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# The psychobiology of post-exercise recovery: an exploratory pilot study on 48-hour plasma irisin change and state anxiety in trained males

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## ABSTRACT

Understanding the interplay between physiological stress and psychological recovery following intense exercise is crucial for optimising training adaptations and well-being. Muscle-derived myokines may represent biological correlates of this process, yet the association between irisin and affective responses during post-resistance-training recovery remains poorly understood. This exploratory study investigated the temporal dynamics of plasma irisin, creatine kinase (CK), and psychological states (state anxiety, positive/negative affect) in eight resistance-trained males before, and at 15 min, 24, and 48 h after a single, high-volume resistance training session controlled via repetitions-in-reserve. The findings suggested a temporal dissociation between muscular stress markers and the irisin response. While plasma CK peaked at 24 h, indicating a marked muscular stress response, plasma irisin exhibited a modest but significant increase only at the 48-hour time point. Importantly, 48-hour changes in plasma irisin were significantly and negatively associated with late changes in state anxiety from 24 to 48 h post-exercise. These preliminary findings suggest that plasma irisin may also exhibit later-phase variation, in addition to its established acute response in other exercise modalities. It is hypothesised that this 48-hour irisin variation may track alongside longer-term homeostatic restoration. These observations are exploratory and hypothesis-generating; rather than acting as a direct causal mediator, 48-hour changes in plasma irisin may be associated with inter-individual trajectories of late state anxiety during recovery, providing a novel framework for future investigations.

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Physical training is widely recognised for its capacity to acutely reduce anxiety, enhance mood, and foster psychological resilience. Indeed, robust evidence within the broader exercise-mental health literature demonstrates the comparative effectiveness of different exercise modalities in regulating negative emotional states and alleviating psychosocial stress (Yu et al., 2022; Zhang et al., 2022). While the macro-level psychophysiological benefits are well-documented (Ekkekakis et al., 2004; Petruzzello et al., 1991), the specific biological mechanisms underlying this psychobiological interplay, however, are not fully elucidated. Skeletal muscle, comprising approximately 40% of total body mass, is increasingly understood not only for its contractile function but also for its role as an active endocrine organ (Pedersen, 2013). Through the secretion of peptides and proteins termed myokines, muscle tissue communicates with other organs, including the brain, thereby influencing systemic processes such as energy metabolism, inflammation, and tissue regeneration (Chow et al., 2022; Pedersen & Febbraio, 2012). These muscle-derived factors have therefore been proposed as potential biological correlates of exercise-related psychophysiological responses, although their specific contribution to affective and anxiety-related changes remains incompletely understood.

Among the identified myokines, irisin has garnered significant attention since its discovery (Boström et al., 2012). Derived from the cleavage of FNDC5, irisin is released into circulation and has been implicated in a range of health benefits, including glucose homeostasis, bone metabolism, and, importantly, cognitive function (Liu et al., 2022). While acute aerobic exercise is known to transiently elevate circulating irisin levels in an intensity-dependent manner (Kraemer et al., 2014; Tommasini et al., 2024; Zare et al., 2025), the precise temporal profile of this response remains incompletely characterised, with peak concentrations reported at varying time points from immediately post-exercise to several hours into recovery (Fox et al., 2018; Kazeminasab et al., 2022; Tommasini et al., 2024).

In contrast to aerobic exercise, the response of irisin to resistance training (RT) remains less defined. RT confers numerous physiological benefits, including increased muscle strength, hypertrophy, and improved insulin sensitivity (Hughes et al., 2018), yet the contribution of myokines to these adaptations requires further clarification. The literature concerning RT and irisin release is both limited and inconclusive. Some studies have reported increases (Huh et al., 2015; Marano et al., 2025; Nygaard et al., 2015; Tsuchiya et al., 2015), while others have found no significant changes (Pekkala et al., 2013). This inconsistency is likely attributable to varied training protocols, participant characteristics, and sampling times. Furthermore, a significant gap exists regarding irisin dynamics in non-plasma matrices like saliva, which could offer a non-invasive alternative for biomarker monitoring (Missaglia et al., 2023).

Previous research has linked physiological stress markers, such as creatine kinase (CK) and myokines, to changes in affective states and perceived anxiety, highlighting the relevance of an integrated psychobiological approach (Hackney, 2006; Pignataro et al., 2022; Uysal et al., 2018). It was hypothesised that an acute increase in stress-related biomarkers might correspond to a temporary decline in positive affect, while recovery-related signals like irisin might be associated with later emotional improvement. Therefore, the primary objective of this exploratory pilot study was to generate initial data and formulate a testable hypothesis regarding the temporal dynamics of irisin following a standardised RT protocol. A secondary objective was to explore the potential interplay between these

biological responses and subjective psychological states (assessed via the PANAS and STAI-Y instruments; Spielberger et al., 1983; Watson et al., 1988) in young, trained individuals.

## Materials and methods

### Participants

A total of eight young, healthy male participants were recruited for the study. Eligibility criteria required participants to be: (a) between 18 and 39 years of age; (b) be free of obesity, as determined by a body fat percentage indicative of a healthy/athletic status (avoiding a strict upper-threshold BMI limit, which is often inaccurate in strength-trained individuals due to high muscle mass); and (c) have a minimum of six months of consistent resistance training experience, defined as at least two sessions per week. Exclusion criteria included any musculoskeletal injuries or medical conditions that could affect performance. None of the participants were current smokers. All participants provided written informed consent to participate in the study and for the publication of the results. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Department of Psychology at the Catholic University of the Sacred Heart of Milan (CERPS) (protocol code no. 36/24).

Descriptive characteristics of the participants are presented in Table 1. Based on the 2021 normative data from the American College of Sports Medicine (ACSM), the ratio between the one-repetition maximum (1RM) on the leg press and body mass (kg) places participants in the “well above average” ( $\geq 90$ th) percentile (American College of Sports Medicine & Liguori, 2021).

### Experimental design and procedures

Participants attended the laboratory on five separate occasions: three familiarisation sessions, one experimental testing session (ETS), and two consecutive follow-up visits for post-exercise sample collection. All sessions were conducted in a private gym under

**Table 1.** Participants’ descriptive characteristics.

| Variables<br>n                    | Mean<br>8 | Std. Deviation<br>/ |
|-----------------------------------|-----------|---------------------|
| Age (years)                       | 24.1      | 2.7                 |
| Height (cm)                       | 175.8     | 7.9                 |
| Body mass (kg)                    | 76.8      | 9.1                 |
| BMI (kg/m <sup>2</sup> )          | 24.9      | 2.6                 |
| Muscle mass (kg)                  | 46.1      | 5.0                 |
| % Muscle mass                     | 60.0      | 3.8                 |
| % Fat mass (FM)                   | 16.3      | 3.9                 |
| % Fat-free mass (FFM)             | 83.7      | 3.9                 |
| Fat mass (kg)                     | 12.5      | 4.1                 |
| Training age (years)              | 5.2       | 2.8                 |
| Ratio 1RM/BM LP                   | 2.71      | 0.35                |
| ACSM’s ratio 1RM/BM LP percentile | 91.0      | 0.0                 |

Values are reported as mean and standard deviation. BM, body mass; BMI, body mass index; FFM, fat-free mass; FM, fat mass; 1RM, one-repetition maximum; LP, leg press.

controlled environmental conditions, with relative humidity maintained below 60% and temperature between 18°C and 22°C.

The familiarisation phase included three sessions designed to prepare participants for the ETS: a ten-repetition maximum (10-RM) test, a session to familiarise participants with the prescribed time-under-tension (TUT) protocol, and a one-repetition maximum (1-RM) test. A minimum of 24 h of rest was observed between each session.

During the first laboratory visit, anthropometric data were collected. Height was measured to the nearest 0.1 cm using a calibrated stadiometer (Seca 217, Hamburg, Germany), and body mass was measured to the nearest 0.1 kg using a precision mechanical scale (Seca Viva 750, Hamburg, Germany). Body composition and hydration status were assessed using bioelectrical impedance analysis (BIA) (Akern BIA 101 BIVA® PRO IPS, Akern s.r.l., Florence, Italy), following standardised protocols (Campa et al., 2023; Kyle et al., 2004).

Following a minimum of 48 h of rest, participants completed the ETS. To minimise confounding variables throughout both the baseline and the 48-hour recovery period, participants were instructed to maintain a consistent sleep schedule and to refrain from consuming alcohol, caffeine, or other stimulants. Importantly, participants were required to refrain from any additional structured physical activity during the entire 48-hour post-exercise follow-up. Furthermore, to ensure comparable nutritional states across time points, participants were instructed to maintain their habitual dietary intake and consume a standardised, replicable meal 2 h prior to each laboratory visit. All sessions took place between 09:00 and 13:00 to reduce circadian variability.

### **Exercise protocol**

The ETS consisted of a full-body resistance training programme. The 10-RM and 1-RM testing sessions used to determine the training load for the ETS were conducted in accordance with ACSM guidelines (American College of Sports Medicine & Liguori, 2021). The protocol included eight exercises performed on isotonic resistance machines: 1. 90° Leg Press; 2. Prone Lat Pulldown; 3. Prone Leg Curl; 4. Seated Chest Press; 5. Leg Extension; 6. Seated Shoulder Press; 7. Standing Biceps Curl (dumbbells); 8. Standing Cable Triceps Extension.

The ETS comprised 30 sets in total: four sets for major upper and lower body muscle groups and three sets for arm exercises. The load was set at 70% of each participant's 1-RM. This intensity was chosen to elicit meaningful mechanical tension and falls within the range recommended to stimulate muscular strength, hypertrophy, and local endurance adaptations (Schoenfeld et al., 2021). All sets were performed with a buffer of two repetitions in reserve (RIR). The 2-1-2-1 TUT protocol was consistently applied to control the repetition duration and standardise the training stimulus (Martins-Costa et al., 2022). Rest intervals between sets were standardised at 90 s. Each session was closely monitored by a trainer to ensure accurate adherence to the protocol in terms of repetition count, rest duration, and movement cadence. The completed external training stimulus was quantified using the number of repetitions completed and the total external session volume load. The number of repetitions completed in each set was recorded for every exercise. Exercise-specific

external volume load was calculated as the total number of repetitions completed for each exercise multiplied by the absolute load used for that exercise. Total external session volume load was calculated as the sum of all exercise-specific external volume loads across the session. For the dumbbell biceps curl, the combined load of both dumbbells was used. Session RPE was not collected; therefore, an sRPE-based internal training-load index could not be calculated.

## Measurements

Plasma and salivary irisin, alongside plasma creatine kinase (CK) levels, were analysed using procedures consistent with Marano et al. (2025), with the full methodological details reported below.

Samples were collected at baseline and at 15 min, 24, and 48 h post-exercise: To ensure standardised salivary collection across all visits, participants were instructed to refrain from eating, drinking (except water), and brushing their teeth for at least 60 min prior to each sampling time point. Before collection, participants rinsed their mouths carefully five times without swallowing to remove food residues and potential contaminants. Unstimulated whole saliva was collected via the passive drool method into sterile 1.5 mL Eppendorf tubes to minimise pre-analytical variability. Saliva samples were processed within 10 min of collection, centrifuged at 11,000 rpm for 4 min, aliquoted, and stored at  $-80^{\circ}\text{C}$  until analysis.

Blood samples were drawn into EDTA-containing tubes and processed within 20 min of collection. Samples were centrifuged at 11,000 rpm for 10 min; plasma was then separated, aliquoted, and stored at  $-80^{\circ}\text{C}$  until analysis. Each plasma and saliva aliquot underwent only one freeze–thaw cycle immediately before analysis.

Plasma and salivary irisin concentrations were measured using a commercial ELISA assay kit (Phoenix Pharmaceuticals, Cat. EK-067-29, Lot No. 611353), according to the manufacturer's instructions. The assay detection limit was 1.29 ng/mL, and the calibration range of the standard curve was 0.01–100 ng/mL. Plasma samples were assayed undiluted, whereas saliva samples were diluted 1:1 with the assay buffer provided in the kit. All samples were analysed in duplicate. The intra-assay and inter-assay coefficients of variation were  $<10\%$  and  $<15\%$ , respectively. All measured values fell within the calibration range, and no values below the limit of detection were observed. Total salivary protein concentration was measured using the BCA Protein Assay Kit (Thermo Scientific), and salivary irisin concentrations were normalised to total protein content.

At 24 and 48 h post-exercise, participants registered their perceived muscle soreness using the Visual Analogue Scale (VAS).

Participants' affective states were assessed at all four time points using the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988). The PANAS is a 20-item self-report instrument measuring positive affect (PA) and negative affect (NA) on a 5-point Likert scale. To facilitate direct interpretation along the original response format, results for both subscales are reported as mean item scores (range 1–5). Higher scores indicate a higher level of the respective affect.

State anxiety was measured at the same four time points using the State subscale of the State-Trait Anxiety Inventory – Form Y (STAI-Y) (Spielberger et al., 1983). This 20-

item self-report scale assesses transient feelings of apprehension and tension on a 4-point Likert scale. Consistent with the PANAS, STAI-Y results are reported as mean item scores (range 1–4). Higher scores reflect a greater level of state anxiety.

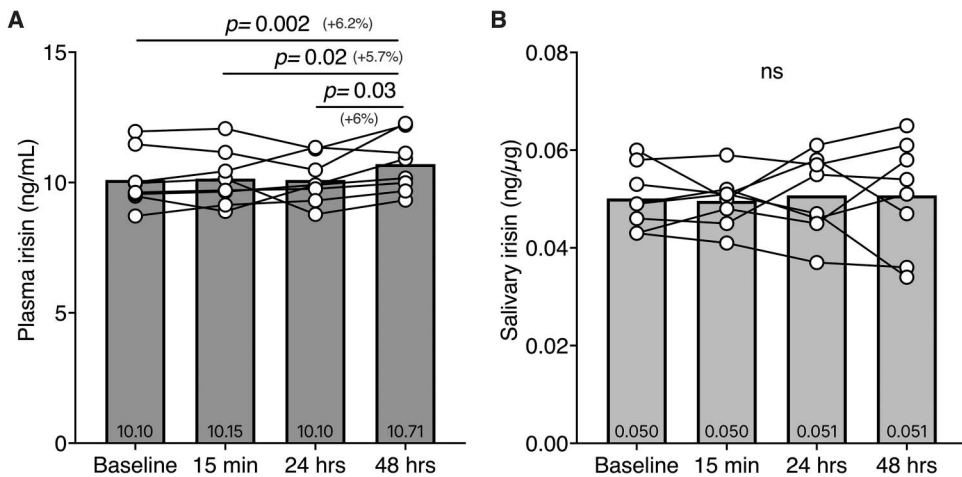
## Statistical analyses

All statistical analyses were considered exploratory and were carried out using JASP (Version 0.19.1) and R (Version 4.4.2). Statistical significance was accepted at  $p < .05$ . Given the small sample size ( $N = 8$ ), non-parametric statistical tests were employed to ensure analytical robustness. Repeated-measures outcomes assessed across more than two time points were analysed using Friedman tests. For each Friedman test, the global test statistic, degrees of freedom, exact  $p$ -value, and Kendall's  $W$  were reported. When a significant omnibus effect was observed, Conover post hoc comparisons with Holm adjustment were performed to identify pairwise differences. For paired outcomes assessed at two time points, Wilcoxon signed-rank tests were used, with the test statistic,  $p$ -value, and matched rank-biserial correlation reported where available. Completed repetitions were analysed to characterise the external training stimulus. Exercise-specific changes in repetitions across sets were examined using Friedman tests, followed by Holm-adjusted Conover post hoc comparisons when a significant omnibus effect was observed. To explore the relationship between biological and psychological responses, correlation analyses were conducted using Kendall's Tau-b. It is acknowledged that a small sample size limits the statistical power to detect small to moderate effects. A sensitivity power analysis (Ditroilo et al., 2025) was conducted a posteriori using G\*Power (version 3.1.9.7). Given  $N = 8$ , the analysis indicated that the study is only sensitive to detecting very large effects (minimum detectable effect size  $f = 0.65$ ) for the primary outcome variable (change in plasma irisin). This result highlights the high uncertainty and wide confidence intervals inherent in the estimation, reinforcing the exploratory nature of the findings.

## Results

### Plasma and salivary irisin

The analysis of plasma irisin revealed a statistically significant change over time, as indicated by the Friedman test ( $\chi^2 F(3) = 9.150$ ,  $p = .027$ , Kendall's  $W = .381$ ). Descriptively, mean plasma irisin values remained stable from baseline ( $10.10 \pm 1.08$  ng/mL) to 15 min ( $10.15 \pm 1.06$  ng/mL) and 24 h post-exercise ( $10.10 \pm 0.90$  ng/mL), before increasing at 48 h post-exercise ( $10.71 \pm 1.11$  ng/mL), corresponding to an approximate 6.2% increase from baseline. Unadjusted Conover post hoc comparisons indicated that plasma irisin values were higher at 48 h than at baseline, 15 min, and 24 h post-exercise (all  $p < .05$ ). After Holm adjustment, only the comparison between 48 h and baseline remained statistically significant ( $p_{\text{Holm}} = .014$ ), whereas the comparisons between 48 h and 15 min ( $p_{\text{Holm}} = .097$ ) and between 48 h and 24 h post-exercise ( $p_{\text{Holm}} = .126$ ) did not remain significant. Figure 1A illustrates these changes. In contrast, salivary irisin did not change significantly over time ( $\chi^2 F(3) = 0.300$ ,  $p = .960$ , Kendall's  $W = .013$ ). Descriptively, salivary irisin values remained essentially unchanged across baseline ( $0.050 \pm$



**Figure 1.** (A) Plasma irisin (ng/mL) and (B) salivary irisin (ng/μg) concentrations at baseline, 15 min, 24, and 48 h post-exercise. Bars represent group means, and dots connected by lines represent individual participant values ( $N = 8$ ). The numerical values displayed within the bars indicate the group mean at each time point. In panel A,  $p$ -values above brackets refer to unadjusted Conover post hoc comparisons following a significant Friedman test involving the 48-h time point; Holm-adjusted post hoc  $p$ -values are reported in the Results section. To accurately reflect inter-individual dynamics, the percentages indicate the mean of individual percentage changes (rather than the percentage change computed from the displayed group means) of the 48-hour value relative to previous time points. In panel B, *ns* indicates no significant effect of time according to the Friedman test.

0.006), 15 min ( $0.050 \pm 0.005$ ), 24 h ( $0.051 \pm 0.008$ ), and 48 h post-exercise ( $0.051 \pm 0.011$ ; Figure 1B).

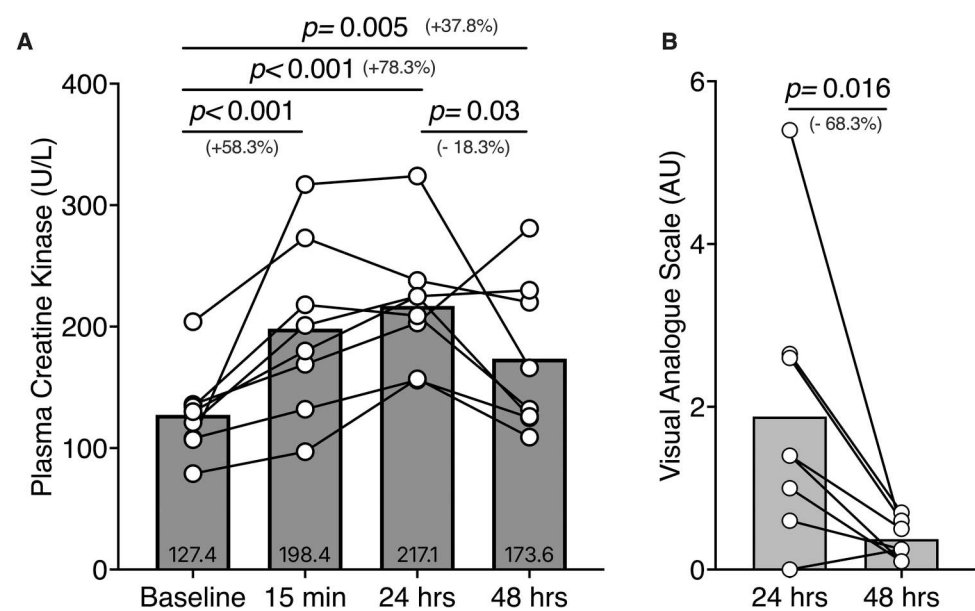
### Plasma creatine kinase and perceived muscle soreness

Plasma CK levels changed significantly over time, as indicated by the Friedman test ( $\chi^2 F(3) = 14.550$ ,  $p = .002$ , Kendall's  $W = .606$ ). Descriptively, CK increased from baseline ( $127.38 \pm 36.19$  U/L) to 15 min post-exercise ( $198.38 \pm 71.63$  U/L; +58.3%), reached its highest mean value at 24 h post-exercise ( $217.13 \pm 52.91$  U/L; +78.3% from baseline), and then declined at 48 h post-exercise ( $173.63 \pm 62.63$  U/L). Holm-adjusted Conover post hoc comparisons showed significantly higher CK values at 15 min ( $p_{\text{Holm}} = .003$ ), 24 h ( $p_{\text{Holm}} < .001$ ), and 48 h post-exercise ( $p_{\text{Holm}} = .018$ ) compared with baseline. The comparison between 24 h and 48 h post-exercise did not remain significant after Holm adjustment ( $p_{\text{Holm}} = .094$ ). These results are shown in Figure 2A.

In parallel with the post-24 h decline in CK, VAS scores for perceived muscle soreness decreased from 24 h ( $1.88 \pm 1.69$  A.U.) to 48 h post-exercise ( $0.38 \pm 0.23$  A.U.), corresponding to a 68.3% reduction. This decrease was statistically significant, as confirmed by a Wilcoxon signed-rank test ( $W = 35.000$ ,  $z = 2.380$ ,  $p = .016$ , matched rank-biserial correlation = .944; Figure 2B).

### Psychological states and psychobiological correlations

Descriptive statistics for the psychological variables -positive affect, negative affect, and state anxiety- across the four time points are reported in Table 2.



**Figure 2.** (A) Plasma Creatine Kinase (CK) levels (U/L) at baseline, 15 min, 24, and 48 h post-exercise, and (B) Visual Analogue Scale (VAS) scores (A.U.) for perceived muscle soreness at 24 and 48 h post-exercise. Bars represent group means, while dots and connecting lines show individual participant values across time points ( $N = 8$ ). The numerical values shown within the bars indicate the group mean at each time point. To accurately reflect inter-individual dynamics, the percentages indicate the mean of individual percentage changes (rather than the percentage change computed from the displayed group means). In panel A,  $p$ -values above brackets refer to unadjusted Conover post hoc comparisons following a significant Friedman test; Holm-adjusted post hoc  $p$ -values are reported in the Results section. Percentages indicate the mean individual percentage change for the indicated comparisons. In panel B, the  $p$ -value refers to the Wilcoxon signed-rank test, and the percentage indicates the mean individual percentage change from 24 h to 48 h.

**Table 2.** Descriptive statistics for psychological variables (reported as mean item scores).

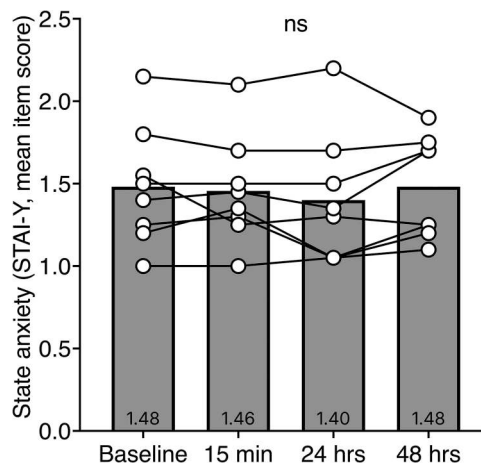
| Variable        | Time     | Mean | SD   | Min  | Max  |
|-----------------|----------|------|------|------|------|
| POSITIVE AFFECT | Baseline | 3.36 | 0.52 | 2.80 | 4.10 |
|                 | 15 min   | 3.45 | 0.56 | 2.80 | 4.30 |
|                 | 24 hrs   | 3.34 | 0.73 | 2.40 | 4.80 |
|                 | 48 hrs   | 3.46 | 0.63 | 2.90 | 4.60 |
| NEGATIVE AFFECT | Baseline | 1.15 | 0.35 | 1.00 | 2.00 |
|                 | 15 min   | 1.14 | 0.35 | 1.00 | 2.00 |
|                 | 24 hrs   | 1.13 | 0.35 | 1.00 | 2.00 |
|                 | 48 hrs   | 1.06 | 0.09 | 1.00 | 1.20 |
| ANXIETY         | Baseline | 1.48 | 0.36 | 1.00 | 2.15 |
|                 | 15 min   | 1.46 | 0.33 | 1.00 | 2.10 |
|                 | 24 hrs   | 1.40 | 0.40 | 1.05 | 2.20 |
|                 | 48 hrs   | 1.48 | 0.31 | 1.10 | 1.90 |

Kendall's Tau-b correlations were performed between the psychological variables at baseline. The results were consistent with theoretical expectations: positive and negative affect were not significantly correlated ( $\tau_b = -.214$ ,  $p = .508$ ), whereas anxiety was

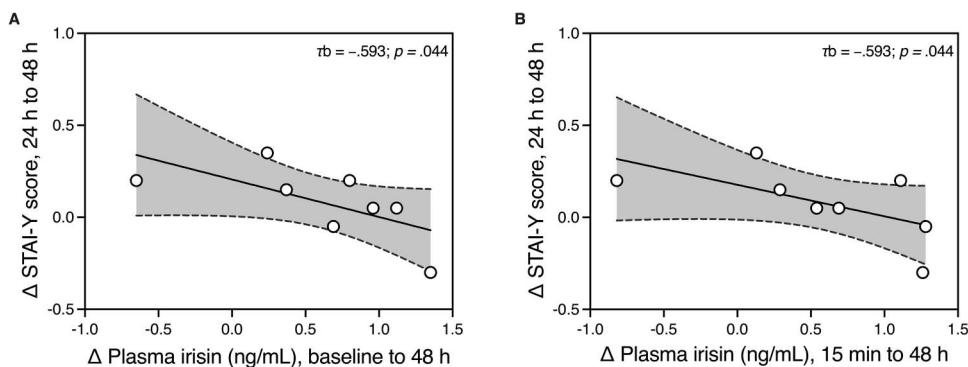
positively associated with negative affect ( $\tau b = .681, p = .033$ ) and negatively associated with positive affect ( $\tau b = -.618, p = .034$ ).

Friedman tests were performed to examine changes in psychological variables across the four time points. No significant time effects were observed for positive affect ( $\chi^2 F(3) = 0.750, p = .861$ , Kendall's  $W = .031$ ), negative affect ( $\chi^2 F(3) = 2.556, p = .465$ , Kendall's  $W = .107$ ), or state anxiety ( $\chi^2 F(3) = 1.775, p = .621$ , Kendall's  $W = .074$ ). These findings indicate that the psychological variables remained relatively stable at the group level across the recovery period. Therefore, the central psychological observation of the present study concerns exploratory associations among inter-individual change scores rather than a consistent sample-level reduction in state anxiety. Individual state-anxiety trajectories across the four assessment time points are illustrated in Figure 3, which highlights marked inter-individual variability around a stable group-level mean.

To generate preliminary hypotheses for future research, exploratory analyses were further conducted on the relationships between changes in biological and psychological variables. An exploratory negative association was observed between the change in plasma irisin from baseline to 48 h post-exercise and the change in state anxiety from 24 h to 48 h post-exercise ( $\tau b = -.593, p = .044$ ). A convergent negative association was also observed between the same 24-to-48-h change in state anxiety and the change in plasma irisin from 15 min to 48 h post-exercise ( $\tau b = -.593, p = .044$ ). In addition, early increases in CK from baseline to 24 h post-exercise were negatively associated with changes in positive affect over the same period ( $\tau b = -.667, p = .024$ ). The complete exploratory correlation matrices are reported in Supplementary Tables S1–S6. Exploratory scatterplots for the key plasma irisin–anxiety change-score associations are shown in Figure 4.



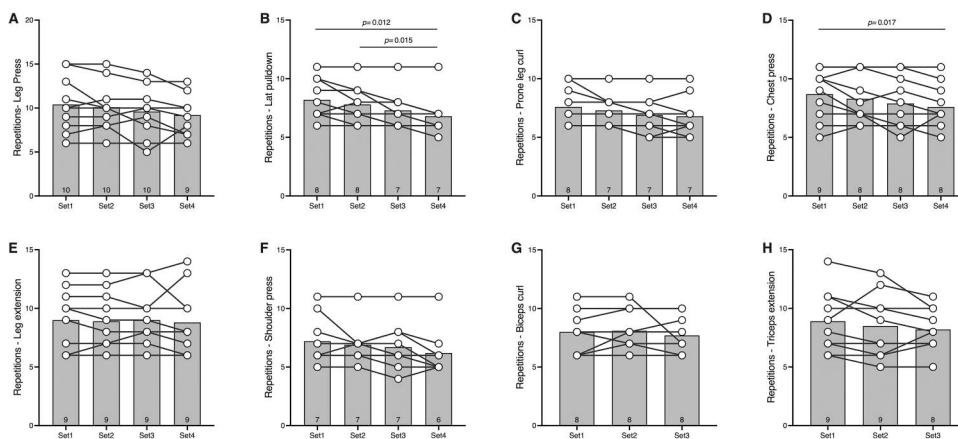
**Figure 3.** Individual state-anxiety trajectories across the recovery period. Bars represent group means (mean item score, range 1–4) of the Spielberger State-Trait Anxiety Inventory–State scale (STAI-Y) at baseline, 15 min, 24, and 48 h post-exercise. White circles connected by thin lines represent individual participant values ( $N = 8$ ). The number reported within each bar indicates the corresponding group mean. “ns” denotes the absence of a significant time effect at the Friedman omnibus test ( $\chi^2 F(3) = 1.775, p = .621$ , Kendall's  $W = 0.074$ ), confirming that state anxiety remained essentially stable at the group level despite marked inter-individual variability.



**Figure 4.** Exploratory scatterplots showing the associations between later-phase changes in plasma irisin up to 48 h and late changes in state anxiety. (A) Association between the change in plasma irisin from baseline to 48 h post-exercise and the change in STAI-Y state anxiety from 24 h to 48 h post-exercise. (B) Association between the change in plasma irisin from 15 min to 48 h post-exercise and the change in STAI-Y state anxiety from 24 h to 48 h post-exercise. Each dot represents one participant ( $N=8$ ). The fitted line and shaded band are shown only to aid visual interpretation of the direction of the association and do not represent the inferential test, which was based on Kendall's Tau-b. These analyses are exploratory and should be interpreted as hypothesis-generating given the pilot nature of the study and the small sample size.

### Training stimulus

The completed resistance-training stimulus is reported in Figure 5. Participants completed  $245.5 \pm 46.7$  repetitions across the 30-set session, corresponding to a total external session volume load of  $19,540.1 \pm 6,131.5$  kg. Repetitions completed across sets were



**Figure 5.** Completed repetitions during the experimental resistance-training session. Bars represent the mean, and individual lines show participant-level responses across sets. Four sets were performed for (A) leg press, (B) lat pulldown, (C) prone leg curl, (D) chest press, (E) leg extension, and (F) shoulder press, whereas three sets were performed for (G) biceps curl and (H) triceps extension. All exercises were performed at 70% of individual 1RM, using a 2-repetitions-in-reserve prescription, a controlled 2-1-2-1 time-under-tension protocol, and 90-s rest intervals between sets. Differences across sets were analysed using Friedman tests, followed by Holm-adjusted Conover post hoc comparisons where appropriate. Horizontal lines with corresponding  $p$ -values indicate significant post hoc pairwise differences between sets.

generally maintained, with significant set effects observed only for lat pulldown ( $\chi^2 F(3) = 10.333$ ,  $p = .016$ , Kendall's  $W = .431$ ) and chest press ( $\chi^2 F(3) = 9.837$ ,  $p = .020$ , Kendall's  $W = .410$ ). Holm-adjusted Conover post hoc comparisons showed fewer repetitions in Set 4 than in Set 1 and Set 2 for lat pulldown ( $p_{\text{Holm}} = .012$  and  $p_{\text{Holm}} = .015$ , respectively), and fewer repetitions in Set 4 than in Set 1 for chest press ( $p_{\text{Holm}} = .017$ ). No significant set effect was observed for leg press ( $\chi^2 F(3) = 6.643$ ,  $p = .084$ , Kendall's  $W = .277$ ), prone leg curl ( $\chi^2 F(3) = 5.763$ ,  $p = .124$ , Kendall's  $W = .240$ ), leg extension ( $\chi^2 F(3) = 1.787$ ,  $p = .618$ , Kendall's  $W = .074$ ), shoulder press ( $\chi^2 F(3) = 5.270$ ,  $p = .153$ , Kendall's  $W = .220$ ), biceps curl ( $\chi^2 F(2) = 0.900$ ,  $p = .638$ , Kendall's  $W = .056$ ), or triceps extension ( $\chi^2 F(2) = 2.545$ ,  $p = .280$ , Kendall's  $W = .159$ ).

## Discussion

The findings from this exploratory pilot study suggest that a single session of high-volume resistance training induces a complex and temporally dissociated psychobiological response in trained males. One of the most noteworthy observations was the convergent negative association between later changes in plasma irisin and late changes in state anxiety. Specifically, greater increases in plasma irisin up to 48 h post-exercise, whether calculated from baseline or from 15 min post-exercise, were associated with more favourable changes in state anxiety from 24 to 48 h. Importantly, the psychological variables did not show significant group-level changes across the recovery period. Thus, the observed irisin–anxiety association should not be interpreted as evidence of a generalised reduction in anxiety after resistance exercise. Rather, it suggests that later plasma irisin changes may be associated with inter-individual differences in late state-anxiety trajectories during recovery. This finding provides a preliminary basis for the hypothesis that the 48-hour irisin variation observed here may be linked to individual trajectories of emotional regulation during the later phase of post-exercise recovery. A complementary temporal pattern was found for CK, whereby early increases in this marker of muscular stress were negatively associated with later positive affect. This suggests that, while acute exercise-induced muscular stress may be associated with transiently less favourable emotional states (Ekkekakis et al., 2004; Watson et al., 1988), a later myokine response within the recovery window may accompany psychological restoration. This interpretation is consistent with broader exercise-related psychophysiological frameworks, according to which physical activity may support emotional stability and autonomic regulation (Zhang et al., 2022; Zou et al., 2018). In addition, preclinical and translational evidence suggests that specific myokines, such as irisin, may contribute to psychological resilience through mechanisms involving brain plasticity and the modulation of anxiety-related circuits (Islam et al., 2018; Lourenco et al., 2019; Pignataro et al., 2022; Villamil-Parra & Moscoso-Loaiza, 2024), although the present findings do not allow any mechanistic inference regarding these processes.

The present findings suggest that resistance training protocols utilising a repetitions-in-reserve buffer can stimulate irisin release. Within the specific time window sampled, plasma irisin increased modestly but significantly at 48 h post-exercise, while salivary irisin remained unchanged. This 48-hour elevation, alongside the concurrent elevation in CK and VAS scores which peaked at 24 h, confirms the protocol induced sufficient muscular stress (Baird et al., 2012).

While these results align with previous studies reporting elevated plasma irisin after resistance training (Huh et al., 2015; Marano et al., 2025; Nygaard et al., 2015; Tsuchiya et al., 2015), the timing of the response appears notably later. Previous reports have documented increases immediately or within a few hours post-exercise (Huh et al., 2015; Marano et al., 2025). In contrast, the data presented here align more closely with the findings of Pekkala et al. (2013), who reported no significant acute differences but did assess irisin levels at 48 and 72 h, also finding no significant increases. However, because the sampling schedule lacked the 1–4 h post-exercise window, a genuinely delayed response cannot be confidently distinguished from a later elevation following an unmeasured earlier acute peak. The modest 48-hour elevation observed in the present study should therefore be interpreted narrowly within the specific timeframes assessed.

Although highly speculative, as these pathways were not measured directly in the current study, a potential molecular hypothesis for this later elevation involves the PGC-1 $\alpha$ 4 isoform. This isoform is specifically induced by resistance training and follows a more delayed kinetic profile than the aerobic-induced PGC-1 $\alpha$ 1 isoform (Koh et al., 2022; Ruas et al., 2012). A hypothesis for future work is that PGC-1 $\alpha$ 4 activation might drive later FNDC5 expression and subsequent irisin secretion, potentially reflecting longer-term muscle repair and remodelling processes. Furthermore, the resistance protocol likely recruited fast-twitch glycolytic fibres, whose molecular characteristics may also influence the timing of the irisin response (Lavi et al., 2022). Although saliva represents a less invasive alternative to venipuncture (Missaglia et al., 2023), the present findings show that no salivary irisin changes were detected under these specific experimental conditions. This result should not be interpreted as evidence that saliva has limited utility for irisin assessment more generally. Rather, further studies are needed to determine whether different resistance-exercise protocols, sampling schedules, normalisation strategies, or analytical approaches may better capture salivary irisin dynamics.

Several limitations of this pilot study must be acknowledged. First, the small sample size ( $N = 8$ ) restricts statistical power and limits the generalisability of the findings. Consequently, the results, particularly the modest yet significant increase in plasma irisin at 48 h and the associated correlations, should be considered preliminary and hypothesis-generating, requiring confirmation in larger, adequately powered studies. Second, the absence of a non-exercise control condition represents a significant limitation. Without a control day, it is not possible to definitively attribute the observed temporal patterns solely to the exercise bout. Normal day-to-day variability, expectancy effects, and repeated-assessment effects may have contributed, particularly to the psychological measures. This limitation does not negate the value of the exploratory findings but restricts the strength of any causal interpretation. Third, the measurement schedule did not include the 1–4 h post-exercise window, a period in which acute irisin peaks have been previously reported (He et al., 2018; Huh et al., 2015). The exploratory irisin-anxiety associations should therefore be interpreted within the temporal resolution of the present sampling schedule, as they identify a cross-window association between 48-hour plasma irisin changes and late state-anxiety trajectories rather than establishing temporal precedence or mechanism. Finally, the study focused exclusively on young, trained males, precluding extrapolation to females, older adults, clinical populations, or untrained individuals. Despite these limitations, the study provides a novel framework for examining the prolonged psychological effects of resistance training.

## Conclusion

This exploratory study provides preliminary evidence that a single bout of resistance training, controlled via a repetitions-in-reserve protocol, was followed by a modest increase in plasma irisin at 48 h post-exercise within the sampled recovery window in young, resistance-trained males. These observations are limited to this specific population and should not be extrapolated to females, older adults, clinical populations, or untrained individuals. Within this specific demographic, this biological response was temporally dissociated from the peak of muscular stress markers and was associated with late changes in state anxiety from 24 to 48 h post-exercise. These findings generate a new, testable hypothesis: 48-hour changes in plasma irisin are inversely associated with inter-individual variations in state anxiety during the extended recovery phase following intense muscular stress. Given the correlational nature of this exploratory pilot study, these data do not establish that irisin functions as a direct biological mediator of emotional regulation, but rather highlight a hypothesis-generating psychobiological association. Future research using larger, more diverse cohorts, non-exercise control conditions, and denser post-exercise sampling should aim to confirm these preliminary associations and clarify whether irisin-related responses are meaningfully linked to psychological recovery trajectories after resistance exercise.

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## Author contributions

CRedit: **Luigi Marano**: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing; **Sara Missaglia**: Investigation, Resources, Writing – review & editing; **Eleonora Martegani**: Investigation, Resources, Writing – review & editing; **Cinzia Di Dio**: Data curation, Formal analysis, Methodology, Software, Writing – review & editing; **Patrizia Catellani**: Formal analysis, Writing – review & editing; **Antonella Marchetti**: Formal analysis, Writing – review & editing; **Michela Vezzoli**: Formal analysis, Methodology, Writing – review & editing; **Daniela Tavian**: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing; **Ferdinando Cereda**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Department of Psychology at the Catholic University of the Sacred Heart of Milan (CERPS) (protocol code no. 36/24). All participants provided written informed consent prior to their participation.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

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

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