

Relation of serum irisin levels to obesity and non-alcoholic fatty liver disease

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ABSTRACT

Background. Irisin is a newly defined myokine which is induced by exercise, which stimulates white fat cells to have the characteristics of brown adipose tissue cell. It thereby causes thermogenesis, energy and weight loss and improvement in insulin sensitivity. These effects of irisin suggest that it may be associated with obesity, insulin resistance and non-alcoholic fatty liver disease (NAFLD).

Methods. The aim of the present study was to determine the relationship of serum irisin levels in obese children with NAFLD. A total of 60 pubertal obese adolescents (age range: 11-18 yrs) as well as age and sex matched 28 healthy children were included in the study. Thirty of obese patients had NAFLD.

Results. The median irisin levels were lower in the obese patients both with and without NAFLD when compared with the control group. NAFLD group had a higher BMI than obese controls, however, the irisin levels were not different between these groups. The irisin levels were negatively correlated with BMI, BMI SDS, waist, hip and arm circumferences, waist/hip ratio, triceps-biceps skinfold thickness and AST, ALT levels in the all study groups. However, it was positively correlated with BMI, BMI SDS and waist and hip circumference in the entire obese group and positively with BMI SDS in the NAFLD subgroup.

Conclusions. Consequently, circulating irisin levels are lower in obese adolescents and negatively correlated with body adiposity. In NAFLD patients, it may be related to steatosis and may decrease with liver damage.

Key words: irisin, obesity, non-alcoholic fatty liver disease, children.

Irisin is a myokine which was first described by Boström et al.¹ in 2012. The peroxisome proliferator-activated receptor- γ coactivator-1' (PGC1)- α expression increases in the muscle cell during exercise, activates the Fibronectin type III domain-containing 5 (FNDC5) gene and forms the FNDC5 protein. As a result of the proteolysis of this protein, irisin is released into circulation. The circulating irisin increases

the expression of uncoupling protein 1 (UCP1) mRNA in subcutaneous white adipose tissue (WAT) cells which is a characteristic of the brown adipose tissue (BAT) cells. The WAT cells gain features of BAT cells (browning). UCP1, a protein found in the inner membrane of the mitochondria, causes protons to escape from the intermembrane space to the matrix and results in heat generation during oxidative phosphorylation.² Irisin is thereby involved in thermogenesis and energy expenditure. Furthermore, increase of irisin levels in circulation was found to be protective against diet-induced weight gain and causes improvement of insulin resistance (IR).¹ The association of irisin levels with physical activity, parameters of glucose and lipid metabolism, obesity and obesity-related morbidities such as type 2 diabetes mellitus (DM), and metabolic

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syndrome (MetS) have been investigated, and inconsistent results have been reported.³⁻²⁰

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children and adolescents, with fat accumulation in the liver without any other diseases or alcohol and drug intake that could cause liver disease. The prevalence is increasing in parallel with the increasing prevalence of obesity.²¹ Although the disease is generally detected as asymptomatic simple steatosis, it may slowly progress to steatohepatitis (NASH), characterized by liver cell damage and inflammation and may eventually lead to cirrhosis and rarely hepatocellular carcinoma through liver fibrosis. IR is primarily responsible for the etiopathogenesis of steatosis in the liver; obesity and dyslipidemia are the other main risk factors.^{22,23} The prevalence of NAFLD is relatively higher in obese patients and patients with type 2 DM. Lifestyle changes such as diet and exercise are recommended in order to lose weight and increase insulin sensitivity for treatment of NAFLD.²⁴ It is known that hepatic steatosis and inflammation may be reduced by physical exercise without any weight loss.^{25,26} As these lifestyle changes are generally not implemented completely, many pharmacological agents are used to prevent liver damage and to reduce the risk factors for NAFLD.^{23,27}

Due to its similar relationship with exercise, obesity and IR which are the major risk factors in NAFLD development, irisin has been suggested to have a role in the development and prognosis of NAFLD by increasing energy expenditure and insulin sensitivity.¹⁷ Contradictory results have been reported on the association between irisin and NAFLD in several previous studies.^{17-20,28,29} To the best of our knowledge, there is no study investigating the relationship between circulating irisin levels and NAFLD in children. Therefore, we aimed to determine whether serum irisin levels are related to anthropometric measurements and metabolic and biochemical parameters in obese children with NAFLD.

Material and Methods

Sixty pubertal patients with exogenous obesity (31 girls, 29 boys), between 11 and 18 years of age admitted to our pediatric endocrinology outpatient clinic were included in the study. Thirty of 60 obese patients had NAFLD. The control group consisted of 28 healthy pubertal children (14 girls, 14 boys) with similar age and gender. The patients with another disease or those using any drugs were excluded from the study.

The study was approved by Eskişehir Osmangazi University Clinical Researches Ethics Committee (Approval no: 148 – 03.06.2016). Children and their families were informed about the objective and methods of the study. Informed consent was obtained from the parents.

Physical examination was performed on all children. Puberty was determined according to the method of Tanner.^{30,31} Body weight (BW) and height were measured. Body mass index (BMI) was calculated by $BW (kg)/height (m)^2$ and compared with the references according to age and gender.³² Any BMI level at and above 95 percentile was considered as obese. The standard deviation scores (SDS) of BW, height and BMI were determined according to age and gender.³² Subcutaneous fat thickness was measured from the triceps and biceps regions by a caliper. The arm circumference was measured from the mid-point of the arm between olecranon and acromion. The waist circumference was measured in the horizontal plane midway between the lowest rib and the iliac crest. The hip circumference was measured over the widest area of the hips. The waist-hip ratio was calculated.

After fasting for one night, two venous serum samples were collected for irisin level and biochemical analyses. Glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and

gamma-glutamyl transferase (GGT) levels were determined with photometric methods by Roche Cobas 8000 analyser, c702 module (Roche Diagnostics GmbH, Penzberg, Germany) autoanalyser. Insulin levels were determined through the electrochemiluminescence immunoassay (ECLIA) method by Roche Cobas 8000 c602 (Roche Diagnostics GmbH, Penzberg, Germany) autoanalyser.

Venous serum samples collected for irisin levels were stored at -80°C until the analysis. Serum irisin levels were measured by commercial ELISA kits (RAG018R, BioVendor Inc., Candler, NC, USA) on VICTOR X3 (PerkinElmer, USA). The sensitivity of the method was 1 ng/ml; the intra-assay CV value was below 8.2%, and the inter-assay CV value was below 9.7%.

The homeostasis model assessment of IR (HOMA-IR) was calculated by using the following formula: fasting insulin level (uIU/ml) x fasting glucose (mg/dl) / 405. A HOMA-IR value above 5.22 in males and above 3.82 in females was considered as IR.³³

A hepatobiliary USG was performed for NAFLD. The hepatosteatosis of the participants was graded as 1, 2, and 3 through the USG.³⁴

Statistical analysis was performed by SPSS 23.0 program (IBM SPSS, Chicago, IL). Variables were expressed as mean ± standard deviations (SD), median (25%-75%), and percentage (%). All variables were assessed for normality and homogeneity of variance by the Shapiro Wilk test. The comparisons were performed through the t-test analysis and One-Way ANOVA when the variable was distributed normally or Mann-Whitney U test and Kruskal-Wallis H test when the variable was not distributed normally. Pearson and Spearman correlation coefficients were conducted for the correlations. Chi-square analysis was used for the analysis of the cross tables. Any p-value below 0.05 was considered statistically significant.

Results

Clinical characteristics and laboratory data of all the study groups are shown in Table I. The BW, BW SDS, BMI, BMI SDS, waist, hip, arm circumference, waist-hip ratio, skinfold thicknesses (triceps and biceps), ALT, TG, insulin and HOMA-IR levels were higher in the obese patients with and without NAFLD when compared to the control group; however, HDL-C was lower ($p < 0.05$). Furthermore BW, BW SDS, BMI, BMI SDS, waist, hip circumference, ALT and GGT levels were higher in the patients with NAFLD than the patients without NAFLD ($p < 0.05$).

The median irisin levels in patients with both NAFLD [5.7 (4.6-6.5) µg/ml] and without NAFLD [5.05 (3.8-5.7) µg/ml] were also lower than in the control group [7.5 (6.5-9.08) µg/ml] ($p < 0.05$). However, no significant difference was shown between obese patients with and without NAFLD ($p > 0.05$) (Fig. 1).

The median irisin levels were not significantly different between the genders in all the study groups ($p > 0.05$).

When the relationship between serum irisin level and other parameters were investigated, a negative correlation was detected between irisin and BMI ($r = -0.53$, $p = 0.001$), BMI SDS ($r = -0.346$, $p = 0.001$), BMI percentile ($r = -0.3$, $p = 0.000$), waist circumference ($r = -0.37$, $p = 0.000$), hip circumference ($r = -0.3$, $p = 0.000$), waist-hip ratio ($r = -0.374$, $p = 0.000$), skinfold thicknesses triceps ($r = -0.36$, $p = 0.001$) and biceps ($r = -0.389$, $p = 0.000$), arm circumference ($r = -0.3$, $p = 0.002$), insulin ($r = 0.24$, $p = 0.02$), glucose/insulin ratio ($r = -0.675$, $p = 0.000$), ALT ($r = -0.4$, $p = 0.000$); and a positive correlation was detected between irisin and HDL-C ($r = 0.43$, $p = 0.000$) in the entire study group including obese and normal-weight group. However, the analysis performed in the subgroups revealed that irisin levels were positively correlated with skinfold thickness

Table I. Clinical characteristics and laboratory results of all study groups*.

	Control Group (n=28)	Obese Patients with NAFLD (n=30)	Obese Patients without NAFLD (n=30)	p value
Gender (F/M)	14/14	12/18	19/11	
Age (Month)	167 (145.5 - 192)	166 (146.2 - 174.2)	173 (154.7 - 186.7)	0.49
Height (cm)	158.2 ± 10.3	159 (155.7- 163.5)	158.2 (151.5 - 163)	0.72
Height SDS	-0.12 ± 0.69	0.67 ± 1.16	-0.44 ± 1.13	0.22
BW (kg)	50.2 ± 9.5	81.1 ± 11.3 ^a	70.6 ± 11.5 ^{b,c}	<0.001
BW SDS	-0.32 ± 0.49	2.6 ± 0.91 ^a	1.94 ± 0.84 ^{b,c}	<0.001
BMI (kg/m ²)	19.7 (18.3 - 21)	31.2 (29.1 - 34.7) ^a	27.7 (26.1 - 29.5) ^{b,c}	<0.001
BMI SDS	-0.27 (-0.47 - -0.02)	2.44 (2.2 - 3.1) ^a	2.09 (2 - 2.23) ^{b,c}	<0.001
Waist (cm)	70.4 ± 6.3	102.2 ± 9.6 ^a	94.1 ± 10.5 ^{b,c}	<0.001
Hip (cm)	87.1 ± 7.8	109.4 ± 8 ^a	104 ± 10.3 ^{b,c}	<0.001
Waist/Hip Ratio	0.81 (0.77 - 0.83)	0.95 (0.89 - 0.98) ^a	0.92 (0.84 - 0.96) ^b	<0.001
Waist/Height Ratio	0.44 (0.42 - 0.45)	0.63 ± 0.06 ^a	0.59 ± 0.06 ^{b,c}	<0.001
Arm Circumference (cm)	23 ± 2.6	31.3 ± 2.77 ^a	29.7 ± 3.62 ^b	<0.001
Skinfold Thickness (Triceps)(mm)	1.1 ± 0.4	3.2 ± 0.87 ^a	3 ± 0.89 ^b	<0.001
Skinfold Thickness (Biceps)(mm)	0.5 (0.4 - 0.8)	2.3 ± 0.72 ^a	2.1 ± 0.89 ^b	<0.001
Glucose (mg/dl)	84.5 (81 - 93.7)	83.2 ± 8.56	87.6 ± 11.22	0.16
Insulin (uIU/ml)	8.5 (6.62 - 13.17)	20.7 (14.68 - 27.88) ^a	17.9 (12.6 - 23.87) ^b	<0.001
Glucose/Insulin Ratio	9.69 (6.74 - 13.04)	3.7 (3.07 - 5.87) ^a	5.21 (3.86 - 6.34) ^b	<0.001
HOMA-IR	1.76 (1.37 - 2.83)	3.88 (2.59 - 5.72) ^a	3.53 (2.7 - 5.95) ^b	<0.001
AST (U/L)	20 (17 - 23)	22 (17.75 - 29)	20.5 (17.75 - 25.25)	0.59
ALT (U/L)	12 (8.2 - 14)	23 (14 - 41.5) ^a	15 (10.75 - 23.25) ^{b,c}	<0.001
GGT (U/L)	9.5 (6.5 - 12.5)	17 (13.75 - 26) ^a	12 (9 - 17) ^c	<0.001
TC(mg/dl)	148 ± 22.4	162.2 ± 32.71	154.9 ± 21.12	0.12
TG (mg/dl)	73.2 (49.2 - 100.2)	122.3 ± 54.72 ^a	116.3 ± 55.75 ^b	0.005
HDL-C (mg/dl)	56.5 (48.7 - 63.7)	43.5 (35.75 - 48.25) ^a	44 (39.75 - 47) ^b	<0.001
LDL-C (mg/dl)	87.3 (65.9 - 102.8)	104.6 ± 25.33 ^a	95.5 ± 17.91	0.009
Irisin (µg/ml)	7.5 (6.5-9.08)	5.7 (4.6-6.5) ^a	5.05 (3.8-5.7) ^b	<0.001

*Parameters with normal distribution were given as mean ± SD. Parameters without normal distribution were given as median (25% -75%). a: p<0.05, comparison between control group and obese patients with NAFLD. b: p<0.05, comparison between control group and obese patients without NAFLD. c: p<0.05, comparison between obese patients with and without NAFLD. NAFLD: non-alcoholic fatty liver disease, BW: body weight, SDS: standard deviation scores, BMI: body mass index, HOMA-IR: the homeostasis model assessment of insulin resistant, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, TC: total cholesterol, TG: triglycerides, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high-density lipoprotein-cholesterol.

(triceps) (r=0.5, p=0.005) and negatively correlated with AST (r=-0.459, p=0.014) in the control group. In the entire obese group, there was a positive correlation between irisin levels and BMI (r=0.327, p=0.01), BMI SDS (r=0.376, p=0.003), waist circumference (r=0.27, p=0.03), hip circumference (r=0.26, p=0.04) and arm circumference (r=0.3, p=0.01). Moreover, the irisin levels were positively correlated with

BMI SDS (r=0.39, p=0.034) and HDL-C (r=0.387, p=0.035) in the NAFLD group. No correlation was found between the irisin levels and other measured parameters in the obese patients without NAFLD (p>0.05).

Five patients with NAFLD had AST and ALT levels above 40 U/L. According to the severity of steatosis, there was grade 3 hepatosteatosis in

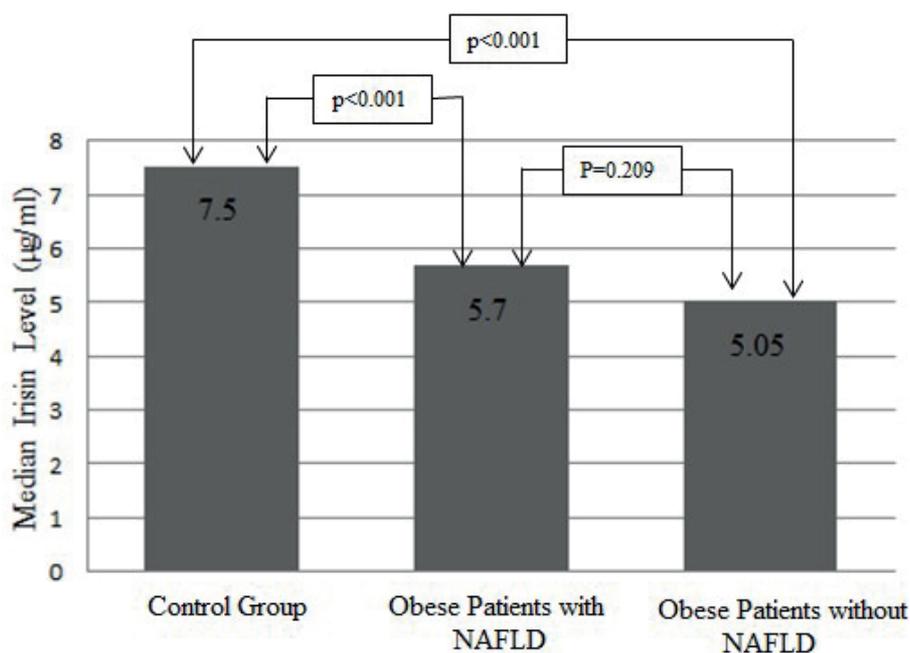


Fig. 1. Median irisin levels in all the study groups.

1 (n=1/30, 3.3%) patient, grade 2 hepatosteatosis in 5 (n=5/30, 16.7%) patients, and grade 1 hepatosteatosis in 24 (n=24/30, 80%) patients.

Insulin resistance was found in 25 patients (n=25/60, 41.7%) including 13 (n=13/60, 52%) patients with NAFLD. The serum irisin levels were not significantly different between the obese patients with and without IR ($p>0.05$).

Discussion

In our study, serum irisin levels were lower in obese patients with and without NAFLD than the controls with normal body weight and negatively correlated with adiposity parameters in all study groups. It was reported in previous studies, that irisin levels in the circulation of obese adults and children were lower similar to our study, higher in some studies and did not change in others.³⁻¹¹ In addition, the irisin levels were negatively correlated with adiposity parameters in some studies and positively correlated in others.^{3-8,13,15,35}

Boström et al.¹ first reported that irisin is secreted from muscle cells as the product of

the *FNDC5* gene. It was later shown that irisin was synthesized in both muscle and adipose tissue.^{9,12,36,37} However, *FNDC5* gene expression in adipose tissue was 100-200 times higher in muscle tissue than adipose tissue in some studies.^{9,12} The elevated irisin levels with a positive correlation with adiposity markers in obese subjects in previous studies suggested that it may also be released from adipose tissue, or it may be a compensatory regulator for maintenance of the energy balance due to increased adipose tissue.^{5,6,12,38} Palacios-Gonzales et al.⁶ found that the irisin level was positively correlated with BMI in obese adults and suggested that circulating irisin in the basal metabolic state has arisen from adipose tissue, and irisin originated from the skeletal muscle is produced during exercise. Huh et al.¹² showed that lean body mass did not change in those who had bariatric surgery however, muscle *FNDC5* mRNA and circulating irisin levels decreased along with the decrease in weight loss and BMI after the surgery. They also indicated that the amount of adipose tissue is a determinant of irisin. However, as in our study, Gonzales-Gil et al.³ showed that circulating irisin levels were lower and negatively correlated with adiposity

markers in obese and MetS patients than those with normal-weight subjects. They found that circulating irisin is determined by the lean-fat ratio rather than the total amount of body muscle and fat mass.

In our study, serum irisin levels were negatively correlated with adiposity parameters in all the study groups and levels were lower in obese patients with and without NAFLD than the control group. These findings could be explained as a result or cause of the ratio of fat to muscle mass. However, the amount of muscle and adipose tissue was not determined. The lower irisin levels in obese patients may not be a result of obesity, but, also a cause of obesity. It is not possible to clarify the comment with this cross-sectional study plan.

Although serum irisin levels were positively correlated with adiposity parameters in the entire obese group including patients with and without NAFLD, levels were not different from obese patients without NAFLD from the patients with NAFLD. These results suggest that there may be a relationship between circulating irisin and steatosis, apart from the associations mentioned above between adipose tissue and irisin or obesity and irisin. It is suggested that there may be an adaptive response for the presence of steatosis. In addition to supporting this statistically insignificant comment, the irisin levels were numerically higher in NAFLD patients than obese controls without NAFLD. This finding may be related to relatively small size of the groups.

As in our study, Polyzos et al.¹⁷ demonstrated that serum irisin levels were lower in obese patients with NAFLD and NASH diagnosed with biopsy and in obese controls than healthy controls. It was not different between the two patient groups. Circulating irisin levels did not correlate with ALT in this study. However, serum irisin levels were higher in patients with a higher degree of portal inflammation. They suggested that the irisin may have a preventive role in portal inflammation. Zhang et al.¹⁸ found that circulating irisin levels are lower in Chinese

obese adults with NAFLD than those without NAFLD. There was no healthy control group in this study. Serum irisin levels were negatively correlated with ALT and AST levels, and its levels increased in parallel with the increase in intrahepatic triglyceride content determined by MRI. Thus, the authors suggested that irisin could have a protective effect from hepatic steatosis.

Contrary to our study, Choi et al.¹⁹ found that circulating irisin levels were higher in those with NAFLD than healthy controls, and those with mild NAFLD than those with severe NAFLD; however, no difference was detected between obese controls and healthy controls. In the NAFLD group, the irisin levels were not different between obese and non-obese patients. However, in the normal control group, serum irisin levels were lower in obese subjects than non-obese subjects. Furthermore, ALT levels were negatively correlated with irisin levels. Although the BMI was higher in patients with NAFLD, it was suggested that the increase of irisin was independent of BMI. Thus, increased irisin levels could be a protective factor for the early stage of NAFLD, and irisin levels decreased with steatosis severity.

Petta et al.²² found in 593 patients with NAFLD who had a liver biopsy with a pre-diagnosis of NASH that neither the serum irisin nor hepatic irisin mRNA levels were not associated with the rs3480 A.G variant and any demographic, anthropometric and metabolic parameters. Both serum irisin levels and hepatic mRNA levels were found to be higher in those with grade 2-3 steatosis, those with NASH, and severe fibrosis. In contrast to these results, Choi et al.¹⁹ reported that serum irisin levels were lower in patients with severe steatosis than those with mild steatosis. In the study by Petta et al.²², it was found that hepatic cell fat accumulation in mice fed with high fat content was not affected by irisin, but it was related to the severity of NAFLD. In the invitro study conducted by these authors, it has been shown that irisin had an expression in hepatic stellate cells which is responsible for collagen synthesis and fibrosis,

and this expression was higher in patients with hepatic fibrosis. These findings indicated that irisin may be associated with extracellular fat accumulation and hepatic fibrogenesis.

NASH is considered as a further form of NAFLD. Furthermore, it is known that elevated serum ALT levels indicate liver cell destruction and are a favored marker indicating the presence of NASH.²¹ In our study, the association between irisin and presence of NASH could not be evaluated because there were only five patients with elevated levels of ALT and AST. However, there was a negative correlation between ALT-AST levels and irisin levels as in previous studies.¹⁸⁻²⁰ This finding suggested that circulating irisin decreases with liver damage besides the relationships between irisin and steatosis. As mentioned above, it is suggested that irisin may be a protective factor for liver injury. However, in the study by Polyzos et al.¹⁷, serum irisin levels were not correlated with ALT levels. On the other hand, Rizk et al.²⁹ found a positive correlation between serum irisin levels and ALT and AST levels. Furthermore, the irisin levels were higher in a group of MetS with normal liver enzymes and with both fatty liver disease and elevated liver enzymes than in the healthy control group and those with elevated liver enzymes compared to patients with normal liver enzyme levels in this study. Thus, they suggested that circulating irisin levels may be affected by hepatic clearance in these patients.

As a result of our study, obese adolescents with and without NAFLD have lower circulating irisin levels and the level decreases with body adiposity. In addition, irisin may be related to steatosis and may decrease with liver damage in NAFLD. On the other hand, NAFLD was not diagnosed through biopsy. This was a limitation of our study. These results as well as the studies mentioned above regarding serum irisin levels in patients with NAFLD, concluded that lower irisin levels may be a risk factor for NAFLD and may also have an association with the severity of NAFLD. In this context, it has been suggested that irisin may have a therapeutic role in

obesity and type 2 DM and NAFLD.^{27,39} Further comprehensive and detailed researches would be useful to clarify the issue.

Ethical approval

The study was approved by Eskişehir Osmangazi University Clinical Researches Ethics Committee (Approval no: 148 – 03.06.2016).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BK, GU; data collection: GU; analysis and interpretation of results: BK, GU, ZKK; draft manuscript preparation: BK, GU. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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