

## Study of Serum Irisin in Patients with Thyroid Dysfunction

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### ABSTRACT

**Background:** irisin, identified as a proteolytic cleavage product of the fibronectin type III domain-containing protein 5 (FNDC5), It is a novel myokine secreted by contracting skeletal muscle, possibly mediating some exercise health benefits via 'browning' of white adipose tissue. Irisin causes a significant increase in total body energy expenditure and resistance to obesity-associated insulin resistance in mice, while controversy still exists concerning irisin origin, regulation and function in humans.

**Aim of the work:** our study aimed to detect relation of thyroid status (hypo, hyperthyroid) with serum irisin, ck and peripheral neuropathy. **Patient and methods:** this study was conducted on 60 candidates consisted of 40 patients with thyroid dysfunction selected from Endocrine outpatient Clinic and Inpatient Department of Ain Shams University Hospitals and 20 healthy volunteers from April 2016 to October 2016. Informed consent was obtained from all participants. Patients of both genders, age more than 18 years old, 20 patients with hypothyroidism, 20 patients with hyperthyroidism and 20 healthy volunteers. We excluded significant renal, hepatic and cardiac disease, severe associated acute illness or depression, pregnant females, diabetes, hypercortisolism, hypocortisolism, muscular or neurological disorders, routinely taking drugs as statins, intensively involved in a sport or any strenuous physical activity. All participants were subjected to full medical history taking, general clinical examination and laboratory investigations as thyroid and thyroid-related hormone concentrations as well as a thyroid ultrasound examination and thyroid scan for patients with hyperthyroidism. Serum irisin level, CK and nerve conduction velocity were measured. **Results:** irisin level was higher in hyperthyroid group than euthyroid group with a significant difference. In comparison with normal NCV candidates and reduced NCV candidates with other variables there was only border line significant difference with irisin p-value. Non significant value of irisin and CK level were detected for diagnosis of hypothyroidism. Irisin has highly significant value for diagnosis of hyperthyroidism with (sen 95%, sp 42.5). Increased BMI is the only independent predictor for hypothyroidism by using multivariable binary logistic regression analysis. Decreased BMI and irisin are independent predictors for hyperthyroidism. **Conclusion:** serum irisin level is one of predictors for hyperthyroidism, on the other hand irisin does not have a significant value to predict hypothyroidism. Serum irisin was found to be higher in patients with hyperthyroidism in comparison with euthyroid participant with non statistically significant difference between hypo and hyperthyroidism. Serum CK level does not have a significant value in diagnosis of thyroid dysfunction (hypothyroidism or hyperthyroidism). Serum irisin level was low in thyroid dysfunction cases with delayed NCV compared to thyroid dysfunction cases and normal NCV with border line significant difference.

**Keywords:** irisin, thyroid dysfunction and nerve conduction.

### INTRODUCTION

Irisin is a newly discovered adipomyokine, which is reported to have a significant influence on the body metabolism and thermogenesis. Other influencing factors on metabolic state are thyroid hormones, which increase heat production and control the energy balance. Due to numerous similarities in action it seems imperative to explore these substances' potential mutual influence on the body<sup>(1)</sup>.

Thyroid hormone signalling regulates crucial biological functions, including energy expenditure, thermogenesis, development and growth. The skeletal muscle is a major target of thyroid hormone signalling. Regulation of the expression and activity of deiodinases constitutes a cell-autonomous, pre-receptor mechanism for controlling the intracellular concentration of T<sub>3</sub>. This

local control of T<sub>3</sub> activity is crucial during the various phases of myogenesis<sup>(2)</sup>.

Hypothyroidism is the most common endocrinal disorder. The variety of end-organ effects and wide range of disease severity from entirely asymptomatic individuals to patients in coma with multisystem failure can make hypothyroidism an elusive clinical entity. Peripheral neuropathy occurs early in hypothyroidism even before other symptoms occur. Hence early detection of peripheral neuropathy in hypothyroidism is necessary for early diagnosis and treatment<sup>(3)</sup>.

Both hypothyroidism and hyperthyroidism may cause signs and symptoms of neuromuscular dysfunction. Hypothyroidism has been associated with the clinical features of myopathy (for example, proximal muscle weakness) mononeuropathy and

sensorimotor axonal polyneuropathy. Hyperthyroidism may cause myopathy and possibly also polyneuropathy. The reported prevalence of these signs and symptoms is variable. In hyperthyroid patients 67% had neuromuscular symptoms, 62% had clinical weakness in at least one muscle group that correlated with FT4 concentrations <sup>(4)</sup>.

Irisin is a novel myokine that promote energy expenditure. It could act on adipocyte metabolism through a novel neural pathway and on the other hand irisin induces neural proliferation and adequate neural differentiation. Lower irisin level may be associated with peripheral neuropathy. Irisin levels associated inversely with insulin resistance <sup>(5)</sup>.

Irisin is identified as a proteolytic cleavage product of the fibronectin type III domain-containing protein 5 (FNDC5), it is a novel myokine secreted by contracting skeletal muscle, possibly mediating some exercise health benefits via 'browning' of white adipose tissue. Irisin causes a significant increase in total body energy expenditure and resistance to obesity-associated insulin resistance in mice, while controversy still exists concerning irisin origin, regulation and function in humans <sup>(6)</sup>. This study aimed to investigate relation of thyroid status (hypo, hyperthyroid) with serum irisin, CK and peripheral neuropathy.

## PATIENTS AND METHODS

Our study was conducted on 60 candidates consisted of 40 patients with thyroid dysfunction and 20 healthy volunteers selected from Endocrine outpatient Clinic and Inpatient Department of Ain Shams University Hospitals from April 2016 to October 2016. Informed consent was obtained from all participants

The study included 3 groups:

**Group A:** 20 patients diagnosed with hypothyroidism

**Group B:** 20 healthy controls

**Group C:** 20 patients diagnosed with hyperthyroidism

### Inclusion criteria

1. Age over 18 years old,
2. Overt hyperthyroid state due to autoimmune thyroid disease, toxic adenoma or toxic multinodular goiter either newly diagnosed or on treatment.
3. Patients with hypothyroid state due to autoimmune disorder (Hashimoto's thyroiditis), post-thyroidectomy or radioiodine therapy.

### Exclusion criteria

1. Significant renal, hepatic and cardiac disease.
2. Severe associated acute illness or depression
3. Pregnant females
4. All patients affected by diabetes, hypercortisolism, hypocortisolism, muscular or neurological disorders, routinely taking any drugs as statins, or intensively involved in a sport or any strenuous physical activity were excluded from the study.

### ALL patients were subjected to:

- 1- Full history taking with full clinical evaluation (BMI, Thyroid and neurological examination).
- 2- Subjects underwent a complete evaluation of thyroid functional state, including thyroid and thyroid-related hormone concentrations (TSH, free triiodothyronine (FT3), free thyroxine (FT4)) and as well as a thyroid ultrasound examination and thyroid scan for patients with hyperthyroidism.
- 3- Serum irisin level
- 4- CK (creatin kinase)
- 5- Nerve conduction study

### 1. Test procedure for measurement of TSH, FT3, FT4: (Elecsys 2010 and Cobas 2011 analyzers. Roche)

#### Assay principle

Electrochemiluminescence immunoassay (ECLIA) for use on the Cobas immunoassay analyzer <sup>(7)</sup>.

#### Specimen collection

1 ml serum or EDTA plasma sample stored at -20°C.

#### Reference range for adults

**TSH** : 0.4-4 MU/I <sup>(7)</sup>.

**FT3** : 1.81-4.06 ng/dl.

**FT4** : 0.8-1.7 ng/dl <sup>(8)</sup>.

### 2. Measurement of serum irisin <sup>(9)</sup>

- Serum: use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g.  
Remove serum and assay immediately or aliquot and store samples at -20°C or -80 °C.
- Sample to be used within 5 days may be stored at 2 – 8 °C, otherwise samples just stored at -20 °C (1 month) or -80 °C (2 months) to avoid loss of bioactivity and contamination.
- Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
- When performing the assay slowly bring samples to room temperature.

Irisin was measured in serum using EIAab<sup>®</sup> Human Fibronectin type III domain- containing protein 5, Irisin, ELIZA kit, Wuhan, China 2016.

**Principle of irisin assay**

This assay is a competitive Enzyme Linked-Immunesorbent Assay (ELISA) for quantitative determination of irisin in human biological fluids (plasma, serum, cell culture supernatant). A polyclonal antibody recognizing native irisin reacts with a series of predetermined recombinant irisin standard proteins or samples under competition in the irisin-coated plate. Their relative reactivity is plotted with that of the standard proteins.

**Detection range:** 5000 ng/mL.

**3. Creatine Kinase Activity Assay Protocol**

- Sample preparation: rapidly homogenize tissue (10 mg) or cells (1 x 10<sup>6</sup>) with 100 µl ice cold CK assay buffer for 10 minutes on ice was centrifuged at 12000 rpm for 5 min and then collection of the supernatant was done with addition of 1-50 µl sample (100 µg) per well. Adjusted final volume was done to 50 µl with CK assay buffer. For positive control, addition of 2-10 µl of positive control into desired well(s) with adjusted final volume to 50 µl with CK assay buffer.

**5. The principals of nerve conduction studies** <sup>(10)</sup>

- NCS involved application of a depolarising square wave electrical pulses to the skin over a peripheral nerve producing: (1) a propagated nerve action potential (NAP) recorded at a distant point over the same nerve; and (2) a compound muscle action potential (CMAP) arising from the activation of muscle fibres in a target muscle supplied by the nerve. In both cases these may be recorded with surface or needle electrodes.
- Surface electrodes are designed to give information about the whole of a muscle stimulated, giving data for the time taken for the fastest axons to conduct an impulse to the muscle and the size of the response.
- Needle electrodes for NCS give very accurate conduction time information, but because they record from only a small area of muscle or nerve, they give poor or, in the case of the latter, more complex information making numerical analysis difficult. However, needle recordings are most

appropriate when severe muscle wasting has occurred, or when the depth of a muscle under study makes a surface recording impossible.

- Nerves may be stimulated through the skin with surface stimulators, or via a needle placed close to a nerve or a nerve root. Spinal root and cerebral cortical stimulation may also be carried out using transcutaneous magnetic stimulation (TMS) dealt with elsewhere in this issue. Thus the full length of the motor pathway may be assessed from cortex to cord, root, neuromuscular junction, and the contractile apparatus. Choice of the stimulation points depends both on the desire to “bracket” above and below the point of a proposed focal lesion and the anatomical availability of the appropriate structure.

• **Normal values for NCS**

- Age matched “normal” values for NCS parameters are either derived from studies of groups of neurologically normal subjects or culled from the literature. Regrettably in the view of the authors the most frequent statistics used are limits of 95% or less frequently 99% confidence limits of a normal group to indicate abnormality of a single parameter <sup>(10)</sup>.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

• **Data Entry and Statistics**

All data will be entered to an IBM compatible PC on MS Excel spread sheet. Analysis was done by using SPSS package system using:

Description of quantitative variables was done in the form of mean and SD.

Description of qualitative variables in the form of frequency and percentages

Significant P-value testing:

P < 0.001 = highly significant test.

P < 0.05 = significant test.

P > 0.05 = non significant

**RESULTS**

**Table 1:** irisin level in the three studied groups

Variable	Hypothyroid (n=20)		Euthyroid (n=20)		Hyperthyroid (n=20)		p-value
	Median	IQR	Median	IQR	Median	IQR	
Irisin (ng/ml)	140.00	87.50 to 145.00	125.00	90.00 to 140.000	145.00†	140.00 to 165.00	<b>0.039</b>

IQR, interquartile range.

†Statistically significant difference versus euthyroid group (Bonferroni-corrected Conover test).

**Table 2:** relation between thyroid hormones, irisin or CK and abnormal NCV

Variable	Normal NCV (n=57)		Mildly delayed NCV (n=3)		p-value¶
	Median	IQR	Median	IQR	
TSH (µIU/ml)	3.2	0.20 to 5.80	60	15.00 to 76.50	.277
FT3 (ng/dl)	2.9	2.33 to 3.80	3.4	1.75 to 6.25	.825
FT4 (µg/dl)	1.42	1.10 to 1.80	2.1	0.75 to 2.63	.599
Irisin (ng/ml)	140	120.00 to 145.00	90	82.50 to 120.00	.051
CK (IU/l)	133	97.50 to 144.78	127.3	119.73 to 182.58	.487

IQR, interquartile range.

¶Mann-Whitney U test.

**Table 3:** receiver-operating characteristic (ROC) curve analysis for the value of serum irisin or CK level for the diagnosis of thyroid dysfunction

Statistic	Marker	
	Irisin	CK
Area under the ROC curve (AUC)	0.705	0.688
p-value (AUC <sub>0</sub> =0.5)¶	<b>0.003</b>	<b>0.011</b>
Best cut-off criterion	>135 ng/ml	>124 IU/l
Sensitivity (%)	72.5	67.5
Specificity (%)	70	65

¶DeLong method.

**Table 4:** receiver-operating characteristic (ROC) curve analysis for the value of serum irisin or CK level for the diagnosis of hyperthyroidism.

Statistic	Marker	
	Irisin	CK
Area under the ROC curve (AUC)	0.739	0.596
p-value (AUC <sub>0</sub> =0.5)¶	<b>0.0001</b>	0.185
Best cut-off criterion	>120 ng/ml	>92 IU/l
Sensitivity (%)	95	100
Specificity (%)	42.5	30

¶DeLong method

**Table 5:** multivariable binary logistic regression analysis for the relation between serum irisin and thyroid dysfunction as adjusted for age, gender and BMI

Variable	Regression coefficient	Standard error	Wald	p-value	Odds ratio	95% confidence limits
Irisin (ng/ml)	0.02	0.01	4.92	<b>0.027</b>	1.02	1.003 to 1.04
Age (yr)	0.0004	0.03	0.0002	0.988	1.00	0.95 to 1.06
Gender (M=0, F=1)	1.02	0.64	2.58	0.109	2.78	0.80 to 9.66
BMI (kg/m <sup>2</sup> )	0.13	0.06	0.23	0.629	1.06	0.83 to 1.36

After adjustment for the effect of age, gender and BMI, irisin was an independent predictor for thyroid dysfunction (odds ratio=1.02; 95% CI=1.003 to 1.04; p-value=.027).

**Table 6:** multivariable binary logistic regression analysis for the relation between serum irisin and hypothyroidism as adjusted for age, gender and BMI

Variable	Regression coefficient	Standard error	Wald	p-value	Odds ratio	95% confidence limits
Irisin (ng/ml)	-0.03	0.001	0.26	0.611	1.00	0.99 to 1.01
Age (yr)	0.02	0.03	0.37	0.544	1.02	0.96 to 1.07
Gender (M=0, F=1)	0.24	0.68	0.12	0.729	1.27	0.33 to 4.80
BMI (kg/m <sup>2</sup> )	0.1	0.015	4.62	<b>0.032</b>	1.37	1.03 to 1.82

After adjustment for the effect of other variables, the BMI was the only independent predictor for hypothyroidism (odds ratio=1.37; 95% CI=1.03 to 1.82; p-value=.032).

**Table 7:** multivariable binary logistic regression analysis for the relation between serum irisin and hyperthyroidism as adjusted for age, gender and BMI

Variable	Regression coefficient	Standard error	Wald	p-value	Odds ratio	95% confidence limits
Irisin (ng/ml)	0.02	0.001	5.96	<b>0.015</b>	1.02	1.004 to 1.04
Age (yr)	-0.01	0.003	0.11	0.743	0.99	0.94 to 1.05
Gender (M=0, F=1)	0.89	0.071	1.57	0.210	2.43	0.61 to 9.76
BMI (kg/m <sup>2</sup> )	-0.28	0.14	4.23	<b>0.040</b>	0.75	0.58 to 0.99

After adjustment for the effect of other variables, the BMI and irisin were the independent predictors for hyperthyroidism (odds ratio=0.75; 95% CI=0.58 to 0.99; p-value=.040),(odds ratio=1.02; 95% CI=1.004 to 1.04;p-value=0.015)respectively.

## DISCUSSION

Thyroid hormone signalling regulates crucial biological functions, including energy expenditure, thermogenesis, development and growth. The skeletal muscle is a major target of thyroid hormone signalling. Regulation of the expression and activity of deiodinases constitutes a cell-autonomous, pre-receptor mechanism for controlling the intracellular concentration of T<sub>3</sub>. This local control of T<sub>3</sub> activity is crucial during the various phases of myogenesis <sup>(2)</sup>.

Both hypothyroidism and hyperthyroidism may cause signs and symptoms of neuromuscular dysfunction. Hypothyroidism has been associated with the clinical features of myopathy (for example, proximal muscle weakness) mononeuropathy, and sensorimotor axonal polyneuropathy. Hyperthyroidism may cause myopathy and possibly also polyneuropathy. The reported prevalence of these signs and symptoms is variable. In hyperthyroid patients 67% had neuromuscular symptoms, 62% had clinical weakness in at least one muscle group that correlated with FT4 concentration <sup>(4)</sup>.

Irisin, identified as a proteolytic cleavage product of the fibronectin type III domain-containing protein 5 (FNDC5), is a novel myokine

secreted by contracting skeletal muscle, possibly mediating some exercise health benefits via ‘browning’ of white adipose tissue. Irisin can cause a significant increase in total body energy expenditure and resistance to obesity-associated insulin resistance in mice, while controversy still exists concerning irisin origin, regulation and function in humans <sup>(6)</sup>.

The present study showed that hyperthyroid group had higher irisin level (median 145) than healthy control group (median 125) with a significant difference with p-value= 0.039

These results are in agreement with those reported by **Tohma *et al.*** <sup>(11)</sup> who studied forty - one hyperthyroid patients and forty four healthy control group and found that serum irisin level was higher in patients with hyperthyroidism than in the control group with high significance difference (p -value= 0.003).

These results also are in agreement with those of **Samy *et al.*** <sup>(12)</sup> through studying serum irisin in wistar rats. They divided them into hypothyroid, hyperthyroid and control groups 10 with rats for each group, irisin level was higher in the hyperthyroid group than the control with a statistical significance (p<0.001).

In the present study, we found borderline negative correlation between irisin and TSH in all the participant groups ( $\rho=-.252$ ) ( $p=0.052$ ).

These results are in agreement with those reported by **Ruchala et al.**<sup>(1)</sup> who studied irisin level in hypothyroid, hyperthyroid compared to the control group (10 person for each group) and found a negative correlation between irisin and TSH level with significant difference  $p$ -value=0.023.

On the other hand, **Altay et al.**<sup>(13)</sup> studied seventy-four patients, 37 were newly diagnosed hypothyroidism, but not started on a treatment yet and the remaining 37 were healthy volunteers, TSH level was positively correlated with irisin with  $p$ -value=0.034.

This discrepancy may be explained by TSH level which is increased in hypothyroidism and causes the differentiation of preadiposites through the receptors in the preadipose tissue and induces adipogenesis. Previously formed adiposites become hypertrophic with adipogenesis and the newly formed adiposities undergo hyperplasy, resulting in increased adipose tissue. Hormones such as leptine, ghrelin, NUCB2/nesfatin-1 and irisin can be synthesized in order to keep the fat distribution in balance in the increased white adipose tissue. In such case, irisin levels can be found to be high with increasing TSH levels<sup>(13)</sup>. On the other hand, there was a statistically non significant correlation between irisin level and TSH serum level as reported by **Samy et al.**<sup>(12)</sup>.

The present study showed that there was no statistically significant correlation between serum irisin level and age of whole population in the 3 groups separately with  $p$ -value 0.347.

On the other hand, this result is not in agreement with those reported by **Altay et al.**<sup>(13)</sup>, their study revealed a statistically negative significant correlation between irisin level and age in whole population and hypothyroid patient with ( $p$ -value=0.028,  $p$ -value=0.036) respectively

This may explain by irisin might be associated with the total skeletal mass which decrease in old age<sup>(13)</sup>.

The present study showed that there is no significant correlation between serum irisin level and CK level with  $p$ -value=0.794.

On the other hand, **Ruchala et al.**<sup>(1)</sup> found that serum irisin level had statistically significant negative correlation with serum CK level with  $p$ -value=0.014.

In another study but different in methodology and groups **Ruchala et al.**<sup>(1)</sup> studied

relation of irisin with hypothyroid patients .The patients were divided into two groups according to duration of disease long lasting auto immune thyroidites and short term overt hypothyroid compared to the healthy volunteer, twelve candidate of each group who revealed negative significant correlation between irisin level and CK level with statistically significant difference with ( $p<0.01$ ). However, **Samy et al.**<sup>(12)</sup> revealed a positive correlation between serum CK and irisin level ( $p$ -value= <0.001).

Difference between studies demonstrated that irisin concentrations may vary according to the thyrometabolic state, which potentially could be related to the degree of muscle damage<sup>(1)</sup>.

The present study showed that serum irisin has positive correlation with FT3 with  $p$ -value=0.023. These results are in agreement with those of **Tohma et al.**<sup>(11)</sup>, their study revealed positive correlation between serum irisin and FT3 level in both groups (hyperthyroid and control) with statistically significant  $p$ -value=0.03. These results are not in agreement with those reported by **Ruchala et al.**<sup>(1)</sup> who found no significant correlation between serum irisin and FT3 level in both two groups. The present study showed that there was a non significant correlation between irisin level and FT4 level. This result is in contrast to that reported by **Ruchala et al.**<sup>(1)</sup> who revealed statistically significant positive correlation between irisin and FT4 with  $p$ -value=0.036. The present study showed that there was a statistically non significant difference between 3 groups as regard CK serum level with  $p=0.855$ . On the contrary **Zybek-Kocik et al.**<sup>(14)</sup> studied ninety seven patients newly diagnosed with overt thyroid dysfunction. Patients in their study were divided into forty eight diagnosed with hypothyroidism and forty nine diagnosed with hyperthyroidism compared to forty healthy volunteer as the control group; hypothyroid group was divided into two sub group according to duration, they found that CK serum level was statistically significant higher in long lasting and short term hypothyroidism compared to hyperthyroid and control groups with  $p$ -value=<0.05.

The present study revealed that CK serum level does not have significant correlation with FT3 level and FT4 level  $p$ -value=0.293,  $p$ -value=0.740 respectively. These results are not in agreement with those of **Ruchala et al.**<sup>(1)</sup> who reported that CK serum level had a significant negative correlation with FT4 and FT3 level with  $p$ -value=<0.0001,  $p$ -value=0.0007 respectively. We also found in the present study a

border line statistically significant difference between normal NCV candidates and reduced NVC candidates as regard irisin level with p-value=0.051. The present study showed that serum irisin is the independent predictor for diagnosis hyperthyroidism with a statistical significance using binary logistic regression with p-value=0.015. These results are in agreement with those reported by **Tohma *et al.***<sup>(11)</sup> who studied serum irisin in forty -one hyperthyroid patients and 44 healthy group and they found that serum irisin was the only a significant factor in graves with significance p-value=0.01. The present study showed that decreased BMI is another independent predictor for hyperthyroidism disease with significance p-value=0.040. We also found that increased BMI is the only predictor factor for diagnosis hypothyroidism by using binary logistic regression with p-value=0.032. These results are in agreement with those reported by **Altay *et al.***<sup>(13)</sup> who revealed that obesity is independent predictor for hypothyroidism. On the other hand, they reported that irisin and FT4 are another independent predictors in hypothyroidism disease, but according to our results irisin and FT4 are not a disease predictors.

## CONCLUSION

Serum irisin level is one of predictors for hyperthyroidism, on the other hand irisin does not have a significant value to predict hypothyroidism. Serum irisin was found to be higher in patients with hyperthyroidism in comparison to euthyroid participant with non statistically significant difference between hypo and hyperthyroidism. Serum CK level does not have a significant value in diagnosis of thyroid dysfunction (hypothyroidism or hyperthyroidism). Serum irisin level was low in thyroid dysfunction cases with delayed NCV compared to thyroid dysfunction cases with normal NCV with border line significant difference.

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