Endokrynologia Polska
DOI: 10.5603/EP.a2016.0030
Tom/Volume 67; Numer/Number 5/2016
ISSN 0423–104X

Time-dependent irisin concentration changes in patients affected by overt hypothyroidism

Zależne od czasu zmiany stężenia irisiny u pacjentów z jawną niedoczynnością tarczycy

Ariadna Zybek-Kocik, Nadia Sawicka-Gutaj, Elżbieta Wrotkowska, Jerzy Sowiński, Marek Ruchała

Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland

Abstract

Introduction: Irisin, a cleaved and secreted part of the transmembrane protein FNDC5, is a recently discovered adipo-myokine that is said to have a significant influence on body metabolism. Changes in thyrometabolic state may also alter the serum irisin level. Since already reported data are not fully consistent, the aim of the present research is to evaluate the time-dependent changes in serum irisin level in patients affected by overt hypothyroidism.

Material and methods: The study involved 36 subjects — two groups of 12 patients with long-lasting (AITD) and short-term (TC) overt hypothyroidism, and a control group (CG) of 12 subjects, matched for age and gender. Serum irisin level, thyrometabolic state, creatine kinase (CK — muscle damage marker), glucose, and insulin concentration were assessed and compared between groups.

Results: The irisin level was significantly lower in AITD than in TC and CG ($p = 0.02$; $p < 0.01$; respectively) patients, with no statistical difference between TC and CG ($p > 0.05$). There was no significant difference between free triiodothyronine and free thyroxine levels in AITD and TC patients ($p > 0.05$). CK concentration was significantly higher in AITD than in CG patients ($p < 0.01$) with no difference between AITD and TC patients ($p > 0.05$) as well as TC and CG patients ($p > 0.05$). Additionally, the CK level negatively correlated with the irisin level ($r = –0.58$; $p < 0.01$).

Conclusions: In conclusion, the irisin concentration changes during thyroid function impairment may be time-dependent. Patients with prolonged hypothyroidism have lower irisin levels that those with short-term disorder. **(Endokrynol Pol 2016; 67 (5): 476–480)**

Key words: *irisin; hypothyroidism; autoimmune thyroid disease; muscle damage*

Streszczenie

Wstęp: Irisina, oczyszczona i wydzielana do krążenia część białka transbłonowego FNDC5, jest niedawno odkrytą adipo-miokiną mającą znaczący wpływ na metabolizm organizmu. Zaburzenia czynności tarczycy mogą zmieniać poziom irisiny w surowicy. W zawiązku z brakiem pełnej zgodności dotychczas uzyskanych danych, celem obecnego badania jest ocena zależności pomiędzy zmianą stężenia irisiny u pacjentów z jawną niedoczynnością tarczycy, a czasem trwania zaburzenia.

Materiał i metody: W badaniu wzięło udział 36 osób — dwie grupy badane po 12 osób z długo trwającą (AITD) oraz krótkotrwałą (TC) jawną niedoczynnością, oraz 12-sto osobowa grupa kontrolna (CG), dopasowana względem płci i wieku. Poziom irisiny, status tyreometaboliczny, stężenie kinazy kreatynowej (CK — marker destrukcji mięśni), glukozy oraz insuliny zostały zbadane oraz porównywane między grupami.

Wyniki: Poziom irisiny był statystycznie niższy w grupie AITD niż w grupie TC i CG ($p = 0,02$; $p < 0,01$; odpowiednio). Różnicy takiej nie zaobserwowano pomiędzy grupą TC i CG ($p > 0,05$). W grupach AITD oraz TC poziom wolnej trijodotyroniny oraz tyroksyny nie różniły się statystycznie ($p > 0,05$). Stężenie CK było znacząco wyższe w grupie AITD niż CG ($p < 0,01$), przy braku różnicy pomiędzy grupami AITD i TC ($p > 0,05$) a także TC i CG ($p > 0,05$). Dodatkowo, wystąpiła negatywna korelacja pomiędzy poziomem CK oraz stężeniem irisiny ($r = –0,58$; $p < 0,01$).

Wnioski: Podsumowując, zmiany stężenia irisiny u pacjentów z niedoczynnością tarczycy mogą zależeć od czasu trwania zaburzenia. Pacjenci z długotrwającą niedoczynnością tarczycy mają niższe stężenie irisiny niż ci, u których zaburzenie trwało krótko.
(Endokrynol Pol 2016; 67 (5): 476–480)

Słowa kluczowe: *irisina; niedoczynność tarczycy; autoimmunologiczna choroba tarczycy; uszkodzenia mięśni*

Introduction

Irisin, a cleaved and secreted part of the transmembrane protein FNDC5 (fibronectin type III domain containing 5), is a recently discovered adipo-myokine that is said to have a significant influence on body metabolism [1–3]. This cytokine, first reported by Boström et al. in 2012, is widely discussed in the literature. Secreted by muscles during physical exercise, it increases thermogenesis by promoting the process of white adipose tissue "browning" [4–8]. Further studies revealed that irisin in not only a myokine, but also a potential adipokine, since *FNDC5*

Prof. Marek Ruchała, M.D., Ph.D., Department of Endocrinology, Metabolism, and Internal Medicine, Poznan University of Medical Sciences, Przybyszewskiego St. 49, 60–355 Poznań, Poland, e-mail: mruchala@ump.edu.pl, phone: + 48 601 748 905; fax: + 48 61 869 16 82

gene expression was also reported in adipose tissue [9–11]. To date, numerous studies have been published evaluating the role of irisin in pathological entities [12, 13] and thyroid dysfunction in metabolic disorders [14–16]. To the best of the authors' knowledge, the first clinical presentation of the thyroid function impairment influence on serum irisin level was published in 2014. The preliminary study by Ruchala et al. [17] states that changes in thyrometabolic state may alter serum irisin level. However, the results obtained by Samy et al. [18], who described serum irisin concentration in rats with short-term drug-induced hyper- and hypothyroidism, were not fully consistent with the previous clinical study. Therefore, the aim of the present research is to evaluate the changes in serum irisin level in long-lasting autoimmune hypothyroidism, short-term hypothyroidism in athyreotic patients after L-thyroxine treatment withdrawal before radioiodine therapy, and a control group with no impairment of thyroid function.

Material and methods

Studied group

The studied group comprised 24 patients (22 women and 2 men) affected by severe overt hypothyroidism, meeting the inclusion criteria, and referred to the Department of Endocrinology, Metabolism, and Internal Medicine. Half of the group constituted newly diagnosed 12 patients with autoimmune thyroid disease, with a presumed long duration of the disorder (AITD). The other 12 subjects were recruited from patients after total thyroidectomy because of differentiated thyroid cancer, who withdrew L-thyroxine four weeks before radioiodine treatment (TC). Inclusion criteria were as follows: age over 18 years, overt hypothyroid state due to autoimmune thyroid disease, or a history of thyroidectomy with no adequate oral L-thyroxine treatment. Exclusion criteria included: any other chronic diseases, particularly metabolic diseases, kidney, heart, muscle, and neurological diseases, as well as those intensively involved in a sport or any strenuous physical activity [19–22]. All patients taking any drugs daily were also excluded from the project. Both groups had similar age and gender distribution.

Control group

A control group (CG) of 12 subjects (11 women and 1 man) with normal thyroid function and no other chronic diseases was engaged, with similar age and gender distribution.

The Bioethical Committee

The Bioethical Committee of Poznan University of Medical Sciences approved the study (Resolution no. 923/13). All participants provided informed written consent to take part in the study.

The study protocol

The study protocol was almost the same as in the research by Ruchala et al. [17] Similarly, all subjects underwent full clinical examination and thyroid ultrasonography. The measured laboratory parameters included irisin level, thyroid and thyroid-related hormone concentrations (thyroid stimulating hormone — TSH, free triiodothyronine — FT3, free thyroxine — FT4), anti-thyroid autoantibody titre assessments (serum anti-thyroid peroxidase antibodies — TPOAb, anti-thyroglobulin antibodies — TgAb), and creatine kinase (CK) level as a biochemical marker of muscle destruction. Additionally, serum fasting glucose and insulin levels were measured. All measurements were performed in venous blood samples collected in a consistent manner after eight hours of fasting. Patients additionally underwent bioelectrical impedance analysis to evaluate their body mass, body mass index (BMI), muscle mass (MM), and fat mass (FM).

The described measurements were performed with the use of the same methods as previously [17]. TPOAb and TgAb levels were assessed by radioimmunological method with commercially available BRAMHS anti-TPO and anti-Tg RIA kits using a scintillation gamma counter (LKB Wallac CliniGamma 1272). Additionally, the glucose level was analysed with the use of a Hitachi Cobas e601 chemiluminescent analyser (Roche Diagnostics), whereas insulin concentration was assessed using an ELISA kit from Phoenix Pharmaceuticals.

Reference ranges

The used reference ranges were as follows: TSH 0.27–4.2 μIU/mL, FT3 3.90–6.70 pmol/L, FT4 11.5–21.0 pmol/L, CK 26.0–140.0 U/L, glucose 70.0–99.0 mg/dl, insulin 2.0–25.0 μIU/mL.

The diagnosis

The diagnosis of overt hypothyroidism was established with TSH level over the normal range and free thyroid hormones concentrations below the normal range. Autoimmune thyroid disease was diagnosed with at least one anti-thyroid autoantibody titre elevated (TPOAb and/or TgAb) and the presence of sonographic signs of chronic inflammation in thyroid ultrasound (decreased, heterogeneous echogenicity, poorly defined hypoechoic areas permeated by fibrous echogenic layers) [23].

Statistical analysis

The gathered data were analysed statistically with the use of STATISTICA software by StatSoft. Normality was analysed by Shapiro-Wilk test, and the equality of variances was assessed with the use of Levene's test. Due to the small number of analysed cases, the difference in normality and equality of variances between

groups, nonparametric tests like Spearman's Rank-Order Correlation and Kruskal–Wallis one-way analysis of variance were applied to evaluate the statistical difference between the groups. In cases of statistically significant differences in Kruskal-Wallis test multiple pairwise comparisons with a Bonferroni correction was be performed. A P-value less than 0.5 indicated statistical significance.

Results

The study included three groups of 12 subjects, that did not differ statistically in gender distribution (11 women and 1 man), body mass, age, BMI, FM, and MM. There was also no statistical difference in serum fasting glucose and insulin level. The statistical difference between the groups was observed in irisin, TSH, fT3, fT4, and CK concentrations. Subsequent post-hoc analysis revealed statistically significant differences in irisin level between AITD and TC, and AITD and CG, and no difference between TC and CG (Fig. 1). TSH level was significantly higher in AITD and TC than in CG, with no difference between AITD and TC. Also, fT3 and fT4 levels were similar in AITD and TC and significantly higher in CG. CK concentration was significantly higher in AITD and CG with no difference between AITD and TC, as well as TC and CG (Table I).

Additionally, in the whole group of involved subjects CK concentration was negatively correlated with irisin level ($r = –0.58$; $p < 0.01$), as well as TSH level ($r = –0.39$; $p = 0.016$). FT3 and fT4 levels were not correlated with irisin concentration.

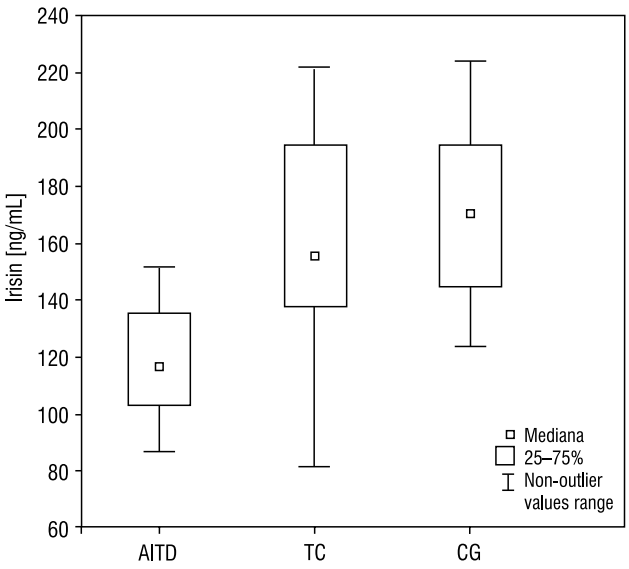

Figure 1. *Irisin levels in patients with long-lasting (AITD) and short-term (TC) overt hypothyroidism and in the control group (CG)*

Rycina 1. *Poziom irisin u pacjentów z długotrwałą (AITD) i krótkotrwałą (TC) jawną niedoczynnością tarczycy oraz w grupie kontrolnej (CG)*

Discussion

The available data concerning the relationship between serum irisin concentrations and thyroid function is limited. In 2014 our research team published a preliminary study evaluating the irisin level in hypothyroid and hyperthyroid patients [17]. It indicated that the irisin concentrations may vary according to the thyrometabolic state, and it may be related to the degree of muscle damage, presented by elevated CK level. Recently, Samy et al. [18] reported the up-regulation of serum irisin in male rats with hyper- and hypothyroidism, which may be associated with oxidative damage and/or myopathy observed in both conditions. In the described study, the drug-induced thyroid impairment lasted for only three weeks. The irisin and CK levels were elevated both in hyper- and hypothyroid rats, with no statistical difference between these groups. In the study by Ruchala et al. [17], most hypothyroid patients were diagnosed with an autoimmune thyroid disease that usually lasts for a couple of months [24]. Due to partial inconsistencies between both studies, the authors of the present study wanted to evaluate whether the described differences in irisin concentrations in hypothyroidism are associated with the duration of the thyroid dysfunction. In the present study, the studied groups with hypothyroidism represent the clinical model of long- and short-term thyroid dysfunction, which was compared with the control group. The obtained data suggests that only long-lasting hypothyroidism significantly decreases the irisin level, while short-term dysfunction does not change irisin concentrations. The wide resemblance between irisin and thyroid hormone functions, with their profound impact on metabolism and energy production, underline the possible influence of thyroid hormones and TSH on irisin levels. Although much lower than in muscles or adipose tissue, some expression of FNDC5 gene was reported in thyroid [5]. However, the difference in irisin levels in the hypothyroid groups is statistically significant despite no statistical difference in their thyrometabolic state (fT3, fT4, TSH). This lack of statistical difference in their thyrometabolic state can be reflected by no correlation between fT3, fT4, and irisin level. Additionally, irisin probably has no in vitro effect on cell proliferation and malignant potential of thyroid cancer cell lines [25]. According to Samy et al. [18], the irisin level may be even transiently elevated after the induction of hypothyroidism due to reported muscle damage and leaks from damaged muscle cells. Disturbance or disintegration of striated muscle tissue with concomitant leakage

Table I. *Median values of performed measurements in patients with long-lasting (AITD) and short-term (TC) overt hypothyroidism and in the control group (CG). The statistical difference between groups is illustrated by the p value. Statistically significant results in Kruskal–Wallis one-way analysis of variance and post-hoc multiple pairwise comparisons with a Bonferroni correction are marked (*). Values of the 25th and 75th percentile are presented in brackets*

Tabela I. *Mediana wartości wykonywanych pomiarów u pacjentów z długotrwałą (AITD) i krótkotrwałą (TC) jawną niedoczynnością tarczycy oraz w grupie kontrolnej (CG). Różnicę statystyczną między grupami zilustrowano wartością p. Statystycznie istotne wyniki w Teście Kruskala-Wallisa i w teście wielokrotnych porównań post-hoc z korektą Bonferroniego są oznaczone symbolem (*). Wartości 25 i 75 percentyla prezentowane są w nawiasach*

	AITD	TC	CG	p	
Irisin [ng/mL]	116.20 (103.20; 135.70)	155.10 (137.65; 195.1)	170.25 (144.95; 194.45)	$p < 0.01$*	AITD vs. TC; $p = 0.02$* AITD vs. CG; $p < 0.01$* TC vs. CG; $p > 0.05$
TSH [pmol/L]	100.00 (82.15; 100.00)	55.98 (34.86; 83.99)	2.33 (1.46; 2.42)	$p < 0.01$*	AITD vs. TC; $p = 0.22$ AITD vs. CG; $p < 0.01$* TC vs. CG; $p < 0.01$*
FT3 [pmol/L]	1.37 (0.45; 2.39)	0.71 (0.46; 1.25)	4.77 (4.45; 5.41)	$p < 0.01$*	AITD vs. TC; $p > 0.05$ AITD vs. CG; $p < 0.01$* TC vs. CG; $p < 0.01$*
FT4 [pmol/L]	2.16 (1.22; 4.80)	2.51 (1.84; 3.89)	15.94 (14.94; 17.73)	$p < 0.0001$*	AITD vs. TC; $p > 0.05$ AITD vs. CG; $p < 0.01$* TC vs. CG; $p < 0.01$*
CK [U/L]	496.50 (241.00; 1669.50)	171.50 (126.00; 306.50)	124.00 (115.00; 130.00)	$p = 0.0006$*	AITD vs. TC; $p = 0.20$ AITD vs. CG; $p < 0.01$* TC vs. CG; $p = 0.39$
Glucose [mg/dL]	89,50 (83.00; 99.00)	91.00 (88.00; 95.50)	87.50 (84.50; 93.50)	$p = 0.39$	
Insulin [μIU/mL]	8.35 (7.00; 9.80)	6.91 (6.60; 8.12)	7.82 (7.19; 13.64)	$p = 0.31$	
Body mass [kg]	65.80 (58.90; 69.35)	70.30 (63.60; 79.55)	67.10 (60.55; 88.00)	$p = 0.59$	
BMI [kg/m^2]	24.20 (22.70; 25.80)	25.60 (23.00; 27.50)	24.90 (21.00; 28.70)	$p = 0.69$	
FM [kg]	20.50 (18.30; 42.00)	22.60 (20.05; 26.65)	21,55 (17.30; 28.75)	$p = 0.94$	
MM [kg]	46.40 (40.70; 52.65)	45.20 (43.90; 48.35)	43.70 (42.45; 48.30)	$p = 0.88$	
Age [years]	45.50 (37.00; 53.50)	46.50 (33.50; 54.00)	43.50 (35.50; 49.50)	$p = 0.86$	

of intracellular muscle constituents into the circulation causes a rise in CK level. The mechanism(s) by which CK is metabolised has not been fully elucidated, and it is likely that the observed serum CK levels reflect complex interactions associated with the energy status and the scale of muscle disturbance [26]. The negative correlation between CK and irisin levels additionally underline a possible relation between irisin level and muscle-damage in patients with overt hypothyroidism. Moreover, in reported muscle biopsies from patients with severe prolonged disorder and significantly elevated CK activity we may observe myopathic features. In patients with short duration of metabolic disturbance and slightly elevated CK concentrations, histological changes were absent in muscle biopsy [27]. The authors hypothesise that myopathy presented by patients with severe, long-term hypothyroidism might impair the function of muscle tissue as a hormone-secreting organ, resulting in a gradual decrease of irisin level. However, these assumptions are a matter of debate and may be

treated only as an introduction to further studies and need confirmation. In the newest study by Ateş et al. [28] the authors analysed the irisin level changes in patients with Hashimoto's thyroiditis. They report that hypothyroid patients had higher irisin levels than the control subjects. However, the described results cannot be compared with our current findings because the average TSH level was much lower in the manuscript by Ateş et al. (13.1 μIU/mL). Moreover, the obesity was not concerned as an exclusion criterion [28]. Since the hypothyroidism group had higher median levels of irisin in obese patients than those in the control group, the results are not reliable.

Nevertheless, the present research has some limitations. The studied groups were small and the measurement has not yet been performed after the restoration of euthyroidism. We were also unable to construct a clinical model of long- and short-term hyperthyroidism. It is worth mentioning a recent discussion about the measurement accuracy of available ELISA kits [29]. However, the studied groups were strictly homogenic in various crucial parameters, and thus constitute a good clinical model of the problem described.

Conclusions

To sum up, irisin levels seem to change with the timespan of hypothyroidism. It may result from initial muscle damage and leaks from damaged muscle cells and following a decrease in irisin production due to prolonged myopathy. Hence, patients with long-lasting hypothyroidism have lower irisin levels than those with short-term disorder. Nevertheless, the presented data are only an introduction to a further exploration of this issue.

Acknowledgements

This research was funded from the Statutory Funds of Department of Endocrinology.

References

1. Polyzos SA, Kountouras J, Shields K et al. Irisin: a renaissance in metabolism? Metabolism 2013; 62: 1037–1044.
2. Huh JY, Mantzoros CS. Irisin physiology, oxidative stress, and thyroid dysfunction: What next? Metabolism 2015; 64: 765–767.
3. Bostrom P, Wu J, Jedrychowski MP et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481: 463–468.
4. Kelly DP. Medicine. Irisin, light my fire. Science 2012; 336: 42–43.
5. Huh JY, Panagiotou G, Mougios V et al. 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 2012; 61: 1725–1738.
6. Zhang Y, Li R, Meng Y et al. Irisin Stimulates Browning of White Adipocytes through Mitogen-Activated Protein Kinase p38 MAP Kinase and ERK MAP Kinase Signaling. Diabetes 2014.
7. Aydin S, Kuloglu T, Kalayci M et al. A comprehensive immunohistochemical examination of the distribution of the fat-burning protein irisin in biological tissues. Peptides 2014; 61: 130–136.
8. Hofmann T, Elbelt U, Stengel A. Irisin as a muscle-derived hormone stimulating thermogenesis--a critical update. Peptides 2014; 54: 89–100.
9. Raschke S, Eckel J. Adipo-myokines: two sides of the same coin--mediators of inflammation and mediators of exercise. Mediators Inflamm 2013; 2013: 320724.
10. Roca-Rivada A, Castelao C, Senin LL et al. FNDC5/irisin is not only a myokine but also an adipokine. PLoS One 2013; 8: e60563.
11. Aydin S. Three new players in energy regulation: preptin, adropin and irisin. Peptides 2014; 56: 94–110.
12. Kuloglu T, Aydin S, Eren MN et al. Irisin: a potentially candidate marker for myocardial infarction. Peptides 2014; 55: 85–91.
13. Aydin S, Kobat MA, Kalayci M et al. Decreased saliva/serum irisin concentrations in the acute myocardial infarction promising for being a new candidate biomarker for diagnosis of this pathology. Peptides 2014; 56: 141–145.
14. Sieminska L, Wojciechowska C, Walczak K et al. Associations between metabolic syndrome, serum thyrotropin, and thyroid antibodies status in postmenopausal women, and the role of interleukin-6. Endokrynol Pol 2015; 66: 394–403.
15. Gierach M, Junik R. The effect of hypothyroidism occurring in patients with metabolic syndrome. Endokrynol Pol 2015; 66: 288–294.
16. Gierach M, Gierach J, Junik R. Insulin resistance and thyroid disorders. Endokrynol Pol 2014; 65: 70–76.
17. Ruchala M, Zybek A, Szczepanek-Parulska E. Serum irisin levels and thyroid function-newly discovered association. Peptides 2014; 60: 51–55.
18. Samy DM, Ismail CA, Nassra RA. Circulating Irisin Concentrations in Rat Models of Thyroid Dysfunction - Effect of Exercise. Metabolism 2015.
19. Liu JJ, Wong MD, Toy WC, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications 2013; 27: 365–369.
20. Moon HS, Dincer F, Mantzoros CS. Pharmacological concentrations of irisin increase cell proliferation without influencing markers of neurite outgrowth and synaptogenesis in mouse H19-7 hippocampal cell lines. Metabolism; 62: 1131–1136.
21. Zhang W, Chang L, Zhang C et al. Irisin: A myokine with locomotor activity. Neurosci Lett 2015.
22. Panagiotou G, Mu L, Na B, Mukamal KJ, Mantzoros CS. Circulating irisin, omentin-1, and lipoprotein subparticles in adults at higher cardiovascular risk. Metabolism 2014; 63: 1265–1271.
23. Ruchala M, Szmyt K, Slawek S et al. Ultrasound sonoelastography in the evaluation of thyroiditis and autoimmune thyroid disease. Endokrynol Pol 2014; 65: 520–526.
24. Maagoe H, Reintoft I, Christensen HE et al. Lymphocytic thyroiditis. I. correlation between morphological, immunological and clinical findings. Acta Med Scand 1977; 201: 299–302.
25. Moon HS, Mantzoros CS. Regulation of cell proliferation and malignant potential by irisin in endometrial, colon, thyroid and esophageal cancer cell lines. Metabolism 2014; 63: 188–193.
26. Baird MF, Graham SM, Baker JS et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. J Nutr Metab 2012; 2012: 960363.
27. Scott KR, Simmons Z, Boyer PJ. Hypothyroid myopathy with a strikingly elevated serum creatine kinase level. Muscle Nerve 2002; 26: 141–144.
28. Ates I, Altay M, Topcuoglu C et al. Circulating levels of irisin is elevated in hypothyroidism, a case-control study. Arch Endocrinol Metab 2016; 60: 95–100.
29. Bostrom PA, Fernandez-Real JM, Mantzoros C. Irisin in humans: recent advances and questions for future research. Metabolism 2014; 63: 178–80.