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Original Article

Lifestyle Behaviors, Adropin, Irisin and Insulin Resistance in Young Iraqi Adults: An Exploratory Mediation Analysis

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Abstract

Introduction: Insulin resistance among young adults is influenced by modifiable behaviors. Irisin, an exercise-responsive myokine, and adropin, a nutrient-regulated hepatokine, may connect lifestyle with glucose homeostasis. We investigated whether a composite lifestyle score was associated with insulin resistance in university students and whether irisin and adropin mediated this association.

Methods: This cross-sectional study enrolled 180 apparently healthy students aged 18–25 years (90 men and 90 women) from the University of Samarra, Iraq, who were selected by stratified random sampling between November 2025 and March 2026. Physical activity (IPAQ-short), sleep quality (PSQI), adherence to the Mediterranean diet (MEDAS), recreational screen time and smoking were combined into a lifestyle score ranging from 0 to 5. Fasting serum irisin, adropin and insulin concentrations were quantified by ELISA, and insulin resistance was estimated using HOMA-IR and the triglyceride–glucose (TyG) index. Multivariable regression, covariate-adjusted parallel bootstrap mediation (exploratory and hypothesis-generating) and internally validated ROC analyses were performed.

Results: Insulin resistance (HOMA-IR ≥ 2.5) was identified in 54 students (30.0%; 95% CI 23.8–37.1%), and its prevalence increased from 8.0% in the healthy lifestyle group to 30.5% in the intermediate group and 52.1% in the unhealthy group. Relative to a healthy lifestyle, the adjusted odds of insulin resistance were 4.69 (95% CI 1.48–14.87) for an intermediate lifestyle and 11.25 (95% CI 3.39–37.32) for an unhealthy lifestyle. Irisin ($P=0.007$) and adropin ($P=0.008$) concentrations rose progressively from the unhealthy to the healthy group. In multivariable regression, the lifestyle score (β = –0.26), adropin (β = –0.24), waist circumference (β = 0.30), family history of diabetes (β = 0.22) and male sex (β = 0.15) were independently associated with HOMA-IR, whereas irisin, although lower in insulin-resistant students and inversely correlated with HOMA-IR in univariate analysis, was not significant (β = –0.08; $P=0.182$). In a cross-sectional exploratory mediation model, which cannot establish temporal order or causality, the two peptides statistically accounted for 29% of the lifestyle–HOMA-IR association (total indirect effect –0.050; 95% CI –0.080 to –0.024), almost entirely through adropin (–0.041; 95% CI –0.069 to –0.019), whereas the indirect effect through irisin was not significant. A model combining irisin, adropin and waist circumference identified insulin resistance with a modest optimism-corrected AUC of 0.739.

Conclusion: Almost one in three Iraqi students showed insulin resistance, and a healthier lifestyle was strongly associated with a lower risk. Adropin, but not irisin, was independently associated with HOMA-IR and accounted statistically, in an exploratory cross-sectional model, for part of this association; this is hypothesis-generating and compatible with, but does not demonstrate, a diet- and sleep-sensitive hepatic pathway. These findings warrant further study of multicomponent campus programs addressing diet, sleep and physical activity, and longitudinal and interventional studies are required to establish temporal order and causality.

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Introduction

Cardiovascular diseases remain the leading cause of death worldwide, accounting for an estimated 19.2 million deaths in 2023, and close to 80% of their burden is attributable to modifiable risk factors; increasing exposure to high body mass index, high fasting plasma glucose and low physical activity has been a major driver of the rise recorded since 1990.[1] Insulin resistance is an early and often clinically silent step along this pathway. Prospective data show that declining insulin sensitivity, accompanied by a compensatory rise in insulin secretion, precedes the diagnosis of type 2 diabetes by several years,[2] and these abnormalities can already be detected in young adults. The problem is particularly relevant to the Middle East and North Africa, a region with some of the highest age-adjusted diabetes prevalence estimates in the world;[3] in Iraq, the prevalence of type 2 diabetes has been reported to range between 8.5% and 13.9%.[4]

Lifestyle is the most important modifiable determinant of this trajectory. In a cohort of nearly 85,000 female nurses followed for 16 years, women who combined several low-risk behaviors, including a healthy body weight, a prudent diet, regular exercise and abstention from smoking, had a relative risk of diabetes of 0.09 compared with the remainder of the cohort, and 91% of incident cases were attributable to behavior patterns that did not conform to this low-risk profile.[5] Individual behaviors also show consistent relationships with insulin sensitivity. One week of experimental sleep restriction reduces insulin sensitivity in healthy men,[6] and selective suppression of slow-wave sleep, without any change in total sleep time, produces a comparable deterioration in young adults;[7] a recent meta-analysis of randomized trials concluded that the duration, quality and timing of sleep are all relevant to insulin sensitivity.[8] Greater sedentary time is associated with a higher risk of incident type 2 diabetes,[9] adherence to a Mediterranean dietary pattern is related to more favorable components of the metabolic syndrome,[10] and smoking promotes insulin resistance and central fat accumulation.[11]

University life is a transitional period in which long-term health habits are consolidated. Among 270 students at a university in Mosul, Iraq, approximately 36% were overweight and 13% obese, half slept 6 hours or less per night and more than half exercised for less than 1 hour per week.[12] In Sulaimani, late bedtimes and bedtime device use were associated with irregular dietary patterns, whereas regular exercise was associated with more regular eating.[13] Among 300 apparently healthy students in Wasit, 41.3% fulfilled the criteria for the metabolic syndrome,[14] and insulin resistance was frequent among those with the syndrome and was related to higher BMI, physical inactivity and dietary habits.[4] In overweight and obese Indian adolescents aged 10–19 years, insulin resistance defined by HOMA-IR ≥ 2.5 reached 62.6%, and low physical activity, poor sleep and longer screen time were more frequent among those affected.[15] Because these behaviors cluster within the same individuals, it is reasonable to study them as a composite lifestyle profile rather than as isolated exposures.

The biological mechanisms that translate lifestyle into insulin sensitivity are only partly understood. Irisin is cleaved from the membrane protein FNDC5 in skeletal muscle; its expression is driven by the exercise-induced coactivator PGC-1α, and in mice it promotes browning of white adipose tissue, increases energy expenditure and improves glucose homeostasis.[16] Mass spectrometry has confirmed that irisin circulates in humans and increases after aerobic interval training,[17] and a meta-analysis of 59 studies reported a positive effect of long-term exercise on circulating irisin (standardized mean difference 0.39), although with considerable heterogeneity.[18] In a randomized trial in overweight women, 8 weeks of exercise and dietary intervention improved HOMA-IR and increased irisin, with exercise having the larger effect on irisin.[19] The human biology of irisin nevertheless remains contested. The specificity of commercial antibodies and immunoassays has been questioned,[20,21] published associations between irisin and insulin resistance are contradictory,[22] and some cross-sectional studies have even reported higher irisin concentrations in individuals with higher HOMA-IR, a finding interpreted as either a compensatory increase or a state of irisin resistance.[23]

Adropin, a 76-amino-acid peptide encoded by the *Enho* gene and expressed mainly in the liver and brain, links nutrient intake to energy homeostasis. Hepatic *Enho* expression is regulated by diet and declines in diet-induced obesity, and adropin treatment attenuates hepatic steatosis and insulin resistance in obese mice independently of adiposity;[24] in diet-induced obese mice, adropin further improves glucose tolerance and muscle insulin signaling and shifts fuel selection toward glucose oxidation.[25] In humans, low adropin concentrations are associated with obesity, ageing and risk factors for the metabolic syndrome,[26,27] and a meta-analysis of 15 observational studies found lower circulating adropin in patients with type 2 diabetes than in controls, albeit with very high heterogeneity.[28] Circulating adropin also appears to be sensitive to diet: it is inversely related to habitual carbohydrate intake in humans,[29] and low plasma adropin predicted weight gain and metabolic dysregulation during high-sugar feeding in macaques.[30] Time-restricted feeding restores circulating irisin and adropin and protects mice from diet-induced insulin resistance, suggesting that meal timing, and not only energy intake, influences both peptides,[31] and hepatic *Enho* expression in mice appears to be under circadian control.[32] Sleep curtailment, in turn, impairs glucose tolerance and insulin sensitivity in young adults,[33-35] but whether part of this effect involves irisin or adropin is unknown.

Human evidence linking both peptides to insulin sensitivity is growing but remains inconsistent. During 60 days of bed rest in healthy young men, irisin and adropin declined in parallel with whole-body and hepatic insulin sensitivity.[36] In type 2 diabetes, poor glycemic control was associated with lower irisin and adropin, and both correlated with HOMA-IR,[37] whereas another study

found no differences across normoglycemia, prediabetes and diabetes, although adropin was higher in participants with a lower waist circumference.[38] Established disease and glucose-lowering treatment may confound these associations.[39] In young adults, irisin was lower in those with hypertriglyceridemia and correlated inversely with the TyG index, but adropin and lifestyle behaviors were not assessed.[40]

Taken together, these observations leave several gaps. Much of the human evidence on irisin and adropin derives from patients with established disease or from experimental interventions, in which the effects of disease and treatment are difficult to separate from those of habitual behavior. Few studies have measured a myokine and a hepatokine in the same participants, even though the two peptides are thought to reflect different tissues and different behavioral inputs. In addition, data from Iraq on how everyday lifestyle relates to insulin resistance in young adults are scarce, and the possibility that these peptides lie on the pathway between lifestyle and insulin resistance has seldom been tested formally.

To our knowledge, no study has examined irisin and adropin together in relation to a composite lifestyle score in apparently healthy university students, or tested whether these peptides mediate the association between lifestyle and insulin resistance. The present study therefore aimed (i) to assess the association of a composite lifestyle score with insulin resistance in students at the University of Samarra, Iraq; (ii) to examine the associations of individual behaviors with circulating irisin and adropin; and (iii) to test the hypothesis that irisin and adropin partially mediate the association between lifestyle and insulin resistance.

Materials and methods

Study design and setting

This analytical cross-sectional study was carried out at the University of Samarra, Salah al-Din Governorate, Iraq, between 1 November 2025 and 1 March 2026, and is reported in accordance with the STROBE statement.[41] Data and biological sample collection were scheduled so as to avoid examination periods, and all questionnaires, anthropometric measurements and fasting blood samples were completed before Ramadan began in mid-February 2026; the remaining weeks were devoted to laboratory analyses and data entry. This timetable was intended to limit the possible influence of seasonal changes and religious fasting on sleep, physical activity and dietary behavior, which could otherwise have affected the variables under study.

Participants and sampling

The study population consisted of full-time undergraduate students. Participants were chosen by stratified random sampling according to college type (scientific or humanities) and sex. Men and women were allocated equally (90 men and 90 women) to ensure adequate representation of both sexes and to permit the evaluation of sex-related differences and interactions; within each sex quota, participants were stratified by college type and then selected by simple random sampling from the official student lists within each stratum.

Eligibility required an age of 18–25 years, full-time enrollment, residence in Samarra or its surroundings for at least 1 year and written informed consent. Students were excluded if they had a previously diagnosed diabetes or thyroid, hepatic, renal or cardiovascular disease; had used corticosteroids, metformin, lipid-lowering drugs or hormonal contraceptives during the preceding 3 months; were pregnant or lactating; had a diagnosis of polycystic ovary syndrome; were engaged in competitive athletic training or following a therapeutic diet; or had experienced an acute infection within the previous 2 weeks.

The sample size was calculated to detect a correlation of $r = 0.22$ between the lifestyle score and HOMA-IR, using Fisher's z transformation with a two-sided α of 0.05 and 80% power, which yielded a minimum of 160 participants. To allow for an anticipated attrition or unusable-sample rate of approximately 11%, the target was increased to 180 participants ($160/0.89 \approx 180$; 90 men and 90 women). Because the study was powered for this primary association, the mediation analysis was considered exploratory.

Questionnaires

A structured questionnaire collected information on age, sex, college, academic year, residence, smoking (cigarettes, waterpipe or electronic cigarettes), energy drink consumption and family history of diabetes and obesity. Physical activity was measured with the Arabic version of the International Physical Activity Questionnaire–Short Form (IPAQ-SF), as previously used among Arab adults,[42] which records the time spent walking and performing moderate- and vigorous-intensity activity during the preceding 7 days; total physical activity was expressed in MET-min/week following the standard IPAQ scoring protocol, and participants were classified as having low, moderate or high activity levels.[43] Sleep quality was evaluated with the 19-item Pittsburgh Sleep Quality Index (PSQI), which generates seven component scores and a global score ranging from 0 to 21, with higher values indicating poorer sleep and scores > 5 denoting poor sleep quality.[44] The Arabic version (A-PSQI), which was developed through forward and back translation and has undergone psychometric evaluation, was used.[45] Dietary habits were assessed with the 14-item Mediterranean Diet Adherence Screener (MEDAS; score range 0–14, higher values indicating greater adherence);[46] Arabic cross-cultural adaptations of the MEDAS have demonstrated acceptable reliability and validity.[47] In some items, the food examples were adapted to commonly consumed local foods without altering the nutritional concept underlying each item. The wine item was retained in order to preserve the original structure of the screener and was scored 0 for non-consumers rather than being replaced. Further information was gathered on fast-food and sugar-sweetened beverage intake, breakfast consumption and the timing of the last meal of the day. Average daily screen time for non-

academic purposes was recorded in hours. Before the main study, the questionnaire and study procedures were pilot-tested in 25 students who were not part of the final sample, in order to evaluate the clarity of wording, the time required for completion and feasibility. The instruments were re-administered after 2 weeks; internal consistency (Cronbach's $\alpha = 0.82$) and test–retest reliability (intraclass correlation coefficient = 0.87) were good.

Composite lifestyle score

One point was awarded for each of five healthy behaviors: physical activity ≥ 600 MET-min/week, PSQI ≤ 5, MEDAS ≥ 9, recreational screen time < 2 hours/day and non-smoking. The resulting score ranged from 0 to 5 and was classified as unhealthy (0-1), intermediate (2-3) or healthy (4-5). MEDAS scores were dichotomized into low/moderate (< 9) and high (≥ 9) adherence; the cut-off of ≥ 9 was adopted as an operational threshold for higher adherence, consistent with its common use in epidemiological studies, including those conducted in university students.[48] The five behaviors were weighted equally, a decision taken a priori for the sake of simplicity and interpretability.

Anthropometric and clinical measurements

Body weight (using a calibrated scale) and height were measured with participants in light clothing and without shoes, to the nearest 0.1 kg and 0.1 cm, respectively, and body mass index (BMI) was calculated. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration, and hip circumference at the widest point over the buttocks, in both cases with a non-elastic tape. Body fat percentage and skeletal muscle mass were estimated by bioelectrical impedance analysis (InBody 270; InBody Co., Ltd., Seoul, Republic of Korea) under standardized conditions with respect to time of day and hydration status as far as practicable. Blood pressure was measured with a validated automated sphygmomanometer (Omron HEM-907; Omron Healthcare Co., Ltd., Kyoto, Japan) and was taken as the mean of two readings obtained 5 minutes apart after rest.

Blood sampling and laboratory analyses

A single 5-mL venous blood sample was collected from each participant between 08:00 and 09:30 after a 10–12-hour overnight fast; participants were asked to refrain from strenuous exercise during the preceding 48 hours. For women, the date of the last menstrual period and cycle regularity were recorded, together with the approximate cycle day at sampling when available. After clotting, serum was separated by centrifugation at 3000 rpm for 10 minutes, divided into aliquots and stored at −80 °C, avoiding repeated freeze–thaw cycles. Serum irisin, adropin and insulin were quantified with commercial human ELISA kits (SunLong Biotech Co., Ltd, China): irisin (catalogue no. SL2020Hu; assay range 0.5–30 ng/mL; sensitivity 0.1 ng/mL), adropin (SL1934Hu; 3–180 pg/mL; 0.5 pg/mL) and insulin (SL0933Hu; 0.3–20 mU/L; 0.05 mU/L). All samples were analyzed in duplicate within a single batch by a laboratory analyst who was blinded to the questionnaire data, in accordance with the manufacturer's instructions, with absorbance read at 450 nm and a separate standard curve on each plate. According to the manufacturer, the intra- and inter-assay coefficients of variation were < 10% and < 12%, respectively, for all three assays. Samples whose absorbance exceeded the upper limit of the insulin standard curve were diluted with sample diluent and re-assayed, and the final concentrations were corrected for the total dilution factor as specified by the manufacturer. Fasting glucose, triglycerides (TG), total cholesterol and HDL cholesterol (HDL-C) were determined by enzymatic colorimetric methods on a Cobas c311 automated analyzer (Roche Diagnostics/Hitachi, Japan) according to the manufacturer's instructions, and high-sensitivity C-reactive protein (hs-CRP) by an immunoturbidimetric method. LDL cholesterol (LDL-C) was measured directly with a homogeneous enzymatic assay (LDL-Cholesterol Gen.3, LDLC3; Roche Diagnostics) on the same analyzer.

Indices of insulin resistance

HOMA-IR was calculated as fasting insulin (mU/L) × fasting glucose (mmol/L) / 22.5,[49] and the TyG index as ln[TG (mg/dL) × fasting glucose (mg/dL) / 2].[50] Insulin resistance was defined as HOMA-IR ≥ 2.5, a threshold widely used in young populations that permits comparison with earlier reports.[15]

Statistical analysis

Analyses were performed with SPSS version 26 and the PROCESS macro.[51] Normality was evaluated with the Shapiro–Wilk test and by visual inspection of histograms and Q–Q plots. Data are presented as mean ± standard deviation, median (interquartile range) or n (%). Lifestyle groups were compared using one-way ANOVA or the Kruskal–Wallis test for continuous variables and the chi-square test for categorical variables; participants with and without insulin resistance were compared using Welch's *t* test, which does not assume equal variances, or the Mann–Whitney *U* test. Correlations were estimated with Pearson's *r* when both variables were approximately normally distributed and with Spearman's ρ for skewed or ordinal variables (such as MET-min/week, TG, insulin, hs-CRP and HOMA-IR), and the *P* values of the correlation matrix were additionally adjusted using the Benjamini–Hochberg false discovery rate procedure.[52] Multiple linear regression was used to identify independent correlates of HOMA-IR, with adjustment for waist circumference, skeletal muscle mass, age, sex and family history of diabetes; multicollinearity was examined using variance inflation factors. Multivariable logistic regression was used to estimate adjusted odds ratios (AOR) for insulin resistance across lifestyle categories, with the healthy category as the reference. Parallel mediation by irisin and adropin of the association between the lifestyle score and HOMA-IR was tested with PROCESS model 4 using 5000 percentile bootstrap samples, adjusting for

the same covariates as in the linear regression model (waist circumference, skeletal muscle mass, age, sex and family history of diabetes); the a (lifestyle → peptide) and b (peptide → HOMA-IR) paths are reported, and indirect effects were considered significant when the 95% bootstrap confidence interval (CI) excluded zero. Receiver operating characteristic (ROC) curves were used to evaluate the ability of irisin, adropin and waist circumference, alone and in combination, to identify insulin resistance. The combined model was entered as predicted probabilities from logistic regression, and the optimism in its AUC was corrected using 1000 bootstrap resamples.[53] A two-sided $P < 0.05$ was regarded as statistically significant. Apart from the correlation matrix, P values were not adjusted for multiple comparisons, and the group comparisons, regression models and mediation analysis should therefore be regarded as exploratory.

The robustness of the association between an unhealthy lifestyle and insulin resistance to unmeasured confounding was examined with the E-value,[54] after the odds ratio had been converted to an approximate risk ratio because insulin resistance was not a rare outcome.

Results

Participant characteristics

Of 235 students invited, 21 declined to participate (participation rate 91.1%) and 214 were screened for eligibility; 29 did not satisfy the inclusion criteria and 185 were enrolled. Five participants were excluded because of incomplete questionnaires or inadequate biological samples, leaving 180 participants (90 men and 90 women; mean age 21.0 years) for analysis (Supplementary Figure S1). Of these, 48 (26.7%) had an unhealthy, 82 (45.6%) an intermediate and 50 (27.8%) a healthy lifestyle profile. Age, sex distribution, skeletal muscle mass and blood pressure did not differ among the groups, whereas BMI, waist circumference and body fat percentage declined progressively from the unhealthy to the healthy group (Table 1). As anticipated from the construction of the score, physical activity, PSQI, MEDAS, screen time and smoking differed markedly among the groups (all $P < 0.05$).

Table 1. Characteristics and lifestyle behaviors of the participants according to composite lifestyle category (n = 180)

Variable	Unhealthy (n = 48)	Intermediate (n = 82)	Healthy (n = 50)	*P*
Age, years	21.3 ± 1.8	21.0 ± 1.9	20.8 ± 1.7	0.393
Men, n (%)	26 (54.2)	39 (47.6)	25 (50.0)	0.768
BMI, kg/m²	25.9 ± 4.2	24.8 ± 3.7	23.9 ± 3.4	0.033
Waist circumference, cm	87.1 ± 10.6	84.3 ± 9.5	81.7 ± 8.9	0.023
Body fat, %	27.8 ± 7.3	25.9 ± 6.7	24.1 ± 6.2	0.027
Skeletal muscle mass, kg	32.6 ± 6.5	33.3 ± 6.2	34.0 ± 6.4	0.551
Systolic BP, mmHg	118.4 ± 10.8	117.1 ± 10.2	116.2 ± 9.9	0.568
Diastolic BP, mmHg	76.3 ± 7.8	75.4 ± 7.3	74.7 ± 7.1	0.562
Physical activity, MET-min/week	480 (320–710)	1040 (690–1650)	1910 (1190–2860)	< 0.001
PSQI score	7.3 ± 2.5	5.9 ± 2.3	4.5 ± 2.0	< 0.001
MEDAS score	5.8 ± 1.7	7.4 ± 1.6	9.5 ± 1.5	< 0.001
Recreational screen time, h/day	4.9 ± 1.9	3.5 ± 1.6	2.3 ± 1.2	< 0.001
Smokers, n (%)	13 (27.1)	12 (14.6)	4 (8.0)	0.033

Data are presented as mean ± SD, median (interquartile range) or n (%). BMI, body mass index; BP, blood pressure; MEDAS, Mediterranean Diet Adherence Screener; PSQI, Pittsburgh Sleep Quality Index

Biomarkers across lifestyle categories

Irisin ($P = 0.007$) and adropin ($P = 0.008$) increased progressively from the unhealthy to the healthy group, whereas fasting insulin ($P = 0.007$), TG ($P = 0.010$), HOMA-IR (2.49, 2.14 and 1.87; $P = 0.005$) and the TyG index ($P = 0.034$) decreased and HDL-C increased ($P = 0.039$) (Table 2). Fasting glucose, total cholesterol, LDL-C and hs-CRP showed non-significant downward trends.

Insulin resistance

Insulin resistance (HOMA-IR ≥ 2.5) was identified in 54 students (30.0%; 95% CI 23.8–37.1%). In comparison with their peers, students with insulin resistance had a lower lifestyle score (1.78 ± 1.22 vs 3.01 ± 1.41; $P < 0.001$), a larger waist circumference (87.00 ± 10.05 vs 83.18 ± 9.50 cm; $P = 0.019$) and lower concentrations of irisin (9.30 ± 3.62 vs 11.03 ± 3.75 ng/mL; $P = 0.004$) and adropin (59.00 ± 25.35 vs 76.07 ± 25.00 pg/mL; $P < 0.001$) (Table 3). Fasting insulin was approximately 63% higher in students with insulin resistance (13.40 ± 3.14 vs 8.21 ± 3.20 mU/L; $P < 0.001$), whereas fasting glucose was only modestly higher (92.00 ± 7.98 vs 89.05 ± 7.40 mg/dL; $P = 0.022$). Students with insulin resistance also had lower HDL-C (45.30 ± 9.38

Table 2. Circulating peptides and metabolic markers according to composite lifestyle category (n = 180)

Variable	Unhealthy (n = 48)	Intermediate (n = 82)	Healthy (n = 50)	*P*
Irisin, ng/mL	9.3 ± 3.6	10.5 ± 3.7	11.7 ± 3.8	0.007
Adropin, pg/mL	62.7 ± 24.9	70.8 ± 25.4	79.1 ± 26.8	0.008
Insulin, mU/L	11.1 ± 4.3	9.7 ± 3.9	8.6 ± 3.4	0.007
Fasting glucose, mg/dL	91.8 ± 8.1	89.9 ± 7.6	88.2 ± 7.1	0.067
Triglycerides, mg/dL	121.7 ± 39.8	108.8 ± 35.6	99.5 ± 31.9	0.010
Total cholesterol, mg/dL	176.4 ± 31.2	169.1 ± 28.7	163.3 ± 27.5	0.085
HDL-C, mg/dL	45.8 ± 9.4	48.1 ± 9.3	50.7 ± 9.7	0.039
LDL-C, mg/dL	106.3 ± 36.0	99.2 ± 34.0	92.7 ± 32.0	0.144
HOMA-IR	2.49 ± 1.08	2.14 ± 0.93	1.87 ± 0.79	0.005
TyG index	8.47 ± 0.46	8.35 ± 0.43	8.24 ± 0.41	0.034
hs-CRP, mg/L	1.42 (0.74–2.54)	1.17 (0.61–2.06)	0.94 (0.48–1.73)	0.083

Data are presented as mean ± SD or median (interquartile range). HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; TyG, triglyceride–glucose; LDL-C, low-density lipoprotein cholesterol (measured directly)

vs 49.46 ± 9.40 mg/dL; $P = 0.008$) and higher total cholesterol (177.00 ± 30.03 vs 166.19 ± 28.50 mg/dL; $P = 0.027$) and LDL-C (109.50 ± 35.04 vs 94.91 ± 33.00 mg/dL; $P = 0.011$), whereas TG ($P = 0.513$) and the TyG index (8.42 ± 0.46 vs 8.32 ± 0.43; $P = 0.182$) did not differ between the two groups. The prevalence of insulin resistance was 8.0%, 30.5% and 52.1% in the healthy, intermediate and unhealthy groups, respectively ($P < 0.001$). After adjustment for age, sex, waist circumference and family history of diabetes, the odds of insulin resistance were 4.69 times higher (95% CI 1.48–14.87; $P = 0.009$) in the intermediate group and 11.25 times higher (95% CI 3.39–37.32; $P < 0.001$) in the unhealthy group than in the healthy group; waist circumference was also independently associated with insulin resistance (AOR 1.04 per cm; 95% CI 1.00–1.08; $P = 0.046$) (Table 4).

Correlations

Irisin correlated positively with physical activity ($\rho = 0.29$; $P < 0.001$) and skeletal muscle mass ($r = 0.24$; $P = 0.002$), and inversely with HOMA-IR ($\rho = -0.26$; $P = 0.001$), the TyG index ($r = -0.18$; $P = 0.017$) and waist circumference ($r = -0.15$; $P = 0.046$). Adropin correlated positively with the MEDAS score ($\rho = 0.22$; $P = 0.003$) and inversely with the PSQI score ($\rho = -0.17$; $P = 0.024$), HOMA-IR ($\rho = -0.28$; $P < 0.001$), the TyG index ($r = -0.21$; $P = 0.005$) and waist circumference ($r = -0.23$; $P = 0.002$). Irisin and adropin were weakly correlated with each other ($r = 0.19$; $P = 0.011$) (Table 5). All 11 correlations remained significant after Benjamini–Hochberg adjustment (largest adjusted $P = 0.046$).

Table 3. Comparison of students with and without insulin resistance (n = 180)

Variable	Without IR (n = 126)	With IR (n = 54)	*P*
Composite lifestyle score	3.01 ± 1.41	1.78 ± 1.22	< 0.001
Waist circumference, cm	83.18 ± 9.50	87.00 ± 10.05	0.019
Irisin, ng/mL	11.03 ± 3.75	9.30 ± 3.62	0.004
Adropin, pg/mL	76.07 ± 25.00	59.00 ± 25.35	< 0.001
Insulin, mU/L	8.21 ± 3.20	13.40 ± 3.14	< 0.001
Fasting glucose, mg/dL	89.05 ± 7.40	92.00 ± 7.98	0.022
HOMA-IR	1.69 ± 0.48	3.25 ± 0.91	< 0.001
Triglycerides, mg/dL	108.44 ± 35.50	112.50 ± 39.09	0.513
HDL-C, mg/dL	49.46 ± 9.40	45.30 ± 9.38	0.008
Total cholesterol, mg/dL	166.19 ± 28.50	177.00 ± 30.03	0.027
LDL-C, mg/dL	94.91 ± 33.00	109.50 ± 35.04	0.011
TyG index	8.32 ± 0.43	8.42 ± 0.46	0.182

Data are presented as mean ± SD. *P* values are from Welch's *t* test. HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance (HOMA-IR ≥ 2.5); LDL-C, low-density lipoprotein cholesterol (measured directly); TyG, triglyceride–glucose index

Table 4. Prevalence of insulin resistance and multivariable logistic regression by composite lifestyle category (n = 180)

Variable	Insulin resistance, n (%)	AOR	95% CI	*P*
Healthy lifestyle	4/50 (8.0)	1.00	Reference	–
Intermediate lifestyle	25/82 (30.5)	4.69	1.48–14.87	0.009
Unhealthy lifestyle	25/48 (52.1)	11.25	3.39–37.32	< 0.001
Age, per year	–	1.02	0.83–1.25	0.873
Male sex	–	1.39	0.69–2.82	0.357
Waist circumference, per cm	–	1.04	1.00–1.08	0.046
Family history of diabetes	–	1.66	0.77–3.56	0.195

All variables were entered simultaneously in a single model. AOR, adjusted odds ratio; CI, confidence interval

Independent correlates of HOMA-IR

In multiple linear regression, waist circumference ($\beta = 0.301$; $P < 0.001$), the lifestyle score ($\beta = -0.257$; $P < 0.001$), adropin ($\beta = -0.235$; $P < 0.001$), family history of diabetes ($\beta = 0.219$; $P < 0.001$) and male sex ($\beta = 0.146$; $P = 0.017$) were independently associated with HOMA-IR, whereas irisin ($\beta = -0.084$; $P = 0.182$), skeletal muscle mass and age were not (Table 6). All variance inflation factors were below 1.30.

Mediation analysis

After adjustment for waist circumference, skeletal muscle mass, age, sex and family history of diabetes, the total effect of the lifestyle score on HOMA-IR was −0.173, and the direct effect after inclusion of both mediators was −0.123. A higher lifestyle score was associated with higher irisin (a1 = 0.467) and adropin (a2 = 6.241), and both peptides were inversely associated with HOMA-IR (b1 = −0.0187; b2 = −0.0066), although only the adropin path was significant. The indirect effect through adropin was significant (−0.0414; 95% CI −0.0689 to −0.0193), whereas that through irisin was not (−0.0087; 95% CI

Table 5. Correlations of irisin and adropin with lifestyle and metabolic variables (n = 180)

Correlation	Coefficient	*P*
Irisin × physical activity	ρ = 0.29	< 0.001
Irisin × skeletal muscle mass	*r* = 0.24	0.002
Irisin × HOMA-IR	ρ = −0.26	0.001
Irisin × TyG index	*r* = −0.18	0.017
Irisin × waist circumference	*r* = −0.15	0.046
Adropin × MEDAS score	ρ = 0.22	0.003
Adropin × PSQI score	ρ = −0.17	0.024
Adropin × HOMA-IR	ρ = −0.28	< 0.001
Adropin × TyG index	*r* = −0.21	0.005
Adropin × waist circumference	*r* = −0.23	0.002
Irisin × adropin	*r* = 0.19	0.011

r, Pearson's correlation coefficient; ρ, Spearman's rank correlation coefficient. Spearman's ρ was applied to correlations involving physical activity (MET-min/week), HOMA-IR and the MEDAS and PSQI scores, and Pearson's *r* to all others. All correlations remained significant after Benjamini–Hochberg false discovery rate adjustment (largest adjusted $P = 0.046$). HOMA-IR, homeostasis model assessment of insulin resistance; MEDAS, Mediterranean Diet Adherence Screener; PSQI, Pittsburgh Sleep Quality Index; TyG, triglyceride–glucose

−0.0259 to 0.0068). The total indirect effect was −0.0501 (95% CI −0.0800 to −0.0238), equivalent to 29.0% of the total effect, of which 23.9% was attributable to adropin and 5.0% to irisin (Table 7, Figure 1).

Discriminative performance

Irisin (AUC 0.608) and waist circumference (AUC 0.638) showed limited, and adropin (AUC 0.706) modest, ability to identify insulin resistance. Discrimination improved when irisin, adropin and waist circumference were combined (apparent AUC 0.755; optimism-corrected AUC 0.739) (Table 8).

Sensitivity to unmeasured confounding

The E-value for the association between the unhealthy lifestyle category and insulin resistance was 6.16 (3.09 for the confidence limit closest to the null). This indicates that an unmeasured confounder would have to be associated with both an unhealthy lifestyle and insulin resistance by a risk ratio of at least 6.16 each, over and above the measured covariates, in order to explain the observed association entirely.

Table 6. Multiple linear regression of the independent correlates of HOMA-IR (n = 180)

Variable	B	95% CI	β	*P*	VIF
Composite lifestyle score	−0.122	−0.186 to −0.059	−0.257	<0.001	1.29
Irisin	−0.019	−0.046 to 0.009	−0.084	0.182	1.11
Adropin	−0.0067	−0.0103 to −0.0030	−0.235	<0.001	1.18
Waist circumference	0.0221	0.0130 to 0.0311	0.301	<0.001	1.12
Skeletal muscle mass	−0.0008	−0.0148 to 0.0132	−0.007	0.914	1.04
Age	0.0116	−0.0365 to 0.0598	0.029	0.634	1.04
Male sex	0.203	0.037 to 0.369	0.146	0.017	1.03
Family history of diabetes	0.338	0.155 to 0.522	0.219	<0.001	1.02

Dependent variable: HOMA-IR. B, unstandardized regression coefficient; β, standardized regression coefficient; CI, confidence interval; VIF, variance inflation factor

Discussion

In this sample of apparently healthy university students in Samarra, almost one in three (30.0%) had insulin resistance according to a conventional HOMA-IR threshold, and its prevalence rose steeply from 8.0% among students with a healthy lifestyle to 52.1% among those with an unhealthy one. After adjustment for age, sex, central adiposity and family history of diabetes, students with an unhealthy lifestyle had approximately elevenfold higher odds of

Table 7. Parallel mediation by irisin and adropin of the association between the composite lifestyle score and HOMA-IR

Path	Effect	95% bootstrap CI
a1: lifestyle score → irisin	0.467	—
a2: lifestyle score → adropin	6.241	—
b1: irisin → HOMA-IR	−0.0187	—
b2: adropin → HOMA-IR	−0.0066	—
Total effect (c)	−0.173	—
Direct effect (c′)	−0.123	—
Indirect effect via irisin	−0.0087	−0.0259 to 0.0068
Indirect effect via adropin	−0.0414	−0.0689 to −0.0193
Total indirect effect	−0.0501	−0.0800 to −0.0238

PROCESS model 4 with 5000 percentile bootstrap samples, adjusted for waist circumference, skeletal muscle mass, age, sex and family history of diabetes. Effects are unstandardized; c and c′ represent the change in HOMA-IR per 1-point increase in the lifestyle score. The total indirect effect corresponds to 29.0% of the total effect (adropin 23.9%; irisin 5.0%). Percentile bootstrap CIs are given for the indirect effects; paths a, b, c and c′ are point estimates from the corresponding regression models

Table 8. Receiver operating characteristic analysis for the identification of insulin resistance

Model	AUC	Optimism-corrected AUC
Irisin	0.608	—
Adropin	0.706	—
Waist circumference	0.638	—
Irisin + adropin + waist circumference	0.755	0.739

AUC, area under the receiver operating characteristic curve. The combined model was entered as predicted probabilities from logistic regression, and its optimism-corrected AUC was estimated with 1000 bootstrap resamples. Optimism correction is not applicable to single markers

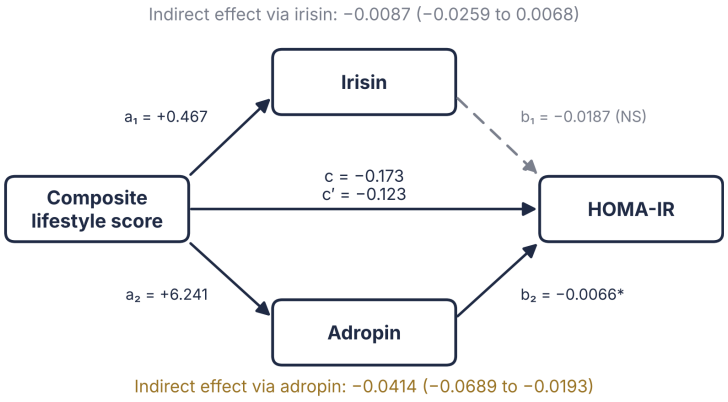


Figure 1. Parallel mediation model of the association between the composite lifestyle score and HOMA-IR through irisin and adropin. Values are unstandardized path coefficients (a, b), total (c) and direct (c′) effects, and indirect effects with 95% percentile bootstrap confidence intervals (5000 samples), adjusted for waist circumference, skeletal muscle mass, age, sex and family history of diabetes. The dashed arrow denotes a non-significant path. *P<0.001; NS, not significant

insulin resistance than those with a healthy lifestyle. A healthier lifestyle was also accompanied by higher circulating irisin and adropin; however, only adropin remained independently associated with HOMA-IR and statistically mediated the lifestyle–HOMA-IR association in an exploratory cross-sectional model, accounting for approximately one-quarter of the total effect.

The graded increase in insulin resistance across lifestyle categories is consistent with evidence that physical inactivity, poor sleep and long screen time cluster with insulin resistance in young people,[15] with experimental data showing that sleep restriction impairs insulin sensitivity in healthy young adults,[6,7,33-35] and with the long-standing observation that combinations of low-risk behaviors are associated with a markedly lower risk of type 2 diabetes.[5] The use of a composite score is justified by the fact that these behaviors are interrelated and probably act through partly overlapping pathways. Prolonged sedentary time is associated with incident diabetes;[9] a Mediterranean-type dietary pattern is related to more favorable components of the metabolic syndrome;[10] insufficient or poor-quality sleep reduces insulin sensitivity, and the timing of sleep also matters;[8] and smoking promotes insulin resistance and central fat deposition.[11] It should also be noted that the physical activity threshold of 600 MET-min/week corresponds approximately to 150 minutes of moderate-intensity activity per week (at about 4 METs), the lower limit of the range recommended by the World Health Organization;[55] a student who earned a point on this item therefore met only a minimum standard.

Using the same HOMA-IR threshold, the prevalence observed here was about half the 62.6% reported in overweight and obese Indian adolescents,[15] which is consistent with the inclusion of students across the full weight range in our sample and with the transient insulin resistance of puberty in that younger cohort. Regional data from comparable young populations point in the same direction. Among 300 students in Wasit, Iraq, 41.3% fulfilled the criteria for the metabolic syndrome,[14] and insulin resistance assessed by HOMA-IR was common among those with the syndrome and was linked to higher BMI, physical inactivity and dietary habits.[4] In 150 young women recruited on a university campus in Qatar, the prevalence of insulin resistance was 22.7% overall, increased with BMI and was already appreciable among non-obese participants (7%–37%), although a lower, population-derived HOMA-IR cut-off of 1.85 was applied.[56] Insulin resistance is, moreover, not confined to individuals with obesity; it is also found in normal-weight and overweight people, in whom it is accompanied by cardiovascular risk factors.[57] Direct comparison of prevalence estimates across studies is nevertheless hampered by differences in HOMA-IR thresholds, since cut-off values vary with sex and age,[58] and by the use of different insulin assays. Even so, a prevalence of 30% among students without known disease is considerable, and the high frequency of overweight, short sleep and inactivity reported among Iraqi students[12,13] suggests that this metabolic burden is not unique to Samarra. The magnitude of the adjusted odds ratios should be interpreted with caution because their confidence intervals were wide; the direction and gradient of the association, however, were consistent with the continuous regression analyses.

An unexpected finding was the discordance between the two surrogate indices. Students classified as insulin resistant by HOMA-IR had markedly higher fasting insulin (by approximately 63%) but only marginally higher fasting glucose (by approximately 3 mg/dL), and their triglycerides and TyG index were similar to those of their peers, although the TyG index still decreased modestly across lifestyle categories. This pattern suggests that insulin resistance at this age is expressed mainly as compensatory hyperinsulinemia, with β-cell secretion still sufficient to keep fasting glucose and triglyceride concentrations within or close to the normal range. Prospective data are consistent with this interpretation, since insulin secretion rises to compensate for declining insulin sensitivity and fasting glycemia deteriorates only later, in the years preceding the diagnosis of diabetes.[2] A similar dissociation was reported in young Qatari women, in whom differences in HOMA-IR were driven mainly by insulin concentrations while glucose levels were comparable.[56] Because the TyG index is derived from fasting glucose and triglycerides, it may detect insulin resistance only once this compensation begins to fail, which would limit its sensitivity in young and predominantly normal-weight populations; although the index has been compared with the euglycemic–hyperinsulinemic clamp,[59] the thresholds derived from such studies may not transfer to young adults with preserved glycemic control. Students with insulin resistance did, however, have lower HDL-C and higher total and LDL cholesterol, indicating that an atherogenic lipoprotein profile may already accompany early hyperinsulinemia before hypertriglyceridemia develops.

Irisin was associated with physical activity and skeletal muscle mass, in line with its origin as a PGC-1α-dependent myokine,[16] with the rise in circulating irisin after aerobic interval training[17] and combined exercise–diet intervention,[19] and with the pooled effect of long-term exercise training.[18] Students with insulin resistance had lower irisin, and irisin correlated inversely with HOMA-IR and the TyG index, which is directionally consistent with the inverse irisin–TyG association reported in young Indian adults[40] and with the parallel decline of irisin and insulin sensitivity during bed rest.[36] However, the association with HOMA-IR lost significance after adjustment for waist circumference, the lifestyle score and other covariates, and the indirect effect through irisin was small and not significant. These findings suggest that, in young adults, circulating irisin mainly reflects physical activity and muscle mass, which are themselves related to adiposity and insulin sensitivity, rather than acting as an independent determinant of insulin resistance. The literature on irisin and insulin resistance is, moreover, far from uniform: in a cross-sectional study of 151 adults, higher irisin was associated with higher HOMA-IR and a

greater risk of the metabolic syndrome, which the authors interpreted as a compensatory increase or an irisin-resistant state,[23] whereas other studies, including ours, have found inverse associations,[37-40] and the effect of exercise on irisin is heterogeneous across trials.[18] Such inconsistencies may reflect differences in age, adiposity, fitness and glycemic status, but they probably also reflect methodological problems. The metabolic benefits of irisin, including adipose browning, improved glucose homeostasis and restoration of muscle PGC-1α expression,[16,60] have been demonstrated mainly in rodents and may not apply at the concentrations found in humans, and the limited specificity of commercial irisin immunoassays[20,21] may have introduced measurement error that attenuated true associations. Even the physiological role of circulating irisin in humans has been questioned,[22] and quantification by mass spectrometry[17] will be needed to resolve these uncertainties.

In contrast, adropin emerged as the more robust correlate of insulin resistance. It was associated with diet quality and sleep rather than with physical activity, which fits its role as a nutritionally regulated hepatokine whose hepatic expression declines in diet-induced obesity,[24] and it remained inversely associated with HOMA-IR independently of waist circumference. This independence from central adiposity is consistent with experimental evidence that adropin attenuates hepatic steatosis and insulin resistance in obese mice independently of body weight,[24] with the improvement in glucose tolerance and insulin signaling after adropin treatment of diet-induced obese mice,[25] with lower adropin in human obesity and metabolic syndrome[26] and with higher adropin in participants with a smaller waist circumference.[38] At the population level, a meta-analysis of 15 observational studies likewise reported lower adropin in type 2 diabetes,[28] although the very high heterogeneity among those studies, and the dependence of absolute values on the assay used, caution against over-interpretation. The positive correlation between adropin and the MEDAS score is in keeping with the sensitivity of plasma adropin to dietary composition: habitual carbohydrate intake is inversely related to circulating adropin in humans,[29] and low adropin predicted greater weight gain and hyperglycemia during high-sugar feeding in macaques.[30] We did not quantify macronutrient intake, but it is conceivable that the better diet quality captured by higher MEDAS scores, which includes a lower intake of sugar-sweetened beverages and commercial sweets, is accompanied by higher adropin; this explanation remains speculative. The association with PSQI is, to our knowledge, novel in young adults. Sleep and adropin have so far been linked only in a small trial of overweight older adults, in which adropin was inversely related to sleep disturbance.[61] Together with animal evidence that time-restricted feeding restores circulating adropin[31] and that hepatic *Enho* expression is under circadian control,[32] our observation raises the possibility that the circadian alignment of eating and sleep contributes to hepatic adropin secretion. Adropin also had a higher AUC for insulin resistance than irisin or waist circumference alone (0.706 vs 0.608 and 0.638, respectively), although these AUCs were not formally compared.

The exploratory mediation analysis is therefore compatible with a hepatic rather than muscular role in linking lifestyle to insulin resistance, but, being cross-sectional, it generates a hypothesis and does not demonstrate this pathway. The two peptides statistically accounted for approximately 29% of the association between the lifestyle score and HOMA-IR, almost entirely through adropin (approximately 24%), whereas the contribution of irisin was small (approximately 5%) and its confidence interval included zero. The weak correlation between the two peptides ($r = 0.19$) indicates that they capture largely distinct biological signals, but only the liver-derived signal appeared relevant to insulin resistance at this age. Mediation estimated from cross-sectional data cannot establish temporal ordering or causality, so the terms indirect effect and mediation are used here in a purely statistical sense, and reverse causation, in which hyperinsulinemia or hepatic insulin resistance itself suppresses adropin secretion, is biologically plausible;[26,36] indeed, circulating adropin in humans responds to metabolic state, including weight loss and meal composition.[29] Moreover, approximately 71% of the association remained direct, indicating that other pathways, such as visceral and hepatic fat accumulation and low-grade inflammation, are likely to be more important; consistent with this, waist circumference was the strongest independent correlate of HOMA-IR.

Male sex and a family history of diabetes were also independently associated with higher HOMA-IR, reflecting the lower insulin sensitivity generally observed in young men than in premenopausal women and the contribution of heritable susceptibility and shared family behaviors. Sex may also modify the adropin axis: in a large human sample, the inverse association between adropin and LDL-C was evident in men but not in women,[32] and we cannot exclude sex-specific differences in how lifestyle influences adropin; although our sample was balanced by sex, it was not powered to test such interactions. From a practical standpoint, neither peptide alone is suitable as a screening test: the combination of irisin, adropin and waist circumference yielded an optimism-corrected AUC of 0.739, indicating acceptable but not strong discrimination, and waist circumference is the only inexpensive component of this model.

Implications for health promotion

These findings support multicomponent health promotion programs in Iraqi universities that address physical activity, sleep hygiene, diet quality and screen time together, because these behaviors cluster within the same students and their combined profile showed a strong, graded association with insulin resistance. Interventions directed at a single behavior may therefore miss much of the potential benefit. Practical components could include campus facilities and timetables that make regular activity feasible, in line with

the World Health Organization recommendation of at least 150 minutes of moderate-intensity activity per week and limited sedentary time;[55] healthier food provision in canteens; education on sleep hygiene and regular bedtimes; strategies to reduce non-academic screen time; and access to smoking cessation support.[11] The link between adropin, diet quality and sleep suggests that nutrition and sleep hygiene deserve particular emphasis alongside physical activity.

Simple screening of new students using waist circumference, complemented by fasting insulin where resources allow, could identify those at higher metabolic risk for targeted counseling, with priority for students with a family history of diabetes; in contrast, the lack of difference in the TyG index between students with and without insulin resistance argues against relying on glucose- and triglyceride-based indices alone in this age group. Prospective cohort studies that repeatedly measure behaviors, adropin and insulin sensitivity, and randomized lifestyle interventions that track changes in adropin, irisin and insulin sensitivity, ideally with mass-spectrometry-based quantification of irisin and standardized adropin assays, are needed to test the causal pathways suggested here.

Strengths and limitations

The strengths of this study include stratified random sampling, the simultaneous measurement of a myokine and a hepatokine, adjustment for skeletal muscle mass and central adiposity, formal mediation analysis with bootstrap inference, and internal validation of the discrimination model. Several limitations merit consideration. First, the cross-sectional design precludes causal inference, and the mediation analysis, for which the study was not specifically powered, should be regarded as exploratory. Second, lifestyle behaviors were self-reported and may therefore be affected by recall and social desirability bias. The IPAQ short form shows only weak agreement with accelerometry in young Arab adults,[62] the MEDAS has not been validated specifically in Iraqi young adults,[47] and, because the wine item was scored 0 for non-consumers, the maximum attainable score was effectively 13; such non-differential misclassification would most probably bias associations towards the null. The composite score also assigns equal weight to the five behaviors, although their metabolic effects are unlikely to be identical. Third, commercial irisin ELISA kits have been criticized for limited specificity,[20] and our irisin concentrations were higher than those measured by mass spectrometry;[17] adropin concentrations likewise vary considerably between assays,[26] so absolute values should not be compared directly across studies. Insulin was measured with a research-grade ELISA rather than a standardized clinical immunoassay, so the HOMA-IR threshold of 2.5, which was derived from clinical assays, may not be directly transferable, and the prevalence estimate should be compared with those of other studies cautiously; population-specific cut-offs also differ by age and sex.[58]

Fourth, HOMA-IR and the TyG index are surrogate measures that classified insulin resistance discordantly in this sample, and neither replaces clamp-based assessment. Fifth, the number of insulin-resistant participants relative to the number of covariates in the logistic model was modest, which resulted in wide confidence intervals for the adjusted odds ratios. Sixth, menstrual cycle phase was recorded only approximately and was not included in the models, and socioeconomic status was not assessed, so residual confounding cannot be excluded, although the E-value of 6.16 (3.09 for the lower confidence limit) for the unhealthy lifestyle category indicates that only a strong unmeasured confounder could fully explain this association. Seventh, we did not measure hepatic fat, liver enzymes or macronutrient intake, which means that the hepatic interpretation of the adropin findings remains speculative, and a single fasting measurement may not capture the dietary and circadian variability of adropin.[29,32] Finally, the study was conducted at a single university with equal allocation by sex, which may limit generalizability to other Iraqi populations.

Several additional methodological points deserve emphasis. The five-item score was constructed with equal weights and fixed cut-offs that were not empirically derived, and no sensitivity analyses (for example, continuous scoring, alternative thresholds, exclusion of smoking or effect-based weighting) were performed; the robustness of the findings to these choices is therefore unknown. The mediation models were adjusted for waist circumference and skeletal muscle mass, which may themselves lie on the pathway between lifestyle and insulin resistance and between physical activity and irisin; such adjustment may have attenuated total and indirect effects and may partly explain the null indirect effect of irisin, and models without these covariates would be informative. Except for the correlation matrix, *P* values were not corrected for multiple testing; sex interactions were not examined, because the study was not powered for them; and the AUCs of the individual markers were not formally compared. The combined AUC of 0.739 is modest and does not support clinical use. Because insulin was measured by a research-grade ELISA, the 30.0% prevalence is assay-dependent, and the discordance between HOMA-IR and the TyG index leaves open the possibility that part of the HOMA-IR classification reflects assay performance rather than true insulin resistance; intra- and inter-assay variability was taken from the manufacturer rather than determined in-house. Equal allocation of men and women, a single institution and the exclusion of students with conditions such as polycystic ovary syndrome or hormonal contraceptive use mean that the prevalence estimate cannot be generalized to the student population. Finally, socioeconomic status, energy intake, hepatic fat and visceral adiposity were not measured, and the timing of the last meal, although recorded, was not modeled despite the nutritional and circadian sensitivity of adropin; residual

confounding is therefore likely.

Conclusion

Among university students in Samarra, Iraq, almost one in three had insulin resistance, and its prevalence rose steeply as the composite lifestyle became less healthy. Higher circulating adropin, but not irisin, independently accounted for part of the association between lifestyle and insulin resistance, an exploratory finding that is compatible with, but does not demonstrate, a hepatic pathway responsive to diet and sleep. Combined promotion of physical activity, adequate sleep and a healthy diet, together with simple screening based on waist circumference, warrants further study as an approach to early metabolic dysfunction in young adults; longitudinal and interventional studies are needed to clarify temporal order and causality.

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Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

The study protocol was reviewed and approved by the Research Ethics Committee of the University of Samarra, Iraq, on 4 September 2025, before the start of data and sample collection. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants, who were free to withdraw at any time without academic consequences and who received their laboratory results; those with abnormal values were referred for medical follow-up. The confidentiality of personal and health information was maintained, and the data were used exclusively for research purposes.

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Supplementary Files

Supplementary File Contains Figures S1.

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