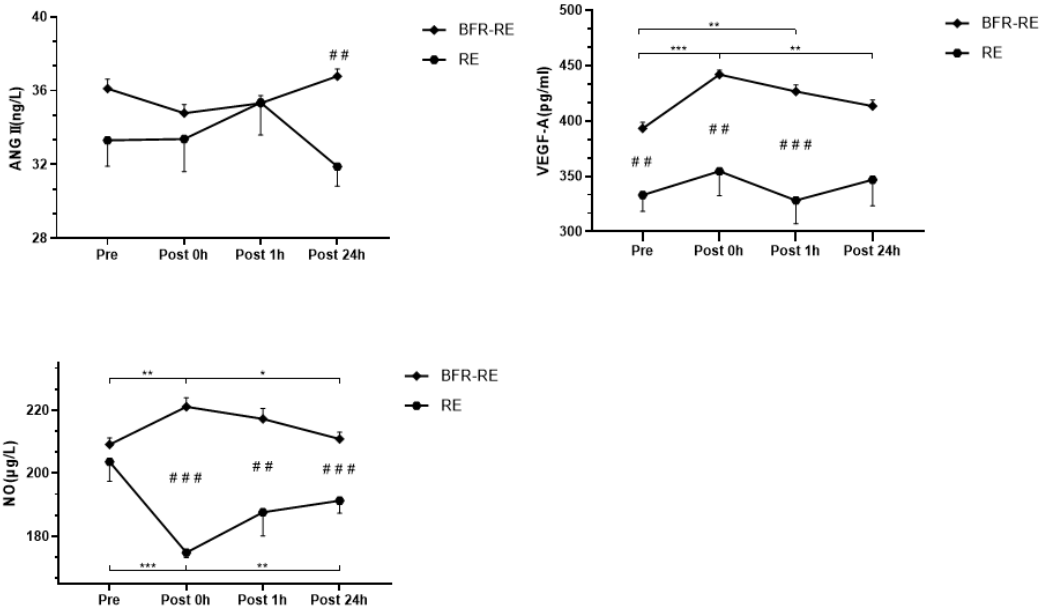

Figure 4. The variations in ANG-II, VEGF-A, and NO

"*": significant difference between time points; "#": significant difference between groups. "*": $p < 0.05$, significant; "**": $p < 0.01$, very significant; "***": $p < 0.001$, extremely significant. "#": $p < 0.05$, significant; "##": $p < 0.01$, very significant; "###": $p < 0.001$, extremely significant.