Role of endogenous irisin, a novel myokine, in cognitive functions and insulin sensitivity in exercised diabetic rats

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Background
Irisin, an exercise-induced myokine, has broad implications for metabolism and energy homeostasis. The aim of this work was to investigate the irisin expression and its receptor and the signaling pathway in relation to cognitive functions to allow for a better understanding of its role in an exercised diabetic model.
Materials and methods
A total of 40 adult male albino rats were divided equally into four groups. The first group included control rats (the control group). The remaining animals were used to establish a type 2 diabetic model and were further divided into the following: the diabetic sedentary group; the chronic exercised diabetic group; and the acute exercised diabetic group. At the end of the study period, the behavioral assessment was carried out for all groups in a T-maze. After scarification, gastrocnemius muscles, whole brains, and abdominal adipose tissues were excised for the measurement of gene expression of muscle *irisin*, brain *irisin receptors*, *brain-derived neurotrophic factor* (*BDNF*), brain MAPK and adipose tissue uncoupling protein 1 (*UCP1*), and MAPK. In addition, insulin sensitivity indices and serum lipid profile were measured for the studied groups.
Results
Muscle irisin expression was significantly elevated in chronic and acute exercised diabetic rats ($P < 0.001$) compared with diabetic sedentary rats. Significant positive correlations existed between the muscle *irisin* mRNA expression patterns compared with the brain *irisin receptors* expression, *BDNF*, brain MAPK ($r =$ 0.878, 0.933, and 0.908, respectively; $P < 0.001$ for all), as well as cognitive performance together with an improvement in insulin sensitivity and lipid profile. On the other hand, although muscle *irisin* was correlated positively with adipose MAPK, it was negatively correlated with *UCP1*.
Conclusion
Our results suggest that raised muscle *irisin* levels for both types of exercise are involved in the improvement of cognitive functions and insulin sensitivity in diabetic rats. However, further studies should clarify its precise role in relation to fat browning.

Keywords:
brain-derived neurotrophic factor, fibronectin type III domain containing protein 5, p38 MAPK, type 2 diabetes mellitus, uncoupling protein 1

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Introduction

Sedentary lifestyle is a worldwide health problem that results in a significant increased risk for morbidity and mortality because of associated comorbidities such as obesity and type 2 diabetes and its implication on cognitive abilities is well known [1,2]. The effect of exercise on preserving or enhancing cognition applies not only under normal conditions but also after a brain injury [3], during aging [4], and in diabetes [1,2].

Exercise increases the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, an integral area for learning and memory [5]. BDNF has a central role in the ability of exercise to enhance cognitive function using molecules involved in synaptic plasticity and cognition [6]. However, the exact mechanisms and physiological pathways responsible for such interrelations are not clearly understood.

Recently discovered, irisin, a PGC-1α (proliferator-activated receptor-gamma coactivator-1α)-dependent myokine, may be an important link between exercise and its benefits on body weight, diabetes, metabolic,

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and cognitive health. Its parent polypeptide, fibronectin type III domain containing protein 5 (FNDC5), is synthesized as a type 1 membrane protein and is then cleaved and shed into the circulation as a highly glycosylated polypeptide of roughly 12 kDa. Irisin appears to act preferentially on the browning of the white fat deposits when elevated in the blood of obese mice through viral vectors. This correlates with improvements in glucose tolerance in obese mice [7].

In their study, Zhang *et al.*[8] showed rather convincingly that these browning effects depend on the activation of extracellular signal-related kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) signaling cascades. Both of these kinases have been implicated previously in the thermogenic actions of other agents on brown fat, including β-adrenergic agonists and fibroblast growth factor 21.

Wrann *et al*. [9] showed that FNDC5 gets elevated by endurance exercise in the hippocampus of mice. Accordingly, it is possible that the supporting role of irisin on cognition is associated with its ability to interface energy metabolism and synaptic plasticity, or it may function to modulate aspects of cognitive function by interacting with the BDNF system.

However, Moon *et al.*[10], who showed that hippocampal neurogenesis was regulated by irisin in a dose-dependent manner, found this response seemingly occurring through a signal transducer and activator of transcription (STAT) 3 but not AMPK and/or ERK signaling pathways.

Thus, it is necessary to characterize irisin and its receptor and the signaling pathway, which will allow for a better understanding of irisin function in the hippocampus and the adipose tissue.

The purpose of the present study was to investigate the influence of endogenous *irisin* expression on memory capacity, in relation to the modulation of the expression of *BDNF* and MAPK activity in the brain, and to clarify its role in glucose homeostasis and its impact on the browning of white fat in chronic and acute exercised diabetic rats.

Materials and methods

Experimental animal

The study was conducted on 40 adult male albino rats (inbred strain) aged ~8 weeks, with their weights ranging from 130–160 g. Rats were inbred in the experimental animal unit, the Faculty of Medicine, Cairo University. They were kept in wire mesh cages at room temperature and under normal dark–light cycles, and were maintained according to the standard guidelines of the Institutional Animal Care and Use Committee. They had free access to laboratory rat chow and tap water throughout the experimental work. Approval for the study was obtained from the Institutional Review Board.

Groups

Animals were randomly divided equally into the following groups:

Group I: the control group

Ten rats were fed with standard rodent diet (6.5% kcal fat), not given any medications, but received intraperitoneal injection of citrate buffer equal in volume to that given to diabetic rats.

The remaining 30 rats were used to establish a type 2 diabetic model as mentioned below. The diabetic rats were further divided randomly into the following subgroups:

Group II: the diabetic (DM) group

Ten diabetic rats were left without exercise till the end of the study. They were killed 6 weeks after induction of diabetes.

Group III: diabetes+chronic exercise (DCEX) group

Ten diabetic rats underwent chronic exercise training for 6 weeks after induction of diabetes.

Group IV: diabetes+acute exercise (DAEX) group

Ten diabetic rats underwent acute exercise session before scarification as described below.

At the end of the study period, the behavioral assessment were carried out for all groups in a T-maze. At the planned time, all rats were subjected to overnight fasting, and blood samples were collected from retro-orbital sinuses immediately before scarification. The right gastrocnemius muscles, entire brains, and subcutaneous adipose tissue of the anterior abdominal wall were excised, immediately placed on dry ice, and stored at −80 °C until the relative gene or protein expression of the following were measured:

(1) In the muscle tissue: *Irisin* (the parent polypeptide – *FNDC5*) gene.
(2) In the brain tissue*: Irisin receptors* and *BDNF* genes together with brain p38 MAPK protein.

(3) In the adipose tissue: *uncoupling protein 1* (*UCP1*) gene, together with the measurement of adipose p38 MAPK protein.

In addition, fasting blood glucose, insulin level and lipid profile in serum were measured, and homeostasis model of assessment for insulin resistance (HOMA-IR) was calculated.

(1) Experimental protocols:
   (a) Induction of type 2 diabetes mellitus (T2DM) in adult male rats:

   Animals were fed a high-fat diet (60% kcal fat) for 2 weeks. Then these rats were subjected to single intraperitoneal injection of freshly prepared streptozotocin (Sigma, St Louis, Missouri, USA) at a dose of 40 mg/kg in ice-cold 0.5 mol/l citrate buffer (pH = 4.5) in fasting state [11]. The diagnosis of diabetes mellitus (DM) was confirmed by measuring fasting blood glucose 1 week after streptozotocin injection. Animals with 200 mg/dl of blood glucose or above were considered diabetic.

   (b) Exercise protocol:
   Animals were made to swim in a tank filled with water maintained at 35 °C. Rats were habituated to the swimming exercise during the first week. Initially, rats swam for 15 min; this swimming time was then increased in 15 min increments daily, until a swimming period of 1 h was achieved. Subsequently, a daily swimming period of 1 h five times/week was maintained for 6 weeks [12].

   At the end of the each exercise session, animals were dried and kept in a warm environment.

   Rats were killed 48 h after the last exercise session to minimize acute effects of exercise [13], where their cognitive functions were carried out before their scarification.
   Acute swimming exercise:
   Before carrying out a forced swim test, rats were habituated to swimming for 10 min/day for 3 days, followed by a 2-day rest to wash out any preconditioning training effects. On the test day, rats were fasted for 12 h, then forced to swim against a load (5% of body weight) fastened to the tail for 60 min, in the same tank conditions as described above for chronic exercise [13]. After the exercise protocol these rats were immediately tested for their cognitive functions and then killed.

   (c) Protocol of cognitive function assessment:

   Cognitive function was assessed for all the study groups by using the spontaneous alternation test in a T-maze. The maze was made from wood. Its dimensions were as follows:

   Start alley: 50 × 16 cm; goal arms (×2): 50 × 10 cm; wall height: 30 cm.

   T-mazes can be used in a variety of ways to assess the cognitive ability of an animal. Animals had to start from the base of the T and were allowed to choose one of the goal arms abutting the other end of the stem. If two trials are conducted in quick succession, on the second trial the rodent tends to choose the arm not visited before, reflecting the memory of the first choice. This is called 'spontaneous alternation'.

   The natural tendency of rats and mice in a T-maze is to alternate their choice of goal arm. They use their 'working memory' – that is, the response on each trial varies according to what they have previously just done. Alternation reflects the motivation of the animal to explore its environment and locate the presence of resources such as food, water, mates, or shelter [14].

   To remove the influence of odor, the maze was cleaned between trials with an alcohol solution after clearing out feces and urine.

   In the present work T- maze alternations was tested by the end of the study; 6 weeks after confirmation of the diabetes to assess the effect of the different treatment protocols, according to Abedinzadeh et al who stated that the time needed for development of the diabetic-associated behavioural changes was three weeks after confirmation of the diabetic state [15].

   Animals were scored for accuracy (arm chosen) and response time for each trial. The whole body of the animal including the tail tip should be in the goal arm for a successful trial.

Each trial lasted for maximum 2 min. If after the 2 min the rat did not enter the goal arm because it chose the wrong arm or was very slow or did not move, according to Deacon [16] it was considered a failed trial.

The percentage of spontaneous alternation in five separate trials and the average time needed to reach the goal arm were measured for each rat.

(2) Biochemical measurements:
   (a) Measurement of fasting plasma glucose level

   Serum glucose in blood samples was measured by using the commercial kit supplied by BioMed (New York, USA) based on the glucose oxidase method [17].
   (b) Measurement of plasma insulin

   Serum insulin levels were analyzed through the enzyme-linked immunosorbent assay using commercial kits (EMD Millipore Corporation, Billerica, Massachusetts, USA) according to the manufacturer's instructions [18].
   (c) HOMA-IR test

   To estimate insulin resistance, the HOMA-IR: insulin resistance index [19] was used, which is calculated as the product of fasting insulin (in μIU) and fasting glucose (in mmol/l) divided by 22.5. A lower index indicates greater insulin sensitivity.

Serum lipid assay

The levels of triglycerides (TG), cholesterol, and high-density lipoprotein (HDL) were determined in the serum of animals from all groups using conventional laboratory methods.

Western blot analysis for brain and adipose p38 MAPK

Total protein was extracted from the tissue using a protein extraction kit. Total protein levels were determined by using the Bradford method. Equal amounts of the protein samples were separated by SDS-PAGE and electro-transferred to PVDF membranes. The membranes were incubated overnight with anti-β-actin antibody (1: 1000 dilution; Thermofisher scientific: Waltham, Massachusetts, USA) and MAPK antibody (1: 1000 dilution; Abcam, Cambridge, Massachusetts, USA) incubated with a mouse anti-rabbit secondary monoclonal antibody conjugated with horseradish peroxidase at room temperature for 2 h. After each incubation, the membranes were washed four times with 10 mmol/l Tris-Cl, pH 7.5, 100 mmol/l NaCl, and 0.1% Tween 20 at room temperature. Chemiluminescence detection was carried out with an Amersham detection kit according to the manufacturer's protocols, and was exposed to a radiographic film. The amount of studied protein was quantified through densitometric analysis of the autoradiograms using a scanning laser densitometer (American BioMed Instrument, Inc., Brooklyn, New York, USA). Results were expressed as arbitrary units after normalization for β-actin protein expression (Fig. 1).

Quantitative real-time reverse transcription PCR analysis

Total RNA was extracted from the tissue using TRIzol Reagent (Invitrogen, San Diego, California, USA). The concentration of total RNA was measured by absorbance at 260/280 nm. The reverse transcription reaction for the first-strand cDNA synthesis was carried out with reverse transcriptase (Bio-Rad, Hercules, California, USA) using 2 μg of total RNA. Real-time PCR was initiated on a Step One Plus Real-time PCR System (ABI Applied Biosystems, San Francisco, USA) using Power SYBR Green PCR

Figure 1

Western blot analysis of protein expression of brain MAPK (a), adipose MAPK (b) and β-actin (c) in different studied groups. DM, diabetic group; DCEX, chronic exercised diabetic group; DAEX, acute exercised diabetic group.

Table 1 Primer sequences used for real-time PCR

Primer	Sequence
Irisin (FNDC5)	Forward: 5′- AAGCACAAGGACTGACTCAAGC-3′Reverse: 5′- CATGTCCTTGATGGCTGGAT-3′
Irisin receptor	Forward: 5′- ATGAAGGAGATGGGGAGGAA-3′Reverse: 5′- GCGGCAGAAGAGAGCTATAACA-3′
BDNF	Forward: 5′- GTGTGACCTGAGCAGTGGGCAAAGGA-3′Reverse: 5′- GGAAGTGGAAGAAACCGTCTAGAGCA-3′
UCP-1	Forward: 5′- GCTCGTAATGCCATTGTCA-3′Reverse: 5′- ACAGTGGCCAGCGCTACTGTA-3′
β-Actin	Forward: 5′- TGTTTGAGACCTTCAACACC-3′Reverse: 5′- TAGGAGCCAGGGCAGTAATC-3′

BDNF, brain-derived neurotrophic factor; FNDC5, fibronectin type III domain containing protein 5, (the irisin parent polypeptide); UCP1, uncoupling protein 1.

Table 2 Comparison of the % of accurate spontaneous alternation and the time consumed/arm entry (s) in T-maze in all studied groups ($n=10$)

	Parameters			
Groups	Control	DM	DCEX	DAEX
% of alternation	76.00±15.78	0.00±0.00*	30.00±10.95$^{*,\#}$	0.00±0.00$^{*,\$}$
Time/arm entry	23.04±6.37	91.04±12.36*	54.60±7.91$^{*,\#}$	120.00±0.00$^{*,\#,\$}$

Values are represented as mean±SD. DAEX, acute exercised diabetic group; DCEX, chronic exercised diabetic group; DM, diabetic group.*Statistically significant compared with corresponding value in the control group ($P<0.05$). $^{\#}$Statistically significant compared with corresponding value in the DM group ($P<0.05$). $^{\$}$Statistically significant compared with corresponding value in the chronic exercised group ($P<0.05$).

Master Mix. The sequences of the primers used are shown in Table 1. The cycling conditions were as follows: 12 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A melting curve analysis was used to confirm the specificity of the PCR product, which was demonstrated as a single peak (data not shown). The expression of β-actin served as the internal control. A comparative C_t method reported previously (analyzing real-time PCR data by the comparative C_t method) was used for analyzing the real-time PCR data [20].

Statistical analysis

The results were analyzed using the SPSS computer software package, version 21 (IBM, New York, New York, USA). Data were presented as mean±SD. Comparison of quantitative variables between the studied groups was carried out using the Kruskal–Wallis test with Wilcoxon signed-rank test according to the result of the Shapiro–Wilk test for normality of distribution. Correlations were calculated by using Spearman's test. Results were considered statistically significant at P value less than or equal to 0.05.

Results

Comparison of the percentage of accurate spontaneous alternation and average time consumed/arm entry (s) in a T-maze in all studied groups

As shown in Table 2, the percentage of alternation significantly decreased ($P<0.001$), whereas the average time consumed significantly increased ($P<0.001$) in the diabetic group compared with the control group. However, in the chronic exercised diabetic group the percentage of alternation significantly increased ($P<0.001$); on the other hand, the time consumed/arm entry significantly decreased ($P<0.001$) compared with the diabetic group.

In acute exercised diabetic group, the percentage of alternation changed insignificantly ($P=1.00$) and the time consumed/arm entry significantly increased ($P<0.001$) compared with the diabetic sedentary group.

Although the percentage of alternation and the average time consumed/arm entry improved in the chronic exercised diabetic group, it was still less than that for the control group.

In the acute exercised diabetic group, the percentage of alternation was significantly lower and the time consumed/arm entry significantly higher than that of both the control and the diabetes+chronic exercise (DCEX) group.

Comparison of fasting serum glucose (mmol/l), insulin (μIU/l) levels, and homeostasis model of assessment for insulin resistance in all studied groups

Table 3 shows that diabetic sedentary rats significantly expressed higher serum glucose, insulin levels, and HOMA-IR ($P<0.001$ for all) compared with controls. All the mentioned parameters were significantly reduced in both types of exercised diabetic rats [$P<0.001$ for glucose and HOMA-IR in both groups, $P=0.006$ and 0.042 for insulin in DCEX and diabetes+acute exercise (DAEX), respectively] compared with DM rats.

Table 3 Comparison of the serum levels of fasting glucose (mmol/l) and insulin (mIU/l) and HOMA-IR in all studied groups ($n=10$)

	Parameters			
Groups	Control	DM	DCEX	DAEX
Glucose	5.27±0.88	11.96±0.95*	8.03±1.50*,#	7.83±1.04*,#
Insulin	10.02±1.45	19.41±4.48*	13.67±2.48#	15.03±2.37*,#
HOMA-IR	2.31±0.22	10.24±2.16*	4.89±1.21*,#	5.15±0.46*,#

Values are represented as mean±SD. DAEX, acute exercised diabetic group; DCEX, chronic exercised diabetic group; DM, diabetic group; HOMA-IR, homeostasis model of assessment for insulin resistance. *Statistically significant compared with corresponding value in the control group ($P<0.05$). #Statistically significant compared with corresponding value in the D group ($P<0.05$).

Table 4 Comparison of serum TGs, cholesterol, and HDL levels in all studied groups, ($n=10$)

	Parameters			
Groups	Control	DM	DCEX	DAEX
TG (mg/dl)	91.36±7.56	126.76±7.70*	108.43±7.77*,#	108.97±11.84*,#
Cholesterol (mg/dl)	145.66±17.66	210.39±15.31*	177.22±12.50*,#	172.41±19.12*,#
HDL (mg/dl)	55.18±4.04	30.81±3.03*	39.53±4.53*,#	42.24±5.11*,#

Values are represented as mean±SD. DAEX, acute exercised diabetic group; DCEX, chronic exercised diabetic group; DM, diabetic group; HDL; high-density lipoprotein; TG, triglycerides. *Statistically significant compared with corresponding value in the control group ($P<0.05$). #Statistically significant compared with corresponding value in the DM group ($P<0.05$).

Although both types of exercises partially but significantly improved serum glucose, insulin, and HOMA-IR, reflecting an improvement in insulin sensitivity, they still significantly varied from the control group, except for insulin ($P<0.001$ for glucose in both groups, $P=0.002, <0.001$ for HOMA-IR in DCEX and DAEX, respectively).

No significant difference existed between the chronic and acute exercised diabetic groups as regards the previous parameters.

Comparison of triglycerides (mg/dl), cholesterol (mg/dl), and high-density lipoprotein (mg/dl) in all studied groups

As shown in Table 4, there was a statistically significant elevation of TG and cholesterol ($P<0.001$ for both) together with a depression in HDL ($P<0.001$) in DM rats compared with controls.

All the measured serum lipids were significantly improved in the DCEX and DAEX groups ($P=0.005$ for TG in both groups, $P=0.008$ and 0.001 for cholesterol in the DCEX and DAEX groups, respectively, and $P=0.006$ and <0.001 for HDL in the DCEX and DAEX groups, respectively) compared with the DM group. Although both types of exercise partially but significantly improved the serum lipid profile, they still significantly varied from that of the control group ($P=0.005$ and 0.002 for TG in the DCEX and DAEX groups, respectively, $P=0.007$ and 0.018 for cholesterol in the DCEX and DAEX groups, respectively, $P<0.001$ for HDL in both groups). Both types of exercise produced comparable effects on serum lipids ($P=1.00$ between both the exercised diabetic groups for all measured lipids).

Comparison of the muscle irisin gene relative expression in all the studied groups

Muscle *irisin* significantly reduced in diabetic rats ($P<0.001$) compared with the control group. It was significantly increased in both types of interventions ($P<0.001$) compared with the DM group. Although both types of exercise significantly partially improved muscle *irisin* mRNA, it was still significantly less than the control group ($P<0.001$ for the DCEX and DAEX groups).

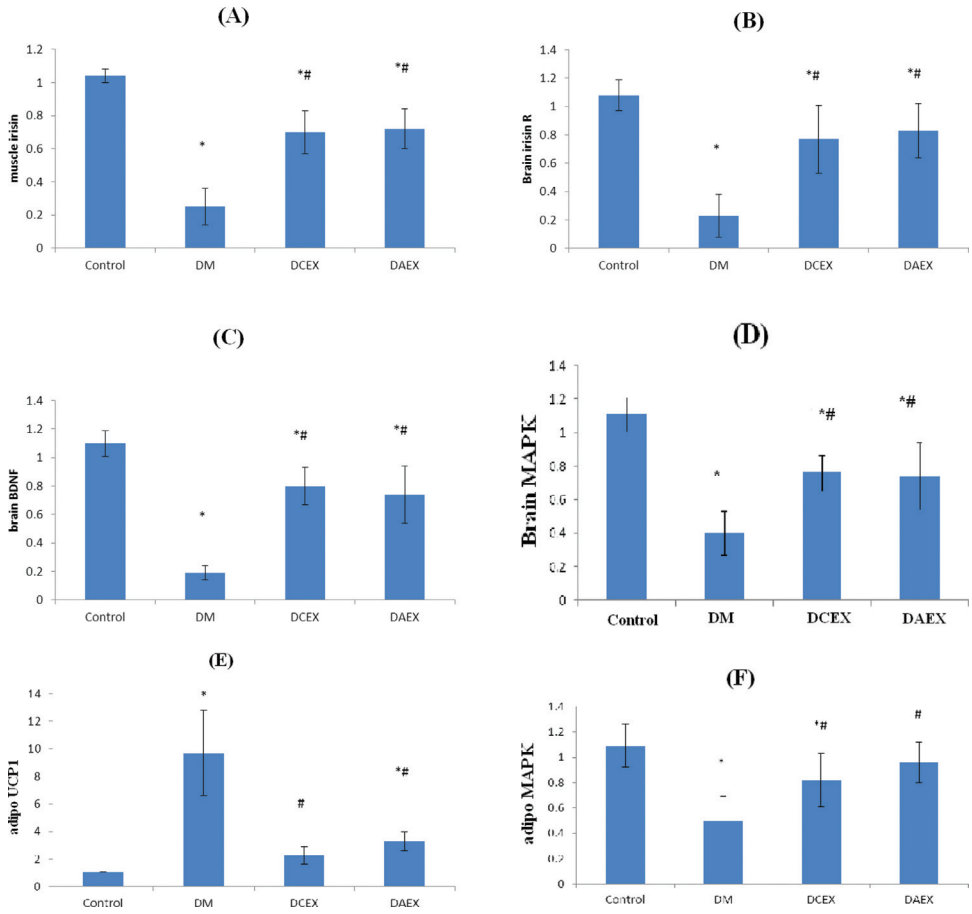
No significant difference existed between the DCEX and DAEX groups, as shown in Fig. 2a.

Comparison of the relative gene expression of brain irisin receptor, BDNF, and MAPK in all studied groups

Fig. 2b–d shows that brain *irisin receptor*, *BDNF*, and MAPK were significantly decreased in the DM group ($P<0.001$ for all) compared with the control group.

They were significantly increased in both types of exercise ($P<0.001$, for brain *irisin receptor* and *BDNF*, and $P=0.001$ for brain MAPK) compared with the DM group. Although both types of exercise significantly partially improved the expression of the above-mentioned genes, it was still significantly less than that in the control group ($P<0.001$ for *BDNF* and MAPK, $P=0.007$ and $P=0.027$ for brain *irisin receptor* in both DCEX and DAEX groups, respectively). But no significant difference was

Figure 2



Comparisons of the relative gene expression of muscle *irisin* (a), brain *irisin receptor* (b), *brain-derived neurotrophic factor (BDNF)* (c), brain MAPK (d), adipose tissue uncoupling protein 1 *(UCP1)* (e), and *adipose* MAPK (f) in all studied groups ($n=10$). Data are represented as mean ± SD. DAEX, acute exercised diabetic group; DCEX, chronic exercised diabetic group; DM, diabetic group.

found between acute and chronic exercised diabetic rats as regards the genes expressed in the brain.

Comparison of the relative gene expression of the adipose tissue UCP1 and MAPK in all the studied groups

As shown in Fig. 2e and f, the diabetic sedentary rats significantly expressed higher adipose *UCP1*mRNA ($P<0.001$) compared with controls. It was significantly reduced in both types of exercised diabetic rats ($P<0.001$) compared with the DM rats.

Although both types of exercise significantly decreased the adipose *UCP1*mRNA in the DAEX group, it still varied significantly from the control group ($P=0.041$).

Adipose tissue MAPK significantly reduced in diabetic rats ($P<0.001$) compared with the control rats. It was significantly increased in both types of interventions ($P=0.024$ and 0.001 in both DCEX and DAEX, respectively) compared with the DM group. Both types of exercise significantly improved adipose MAPK in diabetic rats, it was slightly significantly less than that in the control group ($P=0.05$) specially with the DCEX group.

On the other hand, no significant difference existed between chronic and acute exercised diabetic rats as regards the genes expressed in the adipose tissue.

Significant positive high correlations existed between muscle *irisin* mRNA expression patterns versus brain *irisin receptor*, *BDNF*, brain p38 MAPK ($r=0.878$, 0.933, 0.908, respectively $P<0.001$ for all) as well as cognitive performance tests ($r=0.760$, −0.610 for % of correct alternations and average time consumed in T-maze, respectively; $P<0.001$ for both). In addition, its level was also negatively correlated with serum glucose, insulin, HOMA-IR, TG, and cholesterol ($r=-0.903$, −0.754, −0.898, −0.829, and −0.769, respectively; $P<0.001$ for all), and positively correlated with both HDL and adipose p38 MAPK expressions ($r=0.859$ and 0.771, respectively, $P<0.001$). Interestingly, a negative correlation existed between the muscle *irisin* mRNA expression and adipose *UCP1* ($r=-0.820$, $P<0.001$) in the studied groups. On the other hand,

UCP-1 expression was positively correlated with serum insulin ($r=0.761$, $P<0.001$).

Discussion

We investigated the effect of *irisin* muscle expression on cognitive functions, insulin sensitivity, and browning of white fat in chronic and acute endurance exercised diabetic rats. Our results showed that the raised muscle *irisin* levels for both types of exercise were correlated positively with improvement in cognitive functions and insulin sensitivity, and negatively with *UCP1* (a marker of browning of white fat [8]), whereas its upstream signaling, adipose MAPK [8], was positively correlated with muscle *irisin* in the studied groups.

In the present work, the muscle *irisin* expression levels were lower in T2DM compared with nondiabetic controls. This was in agreement with the results of Liu *et al.*[21], who found that lower-circulating irisin was associated with T2DM. Interestingly, many studies on human patients with diabetes report that they were deficient in irisin compared with their normal counterparts [22,23].

What explains deficiency of irisin in diabetes is the repression of *PGC-1α* mRNA induced by hypermethylation of its promoter, with a subsequent reduction in mitochondrial biogenesis [24] and thus depression of irisin – the PGC-1α-dependent myokine [25]. This way of regulation of the *PGC-1α* gene leads to longer-lasting changes of PGC-1α transcription with a potential relevance for the pathophysiology of diabetes.

In addition, the amounts of PGC-1α in the muscle seem to be regulated by insulin signaling. It was suggested that insulin could decrease PGC-1α activity. The forkhead box class-O (FoxO1) has been shown to bind and stimulate the PGC-1α promoter in the muscle. It was found that insulin inhibits PGC-1α transcription by activating Akt, which phosphorylates and inhibits FoxO1 [26].

In agreement with this view, our findings indicated a negative correlation between muscle *irisin* expression and serum insulin level.

Recent novel findings on the involvement of transforming growth factor (TGF)-b/Smad3 signaling in the pathogenesis of obesity and T2DM have shed some light on its relation to irisin as well [27–29]. Indeed, a novel association between irisin and SMAD3 signaling in skeletal muscle was demonstrated by Tiano *et al.* [30], who found that SMAD3 suppresses FNDC5 and PGC-1α in skeletal muscle cells. Furthermore, other researchers recently demonstrated that TGF-b/Smad3 signaling regulates insulin gene transcription in the pancreatic islet β-cells [31].

In the present study, it has been noted that the level of muscle *irisin* gene increased significantly after endurance exercise with both chronic and acute exercised diabetic rats compared with diabetic sedentary rats. Moreover, *irisin* mRNA levels in the muscle were positively correlated with brain *irisin* receptor expression, *BDNF*, and brain p38 MAPK expressions; in addition, cognitive performance tests correlated positively with the percentage of correct spontaneous alterations and negatively with the average time consumed in a T-maze.

Regarding human irisin, it was shown that *FNDC5* mRNA is increased in the skeletal muscle in some exercise paradigms but not in others [32,33].

In fact, we showed that acute exercised diabetic rats were extremely fatigued after their acute exercise session and exhibited significantly low cognitive results, which were tested immediately following exercise. The observed reduced cognitive performance in these rats despite improvement in the expression of both muscle *irisin* and brain *irisin receptor* could reflect a time for post-translational effects needed for an improvement in cognition. Additional explanations for this finding are as follows: (a) the cognitive test itself in T-maze is not sensitive to the timing of the test to differentiate between extreme fatigue induced in acute exercise and cognitive dysfunction, or (b) the masking effect of acute exercise *per se* as a result of its oxidative injurious effects on the brain. Thus, further research will be required to clarify this point.

Huh *et al.*[34] similarly reported that circulating irisin increases acutely following a sprint-interval exercise bout in healthy humans. Norheim *et al.* [35] also found that the acute and long-term exercise training upregulates the expression of PGC-1α in skeletal and cardiac muscles, which promotes the generation of its downstream FNDC5/irisin.

One important molecular mediator for the beneficial responses in the brain to exercise is the induction of *BDNF* gene. In animal models, BDNF is induced in various regions of the brain with exercise, most robustly in the hippocampus [36]. Thus, we can assume that

exercise induces brain *BDNF* through a PGC-1α/FNDC5 pathway.

Blocking BDNF signaling with anti-TrkB antibodies attenuates the exercise-induced improvement of acquisition and retention in a spatial learning task, as well as the exercise-induced expression of synaptic proteins [6,37].

Surprisingly, endurance exercise induces FNDC5 in the skeletal muscle as well as in the hippocampus in mice [9]. Therefore, further studies with a tissue-specific deletion of *FNDC5* in the skeletal muscle and the hippocampus will help to delineate the effects of skeletal muscle-induced versus hippocampal-induced *FNDC5* on the central BDNF expression, as well as on the improvement of memory by endurance exercise.

Regular exercise has been shown to increase insulin sensitivity and decrease inflammatory markers in people with the metabolic syndrome [38,39].

The hallmark events involved in the development of obesity-linked diabetes include elevation of plasma-free fatty acid, ectopic lipid accumulation in metabolic organs, chronic low-grade inflammation in the adipose tissue, peripheral insulin resistance, chronic hyperglycemia, and eventual pancreatic β-cell dysfunction [40]. This coincides with our findings of dyslipidemia together with insulin resistance in DM rats. In their study, Huh *et al.*[34] found a negative correlation of irisin with age, insulin, cholesterol, and adiponectin levels, indicating a possible compensatory role of irisin in metabolic regulation. Both types of exercise interventions in the current study appeared to improve insulin sensitivity and lipid profile, at least partially, through upregulating *irisin* expression. Taking the results of this study into consideration, irisin appears as a new myokine that improves the facets of T2DM.

In addition, Staiger *et al*. [41] conclude that the *FNDC5* gene also determines insulin sensitivity in humans. Choi *et al.*[23] observed a negative correlation between hemoglobin A1c (HbA1c) and circulating levels of irisin. Furthermore, serum irisin concentrations are also negatively correlated with the BMI [42], and with the TG contents in the liver and liver enzymes in obese adults [43].

Previous reports have suggested that obesity-related insulin resistance stems from lipid oversupply and tissue accumulation of toxic lipid intermediates that impair insulin signaling. Thyfault *et al*. [44] reasoned that muscle contraction might activate hydrolysis and oxidation of intramuscular lipids, thus alleviating 'lipotoxicity' and priming the muscle for enhanced insulin action. In the muscles of obese animals, insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 remained impaired after contraction, whereas phosphorylation of the downstream signaling protein, AS160, was partially restored. These results suggest that acute exercise enables diabetic muscle to circumvent upstream defects in insulin signal transduction throug mechanisms that are more tightly coupled to increased mitochondrial energy metabolism, which include PGC-1α and its induced myokine – irisin.

Recent data have shown that exercise causes an increase in energy expenditure through augmentation in brown fat and the browning of white fat [25,45]. Brown fat can improve type 2 diabetes and metabolic health at least in experimental animals [46]. These cells express UCP1 and have a high mitochondrial content, thereby dissipating chemical energy in the form of heat. The confirmed presence of UCP1+brown fat in humans has added to the interest in finding methods and molecules that can augment energy expenditure through browning of beige fat cells [47–49]. Irisin was of interest because it is induced during exercise in rodents and is at least partially responsible for the browning response observed in white fat during chronic exercise [25].

However, we demonstrated that the adipose *UCP1* expression was significantly elevated in DM and reduced with both types of exercise interventions. In addition, a negative correlation was found between muscle *irisin* and adipose *UCP1* in the studied groups. On the other hand, a positive correlation between serum insulin level and *UCP1* was shown in this phase of experimentally induced T2DM.

Our findings were supported by that of Geloen and colleagues [50,51] who revealed that *UCP1* mRNA and protein concentrations in BAT were regulated by insulin. On the other hand, the increased expression of *UCP1* in DM could reflect the increased turnover of UCP1 for fighting oxidative stress, as UCP1 has been proven not only to increase energy expenditure but also to downregulate ROS (Reactive Oxygen Species) generation. According to Rousset *et al*. [52], UCP1 appears to be involved in the limitation of free radical levels in cells rather than in physiological uncoupling and thermogenesis.

In the current study, a positive correlation existed between muscle *irisin* and adipose MAPK. The results of a study by Zhang *et al.* [8] supported our findings, as they showed that irisin significantly increased the levels of p38 MAPK and ERK in primary adipocytes and 3T3-L1-derived adipocytes. However, they also found that, inhibiting p38 and ERK phosphorylation prevents irisin-induced *UCP-1* expression. Thus, they suggested that p38 and ERK signaling pathways play a central role in the irisin-induced emergence of brown adipocytes. Accordingly, an undetermined signal for MAPK phosphorylation might be inhibited, thus giving rise to depression of *UCP-1* expression, and this could explain the lack of browning effect of irisin in exercised diabetic rats in our study.

Conclusion

Our study presents evidence that the neuroprotective mechanisms triggered by *irisin* expression in exercised diabetic rats is related to the upregulation of brain *irisin* receptor, *BDNF*, and MAPK activity in addition to an improvement in insulin sensitivity. These interactions could determine the capacity of exercise to promote long-lasting modifications in the brain to help cope with challenges. Although optimism should be guarded, irisin may also become a new promising agent employed in the management of neurodegenerative diseases in the near future as well.

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Conflicts of interest

There are no conflicts of interest.

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