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The time course of irisin release after an acute exercise: relevant implications for health and future experimental designs

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Abstract

This study aimed to analyze the acute impact of exercise on serum irisin levels in 22 young (YA, 24.6±3.5 yrs) and in 12 middle-aged male adults (MA, 54.6±5.7 yrs) 15 min and 24 h after an incremental cycling exercise test to exhaustion. ELISA assay was used for serum irisin detection. Circulating irisin increased significantly from baseline (9.0±2.0 ng/ml) to 15 min post-exercise (10.2±2.0 ng/ml, P <0.001), but the greatest increment was detected after 24 h (13.5±2.5 ng/ml, P <0.001) reaching more than 50% of the basal release. Levels were significantly higher in YA (9.7±1.7 to 11.1±1.8 to 14.5±2.2 ng/ml) than MA (7.6±1.6 to 8.7±1.5 to 11.8± 2.2 ng/ml) for all measured time-points (P <0.05). Nevertheless, MA showed a comparable increase in serum irisin levels when compared to YA. These findings highlight the importance of acute physical exercise as a countermeasure against age-related deterioration of skeletal muscle mass and function in both YA and MA.

Key Words: acute physical exercise; irisin; myokine; serum; aging.

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In the past decade, the understanding of skeletal muscle as a secretory organ has significantly increased. Skeletal muscle synthesizes and secretes into the bloodstream a wide range of myokines which exerts beneficial effects on various tissues and organs in an autocrine, paracrine, and endocrine manner. Among them there is irisin, mainly produced in response to muscle contraction. Indeed, physical exercise (PE) activates the Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) which determines the increase of Fibronectin type III domain-containing protein 5 (FNDC5) expression.[1] Irisin is derived from the proteolytic cleavage of the extra cytoplasmatic region of FNDC5 protein and it is consequently released into the peripheral circulation.[1] This myokine may be considered one of main mediators of the beneficial effects of PE on human health, given that it promotes cognition and neurodevelopment by triggering the expression of Brain-Derived Neurotrophic Factor (BDNF) and plays key functions in the whole-body metabolism. It induces the browning of white adipocytes, by increasing the expression of mitochondrial Uncoupling protein 1 (UCP1), improving thermogenesis and weight loss.[2] Moreover, it helps to maintain bone homeostasis,[3] modulates metabolic processes in the liver,[4] enhances glucose metabolism and promotes muscle hypertrophy in human muscle cells.[5,6] A considerable amount of literature has been published on irisin response to an acute bout of exercise in a period ranging from zero minutes to two h after the physical stimulus.[7,8] However, the comparison between studies is complicated due to various type, intensity, and duration

of exercise protocols, as well as heterogeneous study population enrolled. Furthermore, the timing of blood drawing is highly variable after exercise.[7] There seem to be a dose-response relationship between exercise and irisin elevation,[9,10] but contradictory results have been reported considering research that evaluate the irisin response to an incremental exercise until exhaustion. In particular, acute responses immediately (0-15 minutes) after a maximal incremental exercise test have shown either a slight rise[9,11-13] or no effects[14] on serum or plasma irisin levels, while the studies that have measured the amount of circulating irisin at least 30 min following an incremental exercise to exhaustion did not find significant changes in the myokine concentration.[12,13] The existing research seem to suggest that the elevation of irisin following an incremental exercise test to exhaustion is transient, and it is likely that after 24 hours the levels have returned to baseline conditions. However, no studies reported the time course of serum irisin from baseline, soon after as well as 24 hours post the physical exercise.

Given that muscle mass is the main predictor of the circulating irisin concentration in human,[15] the age-related decay in skeletal muscle mass would suggest a reduction in circulating irisin concentration.[16] The relationship between irisin and age has already been discussed by different authors,[11,17] while few studies have examined the impact of acute bout of exercise on irisin release by comparing different age groups, revealing contradictory results.[18-21] In fact, Huh et al.[18] have demonstrated that plasma irisin increases immediately after 45-min of vigorous-to-exhaustive exercise and the increment was similar in both young and older adults. On the contrary, others have reported no significant changes in plasma or serum irisin levels in response to either a single bout of circuit training in both young and middle-aged/older groups of females[19] and males,[20] as well as after 180 minutes of moderate-intensity treadmill walking in both young and older adult groups.[21]

Currently, no previous study has explored the irisin modulation from baseline, immediately and one day after an incremental exercise to exhaustion in a substantial number of healthy male subjects. In addition, there are limited and conflicting information regarding the age-related differences in irisin secretion after an exogenous stimulus. The aims of the present research were to evaluate the impact of a single bout of incremental exercise on a 24 hours' time course of serum irisin levels in healthy male subjects and to compare the acute exercise-induced irisin changes in young and middle-aged adults, thus enhancing the comprehension of the exercise's influence on this myokine.

We hypothesized that an acute stimulus could induce an immediate increase in irisin levels, as well as several hours after exercise. Given the age-related decline in skeletal muscle mass, we also hypothesized that the acute exercise-induced changes in irisin levels would differ between young and middle-aged adults, with younger adults showing a more pronounced increase compared to their older counterparts.

Material and Methods

Participants

Thirty-four healthy males (aged 20-65 yrs) were recruited via notices (recruitment start date: June 8, 2022; recruitment end date: December 12, 2022). Data were collected and analyzed from June to December 2022. The participants were assigned either to the young adult group (YA) (n=22, 24.6±3.5 yrs) or middle-aged adult group (MA) (n=12, 54.6±5.7 yrs). The cutoff point used correspond to 40 yrs and is reasonable for dividing the two groups.[22] This study was conducted in accordance with the ethical principles established in the Declaration of Helsinki. The protocol of the present study was approved by the Ethics Commission of the Università Cattolica del Sacro Cuore of Milan (N° of protocol 28-22) and written informed consent for study participation, permission for personal data treatment and biochemical analysis was obtained from all participants upon enrolment. Individuals with a body mass index (BMI) ≤30 kg/m^2 were involved in the study. To ensure the general health of the subjects, a self-reported history of neurological disorders, musculoskeletal impairment, other motor restrictions which could influence the regular outcome of the study, and current pharmacotherapy, which could alter the results, were set as the exclusion criteria. Participants completed all the assessments in two consecutive days and were instructed to eat a light breakfast and avoid smoke, alcoholic and caffeinated products at least 2 h before the test. Moreover, they were asked to refrain from moderate and vigorous intensity, and long-duration training in the previous 48 h and for the duration of the study post-exercise. Assessments took place at the same time of the day, from 10:00 a.m. to 1:00 p.m., taking into consideration the circadian effects.

Anthropometric and body composition parameters

The analysis of anthropometric and body composition parameters included weight and height measurement, the calculation of BMI, the estimation of body density and percentage of fat mass (FM). weight and height were measured using a mechanical scale (761, SECA GmbH & Co. KG., Hamburg, Germany) and a stadiometer (213, SECA GmbH & Co. KG. Hamburg, Germany), respectively. Percentage of FM was estimated by skinfold thickness using a calibrated skinfold caliper (Harpenden; Baty International, United Kingdom), in accordance with the procedures suggested by the American College of Sports Medicine.[23]

Physical activity

Short form of the Italian version of International Physical Activity Questionnaire was used to evaluate the volume of physical activity over the previous 7 days.[24] Total physical activity (PA_{tot}) was calculated by summing the time spent on walking, moderate, and vigorous intensity activities and was expressed in metabolic equivalent of task (MET)-minutes per week.

Exercise protocol

All the participants performed a maximal incremental test on a cycle ergometer. The exercise test took place in a room

with a relative humidity less than 60% and a temperature ranging from 18°C to 22°C. After 2 min of basal oxygen uptake assessment on the cycle ergometer (LC6 Monark; Vansbro, Sweden and Excalibur Sport, Lode BV, Groningen, Netherlands), the test started at 80 W and, the load was increased by 20 W every minute until volitional exhaustion. The subjects were instructed to maintain a cadence between 85 and 90 revolutions per minute. The exercise protocol included 5 min of cool down with 50 W load and 10 min of passive recovery in a seated position. Heart rate was recorded with an ANT+ heart rate monitor (Garmin, Olathe, USA), whereas respiratory parameters (*i.e.*, oxygen uptake, exhaled carbon dioxide, and pulmonary ventilation) were measured with a breath-by-breath metabolimeter (Quark CPET, COSMED; Rome, Italy and Vyntus Vyntus CPX, Vyaire GmbH, Hochberg, Germany). According to the operating manufacturer's instructions, the turbine flowmeter was calibrated using a 3-liter syringe. In addition, the gas analysis system was calibrated using room air: 21% O_2, 0,03% CO_2 and a certified gas mixture: 16% O_2, 5% CO_2 (Scott Medical ProductsTM, Plumsteadville, PA, USA) prior to each exercise test along with the delay and scrubber calibration. The maximal oxygen uptake, power, and heart rate value reached during the exercise test was considered as peak oxygen uptake ($\dot{V}O_{2peak}$), peak power, and peak heart rate (HR_{peak}).

Biochemical measurements

Participants were asked to arrive to the laboratory after 2 h fast. Three samples (3 ml each one) of peripheral venous blood were drawn using a vacutainer system. The first blood sample was drawn at baseline, the second was collected 15 minutes after reaching maximal exhaustion during the incremental test, and the final blood sample was taken approximately 24 hours later (Figure 1).
Vacutainers were centrifuged at 10,000 rpm for 10 min and serum was separated and stored at –80°C for subsequent analysis. Samples were not diluted and were evaluated in duplicate in 40-well plates. Serum irisin was detected by ELISA Irisin kit (Cat. *EK-067-29*, Phoenix Pharmaceuticals, Burlingame, CA, USA) according to the manufacturer instructions, using the Victor Nivo multimode plate reader (PerkinElmer, Waltham, Massachusetts, USA). Inter- and intra-assay variation for irisin were <15% and <10%, respectively. All samples fell within the provided standard curve.

Statistical analysis

Using the G*Power software, the number of participants required was calculated to be 29, based on an effect size (ES) of 0.31,[25] an α value of 0.05, and a desired statistical power (1-β) of 0.95. However, to account for potential dropouts on the second day of evaluation, we conservatively recruited 34 participants. SPSS software (Version 27) was applied to perform statistical analysis. Normality of the data distribution was tested (Shapiro-Wilk test), data from total sample met the normality assumptions and the power analyses ensure a statistical power necessary to run parametric test. Therefore, one-way repeated measure analysis of variance (ANOVA) was used to identify irisin changes from baseline, Post-hoc Contrasts were conducted to determine significant differences between means of time-points in the total sample. Regarding the data from the age groups, while normality was confirmed, the small sample size and associated lack of statistical power necessitated the use of non-parametric tests. Friedman-test for repeated measures was used to determine differences within group, Bonferroni correction for pairwise comparison was used for data analysis. Wilcoxon signed rank test was performed to test differences within group in irisin variation (Delta). Mann-Whitney U test was used to test differences between groups (YA and MA). Pearson's correlation was used to determine relationship between variables. Since age and some PE parameters differed between groups, partial correlation adjusted for age and peak power was performed to eliminate the strongest confounders. Variation irisin, absolute (Delta) and percent-

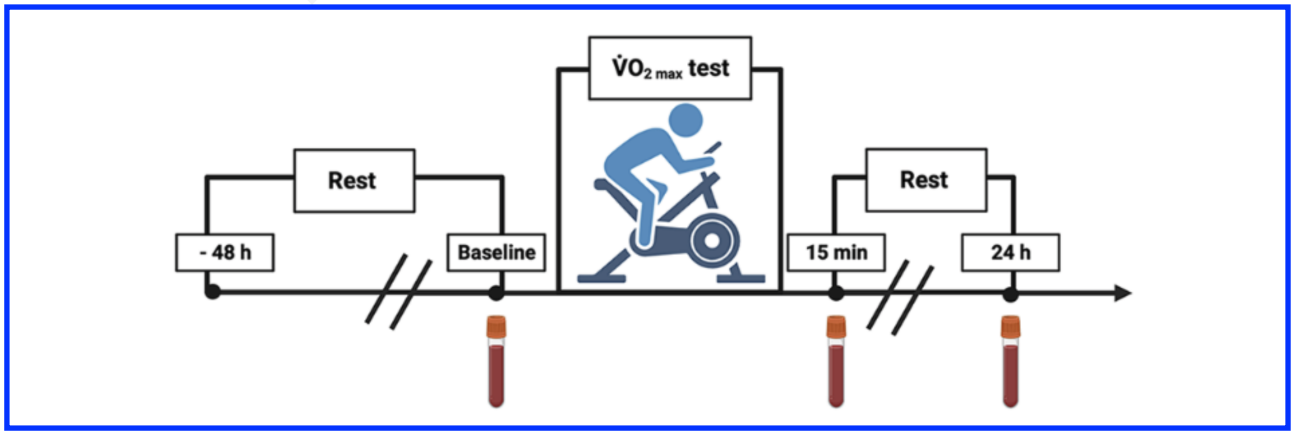

Figure 1. *Schematic flowchart of the experimental protocol. Vacutainers indicate timing of blood sample collection, taken prior (baseline) to incremental exercise ($\dot{V}O_{2max}$ test), immediately (15 min) and 24 h following the exhaustion. Rest indicates refraining from moderate and vigorous intensity, as well as long-duration training, in the 48 h prior to exercise and throughout the duration of the post-exercise study. (Created using BioRender.com).*

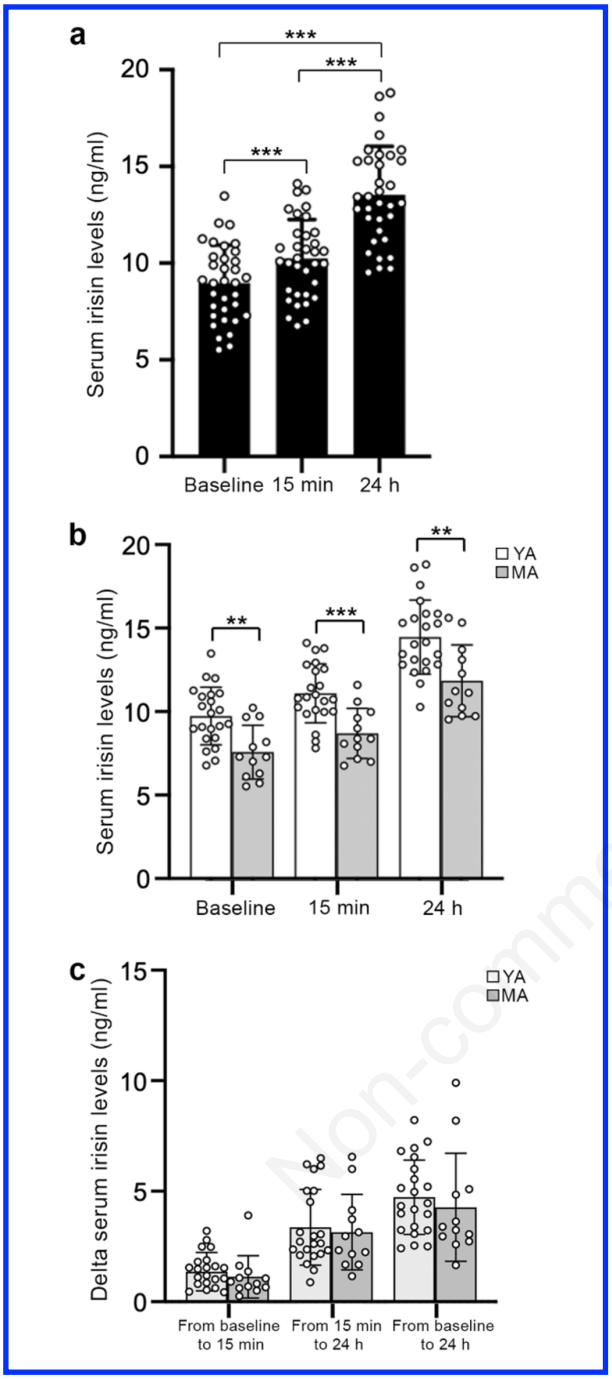

Figure 2. *Circulation irisin concentration after an acute exercise. Serum irisin levels at baseline, 15 min and 24 h post-exercise in 34 participants (black bars) (a) and in two age-groups (b), young adults (YA) (white bars) and middle-aged adults (MA) (grey bars). Change in circulating irisin after the acute bout of incremental exercise in two age-groups, YA (white bars) and MA (grey bars) (c). P-values were obtained using ANOVA for repeated measured to compare baseline and post-exercise irisin levels (a). Mann-Whitney U test was used to verify differences between groups (b, c). Data are expressed as means±SD. **P<0.01; ***P<0.001. (Created by GraphPad).*

age (Delta%), between baseline and post-exercise was computed (Delta=post-exercise irisin level – baseline irisin level), whereas the Delta% in irisin level after acute exercise was calculated using the following equation [(post-exercise irisin level) − (baseline irisin level)/ baseline irisin level] x 100.

Results

Anthropometric characteristics, body composition, physical activity, and PE parameters of 34 participants (22 YA, 12 MA) are reported in Table 1. There were no significant differences between groups in anthropometric characteristics apart from percentage of FM that resulted higher for MA compared to YA. However, according to the ACSM normative fitness categories for percentage of FM,[23] the body composition was considered excellent in both groups. YA and MA were highly active[26] showing a similar PA_{tot} (P=0.943). Regarding PE parameters, significantly lower HR_{peak} and higher peak power were reached by MA during the exercise protocol, while no significant differences in relative $\dot{V}O_{2peak}$ were observed. When compared to the ACSM normative fitness categories for $\dot{V}O_{2max}$,[23] the cardiorespiratory fitness of YA was classified as good, while that of MA was classified as superior.

Biochemical analysis revealed that irisin concentration 15 min and 24 h after the exercise was significantly higher than that detected at baseline in all subjects (P <0.001) (Figure 2a). Participants myokine levels increased (on average) by 15% (9.0±2.0 to 10.2±2.0 ng/ml) 15 min post-exercise, and by 55% (9.0±2.0 to 13.5±2.5 ng/ml) 24 h post-exercise compared with the baseline values. Both YA and MA showed significant increase in irisin concentration from baseline (YA, 9.7±1.7 ng/ml; MA, 7.6±1.6 ng/ml) to 15 min and 24 h post-exercise (YA, 11.1±1.8 ng/ml, P=0.003 and 14.5±2.2 ng/ml, P <0.001; MA, 8.7±1.5 ng/ml, P=0.025 and 11.8±2.2 ng/ml, P <0.001). Serum irisin was significantly higher in YA compared to MA for all measured time points (baseline P=0.002, 15 min $P \leq 0.001$; 24 h P=0.005) (Figure 2b). However, MA individuals displayed a comparable change in serum irisin levels at both 15 minutes and 24 hours after exercise in comparison to their younger counterparts (Figure 2c).

There was no significant correlation between baseline irisin levels and BMI (r=-0.034; P=0.848) or $\dot{V}O_{2peak}$ (r=0.137; P=0.440). Circulating basal irisin was negatively correlated with FM (r= -0.471, P <0.005). However, this parameter did not withstand adjustment to age and peak power in partial correlation analysis (Table 2).

Discussion

This study reported increased irisin levels after an incremental exercise in 34 healthy male adults, providing original evidence that myokine levels enhance immediately after the end of exercise, but, interestingly, the highest concentration occurs one day after exercise. The incremental exercise until exhaustion is a particularly effective stimulus for irisin production, allowing us to shed light both the

quantity and time release of irisin in a homogeneous sample (all males), under well-defined pre- and post-exercise conditions. In literature, a previous study has failed to detect any changes in irisin expression after an acute bout of exhausting exercise.[14] Only some authors documented modest changes of irisin levels immediately following (0-10 min) an acute bout of incremental exercise,[9,11-13] failing to detect post-exercise changes (after 60 and 180 min).[12,13] Moreover, a meta-analysis has stated that irisin increased within 15 min, following an acute exercise.[7] However, authors' assertion was based on studies in which blood draws were taken immediately post-exercise except for one that collected post-exercise samples after 20 min. This time-limited data may have led Fox et al.[7] to conclude that irisin increase is rapid and transient. Our findings clearly show that an acute bout of exhausting cycling induced a significant fast increase in irisin, but also demonstrate a long-drawn process. The fast increase in irisin concentration soon after exercise may be attributed to the proteolytic cleavage of available FNDC5 protein that is localized on the plasma membrane of muscle cells, leading to irisin release into the bloodstream in response to exercise. Differently, *de novo* FNDC5 production, induced by activation of PGC1-α expression, may require a long time (many hours), which could potentially explain the late elevation in circulating irisin reported in our study. The data reported by Norheim et al.[27] appear to support this assumption. They investigated the effect of acute aerobic exercise on PGC1-α and FNDC5 (the irisin precursor) expression in skeletal muscle and they also evaluated the circulating irisin levels in healthy adults, by col-

Table 1. *Participants anthropometric characteristics, body composition, physical activity, and physical exercise parameters.*

	Young adults	Middle-aged adults	*P*-value
Age, yrs	24.6±3.5	54.6±5.7	<0.001
n	22	12	-
Height, m	1.77±0.06	1.81±0.14	0.077
Weight, kg	73.1±9.8	76.53±7.3	0.387
BMI, kg/m^2	23.2±2.4	23.4±2.2	0.773
FM, %	11.4±5.5	16.8±6.1	0.009
PA_{tot}, METs-min/week	3733.6±3296.9	3101.4±1574.5	0.943
HR_{peak}, bpm	189±9	168±13	<0.001
$\dot{V}O_{2peak}$, ml/kg/min	49.0±9.8	44.8±5.1	0.256
$Power_{peak}$, W	276±57	326±24	0.003

Table 2. *Pearson's correlations between baseline irisin levels, body composition, and physical exercise parameters in 34 participants*

	Correlations		Age- and $Power_{peak}$-adjusted correlations	
	Baseline irisin level (ng/ml)		Baseline irisin level (ng/ml)	
	r	*P*	*r*	*P*
BMI, kg/m^2	-0.034	0.848	0.033	0.858
FM, %	-0.471	0.005	-0.264	0.144
$\dot{V}O_{2peak}$, ml/kg/min	0.137	0.440	0.066	0.721

lecting samples before, 0 min, and 2 h post-exercise. They observed that PGC1-α transcription was significantly induced after acute exercise, with a greater increment after 2 h post-exercise. As regard as circulating irisin levels, a transient increase peaking immediately after acute exercise was found by the same authors, with a return to pre-exercise levels after 2 h rest. Consequently, the blood draws performed relatively early during the post-exercise period could have prevented the detection of further release of irisin which would occur in the following hours and that is likely due to new FNDC5 protein expression induced by PGC1-α.

In a previous pilot study, we have observed that serum irisin level returns to baseline only after 48 h from the ending of an acute exercise bout to exhaustion.[28] For this reason, in the present study, to all participants it has been required to abstain from moderate and vigorous intensity, and long-duration physical activity over the 48 h preceding the exercise. Whereas most of the studies reported only 24 h of rest[9,13] or did not specify any information about it.[11,12] Physical activities, carried out in the previous days, may lead to overestimate basal myokines levels and contribute to a reduced increment in response to exercise. This provides important implications even for the design of chronic studies for which it appears necessary to measure basal irisin levels more than 24 hours after the last training session. Furthermore, it is important to note that, except for Zugel et al.[12] and Rodziewicz et al.,[10] all the aforementioned studies included both sexes in the enrolled participants and have reported irisin levels of males and females in combined form. However, it is known that irisin levels are affected by sex[11,17] and there are divergent responses to acute exercise in irisin concentration between males and females.[29,30] Consequently, to exclude between group gender differences, males and females should be considered separately in irisin evaluation. This study enables us to observe the biological variations in this myokine accurately and without confounding factors, albeit to a partial extent due to the absence of a female group.

The second aim of this study was to compare irisin changes induced by acute bout of exercise in two different age groups. We demonstrated that the increase in circulating irisin was similar in both groups, stating, for the first time, the highest and massive change 24 h after a maximal effort. Indeed, the magnitude of average change in irisin was almost 55% that might be considered relevant from a physiological and clinical point of view. Moreover, we observed a comparable increase about 15% (on average) of serum irisin even after 15 min post-exercise in both groups. These results are in accordance with those reported by Huh et al.[18] that have shown a similar increase (about 10%) in circulating irisin immediately after 45-min of vigorous-to-exhaustive exercise in two age groups (young and older adults). While speculative, we can assert that despite aging, muscle cells of physically active older individuals maintain the same responsiveness after acute exercise compared to young individuals, thereby resulting in a similar exercise-induced increase in circulating irisin. In clear contrast to our observations, other researchers[19-21] have observed no change in myokine levels after an exercise session in two age groups. It is likely that the intensity, as well as the type of exercise might have played a role in the blunted response of irisin levels to exercise stimulus. Future studies should further clarify the effect of nature, intensity, and duration of the exercise on irisin production. Moreover, we cannot exclude that the training level of the participants might have had a role in the irisin response to exhaustive exercise. To the best of our knowledge no studies have investigated the influence of training status in irisin response to acute exercise. Future studies should mandatorily explore the extent to which different levels of training can modulate the irisin response to exercise.

Although our age groups showed a similar response of irisin to acute exercise, for each evaluated time point, we found that serum irisin was significantly higher in YA than MA. Our results are in accordance with some previous investigations,[18,21] reporting higher basal irisin levels in the young compared to their older counterparts and in contrast with others.[19,20]

Finally, in the present study, we reported that circulating irisin is negatively associated with the percentage of FM. However, adjustment for age and peak power rendered this significant correlation null, suggesting that the latter may simply reflect the effect of age and cardiorespiratory fitness level.

The findings of our research are subject to some limitations. First, it should be underlined that the present study involved a group of middle-aged healthy men with higher age-related cardiorespiratory fitness in comparison to the younger group, as evidenced by the higher levels of peak power achieved by MA during the maximal exercise test. For this reason, adjustment for peak power was used to eliminate this potential confounder. Second, the portion of subjects from each subgroup of the total sample was not the same and quite small. Finally, additional time point draws would allow to better outline the irisin time-course in response to an incremental bout of exercise and to identify more precisely the irisin peak.

Conclusions

We have provided original evidence that an incremental test to exhaustion causes a relevant increase in circulating irisin one day after the ending of exercise across a wide age range and with a similar extent in both active young and middle-aged adults, likely providing pleiotropic and beneficial effects on health. In our research, we used a maximal oxygen consumption test on a cycle ergometer as exogenous stimulus to increase irisin, however, maximal exercise to exhaustion is not a common daily training. Therefore, future research is needed to understand whether an increase in irisin levels can be achieved through lighter intensity activities or other types of exercise, that can be enjoyed by everyone.

List of abbreviations

PGC1-α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

FNDC5: Fibronectin type III domain-containing protein 5

BDNF: Brain-Derived Neurotrophic Factor
UCP1: mitochondrial Uncoupling protein 1
YA: young adult group
MA: middle-aged adult group
BMI: body mass index
FM: fat mass
PAtot: total physical activity
MET: metabolic equivalent of task
$\dot{V}O_{2peak}$: peak oxygen uptake
HR_{peak}: peak heart rate
$Power_{peak}$: peak power
ANOVA: analysis of variance

Contributions

DT conceived and designed research; ET, SM, PV, CG, ABOS, ANM contributed to the acquisition of data; ET and ABON did the statistical analysis; ET wrote the first draft of the manuscript. All authors contributed to the analysis and interpretation of data, to revising the content of manuscript and they approved the final edited manuscript.

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Ethics approval

The Ethics Committee of the Catholic University of the Sacred Heart approved this study (28-22). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

Informed consent

All patients participating in this study signed a written informed consent form for participating in this study.

Patient consent for publication

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Conflict of interest

The authors declare they have no financial, personal, or other conflicts of interest.

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References

1. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481:463-8.
2. Trettel CDS, Pelozin BRA, Barros MP, et al. Irisin: An anti-inflammatory exerkine in aging and redox-mediated comorbidities. Front Endocrinol (Lausanne) 2023; 14:1106529.
3. Kornel A, Den Hartogh DJ, Klentrou P, Tsiani E. Role of the Myokine Irisin on Bone Homeostasis: Review of the Current Evidence. Int J Mol Sci 2021;22:9136.
4. Canivet CM, Bonnafous S, Rousseau D, et al. Hepatic FNDC5 is a potential local protective factor against Non-Alcoholic Fatty Liver. Biochim Biophys Acta Mol Basis Dis 2020;1866:165705.
5. Huh JY, Dincer F, Mesfum E, Mantzoros CS. Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and metabolism in humans. Int J Obes (Lond) 2014;38:1538-44.
6. Yano N, Zhao YT, Zhao TC. The Physiological Role of Irisin in the Regulation of Muscle Glucose Homeostasis. Endocrines. 2021;2:266-83.
7. Fox J, Rioux BV, Goulet EDB, et al. Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: A meta-analysis. Scand J Med Sci Sports 2018;28:16-28.
8. Kazeminasab F, Sadeghi E, Afshari-Safavi A. Comparative Impact of Various Exercises on Circulating Irisin in Healthy Subjects: A Systematic Review and Network Meta-Analysis. Oxid Med Cell Longe 2022;2022: 8235809.
9. Daskalopoulou SS, Cooke AB, Gomez YH, et al. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. Eur J Endocrinol 2014;171: 343-52.
10. Tsuchiya Y, Ando D, Goto K, et al. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. Tohoku J Exp Med 2014;233:135-40.
11. Löffler D, Müller U, Scheuermann K, et al. Serum irisin levels are regulated by acute strenuous exercise. J Clin Endocrinol Metab 2015;100:1289-99.
12. Zügel M, Qiu S, Laszlo R, et al. The role of sex, adiposity, and gonadectomy in the regulation of irisin secretion. Endocrine 2016;54:101-10.
13. Qiu S, Bosnyák E, Treff G, et al. Acute exercise-induced irisin release in healthy adults: Associations with training status and exercise mode. Eur J Sport Sci 2018;18: 1226-33.
14. Rodziewicz E, Król-Zielińska M, Zieliński J, et al. Plasma concentration of irisin and brain-derived-neurotrophic factor and their association with the level of erythrocyte adenine nucleotides in response to long-term endurance training at rest and after a single bout of exercise. Front Physiol 2020;11:923.
15. Huh JY, Panagiotou G, Mougios V, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 2012;61: 1725-38.
16. Clark BC, Manini TM. Functional consequences of sarcopenia and dynapenia in the elderly. Curr Opin Clin Nutr Metab Care 2010;13:271-6.
17. Ruan Q, Huang Y, Yang L, et al. The effects of both age and sex on irisin levels in paired plasma and cerebrospinal fluid in healthy humans. Peptides 2019;113:41-51.
18. Huh JY, Mougios V, Kabasakalis A, et al. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. J Clin Endocrinol Metab 2014;99:E2154-61.
19. Gmiat A, Micielska K, Kozłowska M, et al. The impact of a single bout of high intensity circuit training on myokines' concentrations and cognitive functions in women of different age. Physiol Behav 2017;179: 290-7.
20. Rioux BV, Brunt KR, Eadie AL, et al. Impact of acute circuit training on irisin in younger and older overweight adults. Appl Physiol Nutr Metab 2021;46: 1248-56.
21. McCormick JJ, King KE, Notley SR, et al. Exercise in the heat induces similar elevations in serum irisin in young and older men despite lower resting irisin concentrations in older adults. J Therm Biol 2022; 104:103189.
22. Lachman ME. Development in midlife. Annu Rev Psychol 2004;55:305-31.
23. Wagner DR, Gibson AL. Health-Related Physical Fitness Testing and Interpretation. In: ACSM's guidelines for exercise testing and prescription. Ed: Liguori, G. 11th ed. Wolters Kluwer, Philadelphia; 2021. pp 116-182.
24. Mannocci A, Di Thiene D, Del Cimmuto A, et al. International Physical Activity Questionnaire: validation and assessment in an Italian sample. Inter J Public Health 2010;7:369-76.
25. Jürimäe J, Purge P, Tillmann V. Serum sclerostin and cytokine responses to prolonged sculling exercise in highly-trained male rowers. J Sports Sci 2021;39: 591-7.
26. Forde C. Scoring the international physical activity questionnaire (IPAQ) exercise prescription for the prevention and treatment of disease. Exercise Prescription for the Prevention and Treatment of Disease. Trinity College Dublin, University of Dublin. 2005.
27. Norheim F, Langleite TM, Hjorth M, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FEBS J 2014;281:739-49.
28. Missaglia S, Tommasini E, Vago P, et al. Salivary and serum irisin in healthy adults before and after exercise. Eur J Transl Myol 2023;33:11093.
29. Scalzo RL, Peltonen GL, Giordano GR, et al. Regulators of human white adipose browning: evidence for sympathetic control and sexual dimorphic re-

sponses to sprint interval training. PLoS One 2014;9: e90696.
30. Wiecek M, Szymura J, Maciejczyk M, et al. Acute anaerobic exercise affects the secretion of asprosin, irisin, and other cytokines - a comparison between sexes. Front Physiol 2018;9:1782.

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