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Original Article

Association between Circulating Irisin and Heart Rate Variability in Anaesthetised Male Wistar Rats

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ABSTRACT

Background: Irisin plays an important role in energy metabolism, thermogenesis, and cardiovascular regulation. Its relationship with autonomic cardiovascular control remains unclear. While, Heart Rate Variability (HRV) is a reliable non-invasive marker to assess autonomic balance, its association with circulating irisin is not yet well defined. **Objectives:** This study aimed to examine the relationship between circulating irisin levels and HRV indices in male Wistar rats, and to assess whether adiposity influences this association. **Materials and Methods:** Electrocardiogram (ECG) recordings were obtained from eighteen male Wistar rats under ketamine-xylazine anaesthesia. HRV was analysed using standard time- and frequency-domain measures. Serum irisin levels and lipid profile parameters were measured. Lee index was calculated. Associations between irisin, HRV indices, and cardiometabolic variables were evaluated using Spearman's correlation, partial correlation (adjusting for Lee index), and multiple linear regression analysis. **Results:** Circulating irisin levels were significantly associated with many HRV parameters in the unadjusted analysis. After adjustment for adiposity and correction for multiple comparisons, only LF power and total power remained significant. In multivariable regression analysis, LF power showed the strongest independent association with irisin levels ($p<0.001$), followed by an inverse association with mean heart rate ($p<0.001$), whereas the Lee index was not statistically significant. **Conclusion:** Circulating irisin is independently associated with HRV-derived indices of autonomic cardiovascular regulation, particularly LF power, under ketamine-xylazine anaesthesia. These findings suggest a potential link between autonomic modulation and irisin, although interpretation is limited by the pharmacologically altered autonomic state and the cross-sectional design.

Keywords: Autonomic Nervous System, Cardiovascular Physiology, Heart rate variability, Irisin, Wistar rats.

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INTRODUCTION

Irisin is a proteolytic cleavage product of Fibronectin type III Domain-Containing Protein 5 (FNDC5).[1] It was initially identified as an exercise-induced myokine and has received more attention for its role in the regulation of energy metabolism,[1] through browning of white adipose tissue, thermogenesis,[2] and insulin sensitivity.[3] Studies have demonstrated that adipose tissue itself can secrete irisin,[2] indicating that it may also function as an adipokine involved in lipid metabolism and overall energy homeostasis.[4] Although circulating irisin concentrations have been linked with several metabolic and cardiovascular risk factors, the reported findings remain inconsistent across studies.[5-9] These discrepancies are said to be due to differences in study populations with various levels of physical activity, sample size, experimental design, and methods used for irisin measurement.

Heart Rate Variability (HRV) is a non-invasive marker of autonomic nervous system function, indicating the dynamic balance between sympathetic and parasympathetic modulation of cardiac activity.[10] It is widely recognised as an early marker of cardiovascular health and physiological adaptability. Reduced HRV has been associated with increased cardiovascular risk as well as adverse metabolic outcomes. Given that both irisin and HRV are influenced by physical activity and metabolic status, a potential link between this metabolic myokine and autonomic regulation is biologically plausible. However, the reports are currently indirect, inferred through exercise physiology, autonomic modulation, inflammation, and metabolic regulation.[11-13] The relationship between circulating irisin levels and HRV parameters remains insufficiently understood.

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Excess adiposity, chronic low-grade inflammation, and neuroendocrine dysregulation are known to contribute to altered irisin secretion and impaired autonomic function.[11,14-16] Individuals with obesity commonly exhibit reduced HRV, reflecting autonomic imbalance, along with disturbances in lipid metabolism. Interestingly, circulating irisin levels in obesity have been reported to be either increased or decreased across studies, indicating a lack of consistent regulatory pattern in the obese state.[17-20] Since irisin is also involved in lipid metabolism, adiposity may act as a potential confounder or mediator in the relationship between irisin and autonomic function.

Experimental animal models offer an effective approach for investigating physiological interactions under well-controlled conditions. In the present study, male Wistar rats were chosen in order to reduce inter-individual variability and to eliminate the potential influence of oestrous cycle-associated hormonal changes seen in female animals, which are known to affect both irisin secretion and autonomic function.[21] Maintaining standardised housing conditions and limiting variations in physical activity also improved the consistency and reliability of the physiological measurements obtained in this model.

Based on previous evidence linking irisin with metabolic and cardiovascular regulation, we proposed that circulating irisin levels may be associated with Heart Rate Variability (HRV) parameters. In addition, adiposity, assessed using the Lee index, was considered a possible factor affecting this relationship. Accordingly, the present study was undertaken to examine the association between circulating irisin levels and HRV parameters in male Wistar rats. The Lee index was also used to assess whether adiposity influenced the relationship between irisin and autonomic regulation.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Post Graduate Dissertation Screening Committee (Approval Number: JIP/Physio/592), Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, and by the Institutional Animal Ethics Committee (Approval Number: PU/CAHF/24IAEC/2019/02), Pondicherry University, Puducherry. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2018) were followed in this study.

Animals and Housing

Eighteen male Wistar rats aged 12 weeks were procured from a certified breeder (Biogen Laboratory Animal Facility, Bangalore, India). Animals were housed in the institutional animal facility under standard laboratory conditions in Perspex cages containing paddy husk bedding. Environmental conditions were maintained at a temperature of 24±1 °C, relative humidity of 50-60%, and a 12-hr light/dark cycle. The animals were acclimatised for a period of two weeks before commencement of the study. Standard pellet diet and water were provided *ad libitum* throughout the experimental period. General health status and activity of the animals were monitored daily.

Anthropometric Measurements

Body weight was measured using a digital weighing balance (Citizen, India) with a sensitivity of 0.5 g. Nose-to-anus length was measured with a flexible non-elastic measuring tape calibrated in millimetres. Lee index was used to assess the level of adiposity in the rats. It is an indirect marker of obesity in rodents and is calculated using the following formula

$$\text{Lee Index} = \frac{\sqrt[3]{\text{Body weight } (g)}}{\text{Nose} - \text{to} - \text{anus length (cm)}}$$

Anaesthesia

Ketamine hydrochloride (50 mg/kg) and Xylazine (5 mg/kg) (KX) were administered to the rats via the intraperitoneal route. Adequate depth of anaesthesia was confirmed prior to ECG recording and blood sample collection. To minimise the severe autonomic and cardiorespiratory depression commonly associated with deep surgical anaesthesia, a low-dose sedative combination of ketamine and xylazine was used. Ketamine is a dissociative anaesthetic with sympathomimetic cardiovascular effects mediated through inhibition of catecholamine reuptake, whereas xylazine is an α2-adrenergic agonist that produces sympatholytic effects by inhibiting norepinephrine release, including bradycardia.[22,23] The combination provides relatively stable conditions for ECG acquisition and HRV analysis. However, the autonomic effects of both agents should be considered during interpretation of the findings.

Electrocardiographic Recording and Heart Rate Variability Analysis

Electrocardiographic (ECG) recordings were obtained using the IX-BIO4-SA small animal ECG instrument (iWorx Systems Inc., Dover, New Hampshire, USA) with needle electrodes. ECG recordings were taken in the morning between 8 am and 11 am using the standard limb lead configuration at the Right Forelimb (RA), Left Forelimb (LA), and Left Hindlimb (LL). ECG signals were recorded at a sampling rate of 1000 Hz, with appropriate

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filters to reduce electrical noise and movement artefacts. After an initial stabilisation period, ECG was recorded continuously for 10 min.

R-R intervals were then extracted using LabScribe software and exported for HRV analysis. The R-R interval series was resampled at 8 Hz using cubic spline interpolation, and HRV analysis was performed using Kubios HRV software (version 3.5, University of Eastern Finland). Time-domain HRV parameters included the Standard Deviation of NN intervals (SDNN) and the Root Mean Square of Successive Differences (RMSSD). Frequency-domain analysis was performed using the Low-Frequency (LF: 0.2-0.8 Hz) and High-Frequency (HF: 0.8-4.0 Hz) bands. Derived parameters included LF power, HF power, total power (ms^2), LF and HF in normalised units, and the LF/HF ratio.

Blood Collection and Biochemical Analysis

Blood samples were collected from the lateral tail vein under anaesthesia. Samples were centrifuged at 3000 rpm for 15 min, following which serum was separated and stored at -80 °C until further analysis.

Serum irisin concentration was measured using a commercially available ELISA kit (KTE101164, Abbiotek Inc., China) according to the manufacturer's protocol. The assay demonstrated intra- and inter-assay coefficients of variation below 10%, as specified by the manufacturer. Serum Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein (HDL) were measured using an automated biochemistry analyser (AU5800, Beckman Coulter, USA) following standard protocols. Very-Low-Density Lipoprotein (VLDL) was calculated as TG/5, and Low-Density Lipoprotein (LDL) was estimated using Friedewald's formula.

Statistical Analysis

Statistical analysis was performed to evaluate the distribution characteristics of metabolic and HRV parameters and to examine the associations between circulating irisin levels, HRV indices, and adiposity.

Descriptive statistics were computed for all variables, including body weight, Lee index, mean heart rate, serum biochemical parameters (irisin, total cholesterol, triglycerides, HDL, LDL, and VLDL), and HRV parameters (SDNN, RMSSD, LF power, LF nu, HF power, HF nu, total power and LF/HF ratio). Data were expressed as mean±Standard Deviation (SD) and median with interquartile range (Q1-Q3), along with 95% confidence intervals. The normality of each variable was assessed using the Shapiro-Wilk test.

The relationship between irisin and normally distributed lipid profile parameters was analysed using Pearson's correlation. Spearman's rank correlation coefficient (rho) was applied to assess the relationship between serum irisin and HRV parameters. To account for potential confounding effects of adiposity, partial correlation analysis was performed controlling for the Lee index.

To identify factors independently associated with circulating irisin, multiple linear regression analysis was conducted with serum irisin levels as the dependent variable and LF power, mean heart rate, and Lee index entered as independent variables. Multicollinearity among predictors was evaluated using the Variance Inflation Factor (VIF). Model performance was assessed using R, R^2, adjusted R^2, Root Mean Square Error (RMSE), F-statistic and p-value.

All statistical analyses were performed using R software (version 4.5.3). A two-tailed p-value<0.05 was considered statistically significant. Where applicable, p-values were adjusted using the Bonferroni correction for multiple comparisons.

RESULTS

Baseline metabolic and physiological characteristics

The descriptive characteristics of metabolic and cardiovascular parameters are presented in Table 1. The mean Lee index was 0.299 with 0.025 standard deviation, indicating minimal variability in adiposity among animals. Lipid profile parameters showed moderate variability, with total cholesterol (49.83±14.96 mg/dL), triglycerides (77.72±28.14 mg/dL), HDL (20.44±7.01 mg/dL), LDL (17±4.60 mg/dL), and VLDL (15.5±5.61 mg/dL). Mean resting heart rate was 410.33±53.28 bpm. All variables in this group were normally distributed (Shapiro-Wilk, $p>0.05$).

Serum irisin levels

Serum irisin levels are summarised in Table 2. The mean concentration was 11.80±1.30 ng/mL, with a median of 11.54 ng/mL (IQR: 11.04-12.35 ng/mL). Irisin showed a non-normal distribution (Shapiro-Wilk W=0.859, $p=0.0116$).

Heart rate variability parameters

HRV indices are presented in Table 3. All parameters showed non-normal distributions (Shapiro-Wilk, $p<0.001$) and are reported as median (IQR). Time-domain measures showed SDNN of 4.9 ms (4.325-5.675 ms) and RMSSD of 7.55 ms (7.175-9.325 ms). In frequency-domain analysis, LF power was 1 ms^2 (0.25-7 ms^2), HF power was 15 ms^2 (13-19.75 ms^2), and total power was 17 ms^2 (14.5-27 ms^2). The LF/HF ratio was low (0.064; 0.038-0.136), indicating parasympathetic predominance.

Correlation between irisin and HRV parameters

In unadjusted analysis (Table 4), irisin showed significant positive correlations with SDNN ($\rho=0.574$, $p=0.0127$), LF power ($\rho=0.745$, $p<0.001$), LF nu ($\rho=0.505$, $p<0.035$), total power ($\rho=0.640$, $p=0.0042$), LF/HF ratio ($\rho=0.505$, $p=0.0346$) and negative correlation with HF power in normalised units, HF nu ($\rho=-0.505$, $p<0.035$). No significant associations were observed with

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RMSSD or HF power in absolute units. After adjustment for Lee index, associations remained significant for LF power (pr_s=0.8, p=<0.0001) and total power (pr_s=0.65, p=0.0391). After Bonferroni correction for multiple comparisons, only LF power and total power remained statistically significant.

Correlation between irisin and lipid profile

No significant correlations were observed between irisin and lipid parameters (Figure 1), including total cholesterol (r=-0.29), triglycerides (r=-0.26), VLDL (r=-0.26), HDL (r=-0.31), and LDL (r=-0.20), all p>0.05.

Multiple Linear Regression Analysis of Independent Associations

Multivariable linear regression analysis (Table 5) showed that LF power of heart rate variability was significantly and positively associated (Figure 2) with serum irisin levels (Standardised β= 0.636, p<0.001). Mean heart rate was significantly and negatively associated (Figure 3) with serum irisin levels (Standardised β= -0.463, p<0.001). The Lee index showed a negative but non-significant association with serum irisin levels (β=-0.150, p= 0.084). Variance inflation factor values for all variables ranged from 1.064 to 1.550, indicating the absence of multicollinearity.

The regression model showed a strong overall fit (Table 6) with an R value of 0.956. The model explained 91.4% of the variance in the outcome variable (R^2=0.914), with an adjusted R^2 of 0.895. The model was statistically significant (F=49.45, p<0.001), and the RMSE was 0.421, indicating a low prediction error.

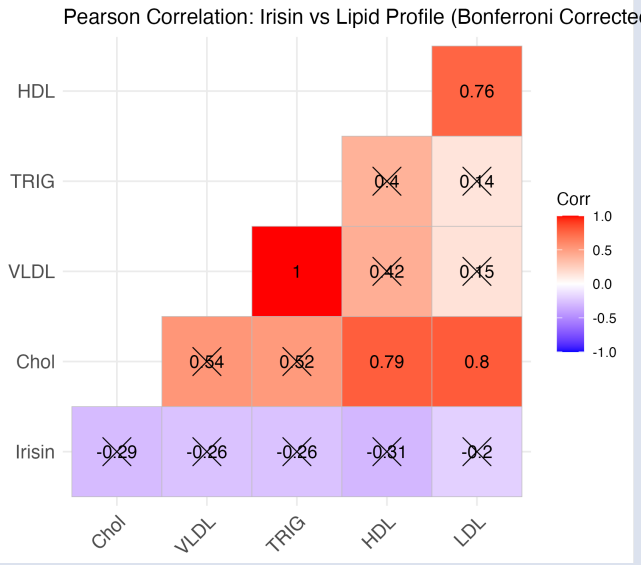

Figure 1: Correlogram: Irisin vs Lipid Profile. Correlation matrix showing Pearson correlations between serum irisin levels and lipid profile parameters in male Wistar rats. Correlation strength is indicated by colour intensity (blue=negative, red=positive). Chol: Total cholesterol (mg/dL). VLDL: Very-low-density lipoprotein (mg/dL). TRIG: Triglycerides (mg/dL). HDL: High-density lipoprotein cholesterol (mg/dL). LDL: Low-density lipoprotein cholesterol (mg/dL). Pearson correlation coefficients with statistically significance after Bonferroni correction (p<0.05) are displayed. For clarity, non-significant associations were masked. Correlation strength is indicated by colour intensity (blue=negative, red=positive).

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Table 1: Baseline anthropometric, metabolic parameters and heart rate of male Wistar rats (n=18).

Variable	Mean±SD	95% CI	Shapiro W	p-value
Weight (g)	285.56±43.26	264.04 - 307.07	0.951	0.444
Lee index	0.299±0.025	0.287 - 0.312	0.920	0.1271
Chol (mg/dL)	49.83±14.96	42.39 - 57.27	0.941	0.2961
VLDL (mg/dL)	15.5±5.61	12.71 - 18.29	0.917	0.1131
TRIG (mg/dL)	77.72±28.14	63.78 - 91.72	0.902	0.0632
HDL (mg/dL)	20.44±7.014	16.96 - 23.93	0.974	0.8632
LDL (mg/dL)	17±4.60	14.71 - 19.29	0.930	0.1976
Mean HR (bpm)	410.33±53.28	383.84 - 436.83	0.906	0.0743

Lee index is a measure of body fat, calculated as the cube root of body weight (g) divided by nose-to-anus length (cm) (values not multiplied by 1000). Chol: Total cholesterol (mg/dL). VLDL: Very-Low-Density Lipoprotein (mg/dL). TRIG: Triglycerides (mg/dL). HDL: High-Density Lipoprotein Cholesterol (mg/dL). LDL: Low-density Lipoprotein cholesterol (mg/dL). Mean HR: mean heart rate in beats per minute (bpm). Values are presented as mean ± Standard Deviation (SD). 95% CI: The 95% Confidence Interval (CI) represents the range of the mean. Normality of distribution was assessed using the Shapiro-Wilk test. A p-value<0.05 indicates deviation from normal distribution.

Table 2: Distribution of circulating irisin levels in male Wistar rats (n=18).

Variable	Mean±SD	Median (Q1-Q3)	Shapiro W	p-value
Irisin (ng/mL)	11.802±1.297	11.54 (11.04 - 12.35)	0.859	0.0116*

Irisin: Serum irisin levels in ng/mL. Values are presented as mean ± Standard Deviation (SD) and median (interquartile range, Q1-Q3). Normality of distribution was assessed using the Shapiro-Wilk test. A p-value<0.05 indicates deviation from normal distribution.

Table 3: Distribution of Heart Rate Variability (HRV) parameters in male Wistar rats (*n*=18).

Variable	Median (Q1-Q3)	Shapiro W	p-value
SDNN (ms)	4.9 (4.325 - 5.675)	0.578	<0.001*
RMSSD (ms)	7.55 (7.175 - 9.325)	0.556	<0.001*
LF (ms^2)	1 (0.25 - 7)	0.591	<0.001*
LF (nu)	5.965 (3.622 - 11.91)	0.727	<0.001*
HF (ms^2)	15 (13 - 19.75)	0.497	<0.001*
HF (nu)	94.03 (88.09 - 96.38)	0.726	<0.001*
TP (ms^2)	17 (14.5 - 27)	0.505	<0.001*
LF/HF	0.064 (0.038 - 0.136)	0.671	<0.001*

SDNN (ms): Standard deviation of the NN intervals, measured in milliseconds. RMSSD (ms): root mean square of successive differences between normal heartbeats, reflecting parasympathetic activity. Frequency-domain analysis was performed using rat-specific frequency bands: Low Frequency (LF: 0.2-0.8 Hz) and High Frequency (HF: 0.8-4.0 Hz). LF (ms^2): low-frequency power in absolute units (ms^2), reflecting predominantly sympathetic and also parasympathetic activity. LF (nu): Low-frequency power in normalised units. HF (ms^2): high-frequency power in absolute units (ms^2), representing parasympathetic activity. HF (nu): High-frequency power in normalised units. TP (ms^2): Total power in absolute units (ms^2); total variance in HRV signal. LF/HF: ratio of low- to high-frequency power, an index of sympatho-vagal balance. Values are presented as median (interquartile range, Q1-Q3). Normality of distribution was assessed using the Shapiro-Wilk test. A p-value<0.05 indicates deviation from normal distribution.

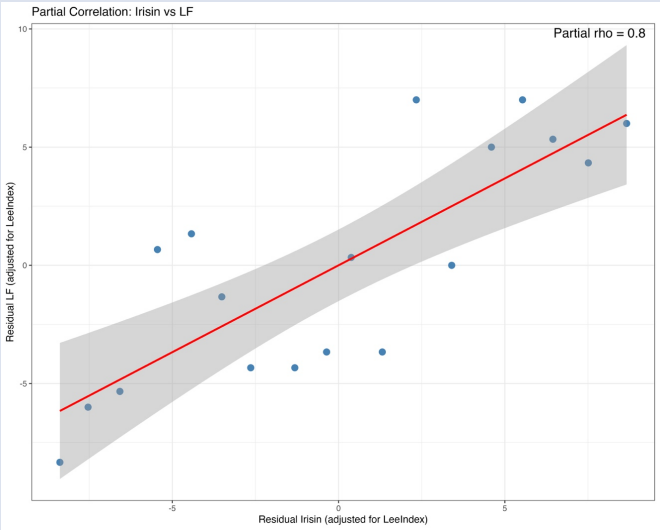

Figure 2: Association between serum irisin and LF power of heart rate variability. Shows the relationship between serum irisin residuals and LF power residuals. The partial correlation coefficient is presented, with a red line representing the linear regression fit with 95% confidence intervals. Associations were assessed using partial correlation after controlling for the Lee index.

DISCUSSION

Baseline Metabolic and Physiological Characteristics

At baseline, the male Wistar rats showed minimal variation in body weight or Lee index. This homogeneity helped minimise the effect of individual adiposity differences on the subsequent analyses. The levels of total cholesterol, triglycerides, HDL, LDL, and VLDL were within the normal range reported for healthy Wistar rats,[24] confirming the absence of baseline metabolic disturbance. In addition, resting heart rate values were consistent with those reported in previous studies on rats,[25] indicating a stable baseline autonomic state across the group. It should also be noted that the ECG recordings were taken under anaesthesia using a ketamine-xylazine combination administered at low doses. Therefore, recorded parameters represent an anaesthetized physiological state rather than true resting wakefulness.

Distribution of Serum Irisin

Serum irisin levels showed a non-normal distribution (median value 11.54 ng/mL) with a relatively narrow interquartile range (11.05-12.35 ng/mL), suggesting limited dispersion despite deviation from normality, indicating relatively consistent circulating levels across animals studied.

Previous studies have reported variable baseline irisin concentrations, ranging from ~1.7 to 10.5 ng/mL.[26,27] In Wistar rats, such variability has been partly due to methodological differences, particularly ELISA-based techniques having potential cross-reactivity with FNDC5-related peptides.[9] It should also be noted that FNDC5 is the precursor of irisin and also has a similar

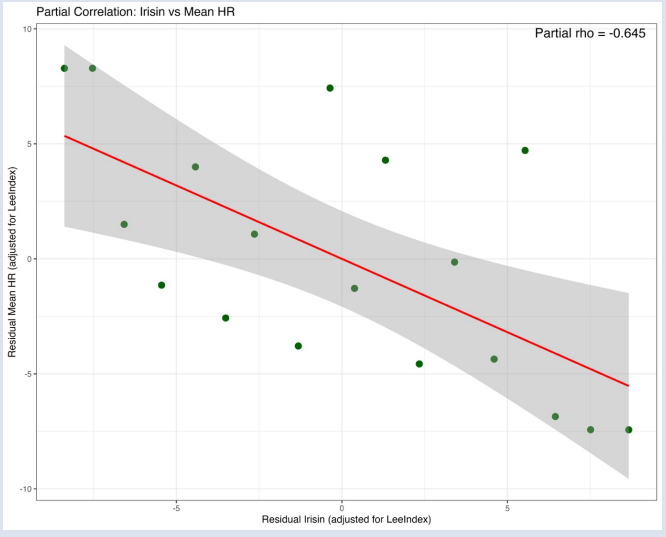

Figure 3: Association between serum irisin and mean heart rate. Shows the relationship between serum irisin residuals and mean heart rate residuals. The partial correlation coefficient is presented, with a red line representing the linear regression fit with 95% confidence intervals. Associations were assessed using partial correlation after controlling for Lee index.

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functional profile, including browning of fat and neuroprotective role, metabolic regulation and cardiovascular control.[12] Given ongoing uncertainty regarding assay specificity, absolute irisin concentrations should be interpreted cautiously.

Association between circulating Irisin level and lipid profile

No significant associations were observed between circulating irisin and lipid profile parameters in the present study (Figure 1). Some clinical studies have suggested complex or non-linear relationships between irisin and lipid metabolism, including differential effects across concentration ranges and populations.[8] Others have reported no clear association between irisin and obesity-related measures.[7,19] There is inconsistency in the literature regarding irisin-lipid relationships, where both positive and null associations have been reported across populations.[8,7,17-20] These findings suggest that the relationship between irisin and lipid profile parameters is influenced by multiple interacting factors, including the complex interplay of genetic, dietary, hormonal, and metabolic determinants of lipid metabolism, and may not be directly determined by lipid status alone.

Heart Rate Variability in Rats

In the present study, HRV parameters (Table 3) showed a non-normal distribution (Table 3) across both time- and frequency-domain indices. They are consistent with prior rodent studies.[28] Time-domain measures (SDNN and RMSSD) were relatively low in magnitude as rapid sinoatrial node firing limits the extent of beat-to-beat variability compared with humans, resulting in compressed HRV ranges even under resting conditions in small animals.[28-30] Nevertheless, preserved RMSSD values indicate maintained short-term variability and residual parasympathetic modulation under experimental conditions.

Frequency-domain analysis revealed a predominance of high-frequency power (median HF (nu), 94.03%) with low LF power (nu) and a low LF/HF ratio (median 0.064), reflecting strong parasympathetic influence on heart rate. The predominance of HF power is likely related to respiratory-linked vagal modulation under controlled laboratory conditions.[31-33] However, xylazine-induced sympatholysis may also contribute to reduced LF power and relative HF dominance.

Frequency-Domain Interpretation and Methodological Context

Because of species-specific cardiovascular physiology, rodent HRV spectral bands differ from human standards, with

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Table 4: Spearman and partial correlation analysis between serum irisin and HRV parameters (*n*=18).

Variable	r_s (Crude)	p (Crude)	pr_s (Adjusted)	p (Bonferroni)
SDNN (ms)	0.574	0.0127 *	0.574	0.1273
RMSSD (ms)	0.401	0.0987	0.408	0.8289
LF (ms^2)	0.745	<0.001 *	0.800	<0.0001*
LF (nu)	0.505	0.0346 *	0.512	0.2844
HF (ms^2)	0.282	0.2569	0.291	1
HF (nu)	-0.505	0.0346 *	-0.512	0.2844
TP (ms^2)	0.640	0.0042 *	0.648	0.0391*
LF/HF	0.505	0.0346 *	0.512	0.2844

SDNN (ms): Standard deviation of the NN intervals, measured in milliseconds; RMSSD (ms): root mean square of successive differences between normal heartbeats; LF (ms^2): low-frequency power in absolute units (ms^2); LF (nu): low-frequency power in normalised units; HF (ms^2): high-frequency power in absolute units (ms^2); HF (nu): high-frequency power in normalised units; TP (ms^2): Total power in absolute units (ms^2); LF/HF: ratio of Low- to High-frequency power. r_s=Spearman's rank correlation coefficient; pr_s=partial Spearman's rank correlation coefficient adjusted for Lee Index; p (Bonferroni)=p-value after Bonferroni correction for multiple comparisons. Statistical significance is defined as $p<0.05$.

Table 5: Multivariable linear regression analysis of factors associated with serum irisin levels (adjusted for mean heart rate and adiposity).

Predictor	B	SE	95% CI	Std. β	t-statistic	p-value	VIF
	18.297	1.686	14.682 - 21.912		10.855	<0.001***	
LF	0.080	0.012	0.053 - 0.106	0.636	6.509	<0.001***	1.550
Mean HR (bpm)	-0.011	0.002	-0.016 - -0.006	-0.463	-4.748	<0.001***	1.541
Lee index	-7.782	4.192	-16.773 - 1.209	-0.150	-1.856	0.0846	1.064

B: Unstandardized-β; SE: Standard Error; 95% CI: 95% confidence intervals; Std.β-Standardized β; LF: low-frequency power in HRV analysis; Mean HR: Mean heart rate in beats per minute; Lee index is a measure of body fat, calculated as the cube root of body weight (g) divided by nose-to-anus length (cm). Multiple linear regression analysis was used to identify independent predictors of serum irisin levels. B: Unstandardized Coefficient, standard errors, 95% Confidence Intervals (CI), and Standardized Coefficient (β), are reported. Variance Inflation Factor (VIF) was used to assess multicollinearity (VIF > 10 indicates significant collinearity). Statistical significance was set at $p<0.05$ (*).

higher frequency cut-offs required due to elevated heart and respiratory rates.[28,31,32] In the present study, HRV values were within previously reported ranges,[28,29] although direct comparison across studies remains limited due to methodological heterogeneity.

Overall, the HRV pattern observed here reflects a pharmacologically influenced autonomic state characterised by strong vagal predominance. LF power represents a mixed autonomic index influenced by baroreflex and combined autonomic modulation, rather than a pure marker of sympathetic activity.[34,35] It should be interpreted as an integrated measure of autonomic regulation under anaesthesia rather than resting physiological tone.

Autonomic Interaction with Irisin

Though the circulating irisin showed significant associations with several HRV indices (Table 4) in the unadjusted analysis, only LF power and total power remained significant after adjusting for adiposity and Bonferroni correction for multiple comparisons. This indicates that body composition may partly influence these relationships.

The lack of a significant relationship with RMSSD and HF power suggests that irisin may not be directly linked to vagal activity under these conditions. Instead, its relationship with LF power reflects an association with integrated autonomic regulation rather than isolated parasympathetic modulation.

The persistence of these relationships (irisin with LF power and total power), despite anaesthesia-driven autonomic shifts (parasympathetic predominance), points to a resilient link between autonomic variability and irisin, though the confounding effects of ketamine-xylazine on baseline autonomic balance must be acknowledged.

Determinants of circulating irisin

Multivariable regression analysis (Table 5) identified LF power as an independent predictor of circulating irisin (standardised β=0.636, p<0.001), whereas mean heart rate showed a significant inverse association (standardised β=-0.463, p<0.001). In contrast, the Lee index was not statistically significant, suggesting a limited independent contribution of adiposity within the present model, which is consistent with the absence of a significant association between irisin and lipid profile.

LF components of heart rate variability reflect a combination of sympathetic and parasympathetic influences and are also linked to Mayer wave-related cardiovascular oscillations and baroreflex-mediated modulation of autonomic outflow.[34,36] Under ketamine-xylazine anaesthesia, external influences such as respiratory movements and thermoregulatory variability are minimised. The opposing autonomic actions of ketamine and xylazine may contribute to partial preservation of reflex cardiovascular regulation during anaesthesia.[37,38]

Within this physiological context, the inverse association between irisin and heart rate may initially appear paradoxical, particularly given the expectation that markers associated with autonomic cardiovascular modulation (such as LF power) would parallel heart rate changes. However, heart rate is not a direct surrogate of autonomic tone alone and is influenced by multiple interacting regulatory systems, including reflex cardiovascular control, metabolic status, and intrinsic sinoatrial node properties.

A plausible explanation for the observed inverse irisin-heart rate relationship is that irisin-associated modulation of vascular or endothelial function may influence reflex cardiovascular dynamics. Irisin has been reported to enhance endothelial nitric oxide bioavailability,[39] which may improve endothelial function and arterial compliance. Since arterial compliance contributes to baroreceptor loading conditions, such vascular effects could indirectly modulate reflex cardiovascular responses. The inverse association between irisin and heart rate may therefore reflect autonomic cardiovascular interactions potentially involving baroreflex-mediated control, which maintains the inverse relationship between arterial pressure and heart rate. However, direct assessment of baroreflex sensitivity would be required to further evaluate this proposed mechanism.

Overall, these findings show a greater influence of autonomic regulation on circulating irisin levels compared with gross body composition under the present experimental conditions. However, given the cross-sectional nature of the study, no causal relationships can be inferred.

Integrated Interpretation Under Anaesthesia

Our findings reveal that individual differences in autonomic function under ketamine-xylazine anaesthesia correlate with circulating irisin levels. Despite an overall shift toward parasympathetic dominance, LF power retained significant predictive value, suggesting that subtle residual autonomic differences may still influence or reflect metabolic signalling pathways.

Table 6: Model summary and goodness-of-fit statistics for the regression model.

Model	R	R^2	Adjusted R^2	RMSE	F-statistic	p-value
Regression Model	0.956	0.914	0.895	0.421	49.45	<0.001*

Model performance was evaluated using multiple regression model statistics. R: multiple correlation coefficient; R^2: coefficient of determination; Adjusted R^2: adjusted coefficient of determination; RMSE: Root Mean Square Error; F-statistic: overall model significance. Statistical significance was set at p<0.05 (*).

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Mean heart rate showed an inverse relationship with irisin, indicating that animals exhibiting stronger bradycardic responses tended to have higher circulating irisin levels. This may reflect differential sensitivity to xylazine-induced sympatholysis and resulting autonomic shifts. However, these findings should be interpreted strictly within the context of an anaesthetised model, and not as evidence of resting physiological autonomic regulation.

Limitations

One important limitation of this study is the use of anaesthetic agents, as they can influence autonomic tone. Anaesthesia was needed to obtain stable ECG recording using subdermal electrodes. But it may have affected the HRV measurements. Since a telemetry system and an isoflurane vaporiser (which has a relatively lesser impact on HRV) were not available, this method was used. The cross-sectional design limits any causal inference between circulating irisin and autonomic indices. The small sample size reduces statistical power and may increase the risk of type II error. As the study was conducted in an animal model, the findings should be applied to humans with caution.

Circulating irisin was measured using an ELISA-based assay, which may also detect FNDC5 or related peptides. Therefore, exact Irisin values should be interpreted carefully.

The study did not measure some metabolic, inflammatory, and cardiovascular autonomic markers such as HOMA-IR, IL-6, and baroreflex sensitivity, which could have given a better understanding of metabolic status and autonomic regulation.

Strengths

Despite these limitations, the study had several strengths. The experimental conditions were kept similar for all rats, including housing, light-dark cycle, and environmental conditions. This helped reduce differences caused by outside factors. Physical activity was also controlled, which helped reduce changes in irisin and autonomic function caused by exercise. Only male rats were used, which reduced differences related to sex hormones that may affect metabolism and autonomic function. In addition, standardised ECG acquisition and validated HRV analysis software improved the reliability and reproducibility of the measurements.

CONCLUSION

This study found that circulating irisin was related to some HRV measures in male Wistar rats. LF power was the strongest predictor of irisin, while mean heart rate showed an inverse relationship with irisin. Body fat and lipid levels were not significantly related to irisin. These findings suggest that irisin may be related to the way the autonomic nervous system controls the heart, independent of body fat and lipid levels. This may help us better understand the relationship between metabolism and autonomic function. Further studies with larger sample sizes, rats without anaesthesia, and direct measurement of baroreflex function are needed to confirm these findings and better understand the reasons behind this relationship.

ABBREVIATIONS

ANOVA: Analysis of Variance; **bpm:** Beats per Minute; **CI:** Confidence Interval; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **ECG:** Electrocardiogram; **ELISA:** Enzyme-Linked Immunosorbent Assay; **FNDC5:** Fibronectin Type III Domain-Containing Protein 5; **HF:** High Frequency; **HDL:** High-Density Lipoprotein; **HRV:** Heart Rate Variability; **IAEC:** Institutional Animal Ethics Committee; **IQR:** Interquartile Range; **KX:** Ketamine-Xylazine; **LDL:** Low-Density Lipoprotein; **LF:** Low Frequency; **LF/HF:** Low-Frequency to High-Frequency Ratio; **RMSE:** Root Mean Square Error; **RMSSD:** Root Mean Square of Successive Differences; **SD:** Standard Deviation; **SDNN:** Standard Deviation of NN Intervals; **TC:** Total Cholesterol; **TG:** Triglycerides; **TP:** Total Power; **VIF:** Variance Inflation Factor; **VLDL:** Very-Low-Density Lipoprotein; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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