sciendo

ENDOCRINE REGULATIONS, Vol. 58, No. 2, 115–128, 2024

doi:10.2478/enr-2024-0013

Plasma irisin and the brain-derived neurotrophic factor levels in sedentary subjects: effect of 8-weeks lifestyle intervention

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Objectives. Sedentary lifestyle increasingly observed in the population contributes to the incremental incidence of obesity, cardiovascular diseases, mental disorders, type 2 diabetes, hypertension, dyslipidemia, and others. Physical inactivity together with an imbalance in caloric intake and expenditure leads to a loss of muscle mass, reduced insulin sensitivity, and accumulation of the visceral fat. Organokines (adipokines, myokines, hepatokines, etc.) serve in the organism for inter-organ communication. However, human studies focused on the exercise-related changes in plasma levels of certain myokines have produced contradictory results. In the present study, we verified a hypothesis that myokine irisin, which is expected to increase in response to physical activity, induces brain-derived neurotrophic factor (BDNF) production and by this way mediates the beneficial effect of exercise on several brain functions.

Subjects and Methods. Women (n=27) and men (n=10) aged 44.5±12.0 years, who were sedentary and overweight/obese (men ≥25%, women ≥28% body fat), participated in the study. The effect of an 8-week intensive lifestyle intervention (150 minutes of moderate physical activity per week, diet modification, and reduction of caloric intake) on the selected organokines (irisin, BDNF) in the context of an expected improvement in cardiometabolic status was examined.

Results. The 8-week lifestyle intervention resulted in a significant ($p<0.05$) reduction in body mass index, body fat, blood pressure, insulin resistance, lipid and liver parameters, and irisin levels ($p<0.001$). However, BDNF increase in the whole group did not reach statistical significance. After the improvement of cardiometabolic parameters, a significant decrease in irisin and increase in BDNF levels were also observed in the subgroup with unsatisfactory (≤5%) body weight reduction. Neither relationship between irisin and BDNF levels, nor effect of age or sex on their levels was observed.

Conclusions. We cannot confirm the hypothesis that exercise-induced irisin may increase the BDNF levels, whereas, the organokine levels in the periphery may not completely reflect the processes in the brain compartments. The observed decrease in irisin levels after 8-week intensive lifestyle intervention program, which was in contrary to its supposed mechanisms of action and dynamics, suggests the presence of several yet undiscovered impacts on the secretion of irisin.

Key words: irisin, BDNF, physical activity, sedentary lifestyle, cardiometabolic improvement

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The obesity and obesity related diseases are considered a global world problem leading to serious health problems. In 2016, about 1.9 billion adults worldwide had body mass index (BMI) >25 kg/m^2, including 650 million adults with obesity. Additionally, almost 400 million children and adolescents are suffering from overweight/obesity (WHO 2022). Based on the recent data (from 2020), more than 2.6 billion people worldwide are obese/overweight with an unfavorable expectation of 4 billion in 2035 (World Obesity Federation 2023).

The mechanisms how the obesity contributes to the development of metabolic syndrome, its features, and complications (insulin resistance, hypertension, diabetes mellitus type 2 (T2DM), dyslipidemia, low-grade inflammation, cardiovascular diseases, nonalcoholic fatty liver disease, obstructive sleep apnea, different types of cancer etc.) are extensively studied (Fasshauer and Bluher 2015).

Physical inactivity and disbalance in the caloric intake and expenditure (in favor of overnutrition) lead to a visceral fat accumulation, obesity, loss of muscle mass, impaired glucose tolerance, decreased insulin sensitivity, and associated diseases (Dirks et al. 2016). Overweight and obese individuals are repeatedly recommended to follow two main health advices that can reduce the risk of health problems: energy intake decrease and physical active increase (Dinas et al. 2014). Disappointing weight loss is often followed by a poor compliance and loss of motivation. However, the beneficial effects of exercise (decreased systolic and diastolic blood pressure, lower waist circumference, improved cardiorespiratory fitness, etc.) are not dependent on the weight loss itself and can be achieved even in the presence of lower-than-expected exercise-induced weight loss (King et al. 2009).

The communication between the tissues and organs in the body, such as adipose tissue, liver, skeletal muscle, immune system, brain, gut, pancreas, vessels, bones, etc., is a complex. The increasing understanding that how the tissues communicate by secretion of various substances, e.g., organokines (adipokines, hepatokines, myokines, etc.) affecting local and distant organs, has proposed the concept of the inter-tissue crosstalk (Meex and Watt 2017). Individual tissues produce relevant organokines. The adipose tissue produces more than 600 adipokines (Lehr et al. 2012), hepatocytes produce more than 500 hepatokines, and the skeletal muscle more than 600 myokines (Gorgens et al. 2015). In addition, many substances are produced in different organs having several sites of origin and many of them are still unidentified.

In the resting state and during the contraction, the skeletal muscle secretes many myokines, which are supposed to mediate exercise-induced beneficial health effects. One of the myokine, which is secreted by skeletal muscle, is irisin. Although initially described as a myokine, irisin has multiple sites of origin, i.e. besides skeletal and cardiac muscles, it is secreted from the adipose tissue, pancreas, kidney, and liver (Bostrom et al. 2012). Irisin induces browning of the subcutaneous fat adipocytes (Bostrom et al. 2012), weight loss with increased energy expenditure, and loss of the visceral adipose tissue (Gonzalez-Gil and Elizondo-Montemayor 2020). Moreover, irisin has also anti-inflammatory and antioxidative properties, which by acting on the hepatocytes can decrease the hepatic steatosis (Gonzalez-Gil and Elizondo-Montemayor 2020). Irisin acts after binding to a recently discovered αV integrin receptor (Kim et al. 2018; Mu et al. 2023).

By demonstrating irisin's beneficial effects on browning of the subcutaneous fat adipocytes, improving energy homeostasis, and obesity in animal and *in vitro* studies (Bostrom et al. 2012; Polyzos et al. 2018), it has been considered to be a promising target for the therapy of obesity. However, human studies have brought several contradictory results, either supporting (Bostrom et al. 2012; Miyamoto-Mikami et al. 2015) or refuting the hypothesis of an increased circulating irisin in response to solitary or regular exercise. The later finding has revealed no change (Kurdiova et al. 2014) or even decreased levels (Qiu et al. 2015) of this myokine after chronic exercise training.

Brain-derived neurotrophic factor (BDNF) is a growth factor ubiquitously expressed in the brain regions related to cognitive functions (Carlino et al. 2013). It is involved in the differentiation of neurons, formation and plasticity of synapses, and processing associated with survival, reparation, and protection of the central nervous system tissues (Benarroch 2015). Its expression is not limited to the nervous system. Its function has been described in multiple non-neural tissues, such as heart, lung, skeletal muscle, adipose tissue, kidneys, vascular system, and blood cells (Esvald et al. 2023).

Decreased BDNF levels are associated with cognitive deficits in elderly population (Shimada et al. 2014) and involved in the pathophysiology of many neurodegenerative disorders including Alzheimer's and Parkinson's diseases, multiple sclerosis, and Huntington's disease (Wang et al. 2016; Prokopova et al. 2017; Ng et al. 2019; Zhou et al. 2021) as well as neuropsychiatric disorders, such as major

depressive disorder (Porter and O'Connor 2022) or schizophrenia (Yang et al. 2019). BDNF levels are also associated with obesity playing an important role in the regulation of energy balance, controlling the appetite, and managing the body weight (Sandrini et al. 2018). On the other hand, higher levels of this neurotrophic factor have been observed after physical activity (Leung et al. 2023).

In the present study, we verified a hypothesis that myokine irisin, which is expected to increase in response to physical activity, induces BDNF production and by this way mediates the beneficial effect of exercise on several brain functions. The aim of the present study was to examine the effect of 8-weeks intensive lifestyle changes (diet and 150 min of moderate physical activity per week) on the organokine irisin levels in context with the expected improvement of the cardiometabolic status. The assumption that the weight loss itself is not a prerequisite for improving the cardiometabolic parameters and that the lifestyle changes alone may lead to a significant improvement in the observed parameters, was also assessed. For this reason, a subgroup of subjects who achieved lower-than-expected weight loss (LWL subgroup) was transferred into the group of volunteers and evaluated separately.

Subjects and Methods

Design of the study. A prospective longitudinal study consisted of an 8-weeks weight loss intervention program including a 30% reduction in intake of weight-maintaining calories and recommended moderate aerobic exercise of 150 min per week. The study, registered on ClinicalTrials.gov under NCT02325804, was performed at the Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia. The project was approved by the Ethic committee of the Bratislava self-governing region (No: 05239/2016/HF). The study was performed in accordance with the Declaration of Helsinki principles. After a comprehensive explanation of the particular tests and detailed instructions concerning the diet and exercise program, the signed informed consent was obtained from all participants before being enrolled in the program.

Participants. The participants of our program, aged 20–69 years were all Caucasian volunteers (men n=10, women n=27) with central obesity, expressed as higher amount of body fat (men ≥25%, women ≥28%) and sedentary lifestyle, assessed using the Slovak version of the Lagerros questionnaire (Lagerros et al. 2006), which is a useful method to estimate the average daily energy expenditure. From the participants who completed the program, available plasma and serum samples of 37 subjects fulfilling the inclusion (age, obesity, and sedentary lifestyle as mentioned above) and exclusion criteria were examined. The exclusion criteria were: current symptoms or treatment of chronic diseases (diabetes mellitus on insulin therapy, any serious endocrine, rheumatic, metabolic, hematologic, pulmonary, liver, cardiovascular disease), malignancies, recent trauma or surgery interfering with the intervention program, pregnancy and breastfeeding, tobacco, alcohol or drug addiction.

Study protocol. The participants were examined twice – after enrolment in the study and after completing the intervention program. The volunteers were asked to fast 12 h prior to the study and avoid making intensive physical exercise 24 h before the examination. The tests were performed at 08:00 a.m. in the outpatient clinic of the internal medicine and diabetes at the Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia.

In all participants, anthropometric measurements (height, weight, waist, and hip circumference) were performed. The percentage of total body fat was examined by bioimpedance method (InBody R20, InBody Co., Ltd., Seoul, South Korea). Blood pressure was measured after at least 5 min rest (Omron). After obtaining a short medical history, resting energy expenditure (REE) and respiratory quotient (RQ) were measured in all participants using an indirect calorimetry (Ergostik, Geratherm Respiratory GmbH, Bad Kissingen, Germany) performed with a face mask with flow sensor. The measurements were performed in a comfortable and thermoneutral environment with attention to standardized resting conditions and exclusion of possible distractions. Data from the indirect calorimetry and the estimated daily energy expenditure were used to calculate the desired caloric intake. Cubital vein was cannulated for blood sampling. The subjects underwent an oral glucose tolerance test (OGTT). After the first baseline blood sampling (0 min), the participants ingested a solution of 75 g glucose in 250 ml water within 3 min. Blood samples were collected in 30 min intervals for 2 h into polyethylene tubes, processed, and serum and plasma aliquots stored at –70°C until analyzed. Cardiorespiratory fitness, expressed as maximal oxygen consumption (VO_2max), was measured using the ramp protocol as described previously (Bajer et al. 2019).

Biochemical analyses and calculations. All standard biochemical parameters (glucose, insulin, lipid parameters, etc.) were assayed in certified hospital laboratory (Synlab Bratislava, Slovakia) using appropriate methods on auto-analyzer Beckman Coulter AU (Beckman Coulter, Inc., Brea, CA, USA). Serum insulin concentrations were measured using Chemiluminescent Microparticle Immunoassay (CMIA; ARCHITECT Immunoassay analyzer, Abbott Laboratories Diagnostics, Lake Forest, IL, USA). Irisin concentration was measured in plasma after 10-fold dilution of the sample according to the manufacturer's recommendation for optimal dilution for the particular experiments using the Human Irisin ELISA Kit (Cusabio, Houston TX, USA) with declared detection range 3.12–200 ng/ml, intra-assay variability <8% and inter-assay variability <10%. BDNF concentrations were measured in plasma using the Human BDNF ELISA Kit (Cusabio, Houston TX, USA) with declared detection range 0.3125–20 ng/ml, intra-assay variability <8% and inter-assay variability <10%. (Cusabio, Houston TX, USA).

Insulin sensitivity/resistance indices were calculated using fasting (HOMA-IR, Matthews et al. 1985) and OGTT-derived serum glucose and insulin concentrations (ISI_{Ced}, Cederholm and Wibell 1990; ISI_{Mat}, Matsuda and DeFronzo 1999). Insulin response to oral glucose load was calculated as Area Under the Curve (AUC) using the trapezoidal rule. The fatty liver index (FLI) was calculated according to the formula proposed by Bedogni et al. (2006) using gamma-glutamyl transferase (GGT), BMI, triglycerides, and waist circumference as variables, with FLI<30 representing low likelihood and FLI≥60 high likelihood of having hepatic steatosis (Bedogni et al. 2006).

Intervention. The intervention of the participants has been described previously (Bajer et al. 2019). Briefly, the subjects underwent an 8-week weight loss intervention program including reduction of caloric intake by 30% of the weight maintenance calories and 150 min per week of moderate to intensive aerobic exercise. The investigators provided individual detailed instructions and counseling about lifestyle changes: personalized nutritional plan prepared using software PLANEAT (www.planeat.sk) and individually tailored plan of physical activity (type, duration, frequency, repetitions). The creation of the personalized nutrition plans and plans for individually tailored physical activity have been described previously (Bajer et al. 2019).

Statistical evaluation. Statistical analysis of the data was performed using the IBM SPSS Statistics version 19 (SPSS Inc., Chicago, IL, USA). The pre- and post-differences in the mean values were analyzed by the Student's paired t-test or the Wilcoxon signed-rank test depending on the normality of the data distributions, which was assessed by the Kolmogorov-Smirnov test. The general linear model (repeated measures analysis of variance, ANOVA) with Student-Newman-Keuls *post hoc* test was used to analyze the differences in plasma insulin response to oral glucose load during the OGTT before and after the intervention. The associations of irisin and BDNF with other anthropometric and cardiometabolic parameters measured were examined using Pearson's or Spearman's correlation depending on the normality of data. Normally distributed data were expressed as mean±SD, while data not normally distributed were expressed as median (interquartile range [IQR]). A p value less than 0.05 was considered to be statistically significant.

Results

The whole group of participants. The available samples of 37 participants (10 men and 27 women) with the mean age of 44.5±12.0 years were used to examine the effect of 8-weeks lifestyle intervention program on the cardiometabolic parameters. Anthropometric, clinical, and laboratory characteristic of the study participants before and after intervention are presented in Table 1.

As expected, the evaluated cardiometabolic parameters improved significantly overall. After 8 weeks of the lifestyle intervention program, the participants in the whole observed group had significantly lower BMI, percentage of the body fat, blood pressure, insulin resistance and lipid and liver parameters. Physical fitness, expressed as VO_2max, improved as well in all subjects (Table 1).

Plasma glucose concentration course during OGTT was comparable before and after the lifestyle intervention program (Figure 1A). The insulin response to the oral glucose load was significantly lower after intervention ($F=16.3$; $p<0.001$) in all subjects evaluated, as shown in Figure 1B.

Irisin levels decreased significantly ($p<0.001$) after the lifestyle intervention (Figure 2A), while BDNF levels increased, however, this increase did not reach statistical significance ($p=0.114$) (Figure 2B) in all examined subjects.

The lower-weight-loss (LWL) subgroup. From 37 volunteers, who completed the intervention study and had sufficient number of samples for all analyses, we identified a subgroup of 17 participants (3 men and

Table 1
General characteristics of all participants before and after 8 weeks of intervention

Parameter	Before intervention (n=37)	After intervention (n=37)	p-value
BMI (kg/m^2)	30.5±4.8	28.6±4.6	<0.001
Body fat (%)	34.8 (31.9–40.6)	31.6 (27.0–36.4)	<0.001
Waist (cm)	98±14	91±12	<0.001
Hip (cm)	111±11	107±10	<0.001
BPsys (mmHg)	125 (116–136)	114 (109–129)	<0.001
BPdia (mmHg)	76±12	72±9	=0.008
Heart rate (1/min)	74±14	70±11	=0.032
Fasting glucose (mmol/l)	4.3±0.6	4.2±0.5	=0.339
Fasting insulin (mIU/l)	7.3 (5.1–9.4)	4.9 (3.7–6.9)	<0.001
HOMA-IR	1.32 (0.90–1.85)	0.86 (0.64–1.37)	<0.001
ISI Cederholm	60±21	62±19	=0.324
ISI Matsuda	7.0±4.2	8.6±3.7	=0.002
AUC insulin	6534 (4083–9998)	5295 (3338–7274)	=0.015
AST (μkat/l)	0.37±0.12	0.35±0.09	=0.168
ALT (μkat/l)	0.30 (0.23–0.41)	0.26 (0.21–0.39)	=0.005
GGT (μkat/l)	0.27 (0.21–0.38)	0.23 (0.20–0.31)	=0.014
FLI	45.2±30.4	29.8±22.7	<0.001
Total cholesterol (mmol/l)	5.16±1.35	4.73±1.04	=0.005
HDL-C (mmol/l)	1.45±0.37	1.38±0.30	=0.066
LDL-C (mmol/l)	3.22 (2.56–3.92)	2.77 (2.41–3.55)	=0.024
Triglycerides (mmol/l)	0.94 (0.62–1.40)	0.79 (0.59–1.13)	=0.045
VO_2max (ml/(kg.min))	25.5±5.9	29.2±5.6	<0.001
Irisin (ng/ml)	236±58	165±79	<0.001
BDNF (ng/ml)	2.56±1.43	3.27±2.63	=0.114

Data are presented as means±SD for parametric variables and median (interquartile range) for nonparametric variables, depending on normality testing. Respective statistical tests are used to express the p-value (Student's paired t-test or the Wilcoxon signed rank test). Abbreviations: AUC – area under curve; BDNF – brain-derived neurotrophic factor; BMI – body mass index; BPdia – blood pressure diastolic; BPsys – blood pressure systolic; FLI – fatty liver index; GGT – gamma-glutamyl transferase; HDL-C – high-density lipoprotein-cholesterol; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; LDL-C – low-density lipoprotein-cholesterol.

14 women with the mean age 46.2±11.4 years), who lost ≤5% of their initial body weight and considered their weight loss insufficient. Their characteristics are presented in Table 2.

Even the lower-than-expected weight loss was significant and accompanied by a significant reduction in body fat percentage, several cardiometabolic parameters also improved after intervention, particularly the systolic blood pressure, fasting and post-load insulin levels as well as FLI, a marker of the fatty liver. The improvement in physical fitness (VO_2max) did not reach statistical significance (Table 2).

In the LWL subgroup, plasma glucose concentration course during OGTT was comparable before and after the intervention program (Figure 1C). The response of insulin to oral glucose load was significantly lower after intervention even in the LWL subgroup (F=8.8; p=0.009, Figure 1D), which reflects the improvement of insulin sensitivity, even the increments in calculated insulin sensitivity indices did not reach statistical significance (Table 2).

Like in the whole participants group, irisin levels decreased significantly (p=0.002) in the LWL subgroup (Figure 2C) after lifestyle intervention program. Remarkably, although the increase in

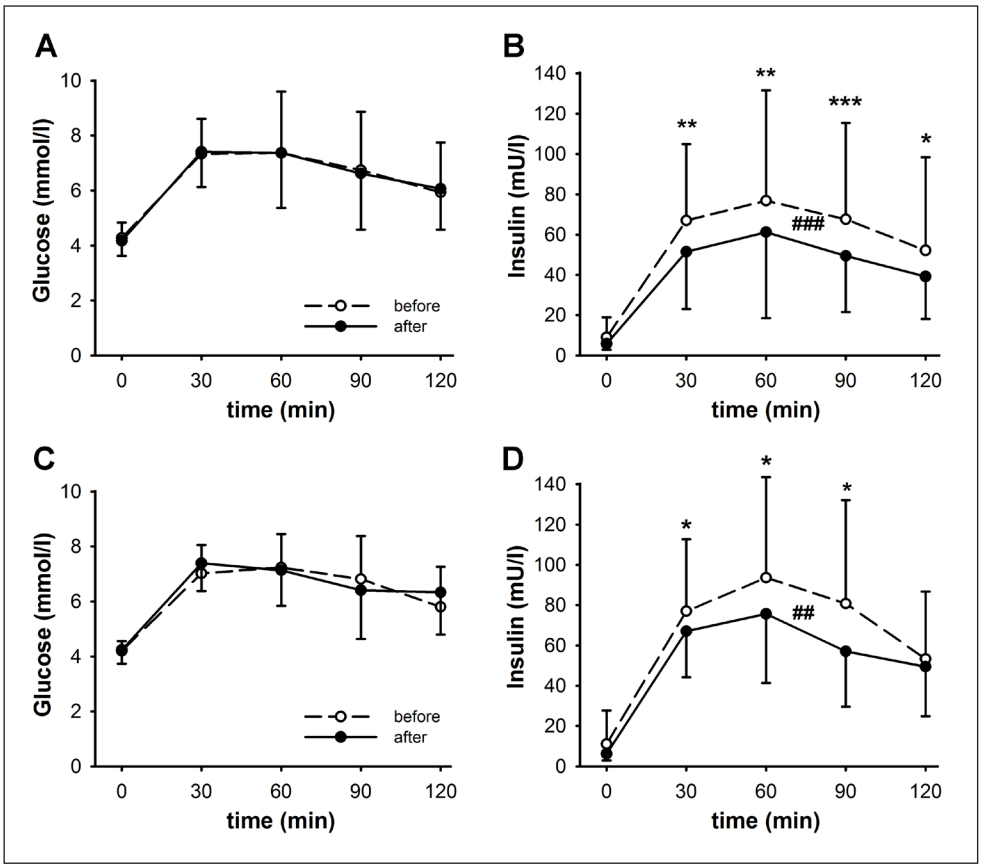

Figure 1. Plasma glucose (A) and insulin (B) concentrations of the oral glucose tolerance test before and after the intervention in the whole group of volunteers (n=37) and plasma glucose (C) and insulin (D) concentrations of the oral glucose tolerance test before and after the intervention in the lower-than-expected weight loss subgroup of volunteers (n=17). Data are presented as mean±SD. Figure 1B – ###p<0.001 between pre- and post-intervention (p<0.001, F=16.305); *p<0.05, **p<0.01, ***p<0.001 for Student-Newman-Keuls post hoc comparisons between pre- and post-intervention data within individual sampling times. Figure 1D – ##p<0.01 between pre- and post-intervention (p=0.009, F=8.787); *p<0.05 for Student-Newman-Keuls post hoc comparisons between pre- and post-intervention data within individual sampling times.

BDNF following the lifestyle modification was not significant in the whole participants group, in the LWL subgroup, a significant BDNF increase (p=0.038) after intervention was observed (Figure 2D).

The gender and the age impact. When analyzing the entire dataset for males and females separately, the pattern of cardiometabolic improvement was comparable in both sexes. There were no significant differences when comparing the changes (deltas) of irisin (p=0.691) and BDNF (p=0.919) after lifestyle intervention between sexes. The men improved significantly in several liver parameters (alanine transaminase – ALT, GGT, FLI), whereas the women in FLI only. In the women, the improvement in FLI reached a lesser extent (p=0.025), probably due to markedly lower initial FLI values.

Being aware of the wide age range of our subjects, we compared the irisin and BDNF levels between the subgroups of individuals younger than 47 years (Y; n=19) and older than 47 years (O; n=18). The decrease of irisin after intervention was significant in both age groups (Y: 238±58 ng/ml vs. 162±78 ng/ml, p<0.001; O: 232±60 ng/ml vs. 168±84 ng/ml, p<0.001) and comparable (delta irisin Y: –76±61 ng/ml vs. O: –64±53 ng/ml, p=0.570). The increase of BDNF after intervention was not significant in both age groups (Y: 2.50±1.42 ng/ml vs. 3.34±2.82 ng/ml, p=0.271; O: 2.63±1.48 ng/ml vs. 3.18±2.48 ng/ml, p=0.325) and comparable (delta BDNF Y: 0.85±2.80 ng/ml vs. O: 0.55±2.00 ng/ml, p=0.739).

Correlation analyses demonstrated that the changes in irisin levels were positively correlated

Table 2
General characteristics of participants of the lower-weight-loss (LWL) subgroup before and after 8 weeks of intervention

Parameter	Before intervention (n=17)	After intervention (n=17)	p-value
BMI (kg/m^2)	29.3±4.4	28.4±4.5	<0.001
Body fat (%)	34.5 (31.1–40.0)	32.0 (28.1–37.4)	<0.001
Waist (cm)	91 (84–102)	88 (80–98)	<0.001
Hip (cm)	109±8	106±8	=0.003
BPsys (mmHg)	127±12	118±11	=0.007
BPdia (mmHg)	77±8	73±6	=0.115
Heart rate (1/min)	74±12	72±9	=0.520
Fasting glucose (mmol/l)	4.2±0.4	4.2±0.5	=0.850
Fasting insulin (mIU/l)	6.3 (5.7–8.3)	5.7 (4.0–6.9)	=0.021
HOMA-IR	1.23 (0.98–1.63)	1.06 (0.72–1.40)	=0.074
ISI Cederholm	59±20	62±21	=0.305
ISI Matsuda	6.8±4.5	7.8±3.4	=0.152
AUC insulin	7506 (5063–11128)	6339 (3950–7520)	=0.027
AST (μkat/l)	0.36±0.10	0.35±0.11	=0.460
ALT (μkat/l)	0.36±0.19	0.31±0.19	=0.084
GGT (μkat/l)	0.28±0.09	0.28±0.08	=0.404
FLI	38.0±24.6	28.6±21.7	=0.003
Total cholesterol (mmol/l)	5.45±1.57	5.24±1.18	=0.271
HDL-C (mmol/l)	1.55±0.44	1.52±0.35	=0.569
LDL-C (mmol/l)	3.28 (2.45–3.84)	3.01 (2.60–3.99)	=0.940
Triglycerides (mmol/l)	1.09 (0.72–1.56)	1.11 (0.57–1.42)	=0.562
VO_2max (ml/(kg.min))	25.2±6.5	26.9±4.9	=0.091
Irisin (ng/ml)	234±61	187±79	=0.002
BDNF (ng/ml)	2.38±1.41	3.46±2.44	=0.038

Data are presented as mean±SD for parametric variables and median (interquartile range) for nonparametric variables, depending on normality testing. Respective statistical tests are used to express the p-value (Student's paired t-test or the Wilcoxon signed rank test). Abbreviations: AUC – area under curve; BMI – body mass index; BPsys – blood pressure systolic; BPdia – blood pressure diastolic; BDNF – brain-derived neurotrophic factor; FLI – fatty liver index; GGT – gamma-glutamyl transferase; HDL-C – high-density lipoprotein-cholesterol; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; LDL-C – low-density lipoprotein-cholesterol.

with the changes in the weight (r=0.456, p=0.007), BMI (r=0.473, p=0.005), and percentage of body fat (r=0.345, p=0.046) and negatively correlated with changes in whole-body insulin sensitivity expressed as ISI Matsuda (r=–0.409, p=0.02) and with improvement in physical fitness expressed as VO_2max (r=–0.532, p=0.007). Changes in plasma BDNF levels were negatively correlated with changes in systolic blood pressure (r=–0.361, p=0.036) and positively with changes in the peripheral insulin sensitivity expressed as ISI Cederholm (r=0.416, p=0.012). No correlation of BDNF with parameters of the obesity (BMI, body fat percentage, waist circumference) or with irisin levels was observed. No significant mutual correlation of parameters of interest (irisin and BDNF), their initial, post-interventional levels or even their changes over time was found (p=0.256–0.800).

Discussion

The present study was aimed to assess the irisin and BDNF levels on the background of improvement of cardiometabolic status in response to the life style counseling in the overweight/obese subjects with the sedentary lifestyle. In our 8-week follow-up study, the participating sedentary subjects lost weight and

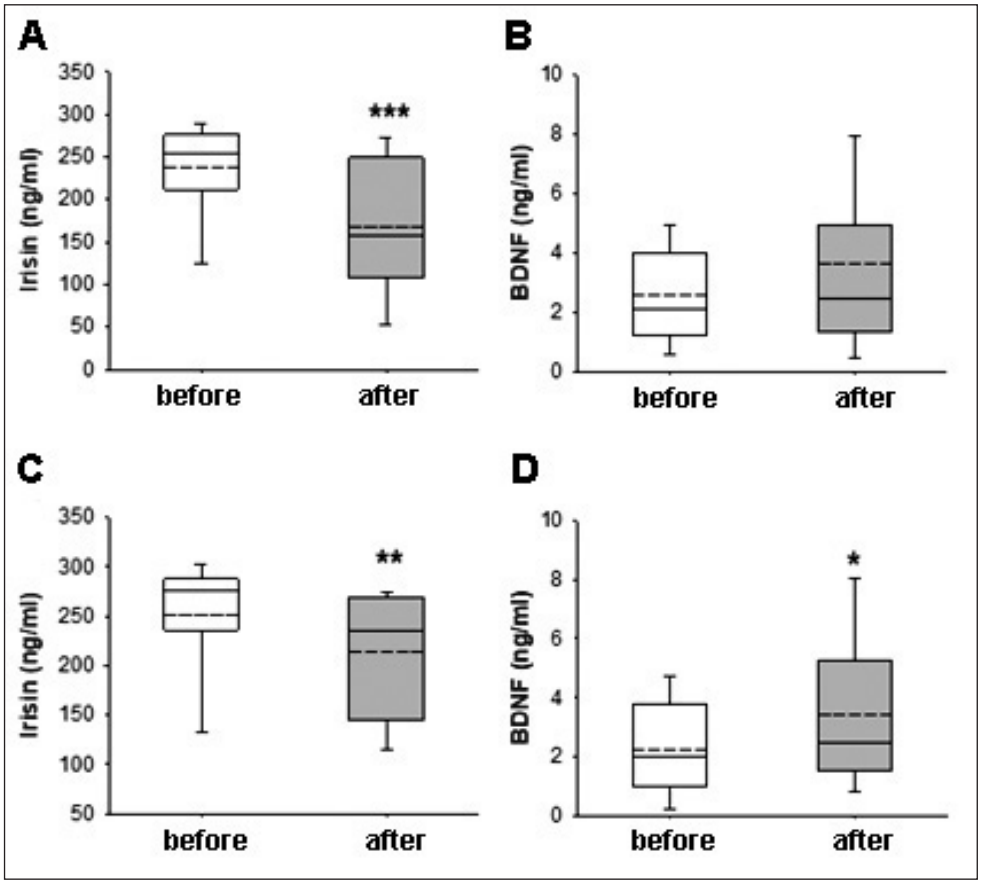

Figure 2. Plasma irisin (A) and brain-derived neurotrophic factor (BDNF) (B) concentrations before and after the intervention in the whole group of volunteers (n=37) and plasma irisin (C) and BDNF (D) before and after the intervention in the lower-than-expected weight loss subgroup of volunteers (n=17). Box-and-whisker plots represent mean and SD. *p<0.05; **p<0.01; ***p<0.001 between pre and post intervention.

favorable changes occurred in metabolic and cardiovascular parameters. These changes were observed even in a subgroup of participants who lost 5% or less of their initial body weight. After 8 weeks of lifestyle intervention, serum irisin levels decreased and correlated with the decrease in the adiposity markers and the increase of the insulin resistance.

It is generally accepted that lifestyle changes play a key role in both the prevention and treatment of obesity and metabolic syndrome (Bajer et al. 2015). The approaches used in the management of the obesity include changes in dietary habits, physical activity, and behavioral modifications. Regular exercise has a beneficial effect on both the cardiometabolic parameters and the mental health (cognitive functions, sleep, mood, etc.) and is known to counteract in the development of obesity, diabetes, cardiovascular diseases, etc. (Ball 2015).

The sedentary lifestyle leads to insulin resistance, dyslipidemia, decreased muscle mass, and increased visceral adipose tissue. It also increases the risk of many diseases, such as T2DM, cardiovascular disease, cancers, etc. Several mechanisms have been assumed to trigger the beneficial effects of physical activity, such as improved cardiorespiratory fitness (VO_2max), decreased adiposity and circulating lipids, decrease in inflammatory parameters, energy expenditure, and weight loss (Whitham and Febbraio 2016).

The FLI (Bedogni et al. 2006) is a validated biomarker for the diagnosis of fatty liver (Foschi et al. 2021) and its levels ≥60 can identify not only subjects with the liver steatosis, but also a high cardiometabolic risk (Carli et al. 2023). Our lifestyle intervention led to a significant decrease of this parameter. This means that in 38% of study participants high risk of

liver steatosis was identified in the initial examinations. However, after intervention, the percentage of subjects with high risk decreased to 8%. Similar pattern was observed in the LWL subgroup, where the percentage of subjects with high liver steatosis risk decreased from 24% to 12% after intervention.

Besides the positive effect of exercise on the cardiometabolic status, physical activity has been suggested to improve sleep, memory, cognitive functions, dementia, and depression by increased BDNF production (Inyushkin et al. 2023). In our study, we did not find any significant changes in plasma BDNF levels after intervention program or its correlation with the age or obesity markers. Several human studies have been focused on the changes in BDNF levels in subjects with/without obesity, depending on the intensity and duration of the physical activity as well as the age of the participants. Acute exercise leads to a transient increase in serum BDNF concentrations in healthy active males (Rojas Vega et al. 2006) and active and sedentary females (Nofuji et al. 2012). However, some authors have reported a transient elevation of circulating BDNF levels in the elderly sedentary/inactive, but not active elderly or young individuals (Maderova et al. 2019), especially women (Alizadeh and Dehghanizade 2022).

On the other hand, there are inconsistent data regarding the baseline levels in active vs. sedentary, obese vs. non-obese individuals or regarding the effect of long-term training program on BDNF levels. Three-month training did not induce any changes in resting serum or plasma BDNF levels in elderly subjects and those levels were comparable with young lean active individuals (Maderova et al. 2019). The chronic exercise did not affect the BDNF levels in the obese children (Rodriguez-Ayllon et al. 2023). Other authors have found higher baseline serum BDNF levels in active compared to inactive obese females (Alizadeh and Dehghanizade 2022) or even inverse relationship between serum BDNF levels and physical fitness (expressed as VO_2max) has been reported (Currie et al. 2009).

Similarly, reduced circulating BDNF levels have been reported in obese individuals (Alomari et al. 2020; Katuri et al. 2021). Other authors have reported increased circulating levels of BDNF in subjects with obesity (Golden et al. 2010; Slusher et al. 2015). Additionally, a meta-analysis of ten studies have revealed comparable circulating BDNF levels in obese and control subjects (Sandrini et al. 2018). One of the explanations for the considerable differences in the literature could be in representation of different BDNF pools in serum and plasma samples. In serum, BDNF released from activated platelets during clotting is present (Maderova et al. 2019) and this level depends on the clotting time during blood sample processing (Currie et al. 2009). On the other hand, plasma levels represent a biologically available BDNF pool (Maderova et al. 2019). Another point is that the circulating BDNF levels in the periphery do not adequately reflect the BDNF release from the CNS, e.g. its main production site (Seifert et al. 2010). Several authors have suggested that the exercise-induced irisin stimulates secretion of BDNF in the brain leading to a cognitive improvement (Jo and Song 2021; Babaei et al. 2023; Inyushkin et al. 2023). Our results based on the plasma irisin and BDNF levels cannot confirm this hypothesis. However, it is possible that the organokine levels measured in the peripheral circulation do not adequately reflect the processes in the brain (Seifert et al. 2010).

Bostrom et al. (2012) have discovered irisin as an exercise-induced myokine cleaved from the transmembrane protein fibronectin type III domain-containing 5 (FNDC5) and hypothesized its role in mediating the positive effects of exercise on browning of the white adipose tissue, increasing energy expenditure, weight loss, and improving glucose metabolism. Several authors have supported this hypothesis by the finding of increased irisin levels after acute exercise bouts but not training or prolonged exercise (Huh et al. 2012; Kraemer et al. 2014; Norheim et al. 2014). Other authors also failed to find significant changes in circulating irisin in response to long-term exercise (Hecksteden et al. 2013; Pekkala et al. 2013; Kurdiova et al. 2014; Miazgowski et al. 2021).

Only few years after the discovery of irisin and postulating its features as a skeletal muscle-derived exerkine responsible for metabolic improvement (Bostrom et al. 2012), the antibodies used for its detection in several studies have been investigated in detail (Albrecht et al. 2015). The research revealed that in more than 80 studies, the methods used to detect irisin did not measure irisin itself, but some other cross-reacting proteins. The amino acid sequence, which was the target of antibodies used in the kits, was actually not part of the circulating irisin, not even in the case of the original study (Albrecht et al. 2015). However, this did not eliminate the discrepancies in the exercise-induced irisin secretion pattern.

There were several attempts to elucidate the contradictory data reported, e.g. the lack of validation of detection methods used to report irisin concentrations with variations by order of magnitude (0.01–2000 ng/ml), presence of glycosylated irisin

molecule (Albrecht et al. 2015; Gamas et al. 2015; Maak et al. 2021). Irisin over-time degradation in frozen samples obtained before exercise intervention led to a false increase of irisin levels after several weeks of training (Hecksteden et al. 2013) or the mutation of the start codon of the gene encoding the precursor of irisin present in humans, but not rodents (Raschke et al. 2013), were suspected as well. Other authors have suggested different spectrum of myokines secreted from the insulin-resistant and insulin-sensitive exercising skeletal muscles (Kurdiova et al. 2014) supporting the hypothesis of irisin downregulation in the insulin-resistant muscle.

The finding that chronic exercise is not related to the increased irisin levels, led to a suggestion of a presence of an adaptive mechanism in active subjects, making them more sensitive to irisin action (Huh et al. 2014; Gamas et al. 2015). The controversies are even more prominent when it comes to irisin concentrations in relation to obesity, insulin resistance, and other pathological conditions (Perakakis et al. 2017; Polyzos et al. 2018).

Besides skeletal muscles, the adipose tissue is the second most important site of irisin secretion (Perakakis et al. 2017), however, only with a minor contribution to the circulating pool (Huh et al. 2012; Kurdiova et al. 2014), which however, might become substantial in obesity due to higher fat mass (Polyzos et al. 2018). Different regulation of irisin secretion has been assumed in obesity or metabolic disarrangement (Gamas et al. 2015). Considering irisin an exercise-induced myokine, the findings of increased irisin levels in the subjects with obesity, insulin resistance, metabolic syndrome, and associated features are against all odds. Several studies have described a positive association of irisin levels and markers of obesity, such as BMI, body fat percentage, waist circumference (Crujeiras et al. 2015; Polyzos et al. 2018; Jia et al. 2019; Gonzalez-Gil and Elizondo-Montemayor 2020) or have reported increased irisin in metabolic syndrome and its positive association with insulin resistance and cardiovascular risk (Park et al. 2013).

Additionally, weight loss achieved either by energy restriction, surgical intervention (Crujeiras et al. 2015) or dietary and physical activity interventions (Tok et al. 2021) led to decreased irisin concentrations in circulation. Similarly, we also found a positive correlation between changes in irisin levels and changes in body composition (BMI, weight, body fat percentage), although our subjects were not extremely obese, but predominantly sedentary. Furthermore, in our study, the irisin decrease correlated with an increase in the insulin sensitivity. However, this is not in agreement with studies that found increased irisin levels in obese patients following exercise-induced weight loss (Merawati et al. 2023). This could lead to an assumption that patients with T2DM could have even higher levels of irisin. However, several studies have proven the opposite (Vecchiato et al. 2022) suggesting chronic hyperglycemia and hyperlipidemia being the triggering factor for the "switch" from high irisin secretion in obesity to low irisin secretion in T2DM (Perakakis et al. 2017).

A possible explanation for the elevated irisin levels in obesity might be the development of "irisin resistance", similar to insulin or leptin resistance seen in obese subjects (Parks et al. 2013). Elevated irisin in the obesity and metabolic syndrome might be seen as a compensatory mechanism aimed at overcoming the irisin resistance, maximizing energy usage, and achieving metabolic homeostasis (Perakakis et al. 2017; Arhire et al. 2019).

Some studies have reported association of circulating irisin levels with age, however, with opposite outcomes (Trettel et al. 2023), e.g. baseline irisin levels have been reported to be lower in older individuals (Huh et al. 2014) or a positive correlation between age and irisin levels in plasma and cerebrospinal fluid has been reported (Ruan et al. 2019). We did not observe any relationship of irisin levels with age, even having a broad age range (20–69 years) of our participants. Therefore, we assume that the age itself is not the one of the main factors influencing the circulating irisin levels. Other factors, such as muscle mass, body fat percentage, physical fitness, nutritional status, accompanying diseases, which strongly correlate with age, might be better candidates.

Study limitations. The present study outcomes have some limitations. Subjects of a wide age range (20–69 years) and an unbalanced gender ratio (10 men, 27 women) were enrolled in the study. However, we did not observe any impact of gender or age on the parameters of interest. Regarding the organokine levels in the periphery, they may not completely reflect the processes in the brain compartments. Different types of exercise were applied according to the individual preferences (as described by Bajer et al. 2019). Some participants were taking medications for comorbidities, which might influence the study results. Finally, different adherence of our participants to the intervention program might also affect the data.

Conclusions. The regulation of irisin secretion and its action is not that simply like in rodents and many other variables may influence the irisin levels in

humans. Thus, the age, sex, body composition, health status, and type of duration, frequency, and intensity of physical activity may play a substantial role (Huh et al. 2014; Buscemi et al. 2018; Gonzalez-Gil and Elizondo-Montemayor 2020). The lifestyle intervention, including the diet and the physical activity, is an effective way to improve the cardiometabolic status. The finding of decreased irisin after intervention and its correlations with markers of adiposity support the hypothesis of the presence of "irisin resistance". The lifestyle changes, even without remarkable weight loss, were able to induce positive cardiometabolic changes in sedentary subjects, which leads to the assumption that health benefits of the lifestyle changes exceed the benefits of weight loss alone. This supports the current theory that physical inactivity is being one of the leading risk factors of the cardiovascular diseases. However, the exact mechanisms of irisin secretion and action need to be further studied.

Acknowledgement

The authors express appreciation to the volunteers for their participation in the study. The study was supported by the grants: APVV-22-0047 (Slovak Research and Development Agency) and VEGA 2/0105/24.

Conflict of interest: *The authors declare no conflict of interest.*

References

Albrecht E, Norheim F, Thiede B, Holen T, Ohashi T, Schering L, Lee S, Brenmoehl J, Thomas S, Drevon CA, Erickson HP, Maak S. Irisin - a myth rather than an exercise-inducible myokine. Sci Rep 5, 8889, 2015.

Alizadeh M, Dehghanizade J. The effect of functional training on level of brain-derived neurotrophic factor and functional performance in women with obesity. Physiol Behav 251, 113798, 2022.

Alomari MA, Khabour OF, Alawneh K, Alzoubi KH, Maikano AB. The importance of physical fitness for the relationship of BDNF with obesity measures in young normal-weight adults. Heliyon 6, e03490, 2020.

Arhire LI, Mihalache L, Covasa M. Irisin: a hope in understanding and managing obesity and metabolic syndrome. Front Endocrinol (Lausanne) 10, 524, 2019.

Babaei P, Hosseini F, Damirchi, Mehdipoor M. Mediatory role for irisin/BDNF signaling in the protective effects of combined MSROM and aerobic training against metabolic risk factors in postmenopausal women. Sport Sci Health 19, 979–985, 2023.

Bajer B, Vlcek M, Galusova A, Imrich R, Penesova A. Exercise associated hormonal signals as powerful determinants of an effective fat mass loss. Endocr Regul 49, 151–163, 2015.

Bajer B, Radikova Z, Havranova A, Zitnanova I, Vlcek M, Imrich R, Sabaka P, Bendzala M, Penesova A. Effect of 8-weeks intensive lifestyle intervention on LDL and HDL subfractions. Obes Res Clin Pract 13, 586–593, 2019.

Ball D. Metabolic and endocrine response to exercise: sympathoadrenal integration with skeletal muscle. J Endocrinol 224, R79–R95, 2015.

Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 6, 33, 2006.

Benarroch EE. Brain-derived neurotrophic factor: Regulation, effects, and potential clinical relevance. Neurology 84, 1693–1704, 2015.

Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481, 463–468, 2012.

Buscemi S, Corleo D, Buscemi C, Giordano C. Does iris(in) bring bad news or good news? Eat Weight Disord 23, 431–442, 2018.

Carli F, Sabatini S, Gaggini M, Sironi AM, Bedogni G, Gastaldelli A. Fatty liver index (FLI) identifies not only individuals with liver steatosis but also at high cardiometabolic risk. Int J Mol Sci 24, 14651, 2023.

Carlino D, De Vanna M, Tongiorgi E. Is altered BDNF biosynthesis a general feature in patients with cognitive dysfunctions? The Neuroscientist 19, 345–353, 2013.

Cederholm J, Wibell L. Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res Clin Pract 10, 167–175, 1990.

Currie J, Ramsbottom R, Ludlow H, Nevill A, Gilder M. Cardio-respiratory fitness, habitual physical activity and serum brain derived neurotrophic factor (BDNF) in men and women. Neurosci Lett 451, 152–155, 2009.

Crujeiras AB, Pardo M, Casanueva FF. Irisin: 'fat' or artefact. Clin Endocrinol (Oxf) 82, 467–474, 2015.
Dinas PC, Markati AS, Carrillo AE. Exercise-induced biological and psychological changes in overweight and obese individuals: A review of recent evidence. International Scholarly Research Notices 2014, 964627, 2014.
Dirks ML, Wall BT, van de Valk B, Holloway TM, Holloway GP, Chabowski A, Goossens GH, van Loon LJ. One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation. Diabetes 65, 2862–2875, 2016.
Esvald EE, Tuvikene J, Kiir CS, Avarlaid A, Tamberg L, Sirp A, Shubina A, Cabrera-Cabrera F, Pihlak A, Koppel I, Palm K, Timmusk T. Revisiting the expression of BDNF and its receptors in mammalian development. Front Mol Neurosci 16, 1182499, 2023.
Fasshauer M, Bluher M. Adipokines in health and disease. Trends Pharmacol Sci 36, 461–470, 2015.
Foschi FG, Conti F, Domenicali M, Giacomoni P, Borghi A, Bevilacqua V, Napoli L, Berardinelli D, Altini M, Cucchetti A, Ercolani G, Casadei-Gardini A, Bellentani S, Gastaldelli A, Tiribelli C, Bedogni G, Group BS. External validation of surrogate indices of fatty liver in the general population: the Bagnacavallo study. J Clin Med 10, 520, 2021.
Gamas L, Matafome P, Seiça R. Irisin and myonectin regulation in the insulin resistant muscle: implications to adipose tissue: muscle crosstalk. J Diabetes Res 2015, 359159, 2015.
Golden E, Emiliano A, Maudsley S, Windham BG, Carlson OD, Egan JM, Driscoll I, Ferrucci L, Martin B, Mattson MP. Circulating brain-derived neurotrophic factor and indices of metabolic and cardiovascular health: data from the Baltimore Longitudinal Study of Aging. PLoS One 5, e10099, 2010.
Gonzalez-Gil AM, Elizondo-Montemayor L. The role of exercise in the interplay between myokines, hepatokines, osteokines, adipokines, and modulation of inflammation for energy substrate redistribution and fat mass loss: A review. Nutrients 12, 1899, 2020.
Gorgens SW, Eckardt K, Jensen J, Drevon CA, Eckel J. Exercise and regulation of adipokine and myokine production. Prog Mol Biol Transl Sci 135, 313–336, 2015.
Hecksteden A, Wegmann M, Steffen A, Kraushaar J, Morsch A, Ruppenthal S, Kaestner L, Meyer T. Irisin and exercise training in humans - results from a randomized controlled training trial. BMC Med 11, 235, 2013.
Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, Mantzoros CS. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 61, 1725–1738, 2012.
Huh JY, Mougios V, Kabasakalis A, Fatouros I, Siopi A, Douroudos II, Filippaios A, Panagiotou G, Park KH, Mantzoros CS. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. J Clin Endocrinol Metab 99, E2154–E2161, 2014.
Inyushkin AN, Poletaev VS, Inyushkina EM, Kalberdin IS, Inyushkin AA. Irisin/BDNF signaling in the muscle-brain axis and circadian system: A review. J Biomed Res 38, 1–16, 2023.
Jia J, Yu F, Wei WP, Yang P, Zhang R, Sheng Y, Shi YQ. Relationship between circulating irisin levels and overweight/obesity: A meta-analysis. World J Clin Cases 7, 1444–1455, 2019.
Jo D, Song J. Irisin acts via the PGC-1α and BDNF pathway to improve depression-like behavior. Clin Nutr Res 10, 292–302, 2021.
Katuri RB, Gaur GS, Sahoo JP, Bobby Z, Shanmugavel K. Association of circulating brain-derived neurotrophic factor with cognition among adult obese population. J Obes Metab Syndr 30, 163–172, 2021.
Kim H, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, Zhou C, Chou J, Parkman VA, Novick SJ, Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM, Gerber KK, Mattheis L, Chen W, Tu H, Bouxsein ML, Griffin PR, Baron R, Rosen CJ, Bonewald LF, Spiegelman BM. Irisin mediates effects on bone and fat via αV integrin receptors. Cell 175, 1756–1768, 2018.
King NA, Caudwell PP, Hopkins M, Stubbs JR, Naslund E, Blundell JE. Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. Am J Clin Nutr 90, 921–927, 2009.
Kraemer RR, Shockett P, Webb ND, Shah U, Castracane VD. A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. Horm Metab Res 46, 150–154, 2014.
Kurdiova T, Balaz M, Vician M, Maderova D, Vlcek M, Valkovic L, Srbecky M, Imrich R, Kyselovicova O, Belan V, Jelok I, Wolfrum C, Klimes I, Krssak M, Zemkova E, Gasperikova D, Ukropec J, Ukropcova B. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J Physiol 592, 1091–1107, 2014.

Lagerros YT, Mucci LA, Bellocco R, Nyren O, Balter O, Balter KA. Validity and reliability of self-reported total energy expenditure using a novel instrument. Eur J Epidemiol 21, 227–236, 2006.

Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clin Appl 6, 91–101, 2012.

Leung WKC, Yau SY, Suen LKP, Lam SC. Effect of exercise interventions on brain-derived neurotrophic factor expression in people with overweight and obesity: protocol for a systematic review and meta-analysis. BMJ Open 13, e076118, 2023.

Maak S, Norheim F, Drevon CA, Erickson HP. Progress and challenges in the biology of FNDC5 and irisin. Endocr Rev 42, 436–456, 2021.

Maderova D, Krumpolec P, Slobodova L, Schon M, Tirpakova V, Kovanicova Z, Klepochova R, Vajda M, Sutovsky S, Cvecka J, Valkovic L, Turcani P, Krssak M, Sedliak M, Tsai CL, Ukropcova B, Ukropec J. Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly. Neuropeptides 78, 101961, 2019.

Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22, 1462–1470, 1999.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419, 1985.

Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nat Rev Endocrinol 13, 509–520, 2017.

Merawati D, Sugiharto, Susanto H, Taufiq A, Pranoto A, Amelia D, Rejeki PS. Dynamic of irisin secretion change after moderate-intensity chronic physical exercise on obese female. J Basic Clin Physiol Pharmacol 34, 539–547, 2023.

Miazgowski T, Kaczmarkiewicz A, Miazgowski B, Kopec J. Cardiometabolic health, visceral fat and circulating irisin levels: results from a real-world weight loss study. J Endocrinol Invest 44, 1243–1252, 2021.

Miyamoto-Mikami E, Sato K, Kurihara T, Hasegawa N, Fujie S, Fujita S, Sanada K, Hamaoka T, Tabata I, Iemitsu M. Endurance training-induced increase in circulating irisin levels is associated with reduction of abdominal visceral fat in middle-aged and older adults. PLoS One 10, e0120354, 2015.

Mu A, Wales TE, Zhou H, Draga-Coleta SV, Gorgulla C, Blackmore KA, Mittenbuhler MJ, Kim CR, Bogoslavski D, Zhang Q, Wang ZF, Jedrychowski MP, Seo HS, Song K, Xu AZ, Sebastian L, Gygi SP, Arthanari H, Dhe-Paganon S, Griffin PR, Engen JR, Spiegelman BM. Irisin acts through its integrin receptor in a two-step process involving extracellular Hsp90α. Mol Cell 83, 1903–1920, 2023.

Ng TKS, Ho CSH, Tam WWS, Kua EH, Ho RC. Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): A systematic review and meta-analysis. Int J Mol Sci 20, 257, 2019.

Nofuji Y, Suwa M, Sasaki H, Ichimiya A, Nishichi R, Kumagai S. Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects. J Sports Sci Med 11, 83–88, 2012.

Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, Gulseth HL, Birkeland KI, Jensen J, Drevon CA. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FEBS J 281, 739–749, 2014.

Park KH, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, Tsoukas MA, Geladari EV, Huh JY, Dincer F, Davis CR, Crowell JA, Mantzoros CS. Circulating irisin in relation to insulin resistance and the metabolic syndrome. J Clin Endocrinol Metab 98, 4899–4907, 2013.

Pekkala S, Wiklund PK, Hulmi JJ, Ahtiainen JP, Horttanainen M, Pollanen E, Makela KA, Kainulainen H, Hakkinen K, Nyman K, Alen M, Herzig KH, Cheng S. Are skeletal muscle FNDC5 gene expression and irisin release regulated by exercise and related to health? J Physiol 591, 5393–5400, 2013.

Perakakis N, Triantafyllou GA, Fernandez-Real JM, Huh JY, Park KH, Seufert J, Mantzoros CS. Physiology and role of irisin in glucose homeostasis. Nat Rev Endocrinol 13, 324–337, 2017.

Polyzos SA, Anastasilakis AD, Efstathiadou ZA, Makras P, Perakakis N, Kountouras J, Mantzoros CS. Irisin in metabolic diseases. Endocrine 59, 260–274, 2018.

Porter GA, O'Connor JC. Brain-derived neurotrophic factor and inflammation in depression: Pathogenic partners in crime? World J Psychiatry 12, 77–97, 2022.

Prokopova B, Hlavacova N, Vlcek M, Penesova A, Grunnerova L, Garafova A, Turcani P, Kollar B, Jezova D. Early cognitive impairment along with decreased stress-induced BDNF in male and female patients with newly diagnosed multiple sclerosis. J Neuroimmunol 302, 34–40, 2017.

Qiu S, Cai X, Sun Z, Schummann U, Zugel M, Steinacker JM. Chronic exercise training and circulating irisin in adults: A meta-analysis. Sports Medicine 45, 1577–1588, 2015.

Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, Jung R, Wisloff U, Tjonna AE, Raastad T, Hallen J, Norheim F, Drevon CA, Romacho T, Eckardt K, Eckel J. Evidence against a beneficial effect of irisin in humans. PLoS One 8, e73680, 2013.

Rodriguez-Ayllon M, Plaza-Florido A, Mendez-Gutierrez A, Altmae S, Solis-Urra P, Aguilera CM, Catena A, Ortega FB, Esteban-Cornejo I. The effects of a 20-week exercise program on blood-circulating biomarkers related to brain health in overweight or obese children: The Active Brains project. J Sport Health Sci 12, 175–185, 2023.

Rojas Vega S, Struder HK, Vera Wahrmann B, Schmidt A, Bloch W, Hollmann W. Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. Brain Res 1121, 59–65, 2006.

Ruan Q, Huang Y, Yang L, Ruan J, Gu W, Zhang X, Zhang Y, Zhang W, Yu Z. The effects of both age and sex on irisin levels in paired plasma and cerebrospinal fluid in healthy humans. Peptides 113, 41–51, 2019.

Sandrini L, Di Minno A, Amadio P, Ieraci A, Tremoli E, Barbieri SS. Association between obesity and circulating brain-derived neurotrophic factor (BDNF) levels: Systematic review of literature and meta-analysis. Int J Mol Sci 19, 2281, 2018.

Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknecht B, Adser H, Jakobsen AH, Pilegaard H, Nielsen HB, Secher NH. Endurance training enhances BDNF release from the human brain. Am J Physiol Regul Integr Comp Physiol 298, R372–377, 2010.

Shimada H, Makizako H, Doi T, Yoshida D, Tsutsumimoto K, Anan Y, Uemura K, Lee S, Park H, Suzuki T. A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly. Front Aging Neurosci 6, 69, 2014.

Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj A, Huang CJ. Brain-derived neurotrophic factor and substrate utilization following acute aerobic exercise in obese individuals. J Neuroendocrinol 27, 370–376, 2015.

Tok O, Kisioglu SV, Ersoz HO, Kahveci B, Goktas Z. Effects of increased physical activity and/or weight loss diet on serum myokine and adipokine levels in overweight adults with impaired glucose metabolism. J Diabetes Complications 35, 107892, 2021.

Trettel CDS, Pelozin BRA, Barros MP, Bachi ALL, Braga PGS, Momesso CM, Furtado GE, Valente PA, Oliveira EM, Hogervorst E, Fernandes T. Irisin: An anti-inflammatory exerkine in aging and redox-mediated comorbidities. Front Endocrinol (Lausanne) 14, 1106529, 2023.

Vecchiato M, Zanardo E, Battista F, Quinto G, Bergia C, Palermi S, Duregon F, Ermolao A, Neunhaeuserer D. The effect of exercise training on irisin secretion in patients with type 2 diabetes: a systematic review. J Clin Med 12, 62, 2022.

Wang Y, Liu H, Zhang BS, Soares JC, Zhang XY. Low BDNF is associated with cognitive impairments in patients with Parkinson's disease. Parkinsonism Relat Disord 29, 66–71, 2016.

Whitham M, Febbraio M. The ever-expanding myokinome: discovery challenges and therapeutic implications. Nat Rev Drug Discov 15, 719–729, 2016.

World Health Organization (WHO). World Obesity Day 2022 – Accelerating action to stop obesity. WHO, 2022.

World Obesity Federation (WOF). World Obesity Atlas 2023. WOF, 2023.

Yang Y, Liu Y, Wang G, Hei G, Wang X, Li R, Li L, Wu R, Zhao J. Brain-derived neurotrophic factor is associated with cognitive impairments in first-episode and chronic schizophrenia. Psychiatry Res 273, 528–536, 2019.

Zhou Z, Zhong S, Zhang R, Kang K, Zhang X, Xu Y, Zhao C, Zhao M. Functional analysis of brain derived neurotrophic factor (BDNF) in Huntington's disease. Aging (Albany NY) 13, 6103–6114, 2021.