JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2016, 67, 2, 195-203
www.jpp.krakow.pl

Original articles

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THE EXERCISE-INDUCED MYOKINE IRISIN DOES NOT SHOW AN ASSOCIATION WITH DEPRESSIVENESS, ANXIETY AND PERCEIVED STRESS IN OBESE WOMEN

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Irisin has recently been proposed to act as a myokine secreted from skeletal muscle following exercise and to exert several health-beneficial effects, although its role is far from being established. In contrast to the growing body of literature on the biological regulation and function of irisin, there is no evidence on potential associations with mental functions. Since physical activity has been reported to have favorable impact on mental functions, we investigated the association of irisin with depressiveness, perceived stress, and anxiety as well as eating disorder symptoms in obese women. We included 98 female obese inpatients (age: mean ± S.D. 43.9 ± 12.5 years; body mass index 49.2 ± 8.3 kg/m^2) covering a broad spectrum of psychopathology. Depressiveness (PHQ-9), perceived stress (PSQ-20), anxiety (GAD-7), and eating disorder symptoms (EDI-2) were assessed psychometrically. Plasma irisin obtained at the same time was determined by ELISA. Irisin did not correlate with depressiveness (r = –0.03, P = 0.80), anxiety (r = 0.14, P = 0.17) and perceived stress (r = –0.14, P = 0.18) as well as eating disorder symptoms in general (r = –0.09, P = 0.39). No correlation of irisin was observed with any subscales of the PSQ-20 and EDI-2 (after Bonferroni correction). In conclusion, irisin is not associated with depressiveness, anxiety and perceived stress in female obese patients. These results do not support the assumption of irisin being involved in psychoendocrine pathways of the regulation of depression or other mental functions such as anxiety and perceived stress.

Key words: *activity, depression, eating disorder, exercise, fibronectin type III domain containing 5, myokine, psychobiology, psychoneuroendocrine, stress*

INTRODUCTION

Irisin has recently been described as a peptide hormone derived from skeletal muscles and cleaved from fibronectin type III domain containing 5 (FNDC5) in response to an induction by peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC1-α) caused by exercise (1). It was suggested to be involved in the regulation of energy expenditure by driving the browning of subcutaneous white adipose tissue into beige fat cells through stimulation of uncoupling protein (UCP1) in mice (1). Due to these potential anti-obesity and anti-diabetic effects it received great attention as a possible pharmacological treatment strategy (2 – 5) as also discussed with regards to PPAR-inducing substances (6).

Depressiveness has been repeatedly reported to be reduced following exercise programs (7-9). A review of 37 meta-analyses reported significant effects of exercise on depression with greater effects in patients as compared to non-clinical subjects (10). Conversely, physical inactivity has been shown to be associated with an increased risk of mental disorders such as depression and anxiety (11-14). There are several neurobiological explanations for the beneficial effect of exercise on mental disorders including alterations in the serotonergic system (15), modifications of the hypothalamus-pituitary-adrenal axis (16) or induction of neurotrophic growth factors (17-19). In this line, central as well as peripheral FNDC5 have been shown to induce the expression of brain-derived neurotrophic factor (BDNF) in the murine hippocampus in an exercise-dependent manner (20). Since BDNF has been linked to the alleviation of depressive symptoms (21), also irisin as the cleaved part of FNDC5 might be associated with depressive symptoms.

However, several studies were unable to confirm the relationship between physical activity and irisin in rodents (22) and more prominently in humans (19, 23-32), leading to a debate on the physiological function of irisin in general and more specifically on its relevance in humans (25, 33). Due to these inconsistencies, one might assume that irisin's primary function is yet to be established.

In line with this assumption, several other peptides once ascribed to one particular function were later shown to exert additional functions or even one very different primary function. Cholecystokinin has initially been shown to be involved in gallbladder contraction (34) and later been identified as an integral part of the regulation of hunger and satiety (35) but also the modulation of anxiety (36). Other pleiotropic peptides include nesfatin-1 which was initially described as an anorexigenic modulator of food intake (37) and subsequently shown to play a role in the stress response and the regulation of anxiety as well

(38, 39). Ghrelin has been initially described to be a hunger-inducing hormone (40) but was later on demonstrated to play a role in additional gastrointestinal functions (41) and the regulation of mood (42) and stress (43). Likewise, also irisin has been subsequently implicated in neuronal processes (44) and suggested to mediate beneficial exercise-induced effects on cognitive functions (20). The only study on irisin and psychosocial factors to date associated early life adversity positively with increased levels of circulating irisin (45).

In the present study, we therefore investigated the association of circulating irisin with psychometrically measured depressiveness to examine whether irisin might play a role in the psychoendocrinology of depression. We further also studied anxiety and perceived stress as additional psychological constructs related to depressiveness. To exclude sex-related effects previously shown to play a role for irisin (46) we only examined women and due to the study of obese inpatients we examined potential interactions between irisin and disordered eating behavior. Lastly, analyses of body composition, physical activity and energy expenditure were performed.

MATERIALS AND METHODS

Subjects

In the present study 98 female obese inpatients with a mean body mass index (BMI) of 49.2 ± 8.3 kg/m^2 (range 30.3 kg/m^2 – 73.5 kg/m^2) were consecutively enrolled from September 2010 to March 2013 after admission to the Department of Psychosomatic Medicine at Charite - Universitaetsmedizin Berlin. Inclusion criteria for the study encompassed female sex and a BMI of over 30 kg/m^2. Patients under the age of 18 years, pregnant, with a diagnosis of current malignoma or psychotic disorders, hypercortisolism or disturbed thyroid function were excluded.

The study was approved by the institutional ethics committee of the Charite - Universitaetsmedizin Berlin (protocol number: EA1/114/10) and all patients gave written informed consent. All investigations were conducted in accordance with the Declaration of Helsinki.

Laboratory analyses

Blood samples were taken between 07:00 – 08:00 in the morning after an overnight fast within 3 days of admission to the ward. The patients were advised not to smoke, eat or exercise in the morning until blood withdrawal, while drinking of small amounts of water was permitted. The venous blood was collected in precooled EDTA tubes containing aprotinin (1.2 Trypsin Inhibitory Unit per 1 ml blood; ICN Pharmaceuticals, Costa Mesa, CA, USA) for peptidase inhibition which were placed back on ice immediately after blood withdrawal and centrifuged at 4°C for 10 min at 3000 × g. Plasma samples were stored at –80°C after separation until further processing. At the day of the measurement, samples were diluted 1:10 and irisin plasma levels were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA, catalog # EK-067-16, Phoenix Pharmaceutical, Inc., Burlingame, CA, USA) as used before in our previous study (23). All samples were processed in one batch (intra-assay variability < 2%).

Anthropometric indices, measurement of body composition, physical activity and energy expenditure

Body weight and height were assessed at the same day as the blood sampling between 07:00 – 08:00 am and BMI was calculated as kg/m^2.

The bioelectrical impedance analyses (BIA) were also performed on the day of the blood sampling between 10:30 am and 01:00 pm after a fasting period of at least two hours using a bioelectrical impedance analyzer (Nutrigard-M, Data Input, Darmstadt, Germany) under standardized conditions as described before (47).

Physical activity and energy expenditure were continuously measured for three consecutive days (from Friday to Sunday) including the day of blood withdrawal using a portable multisensory armband device (SenseWear™ PRO3 armband; BodyMedia, Inc., Pittsburgh, PA, USA) as described in detail previously (23). Resting energy expenditure (REE) which is required for calculation of non-exercise activity thermogenesis (NEAT) (48) was calculated using an equation for obese subjects of Muller *et al.* (49). Energy expenditure of more than five metabolic equivalents of tasks (MET; physiological measure expressing the energy cost of physical activities) was classified as exercise-related activity thermogenesis (EAT) in accordance with findings by Ainsworth *et al.* (50).

Psychometric questionnaires

All psychometric measurements and *ad hoc* questions on socioeconomic status were determined with electronic devices given to the patients at the time of blood sampling using self-assessment questionnaires that are well established, suitable and easy to apply for the measurement of the perceived burden of symptoms in clinical settings. Patients without psychometric data within 5 days after blood withdrawal were excluded.

The PHQ-9, a broadly used 9-item subscale of the Patient Health Questionnaire (PHQ) with scores ranging from 0 to 27, was employed to determine depressiveness (51). We used the German version (52). For the current population Cronbach's alpha was calculated as 0.88.

For the measurement of anxiety we administered the German version of the GAD-7 (53), a 7-item subscale of the Patient Health Questionnaire for the assessment of generalized anxiety which is also sensitive for panic symptoms, social anxiety, and posttraumatic stress (54). Scores on this scale range from 0 to 21. Cronbach's alpha for the present sample was 0.90.

Perceived stress was measured by the revised German 20-item version (PSQ-20) (55) of the perceived stress questionnaire (PSQ) (56). The PSQ-20 emphasizes the subjective perception of stress and consists of four subscales assessing 'worries', 'tension', and 'joy' as stress responses and 'demands' as a perception of external stressors. Calculated mean values between 0 and 1 were extrapolated to scores ranging from 0 to 100. Cronbach's alpha for the four subscales ranged from 0.80 to 0.89 and was 0.76 for the total score.

Eating disorder symptoms were assessed using the German version (57) of the short form of the 2nd version (58) of the Eating Disorder Inventory (EDI-2) (59). This is also a self-report instrument with 64 items on 8 subscales measuring 'drive for thinness', 'bulimia', 'body dissatisfaction', 'ineffectiveness', 'perfectionism', 'interpersonal distrust', 'interoceptive awareness', and 'maturity fears'. The values obtained from all scales were extrapolated to scores ranging from 0 to 100. Cronbach's alpha of the EDI-2 total score in our study was 0.91 with the eight subscales ranging from 0.64 to 0.90.

Statistical analyses

Distribution of the data was determined by the Kolmogorov-Smirnov test and differences between groups were calculated using t-tests for group differences in irisin, psychometric variables as well as demographic characteristics. Crosstabs followed by χ^2-tests were used to determine group differences

for socioeconomic characteristics, comorbidities and medications. Depending on the distribution of the data, correlations were determined by Pearson's or Spearman's analyses. Differences between groups and correlations were considered significant when $P < 0.05$. Bonferroni corrections for multiple testing were applied for psychometric measurements and measures of body composition, energy expenditure and physical activity. All data are expressed as mean ± standard deviation (S.D.). Statistical analyses were conducted using SigmaStat 3.1 (Systat Software, San Jose, CA, USA) and IBM SPSS Statistics 23 (IBM Corp, Armonk, NY, USA).

RESULTS

Demographic, socioeconomic and medical characteristics

Demographic and socioeconomic characteristics of the study population are presented in *Table 1*. Concerning comorbidities, 16% of the study population had a diagnosis of binge eating disorder and 1% of bulimia nervosa. A total of 39% suffered from sleep-related breathing disorders as sequelae of obesity. With regard to the metabolic syndrome, 22% had a type 2 diabetes, 6% displayed impaired fasting glucose and 25% an insulin resistance. More than half (52%) of patients had a diagnosis of arterial hypertension, 46% of hypercholesterinemia, 16% of hypertriglyceridemia and 36% of hyperuricemia. Fatty liver disease was observed in 56% of the study population (*Table 1*). Except for hypertriglyceridemia, there were no significant differences between the subgroups of low and high depressiveness as shown in *Table 1*. With respect to antidiabetic medication, 7% were on insulin, 4% had a medication of DPP-4-inhibitors or GLP-1-analogues, which all were in the high depressiveness subgroup (significant difference to the low depressiveness subgroup) and 22% took other antidiabetic medications, mostly metformin (*Table 1*). A total of 40% were treated with psychopharmacological medications which was significantly more frequent in the high depressiveness subgroup (*Table 1*).

Irisin is not associated with depressiveness and anxiety

The study population covered a broad range of psychopathology with depressiveness scores (PHQ) ranging from 0 to 25 (maximum of 27) and anxiety scores (GAD-7) from 0 to 21 (maximum of 21). Per definition, patients with higher depressiveness displayed elevated depressiveness scores compared to patients with lower depressiveness (*Fig. 1A*). No differences in plasma levels of irisin were observed between the low and high depressiveness subgroups (*Fig. 1B*). Likewise, no correlations were observed between circulating irisin with depressiveness (PHQ-9; $r = -0.03$, $P = 0.80$; *Fig. 2A*, *Table 2*) or with anxiety (GAD-7; $r = 0.14$, $P = 0.17$; *Fig. 2B*, *Table 2*). Similarly, we could not detect significant correlations of irisin with depressiveness (PHQ-9) or anxiety (GAD-7) in the subgroups of patients with or without type 2 diabetes (*Table 2*).

Table 1. Demographic and socioeconomic characteristics, comorbidities and medication of study patients.

Parameter	Whole Sample (n=98)	Low depressiveness (n=49)	High depressiveness (n=49)	P (low *vs.* high depressiveness)
Demographic characteristics				
Age (years)	43.9 ± 12.5	44.1 ± 11.7	43.7 ± 13.5	0.89[a]
BMI (kg/m^2)	49.2 ± 8.3	48.5 ± 7.5	49.9 ± 9.1	0.40[a]
Socioeconomic characteristics				
Living in a partnership	46 (46.9%)	21 (42.9%)	25 (51.0%)	0.46[b]
Level of education				0.77[b]
university entrance diploma ('Abitur')	17 (17.3%)	10 (20.4%)	7 (14.3%)	
vocational diploma ('Fachabitur')	3 (3.1%)	2 (4.1%)	1 (2.0%)	
secondary education certificate ('Mittlere Reife')	42 (42.9%)	18 (36.7%)	24 (49.0%)	
basic school qualification ('Hauptschulabschluss')	22 (22.4%)	11 (22.4%)	11 (22.4%)	
without school-leaving qualification	9 (9.2%)	5 (10.2%)	4 (8.2%)	
Currently employed	42 (42.9%)	24 (49.0%)	18 (36.7%)	0.18[b]
Unemployment during past 5 years	39 (39.8%)	18 (36.7%)	21 (42.9%)	0.59[b]
Comorbidities				
Binge eating disorder	16 (16.3%)	5 (10.2%)	11 (22.4%)	0.10[b]
Bulimia nervosa	1 (1.0%)	0 (0.0%)	1 (2.0%)	0.32[b]
Sleep-associated breathing disorder	38 (38.8%)	15 (30.6%)	23 (46.9%)	0.10[b]
Type 2 diabetes mellitus	22 (22.4%)	11 (22.4%)	11 (22.4%)	1.00[b]
Impaired fasting glucose	6 (6.1%)	5 (10.2%)	1 (2.0%)	0.09[b]
Insulin resistance (with preserved glycemic control)	24 (24.5%)	13 (26.5%)	11 (22.4%)	0.60[b]
Arterial hypertension	51 (52.0%)	27 (55.1%)	24 (49.0%)	0.54[b]
Hypercholesterinemia	45 (45.9%)	20 (40.8%)	25 (51.0%)	0.31[b]
Hypertriglyceridemia	16 (16.3%)	3 (6.1%)	13 (26.5%)	**<0.01[b]**
Hyperuricemia	36 (36.7%)	14 (28.6%)	22 (44.9%)	0.09[b]
Fatty liver disease	55 (56.1%)	26 (53.1%)	29 (59.2%)	0.54[b]
Medication				
Insulin	7 (7.1%)	4 (8.2%)	3 (6.1%)	0.70[b]
DPP-4-antagonists/GLP-1-analogues	4 (4.1%)	0 (0.0%)	4 (8.2%)	**0.04[b]**
Other antidiabetics	22 (22.4%)	8 (16.3%)	14 (28.6%)	0.15[b]
Psychopharmacological treatment	39 (39.8%)	14 (28.6%)	25 (51.0%)	**0.02[b]**

Statistical analyses: Kolmogorov-Smirnov-test for normal distribution; [a] t-tests, data expressed as mean ± standard deviation; [b] χ^2-tests. Significant differences ($P < 0.05$) are displayed in bold.
Abbreviations: BMI, body mass index; DPP-4, dipeptidylpeptidase-4; GLP-1, glucagon-like-peptide-1.

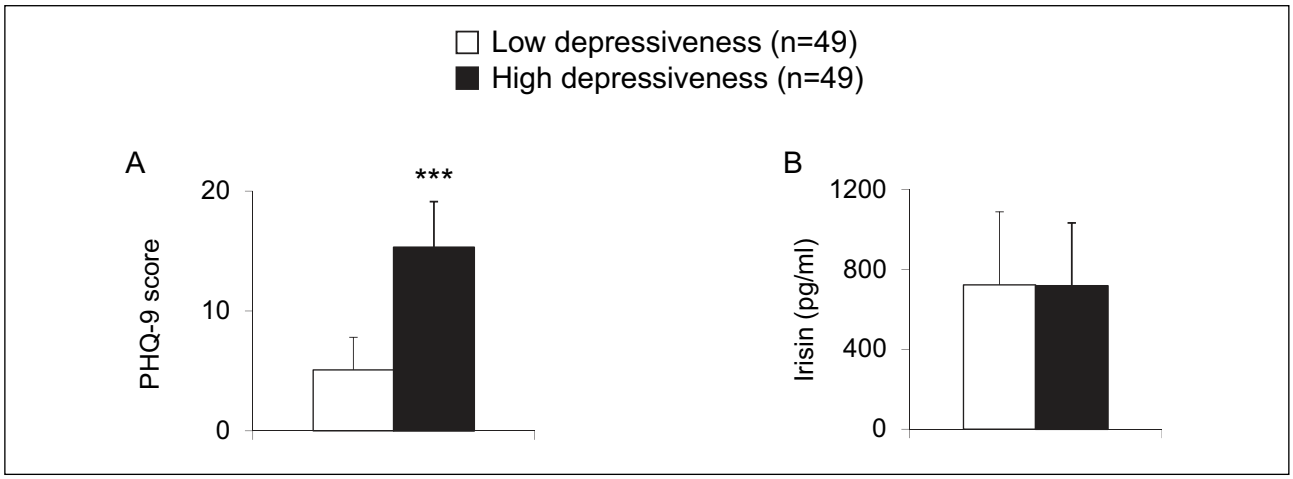

Fig. 1. Depressiveness score and plasma irisin levels in patients with high *vs.* low depressiveness scores. Per definition, patients with higher depressiveness displayed elevated depressiveness scores compared to patients with lower depressiveness (A). No differences of plasma irisin levels were observed between the low and the high depressiveness subgroups (B). Data are expressed as mean ± S.D. *** P < 0.001. *Abbreviation:* PHQ-9 score, patient health questionnaire score, 9 items.

Table 2. Correlation of plasma irisin with psychometric parameters in the whole sample and subgroups with and without type 2 diabetes.

	Whole sample (n = 98)		**With type 2 diabetes (n = 22)**		**Without type 2 diabetes (n = 76)**	
Parameter	***r***	**P**	***r***	**P**	***r***	**P**
Depressiveness						
PHQ-9	–0.027	0.795	–0.860	0.705	–0.007	0.951
Anxiety						
GAD-7	0.141	0.168	–0.178	0.428	0.139	0.233
Stress						
PSQ-20 total	–0.138	0.181	–0.266	0.232	–0.139	0.237
worries	–0.049	0.638	–0.318	0.149	–0.025	0.832
tension	–0.111	0.282	–0.274	0.217	–0.103	0.383
joy	0.080	0.441	0.049	0.829	–0.014	0.904
demands	–0.243	0.017*	–0.175	0.436	–0.279	0.016*
Eating disorder symptoms						
EDI-2 total	–0.091	0.391	–0.434	0.056	–0.091	0.448
drive for thinness	–0.214	0.042*	–0.493	0.027*	–0.134	0.263
bulimia	–0.127	0.227	–0.266	0.256	–0.200	0.093
body dissatisfaction	0.008	0.937	0.017	0.944	0.041	0.733
ineffectiveness	–0.057	0.592	–0.305	0.190	–0.062	0.607
perfectionism	–0.071	0.500	–0.175	0.461	–0.041	0.732
interpersonal distrust	0.041	0.702	–0.065	0.784	0.041	0.735
interoceptive awareness	0.070	0.506	–0.343	0.139	–0.059	0.624
maturity fears	0.041	0.698	–0.143	0.548	0.029	0.809

Statistical analyses: test for normal distribution with Kolmogorov-Smirnov-test, correlation analyses with Pearson's or Spearman's *r*. *Significant correlation is lost after Bonferroni correction for multiple testing (for PSQ subscales required P < 0.0125, for EDI-2 subscales P < 0.0062). *Abbreviations*: EDI-2, Eating Disorder Inventory; GAD-7, Generalized Anxiety Disorder questionnaire; PHQ-9, Patient Health Questionnaire; PSQ-20, Perceived Stress Questionnaire.

Irisin is not associated with perceived stress

Similar to the levels for depressiveness, the study population also displayed a broad spectrum of stress levels (PSQ-20 scores from 8 to 98; maximum of 100). In the whole sample as well as after division of the sample into two subgroups with and without type 2 diabetes (*Table 2*), we did not detect an association of plasma irisin levels with perceived stress (PSQ-20; $r = –0.14$, P = 0.18; *Fig. 2C*, *Table 2*) in general. This was also reflected by the lack of associations of circulating irisin with the PSQ-20 subscales 'worries' ($r = –0.049$, P = 0.638; *Table 2*), 'tension' ($r = –0.111$, P = 0.282; *Table 2*) and 'joy' ($r = 0.080$, P = 0.441; *Table 2*). The negative correlation of irisin with the subscale 'demands' ($r = –0.24$, P = 0.02; *Table 2*) was lost after Bonferroni correction for multiple testing (required P < 0.0125).

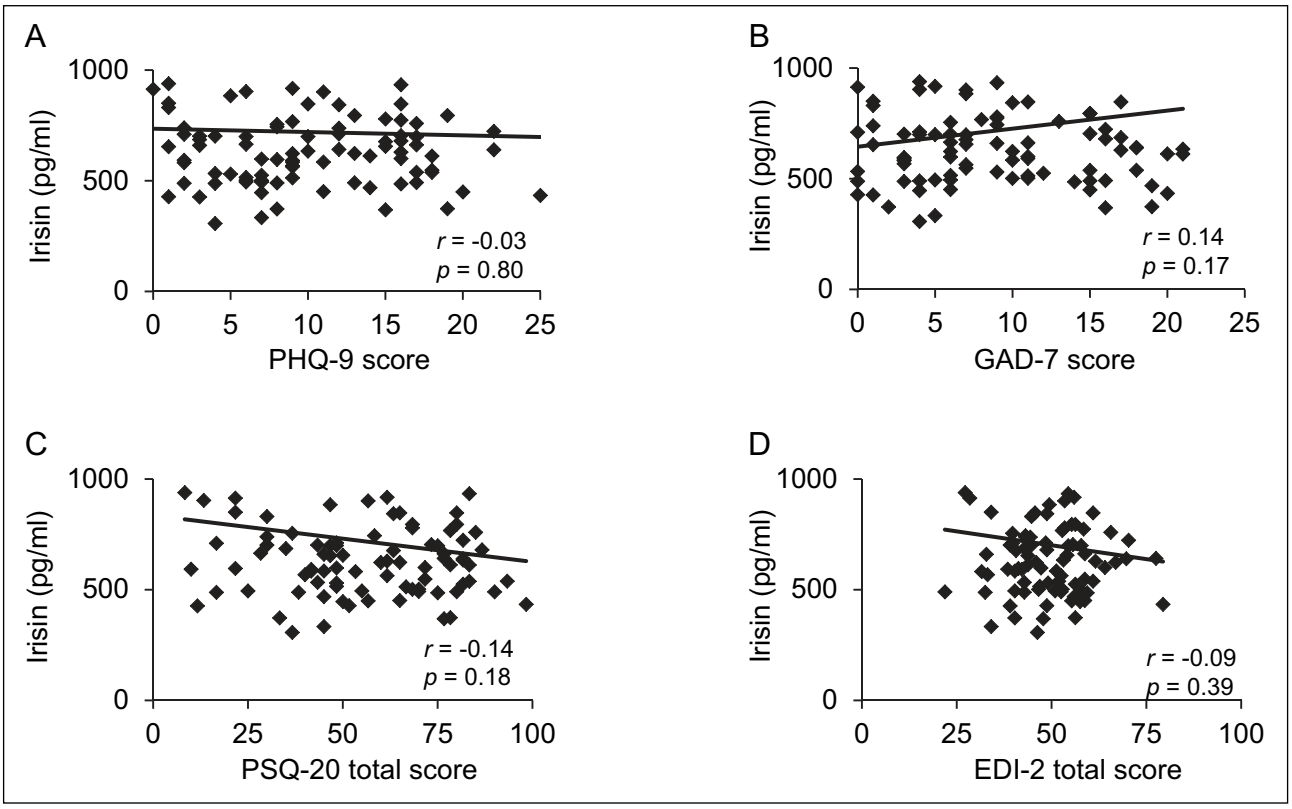

Fig. 2. Irisin plasma levels do not show an association with depressiveness, anxiety, perceived stress and eating disorder symptoms in obese women. The study population of obese women (n = 98) did not show a correlation between irisin and PHQ-9 (A) and GAD-7 scores (B) as well as PSQ-20 (C) and EDI-2 (D) total scores. Values for *r* and *P* are indicated in each correlation graph.
EDI-2, Eating Disorder Inventory; GAD-7, Generalized Anxiety Disorder questionnaire; PHQ-9, Patient Health Questionnaire; PSQ-20, Perceived Stress Questionnaire.

Table 3. Correlation of irisin plasma levels with measures of body composition, energy expenditure and physical activity.

	Whole Sample (n=98)		Low depressiveness (n=49)		High depressiveness (n=49)	
	r	P	*r*	P	*r*	P
Body mass index (kg/m^2)	–0.173	0.088	–0.233	0.107	–0.121	0.407
Bioelectrical impedance analysis (n=94)						
Total body water (l)	–0.059	0.573	–0.057	0.708	–0.051	0.729
Fat mass (kg)	–0.170	0.102	–0.149	0.323	–0.191	0.193
Fat free mass (kg)	–0.057	0.582	–0.055	0.716	–0.052	0.727
Body cell mass (kg)	–0.041	0.692	0.009	0.954	–0.408	0.748
Extracellular mass (kg)	–0.047	0.654	0.013	0.934	–0.002	0.991
Resting energy expenditure equation for obesity (n=98)						
Resting energy expenditure (kcal/kg/day)	0.201	0.047*	0.203	0.162	0.180	0.215
Portable multisensory armband device (n=29)						
Number of steps/day	–0.045	0.818	–0.203	0.507	0.091	0.738
Metabolic equivalents of tasks/day	0.004	0.984	–0.148	0.629	0.090	0.739
Total energy expenditure (kcal/kg/day)	0.036	0.852	–0.032	0.916	0.060	0.825
Exercise-related activity thermogenesis (kcal/kg/day)	–0.152	0.431	–0.675	0.011*	0.088	0.746
Non-exercise activity thermogenesis (kcal/kg/day)	–0.001	0.997	–0.051	0.868	–0.043	0.874

Statistical analyses: test for normal distribution with Kolmogorov-Smirnov-test, correlation analyses with Pearson's or Spearman's *r*. Resting energy expenditure was calculated according Muller *et al.* (46); *Significant correlation, lost after Bonferroni correction for multiple testing (for bioelectrical impedance analysis required P < 0.01, for energy expenditure and physical activity required P < 0.0083).

Irisin is not associated with eating disorder symptoms

The study population of obese women also showed a broad spectrum of eating disorder symptoms as reflected by the total EDI-2 scores (from 22 to 79; maximum of 100). Similar to the findings on perceived stress, no correlations of plasma irisin with eating disorder symptoms in general (EDI-2; $r = –0.09$, P = 0.39; *Fig. 2D*) and the EDI-2 subscales 'bulimia' ($r = –0.127$, P = 0.227; *Table 2*), 'body dissatisfaction' ($r = 0.008$, P = 0.937; *Table 2*), 'ineffectiveness' ($r = –0.057$, P = 0.592; *Table 2*),

'perfectionism' (r = –0.071, P = 0.500; *Table 2*), 'interpersonal distrust' (r = 0.041, P = 0.702; *Table 2*), 'interoceptive awareness' (r = 0.070, P = 0.506; *Table 2*), and 'maturity fears' (r = 0.041, P = 0.698; *Table 2*) were observed. The negative correlation of irisin with the EDI-2 subscale 'drive for thinness' (r = –0.214, P = 0.042; *Table 2*) was lost after Bonferroni correction for multiple testing (required P < 0.00625). These findings persisted when patients with and without type 2 diabetes were analyzed separately (*Table 2*).

Irisin is not significantly associated with BMI and measures of body composition, physical activity and energy expenditure

As depicted in *Table 3*, no correlation could be observed for irisin plasma levels with BMI and different indices of body composition as total body water, fat mass, fat free mass, body cell mass and extracellular mass. A significant correlation of irisin with resting energy expenditure (REE; r = 0.201, P = 0.047; *Table 3*) was lost after Bonferroni correction for multiple testing (required P < 0.0083). Similarly, the significant correlation of irisin with exercise-related activity thermogenesis (EAT) in the low depressiveness subgroup (r = –0.675, P = 0.011; *Table 3*) was lost after Bonferroni correction (required P < 0.0083). There were no associations of circulating irisin with activity measures such as steps per day or measures of energy expenditure such as metabolic equivalents of tasks (MET), total energy expenditure (TEE) or non-exercise activity thermogenesis (NEAT; *Table 3*).

DISCUSSION

Physical activity has been reported to induce the expression of irisin (1) as well as to exert a favorable impact on depression (9) and cognitive functions (60, 61). So far, little is known on a potential association of irisin with psychological aspects that might give rise to irisin being a link between physical activity and mental functions. However, in the present study we did not detect an association of irisin plasma levels with psychometrically measured depressiveness (PHQ-9) and anxiety (GAD-7) in female obese patients. We also did not observe associations between circulating irisin with perceived stress (PSQ-20) and eating disorder symptoms (EDI-2). In addition, there were no robust associations of BMI and measures of body composition, physical activity or energy expenditure with irisin plasma levels.

Here, we studied a population with a very broad spectrum of psychopathology in terms of depressiveness and anxiety as well as subjectively perceived stress levels. Therefore, it seems to be unlikely that irisin is involved in the regulation of these emotional states in obese women. The scores of behaviors, attitudes and emotions related to eating disorder symptoms as assessed by the EDI-2 also exhibited a wide range so that an involvement of irisin in the psychopathology of disordered eating behavior - at least in obese women - is also unlikely.

Wrann *et al.* reported FNDC5, the precursor of irisin, to be involved in a pathway linking exercise with increased hippocampal expression of BDNF (20). Since BDNF has been linked to depression and anxiety with lower BDNF levels observed under conditions of depression (62) and anxiety (63), one might hypothesize that circulating irisin levels are related to the degree of depressiveness and anxiety. The aim of the present study therefore was to relate irisin itself with depressiveness and contribute to the suggestion of irisin being involved in a pathway linking physical activity with BDNF (20) and subsequently depressive symptoms. The present data, however, do not support the assumption of irisin being associated with depressiveness. This could be due to several reasons.

First, the studies successfully linking irisin with physical activity report on physical exercise with an emphasis on acute exercise as opposed to chronic exercise or chronically elevated physical activity (24, 26, 28, 29). However, it is unclear whether also daily physical activity increases circulating irisin levels. The patients enrolled in the present study were not subjected to a specific exercise program. Therefore, in the present obese inpatient study population most likely there was too little exercise activity and only regular daily physical activity was practiced which did not contribute significantly to circulating irisin levels. In line with this assumption, in a subset of the current study population no correlation of irisin with the daily step count was observed. In addition, there was no correlation of irisin with EAT in the whole sample and though there was an association of EAT with irisin in the low depressiveness group, this was in a very small subgroup (n = 13) and the association was lost after correction for multiple testing. Corresponding to these data, in a previous study we also did not find an association of irisin with daily physical activity in a population suffering from anorexia nervosa and exhibiting a broad spectrum of physical activity (23). Thus, the impact of daily physical activity on the expression of irisin might be too small or even lacking to detect differences in psychological endpoints such as depression or anxiety. This assumption is supported by one study that did not detect an effect of daily physical activity as measured with a portable armband device on cognitive functions in obese pre-bariatric surgery patients (64).

Second, the present study was conducted in obese patients and irisin has been previously shown to be positively correlated with body mass index (47). Though, one has to note that this positive association with BMI was not observed in the present study, likely to the smaller body mass index range studied here compared to our previous study (47). However, the assumed positive effect of irisin on mental functions *via* the proposed BDNF pathway (20) should be detected preferably in obese subjects with their high body mass indices. Yet, since obese patients, and particularly severely obese as studied here, are a special subgroup it is possible that other influencing factors such as inflammation (65) confound an association between irisin and depressiveness that might exist in normal weight populations. In addition, one study conducted in young men and women showed an increase of BDNF but not irisin serum levels after a three-months fitness training program (19) which does not support the hypothesis of FNDC5/irisin being involved in the expression of BDNF (20) and its beneficial effects on brain functions.

Third, one study reported higher irisin plasma levels in a group with the highest early life adversity scores (45). This tertile also displayed significantly higher depression scores on the Beck Depression Inventory (BDI) than the two tertiles with lower scores of adversity (45) which would rather suggest a positive association of irisin and depression. Consequently, the authors even proposed irisin as possible psychobiological mediator between early life adversity and obesity (45). Taken together, although we did not detect an association between irisin and depressiveness in female obese patients, this possible link warrants further investigations in larger and different patient populations such as male or normal weight subjects, patients with a diagnosis of major depression *vs.* healthy subjects, and depressive patients before and after psychological or drug treatment of depression.

The present study entails several limitations that have to be taken into account. In the present study, we used a commercial ELISA kit to measure irisin levels (# EK-067-16, Phoenix Pharmaceutical, Inc.). Yet, in light of the controversial data on the influence of irisin in the regulation of exercise, the validity of the measurement of irisin using commercially available ELISA kits has been critically discussed. The antibodies used in

several commercially available kits were reported to exhibit cross-reactivity with other proteins and therefore might not only reflect circulating irisin (66). However, the kit used in the present study was not included in this controversy and a recent study argued in favor of several commercial ELISA kits with close inter-kit correlation (67). In addition, the occurrence of irisin *per se* has been questioned (66). Yet, using mass spectrometry irisin was recently convincingly detected in the circulation of humans (68). Therefore, although uncertainties remain, we feel that the use of several commercial ELISA kits is justified to assess circulating irisin levels. Next, unlike observed measures, self-assessment questionnaires as used in this study might be affected by inaccurate self-reporting. However, observed assessments might be biased as well by interpersonal misjudgments. Moreover, the questionnaires used are well validated (51, 54, 55, 59). Furthermore, psychopharmacological treatment present in 40% of the study population should exert effects on the mental functions measured with psychometric questionnaires. Nonetheless, as we compared the present state of psychopathology at a given time and correlated these parameters with plasma levels of irisin, the confounding effect of psychopharmacological medication on irisin levels should merely correspond to the amount of alleviation of depressive symptoms through a certain medication. Lastly, interpretation of the study's results should also consider that larger sample sizes would be desirable, no healthy control group has been employed and generalizability to men or outpatients is limited since female obese inpatients were studied.

In conclusion, in the present study conducted in female obese patients, irisin plasma levels were not associated with depressiveness, anxiety and perceived stress. Therefore, an involvement of circulating irisin in the regulation of depressive or anxious emotional states as well as the stress response is unlikely - at least in obese women.

Acknowledgements: We thank Karin Johansson and Christina Hentzschel for their help with the clinical measurements and blood withdrawals as well as Petra Busse and Reinhard Lommel for their laboratory work, and Florian Bruckbauer and Friederice Schroder for data collection and maintaining the database. The study was supported by the German Research Foundation STE 1765/3-1 (A.S.) and Charite University Funding UFF 88-226-168 (A.S., T.H.).

Conflicts of interests: Non declared.

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Received: November 17, 2015
Accepted: March 21, 2016

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