

Research Article

Effects of six weeks of metabolic conditioning exercise on serum IGF-1 and GPX responses in active young adults: A randomized controlled trial

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Abstract

The present study aimed to investigate the effect of a six-week metabolic conditioning training program on serum levels of insulin-like growth factor-1 (IGF-1) and glutathione peroxidase (GPX) in physically active young adults. This randomized controlled trial employed a pre-test–post-test design with a control group. A total of 23 physical education students (aged 18–24 years) were purposively recruited and randomly assigned to an exercise group ($n = 12$) or a control group ($n=11$). The exercise group participated in a six-week metabolic conditioning circuit training program (three sessions per week, 18 sessions total), with progressive overload in volume and intensity. The control group maintained their usual daily activities. Blood samples were collected before and after the intervention to measure IGF-1 and GPX concentrations using validated ELISA kits. The primary between-group analysis using ANCOVA, after adjusting for baseline values, revealed no statistically significant difference between the groups in post-test IGF-1 levels ($F(1,20)=1.339$, $p=0.261$, $\eta^2=0.063$) or in post-test GPX levels ($F(1, 20)=0.682$, $p=0.419$, $\eta^2=0.033$). A secondary within-group analysis showed a significant decrease in IGF-1 within the exercise group ($p=0.047$); however, this finding must be interpreted with caution given the negative primary between-group comparison. No significant within-group changes were observed for GPX ($p>0.05$). Anthropometric measures (weight and BMI) remained stable in both groups throughout the study. Based on the primary between-group comparisons, these findings indicate that six weeks of metabolic conditioning does not produce statistically significant changes in serum IGF-1 or GPX levels compared to a control condition in physically active young adults.

Key Words: Metabolic conditioning program, Insulin-like growth factor-1, Glutathione peroxidase, Antioxidant enzyme

Introduction

Metabolic conditioning exercise has emerged as a time-efficient training modality that combines high-intensity aerobic and resistance exercises in circuit-based formats to elicit comprehensive physiological adaptations (Alsamir Tibana et al., 2019). This training approach, characterized by progressive overload in volume and intensity with reduced rest intervals, targets multiple energy systems simultaneously and has been shown to improve cardiovascular fitness, muscular strength, and metabolic health markers in young adults (Mikeska, 2014). The biochemical responses to metabolic conditioning, particularly alterations in anabolic and antioxidant systems, remain an area of active investigation given the complex interplay between exercise-induced stress and adaptive signaling (Leeuwenburgh et al., 1997; Xie et al., 2025).

Insulin-like growth factor-1 (IGF-1) serves as a critical mediator of exercise-induced anabolic responses, playing pivotal roles in muscle protein synthesis, tissue repair, and metabolic regulation (Kwon et al., 2025). Circulating IGF-1 levels are influenced by exercise modality, intensity, duration, and training status, with systematic reviews indicating that chronic exercise interventions lasting beyond 8 weeks typically increase serum IGF-1 in healthy adults (Chu et al., 2025). However, the temporal dynamics of IGF-1 adaptation remain controversial, as some studies report no significant changes following shorter intervention periods, particularly in already active populations where baseline levels may be near optimal (Chu et al., 2025; Meyer et al., 2024).

Glutathione peroxidase (GPX), a key antioxidant enzyme in the endogenous defense system, catalyzes the reduction of hydrogen peroxide and lipid peroxides, thereby protecting cellular structures from oxidative damage (Jomova et al., 2024). Regular exercise training has been demonstrated to upregulate GPX activity through repeated exposure to exercise-induced reactive oxygen species, which serve as signaling molecules triggering antioxidant gene expression (Wibawa et al., 2025). Nevertheless, the magnitude and time course of GPX

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adaptation appears to depend on training duration, with meta-analytic evidence suggesting that significant increases in GPX activity typically require interventions exceeding 4–6 weeks, particularly in previously trained individuals (Xie et al., 2025). Given the limited evidence regarding metabolic conditioning effects on these specific biomarkers in active young adults, the present study aimed to investigate the impact of a six-week metabolic conditioning program on serum IGF-1 and GPX concentrations using a controlled pre–post intervention design.

Materials and methods

Study design

This applied, randomized controlled trial used a pre-test–post-test design with a control group. Following the pre-test assessment of the participants' physical characteristics, eligible participants were randomly allocated to either an exercise group or a control group. The exercise group participated in a six-week metabolic conditioning training program, whereas the control group continued its usual daily activities and did not participate in any organized exercise program during the study period.

Ethical considerations

All procedures involving human participants were conducted in accordance with the ethical principles of human-subject research. Before participation, the study objectives, procedures, potential benefits, and possible risks were explained to the participants. Participation was voluntary, and written informed consent was obtained from all participants.

The participants were informed that their personal information and assessment results would remain confidential and would be used only for scientific purposes. They were also informed of their right to withdraw from the study at any stage without consequences. Eligibility and exclusion criteria were applied to protect participant safety, and all training sessions were supervised by the researcher. Warm-up, cool-down, and safety procedures were implemented to reduce the risk of injury. The study was approved by the ethical committee of Islamic Azad University, Tehran, Iran, under approval code IR.IAU,SRB.REC.1404.365.

Participants

The study population consisted of physical education students. A total of 23 participants were recruited voluntarily through purposive sampling. Initially, all volunteers underwent body-composition assessment using a body analyzer (Anea BIA, Iran). The device provides a composite "Physical Fitness Index" (PFI) score ranging from 0 to 100, calculated from bioelectrical impedance parameter include muscle mass, body fat percentage

, and phase angle. According to the manufacturer's normative database, a PFI score ≥ 70 is classified as indicative of a healthy, physically active body composition profile. Therefore, only volunteers who obtained a score of 70 or higher proceeded to the next stage of the study.

The inclusion criteria were an age of 18–24 years, no tobacco or alcohol use, no underlying disease or musculoskeletal injury, self-reported adherence to a healthy diet (verified solely via verbal confirmation at the time of recruitment; no formal dietary logs, food frequency questionnaires, or objective nutritional biomarkers were used to monitor or verify dietary compliance during the study period), and avoidance of fast food, energy drinks, and sugar-sweetened beverages. The participants' body weight ranged from 52.3 to 97.8 kg, height ranged from 171 to 191 cm, and BMI ranged from 17.9 to 27.1 kg/m².

After eligibility was confirmed, the participants were randomly assigned to an exercise group ($n=12$) or a control group ($n=11$). The exclusion criteria were failure to maintain the inclusion criteria, absence from more than one training session, and occurrence of any injury during the study. The sample size was determined using G*Power software (version 3.1) for a two-group ANCOVA design with the pre-test value as the covariate. The following parameters were used: anticipated large effect size (Cohen's $f=0.40$), $\alpha=0.05$, power $(1-\beta)=0.80$, and a strong expected correlation ($r\geq 0.50$) between pre-test and post-test measures, which is typical for repeated physiological measurements. This calculation yielded a minimum total sample size of 22 participants (11 per group). Allowing for a 10% attrition rate, we recruited 23 participants, with 12 allocated to the exercise group and 11 to the control group.

Anthropometric measurements

Body weight and BMI were assessed before and after the six-week intervention using a body analyzer (Anea BIA, Iran). Height was recorded in centimeters, and body weight was recorded in kilograms. BMI was calculated in kilograms per square meter (kg/m²). The same measurement procedures and equipment were used at both assessment stages.

Metabolic conditioning training protocol

The metabolic conditioning training protocol was designed according to the model proposed by Michael Jett and colleagues (2013) and was modified and adapted according to the participants' fitness levels, characteristics, and the objectives of the study (Jett & Swank, 2013). The intervention lasted six weeks and consisted of three sessions per week, resulting in 18 training sessions.

A progressive-overload principle was applied throughout the pro-

-gram. Training volume was increased every two weeks, while the rest interval between sets was reduced. Exercise intensity and external load were determined using the Movement Competency Screen, as described by Wright and colleagues (2016) (Wright & Laas, 2016), to ensure that the training demands were appropriate for the participants' movement abilities. All participants were classified at level 6; therefore, they were considered capable of performing high-intensity exercises with greater training loads and shorter rest intervals. Each training session was performed as a circuit consisting of seven stations. After completing one station, the participants proceeded to the next station following the prescribed rest interval. The equipment included a 7-cm step, a 7-kg medicine ball, two 15-kg dumbbells, a SEG stationary bicycle, a metronome, a stopwatch, and a measuring tape.

During weeks 1 and 2, each exercise was performed for 2 sets, with a 60-s rest interval between sets. During weeks 3 and 4, the number of sets increased to 3, and the rest interval decreased to 45 s. During weeks 5 and 6, the exercises were performed for 4 sets, with a 30-s rest interval between sets. The circuit consisted of the following seven stations:

1. Skipping: Four repetitions over a distance of 20 m were performed in each set throughout the six-week program. The number of sets increased from 2 to 4 according to the progression of the program.
2. Step-up exercise: The exercise was performed on a 7-cm step for 1 min using a metronome and a 1:1 rhythm, corresponding to one step up and one step down. The duration remained 1 min throughout the program, while the number of sets increased from 2 to 4.
3. Dumbbell hang squat: Eight repetitions were performed in each set using a 15-kg dumbbell. The number of sets progressively increased from 2 to 4.
4. Medicine-ball lunge: One repetition over a distance of 20 m was performed in each set. The number of sets followed the progressive increase from 2 to 4 sets.
5. Push-up: Eight repetitions were performed in each set.
6. Medicine-ball overhead slam: Ten repetitions were performed in each set using a 7-kg medicine ball.
7. Stationary cycling: The exercise was performed for 2 min during each stage. The cycling resistance increased progressively from level 4 during weeks 1 and 2, to level 5 during weeks 3 and 4, and to level 6 during weeks 5 and 6.

Before each training session, the participants performed approximately 10 min of general and specific warm-up, including light jogging, dynamic stretching, and joint-mobility exercises. At

the end of each session, approximately 5–10 min of cool-down consisting of slow walking and static stretching was performed.

All training sessions were conducted under the direct supervision of the researcher. The participants were instructed to avoid strenuous physical activity outside the prescribed program, tobacco, alcohol, energy drinks, and major dietary changes throughout the study period.

Blood sampling and biochemical measurements

Blood samples were collected at the pre-test and post-test stages to assess IGF-1 and GPX (in a fasting state). To minimize the possible influence of acute exercise, post-test blood sampling was performed 48 hours after the final training session, under the same pre-analytical conditions as the pre-test sampling, including the same time of day (between 8:00 and 10:00 a.m.) and comparable pre-sampling restrictions (identical fasting duration of 10–12 hours and avoidance of strenuous physical activity for 48 hours prior to both sampling time points). The 48-hour interval was selected to allow for the resolution of acute exercise-induced transients in circulating biomarkers while preserving the chronic adaptations induced by the six-week training program.

Blood samples were collected into appropriate collection tubes and allowed to clot when serum was required. The samples were then centrifuged, and the separated serum was transferred into labeled tubes. Serum samples were stored at an appropriate temperature below zero until biochemical analysis, and repeated freeze–thaw cycles were avoided. IGF-1 (Karmaniparsgen, Iran) and GPX (KPG-GPXNV, Karmaniparsgen, Iran) concentrations were measured using validated commercial assay ELISA kits according to the manufacturers' instructions. The assay performance characteristics provided by the manufacturer were as follows: for IGF-1, the detection range was 25–800 ng/mL with a sensitivity of 2.5 ng/mL, an intra-assay coefficient of variation (CV) of <5.0%, and an inter-assay CV of < 8.0%; for GPX, the detection range was 20–200 U/L with a sensitivity of 3.0 U/L, an intra-assay CV of <6.0%, and an inter-assay CV of < 9.0%. All samples were processed in a single analytical batch for each biomarker to minimize inter-assay variability.

Statistical analysis

Data were analyzed using SPSS software, version 26. Descriptive statistics were used to report the study variables as mean $\pm$ standard deviation. The Shapiro–Wilk test was used to assess the normality of data distributions, and Levene's test was used to evaluate the homogeneity of variances.

The primary analytical comparison was the between-group difference at post-test, assessed using analysis of covariance (ANCOVA), with the corresponding pre-test value entered as the

Table 1. Physical characteristics of the participants at the two assessment stages.

Variable	Time	Control group	Exercise group
Age (year)	—	20.45 ± 2.16	19.83 ± 1.57
Height (cm)	—	181.45 ± 4.43	181.41 ± 6.78
Weight (kg)	Pre-test	75.50 ± 10.47	74.76 ± 12.12
	Post-test	75.46 ± 10.74	74.55 ± 11.33
BMI (kg/m²)	Pre-test	22.93 ± 2.32	22.38 ± 2.97
	Post-test	22.88 ± 2.33	22.29 ± 2.58

covariate. Secondary, within-group changes between the pre-test and post-test stages were examined using paired-samples t-tests. Statistical significance was set at $p \leq 0.05$. Graphs were prepared using GraphPad Prism, version 9.

The statistical procedures are consistent with reporting practice for pre-test–post-test designs in randomized controlled trials. The trial was not registered in a clinical trial registry; this is acknowledged as a limitation. The G*Power parameters used for sample size calculation should be reported as follows: effect size $f=0.25$, $\alpha=0.05$, power $(1-\beta)=0.80$, which yielded a minimum sample size of 22 participants (11 per group), consistent with the 23 participants enrolled.

Results

Anthropometric measurements

The anthropometric and physical characteristics, including age, height, weight, and body mass index (BMI), are presented as mean±standard deviation in Table 1.

Like Growth Factor-1

The results indicated that six weeks of metabolic conditioning exercise did not produce a statistically significant between-group effect on insulin-like growth factor-1 (IGF-1) in active young adults when assessed as the primary ANCOVA comparison. Descriptive statistics and within-group results for IGF-1 at the pre-test and post-test stages are presented in Table 2.

The secondary within-group analysis showed no significant change in IGF-1 in the control group ($p=0.615$), whereas a significant decrease was observed in the exercise group ($p=0.047$). The Levene's test indicated that the homogeneity of variances assumption was satisfied for IGF-1 ($p=0.433$). The primary between-group analysis using analysis of covariance (ANCOVA), adjusted for pre-test IGF-1 values, showed no statistically significant difference between the control

Table 2. Descriptive statistics and within-group comparisons of IGF-1 levels in the control and exercise groups.

Variable	Time	Control group	Exercise group
IGF-1	Pre-test	189.90 ± 46.12	194.83 ± 30.80
	Post-test	185.81 ± 58.97	177.91 ± 34.35
p-value	—	0.615	0.047

and exercise groups at the post-test stage, $F(1,20)=1.339$, $p=0.261$, $\eta^2=0.063$. The ANCOVA results are presented in Table 3.

After adjusting for the effect of the pre-test values, the primary between-group comparison confirmed no statistically significant difference between the study groups in post-test IGF-1 values ($p=0.261$). The means and standard deviations for IGF-1 are presented in Figure 1.

Glutathione peroxidase

The results indicated that six weeks of metabolic conditioning exercise did not have a statistically significant between-group effect on glutathione peroxidase (GPX) in active young adults when assessed as the primary ANCOVA comparison. Descriptive statistics and within-group results for GPX at the pre-test and post-test stages are presented in Table 4.

The secondary within-group analysis showed no significant change in GPX in either the control group ($p=0.441$) or the exercise group ($p=0.390$).

For GPX, Levene's test indicated that the homogeneity of variances assumption was satisfied ($p=0.381$). The primary between-group analysis using ANCOVA, adjusted for pre-test GPX values, showed no statistically significant difference between the control and exercise groups at the post-test stage, $F(1,20)=0.682$, $p=0.419$, $\eta^2=0.033$. The ANCOVA results are presented in Table 5.

After adjusting for the effect of the pre-test values, the primary between-group comparison confirmed no statistically significant difference between the study groups in post-test GPX values ($p=0.419$). The means and standard deviations for GPX are presented in Figure 2.

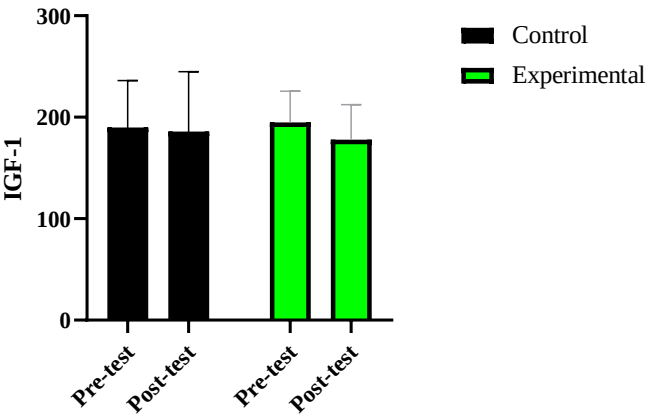


Figure 1. Mean ± standard deviation of IGF-1 concentrations in the control and exercise groups at the pre-test and post-test stages.

Table 3. Analysis of covariance (ANCOVA) results for post-test IGF-1 levels after adjustment for pre-test values.

Source	Sum of squares	df	Mean square	F	Sig.	Effect size
Pre-test covariate	33435.349	1	33435.349	46.654	0.001	0.700
Groups	959.338	1	959.338	1.339	0.261	0.063
Error	14333.204	20	716.660	—	—	—

Table 4. Descriptive statistics and within-group comparisons of GPX levels at pre-test and post-test

Variable	Time	Control group	Exercise group
GPX	Pre-test	63.62 ± 1.16	64.37 ± 1.55
	Post-test	62.43 ± 4.61	63.43 ± 1.23
p-value	—	0.441	0.390

Table 5. Analysis of covariance results for glutathione peroxidase (GPX) between the control and exercise groups.

Source	Sum of squares	df	Mean square	F	Sig.	Effect size
Pre-test covariate	3.011	1	3.011	0.265	0.612	0.013
Groups	7.743	1	7.743	0.682	0.419	0.033
Error	227.065	20	11.353	—	—	—

Discussion

The primary finding of the present study, based on the between-group ANCOVA comparison, was that six weeks of progressive metabolic conditioning training did not result in a statistically significant difference between groups in serum IGF-1 or GPX levels. Although a secondary within-group analysis revealed a significant reduction in IGF-1 within the exercise group ($p=0.047$), after adjustment for pre-test values using ANCOVA, the between-group difference in post-test IGF-1 levels did not reach statistical significance ($F(1,20)=1.339$, $p=0.261$, $\eta^2=0.063$). This discrepancy between within-group and between-group analyses warrants careful interpretation and suggests that the observed reduction in IGF-1 within the exercise group may be partially attributable to baseline differences rather than a genuine training effect. The modest effect size ($\eta^2=0.063$) further indicates that the training intervention accounted for only a small proportion of the variance in post-test IGF-1 levels.

The direction of the IGF-1 response observed in the present study aligns with several previous investigations that have reported reductions in total IGF-1 following high-intensity or high-volume exercise training. Khani (2021) demonstrated that eight weeks of polarized training significantly decreased total IGF-1 levels in active young males, despite concomitant increases in free IGF-I (Khani, 2021). Similarly, a study examining six weeks of wrestling-based circuit exercise in well-trained wrestlers reported significant decreases in both growth hormone and IGF-1 (Rashidlamir et al., 2011). These findings collectively suggest that exercise protocols characterized by high metabolic demand and substantial training volume may suppress total circulating IGF-1, potentially through mechanisms involving enhanced IGF-1 clearance, reduced hepatic synthesis, or altered binding protein dynamics. Conversely, other investigations have reported increases in IGF-1 following circuit training. For instance, a 12-week circuit training program in obese middle-aged women significantly increased IGF-1 levels (Son et al., 2017), and high-intensity circuit training has been associated with elevated IGF-1 in certain populations. These contrasting findings likely reflect differences in participant characteristics (age, adiposity, baseline fitness), training duration, and exercise prescription. The present study's participants were young, physically active, normal-weight individuals with presumably well-functioning GH-IGF-1 axes; in such populations, the anabolic stimulus of exercise may not elicit the same magnitude of IGF-1 elevation observed in older or obese individuals with lower baseline levels.

With respect to glutathione peroxidase, the results of the present study were unequivocal: neither within-group nor between-group analyses revealed any statistically significant changes in serum GPX levels following the six-week metabolic conditioning intervention. The ANCOVA results confirmed the absence of a training effect ($F(1, 20)=0.682$, $p=0.419$, $\eta^2=0.033$), with the pre-test covariate also failing to significantly predict post-test GPX values ($p=0.612$). The lack of GPX response in the present study is inconsistent with the broader literature suggesting that exercise

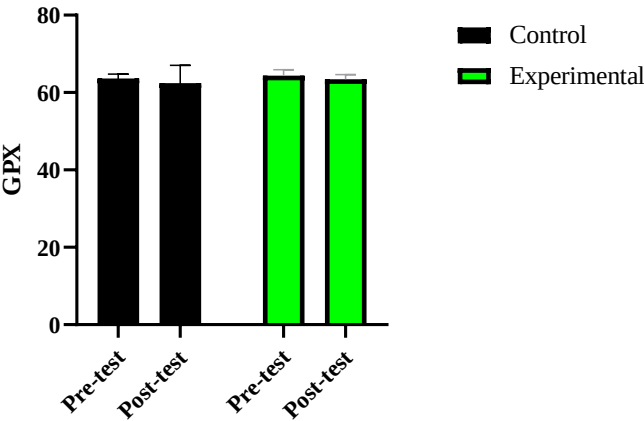


Figure 2. Mean ± standard deviation of glutathione peroxidase (GPX) levels in the control and exercise groups before and after the six-week metabolic conditioning training program.

training enhances antioxidant enzyme activity (Xie et al., 2025). However, several factors may explain this discrepancy. First, the meta-analysis by Xie et al. (2025) reported that only resistance exercise significantly increased GPX activity, whereas high-intensity exercise did not (Xie et al., 2025). Although metabolic conditioning incorporates resistance-type movements, its circuit-based, high-intensity nature with short rest intervals may not provide the same stimulus as traditional resistance training for GPX upregulation. Second, the same meta-analysis found that exercise interventions lasting more than 16 weeks showed no significant impact on GPX activity, suggesting that the relationship between training duration and antioxidant adaptation may be nonlinear. The six-week duration of the present intervention may have been insufficient to elicit measurable changes in serum GPX, particularly in young, healthy individuals with already competent antioxidant defense systems. Third, the intensity and mode of exercise are critical determinants of GPX response; moderate-to-low intensity exercise performed at least three times per week for more than 16 weeks demonstrated the greatest efficacy in enhancing antioxidant enzyme activity. The present protocol, while progressive in nature, may not have achieved the optimal intensity–duration combination for GPX induction.

The differential responsiveness of IGF-1 and GPX to the metabolic conditioning intervention raises important questions about the specificity of training adaptations. IGF-1, as a systemic hormone with rapid turnover (Arai et al., 2001), may be more sensitive to acute and chronic changes in metabolic and energy status, whereas GPX, as an intracellular enzymatic antioxidant, may require more prolonged or specific training stimuli to manifest measurable changes in circulating levels. It is also noteworthy that serum GPX may not fully reflect tissue-specific antioxidant adaptations; previous research has demonstrated that exercise training can increase GPX activity in skeletal muscle mitochondria without corresponding changes in serum levels. Additionally, the regulation of GPX is complex and involves not only transcriptional upregulation but also selenium availability and post-translational modifications. The present study did not control for dietary selenium intake, which could have influenced baseline GPX levels and the responsiveness to training.

This study has several limitations that should be acknowledged. First, the small sample size ($n = 23$) limits the statistical power and generalizability of the findings. Although the study was adequately powered to detect large effect sizes (Cohen's $f \geq 0.40$), the sample size is insufficient to detect small-to-moderate effect sizes, which are often biologically relevant. Therefore, the null findings for both IGF-1 and GPX may reflect a type II error (insufficient power) rather than a true absence of training-induced adaptation, and these results should be interpreted with appropriate caution. Second, the six-week intervention duration

may have been insufficient to induce measurable changes in the biomarkers examined. Third, the lack of clinical trial registration and the absence of dietary controls (particularly for selenium intake, which influences GPX activity) represent additional limitations. Fourth, serum GPX levels may not fully reflect tissue-specific antioxidant adaptations. Fifth, the study was powered based on a strong anticipated correlation between pre- and post-test values (due to the ANCOVA design); however, if the actual correlation is weaker than expected, the effective power would be reduced, further emphasizing the need for larger confirmatory studies. Sixth, although we standardized the post-test blood sampling interval to 48 hours after the last training session, we cannot completely rule out residual acute or delayed-onset effects of the final exercise bout on circulating IGF-1 and GPX. A longer washout period (e.g., 72 hours) might have been preferable to ensure full return to baseline; however, this must be balanced against the risk of detraining effects. Seventh, although we controlled for fasting duration (10–12 hours overnight) and time of day for all blood collections, we did not systematically record or standardize dietary macronutrient composition (particularly protein and carbohydrate intake) in the 24–48 hours preceding the assessments. Because nutritional intake can acutely modulate IGF-1 and its binding proteins, this represents a potential confounder. Eighth, the inclusion criterion requiring "adherence to a healthy diet" was based solely on self-report and verbal confirmation at the time of recruitment, without the use of dietary logs, food frequency questionnaires, or objective biomarkers (e.g., serum albumin, prealbumin, or specific nutrient levels such as selenium) to objectively verify compliance. Consequently, variations in individual dietary intake particularly of protein, carbohydrates, and selenium may have influenced serum IGF-1 and GPX levels independently of the exercise intervention, representing a potential unmeasured confounder that could obscure true training effects or create spurious findings. Ninth, while our protocol implemented progressive overload through increased training volume (sets) and density (reduced rest intervals), it did not incorporate true intensity progression—specifically, increasing the external load or movement difficulty for certain exercises (push-ups and medicine-ball slams). As a result, the mechanical and metabolic stimulus for these particular stations may have plateaued after the initial weeks of the intervention, potentially attenuating the overall training stimulus.

The absence of individualized, graded load progression may therefore have limited the magnitude of physiological adaptations, including those involving the IGF-1 and antioxidant systems. This is particularly relevant given that traditional resistance training which consistently demonstrates load progression has been shown to more reliably upregulate GPX activity than fixed-load circuit protocols (Xie et al., 2025). Tenth, we did not employ any objective or subjective measures to

quantify the internal training load during the exercise sessions, such as heart rate monitoring, session ratings of perceived exertion (sRPE), blood lactate analysis, or training load metrics (e.g., TRIMP-Training Impulse).

While we prescribed the external load (sets, repetitions, rest intervals, cycling resistance levels) and ensured direct supervision of all training sessions to confirm adherence to the protocol structure, the absence of these intensity-verification tools prevents us from confirming that the prescribed program consistently elicited the intended metabolic and cardiovascular stress in each participant across all sessions. Individual variations in effort, fitness level, and daily physiological state could have resulted in significant heterogeneity in the actual training stimulus, thereby potentially masking group-level effects on IGF-1 and GPX. Furthermore, without objective intensity data, we cannot rule out the possibility that some participants trained at intensities below the threshold necessary to induce biochemical adaptations, or that the overall dose of exercise was insufficient relative to the participants' baseline fitness levels. Eleventh, although we now report that no participants were excluded for attendance reasons and that the exercise group demonstrated 100% adherence (all participants attended all 18 sessions), this information was not presented in the original submission. The absence of adherence reporting prevents readers from fully assessing the fidelity of the intervention. While our retrospective reporting confirms perfect attendance, the lack of prospective adherence data documentation in the original manuscript represents a reporting limitation that could have been avoided. We recommend that future studies, in accordance with CONSORT guidelines, prospectively define and report adherence rates, including the number of sessions completed per participant, reasons for non-attendance or dropout, and the impact of adherence on outcome measures (e.g., per-protocol vs. intention-to-treat analyses). Twelfth, while we have now reported the manufacturer-provided assay validation characteristics (including intra-assay and inter-assay CVs, detection ranges, and sensitivities) for the ELISA kits used to measure IGF-1 and GPX, we did not perform independent internal validation of these assays in our laboratory. Specifically, we did not run control samples or quality-control standards in parallel, nor did we perform duplicate measurements for each sample to confirm the manufacturer-reported precision parameters under our specific laboratory conditions. The reported CVs were derived from manufacturer data and may not fully reflect the actual assay performance in the context of our sample handling, storage, and processing conditions. Additionally, we did not include spike-and-recovery tests or linearity dilution assessments to verify assay accuracy for our specific sample matrix (human serum). The absence of internal validation data limits the reader's ability to independently assess the reliability and precision of our biochemical measurements.

Thirteenth, and perhaps most critically for the interpretation of the GPX findings, we did not assess or control for dietary selenium intake, a trace mineral that serves as an essential cofactor for glutathione peroxidase enzymes. Selenium is incorporated into GPX as selenocysteine at the enzyme's active site, and selenium status is a primary determinant of both basal GPX expression and the enzyme's capacity to respond to exercise-induced oxidative stress. Inadequate selenium availability can limit GPX upregulation even when the exercise stimulus is sufficient, whereas selenium-replete individuals may exhibit greater training-induced antioxidant adaptations. We did not administer selenium-specific food frequency questionnaires, dietary recalls, or serum/plasma selenium concentration assays to assess baseline or post-intervention selenium status. This omission is particularly significant given that our participants were recruited from Iran, where dietary selenium intake can vary considerably depending on regional differences in soil selenium content, food sources, and dietary habits. Consequently, inter-individual variability in selenium status may have substantially influenced the GPX responsiveness to the metabolic conditioning intervention, potentially masking a true training effect or contributing to the null findings for GPX. The absence of selenium assessment is therefore a major confounder that limits the interpretability of our GPX results. Future studies investigating exercise effects on antioxidant enzymes must incorporate comprehensive selenium assessment, including dietary intake records (e.g., 3-day food diaries or food frequency questionnaires validated for selenium-containing foods) and objective biomarkers of selenium status (e.g., serum/plasma selenium, selenoprotein P, or erythrocyte GPX activity), to ensure that selenium sufficiency is controlled for and that training-induced GPX adaptations can be accurately attributed to the exercise intervention rather than to nutritional status.

Future studies should incorporate systematic, individualized load progression across all exercise stations (e.g., adding weight vests for push-ups, increasing medicine ball mass, or advancing to more difficult exercise variations) to ensure a sustained adaptive stimulus throughout the intervention period. Future research should also incorporate standardized dietary tracking (e.g., 3-day dietary recalls or weighed food records) and consider nutritional biomarker analyses to strengthen internal validity. In particular, future studies on exercise-induced GPX adaptation must include selenium-specific dietary assessments (e.g., food frequency questionnaires for selenium-rich foods) and objective selenium status biomarkers (serum/plasma selenium, selenoprotein P) to control for this critical confounder and to ensure that GPX responsiveness reflects true training effects rather than baseline or exercise-induced changes in nutritional status. Future research should also clearly document the post-exercise sampling interval and consider incorporating multiple

post-test time points to capture the temporal dynamics of biomarker responses. Future studies should report adherence data systematically, including individual session attendance, reasons for any non-attendance, and the relationship between adherence and biochemical outcomes. Furthermore, future investigations should employ rigorous internal assay validation procedures, including duplicate measurements, quality-control samples, and laboratory-specific precision estimates, to ensure the reliability of biochemical data and to facilitate comparability across studies.

Conclusion

In conclusion, the present study demonstrated that six weeks of progressive metabolic conditioning training did not produce a statistically significant between-group effect on serum IGF-1 or GPX levels in physically active young adults, despite a significant within-group reduction in IGF-1 in the exercise group. These findings suggest that metabolic conditioning, as prescribed in this protocol, may not be of sufficient duration or intensity to induce robust changes in these biochemical markers in healthy, young, physically active individuals. The lack of GPX response further indicates that the antioxidant enzyme system may require more prolonged or differently structured training stimuli to exhibit measurable adaptation. While metabolic conditioning remains a popular and effective training modality for improving cardiorespiratory fitness and metabolic efficiency, its effects on systemic hormonal and antioxidant markers appear to be modest in this population over a six-week period. Further research with larger sample sizes, extended intervention periods, and comprehensive biochemical assessments is warranted to elucidate the full spectrum of physiological adaptations to metabolic conditioning and to establish evidence-based guidelines for its application in health and performance contexts.

What is already known on this subject?

Existing evidence indicates that exercise training can modulate circulating IGF-1, although the direction of this response is highly dependent on training modality, intensity, duration, and the baseline characteristics of the population. Furthermore, the endogenous antioxidant enzyme GPX is known to be responsive to physical activity; however, its upregulation appears to be exercise-mode specific, with traditional resistance training yielding more consistent effects than high-intensity or high-volume protocols. Despite the widespread popularity of metabolic conditioning as a training strategy designed to optimize energy system utilization, its specific effects on hormonal and antioxidant biomarkers remain poorly characterized in the scientific literature,

representing a significant gap in knowledge.

What this study adds?

The present study provides the first controlled experimental evidence specifically examining the concurrent effects of a progressive six-week metabolic conditioning program on both IGF-1 and GPX in physically active young adults. Our findings underscore that, based on the primary between-group comparisons, six weeks of metabolic conditioning does not induce significant changes in these biomarkers relative to a control condition. We contribute a nuanced observation: although a secondary within-group reduction in IGF-1 was observed following the intervention, this effect was not statistically superior to the control group after adjusting for baseline values, suggesting that the within-group change may reflect baseline variability rather than a genuine training adaptation. Importantly, the absence of any significant change in serum GPX levels highlights that six weeks of metabolic conditioning, as prescribed in this protocol, may not constitute a sufficient stimulus to trigger measurable antioxidant enzyme adaptations. This underscores the necessity for longer intervention periods and more comprehensive biochemical assessments to fully elucidate the physiological impacts of this training modality.

Organ Cross-Talk Tips:

- The significant within-group reduction in serum IGF-1 after metabolic conditioning may reflect increased hepatic clearance or reduced hepatic synthesis, suggesting liver-derived IGF-1 is modulated by the metabolic stress of muscle contractions during high-intensity circuit training.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest the authors declare that there is no conflict of interest in the present research.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved

by the Ethical Committee of Islamic Azad University, Tehran, Iran (approval code: IR.IAU,SRB.REC.1404.365).

Informed consent Written informed consent was obtained from all individual participants included in the study after a full explanation of the study objectives, procedures, potential benefits, and possible risks. Participants were informed of their right to withdraw at any stage without consequences.

Author contributions

Conceptualization: N.S.; Methodology: F.Gh.; Software: H.A.; Validation: M.Gh.; Formal analysis: F.N.; Investigation: N.S.; Resources: M.Gh.; Data curation: F.Gh.; Writing -original draft: N.S.; Writing-review & editing: H.A.; Visualization: F.N.; Supervision: F.Gh.; Project administration: H.A.; Funding acquisition: N.S..

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