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Research Article

Assessment of Iron Metabolism-Related Parameters in Obese Children

Mustafa Metin Donma[1]*, Zeynep Ersöz Güngör[2], Ahsen Yılmaz[3], Savas Guzel[3], Orkide Donma[4]

[1]Department of Pediatrics, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey
[2]Ministry of Health, Hayrabolu State Hospital, Department of Pediatrics; Tekirdag, Turkey
[3]Department of Biochemistry, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey
Department of Medical Biochemistry, Cerrahpasa Medical Faculty, Istanbul University Cerrahpasa, Istanbul, Turkey

***Corresponding author:**
Mustafa Metin Donma,
Address: Tekirdag Namik Kemal University, Faculty of Medicine, Department of Pediatrics; Tekirdag, Turkey
Tel: 0090 2822505631
Email: mdonma@gmail.com, mdonma@nku.edu.tr

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Abstract

Objectives: The aim of the study was to assess the possible associations among biochemical parameters that may be correlated with the possible mechanisms of iron metabolism in healthy children with normal body mass index (BMI), along with morbid obese (MO) children with and without metabolic syndrome (MetS).

Methods: To this end, children aged 6-18 years with no history of any acute or chronic diseases were selected as the population of this prospective case-control study. Thirty MO children (with BMI higher than 99th percentile and without MetS findings), 28 MO children (with BMI higher than 99th percentile and with MetS), and 30 healthy children (with BMI values between 15th and 85th percentiles) participated in the study. Then, anthropometric measurements were recorded, followed by performing the complete blood count and serum iron profile. In addition, ferritin, transferrin, hepcidin, irisin, ferroportin, brain-derived neurotrophic factor (BDNF), WISP1, and PTP1/fortilin levels were measured using ELISA. Finally, statistical analyses were performed and $P<0.05$ was considered as the level of statistical significance.

Results: Significant differences were obtained among the groups regarding anthropometric measurements, blood pressures, triacylglycerols, and high-density lipoprotein cholesterol levels. Further, there was a tendency toward an iron deficiency in both MO groups while an increase in ferritin levels was significant in the MetS group. However, BDNF, hepcidin, and ferroportin demonstrated no significant difference among the groups. Eventually, although the above-mentioned parameters were statistically insignificant, fortilin levels indicated a gradual decrease whereas irisin levels represented an increase from control group toward morbid obesity and MetS.

Conclusion: In our study, obesity severity and the tendency toward iron deficiency were in accordance with each other. Particularly, different WISP-1 levels in the groups may help predict future complications, along with its use in diagnosing obesity.

Keywords: Obesity, Metabolic syndrome, Iron metabolism

Background

Nowadays, childhood obesity is considered as a severe health problem with an increasing prevalence. Particularly, the possibility of developing chronic diseases such as diabetes, cardiovascular problems, and cancer is extremely higher in morbid obese (MO) children, whose body mass index (BMI) percentiles are above 99 in terms of gender and age, compared to the remaining population.

Obesity and iron metabolism-related problems may negatively affect the physical growth and mental development of the children, both of which are regarded as the leading health problems throughout the world including Turkey (1,2). Therefore, the current study mainly sought to evaluate the possible associations between obesity in children and the most recent biochemical parameters such as irisin, wingless-related integration site 1 (Wnt1) inducible signalling pathway protein (Wisp1), fortilin, hepcidin, and brain-derived neurotrophic factor (BDNF)-, which may have a close relationship with iron metabolism. To the best of our knowledge, previous studies have not extensively delved into the issue, particularly in the paediatric population. Accordingly, it was attempted to make some contributions to include a healthier population in terms of the physical and mental aspects, along with the possible mechanisms.

Iron is confirmed to be an essential micronutrient which participates in numerous biological and cellular processes such as haemoglobin-mediated oxygen transport in addition to the syntheses of DNA, neurotransmitters, hormones, and cell proliferation and growth. Failure to maintain iron homeostasis in the body is associated with various disease states, including cancer, cardiovascular

diseases, and diabetes mellitus, as well as infections and neurodegenerative disorders. Iron excess and deficiency are both related to the central nervous system (CNS) pathologies as well.

Inorganic iron and heme could both induce Wnt signalling, which is viewed as the major oncogenic signalling pathway underlying colorectal carcinogenesis. Abnormal Wnt/β-catenin signalling is also associated with many diseases including cancer, obesity, degenerative disorders, and cardiovascular diseases. In addition, iron can affect Wnt signalling in tumours with Apc mutation, which is a critical mutation in the development of colorectal cancer. Several previous studies reported an increase in the tumorigenic and metastatic potential and contribution regarding enhancing the tumour growth, as well as the existence of a clear link between iron and Wnt signalling. Further, it is found that iron chelation can regulate the Wnt signalling pathway and its canonical roles in development, tumorigenesis, and metastatic progression (3-9).

According to evidence, fortilin, also known as translationally controlled tumour protein, potently promotes glioma cell proliferation and tumour growth by interacting with transcriptional factor 4 and activating Wnt signalling pathway, and finally, increasing the expression of cell cycle proteins (10-12).

Wisp genes are upregulated in mammary epithelial cells which are transformed by the Wnt-1 oncogene. Likewise, Wisp1 is introduced as a novel adipokine, overexpressed in the visceral fat in obese individuals and associated with insulin resistance and adipose tissue inflammation, as well as a novel link between obesity and inflammation. Wisp proteins exert different biological functions in various human malignancies as well (13-15).

Iron deficiency is known to have deleterious effects on CNS development and function, leading to cognitive and behavioural deficits. BDNF is a key molecule in CNS due to its role in neuronal development and its importance as a modulator of synaptic function and plasticity. The effect of decreased iron concentrations on the expression of growth factors such as BDNF has recently received importance. Variations in the expressions of specific growth factors may lead to psychomotor and developmental problems and iron deficiency may induce these deficits by decreasing the expression and function of BDNF (16).

Irisin-BDNF axis is a pathway, which affects some neurobehavioral mechanisms. Irisin may contribute to the ability of BDNF to influence mood and anxiety and the effect of irisin is more pronounced if the serum BDNF level is higher (17).

Transferrin is a protein that transports iron in blood circulation and ferritin is the iron-storage protein and an acute-phase protein. Both transferrin and ferritin are considered as the members of antioxidative defensive family in the body since both grasp excess free iron, which is known to be involved in free radical formation reactions. Furthermore, ferroportin is a protein that exports iron from reticuloendothelial macrophages and enterocytes. Moreover, hepcidin is a hormone that induces the degradation of ferroportin and regulates cellular iron efflux by binding to ferroportin and inducing its internalization. (18,19).

Considering the above-mentioned background, the current study was conducted to investigate the associations among the parameters thought to be related to the iron metabolism, and within this context, explain possible mechanisms in children with normal BMI, as well as morbid obese (MO) children with and without metabolic syndrome (MetS).

Methods

The study was performed on a total of 88 children whose ages varied between 6 and 18 years and who were divided into three groups as follows.

- Group 1 (C: Control): Thirty healthy children with BMI values between 85th and 15th percentiles;
- Group 2 (MO): Thirty MO children without MetS with age- and gender-adjusted BMI values above the 99th percentile;
- Group 3 (MO+MetS): Twenty-eight MO children with MetS with age- and gender-adjusted BMI values above the 99th percentile.

Written informed consent was obtained from the parents of the children. After a detailed physical examination, children, consulting with Namik Kemal University, Faculty of Medicine, Research and Training Hospital Ambulatory Clinics of Pediatrics Department were included in the study. On the other hand, children with severe congenital anomalies, chronic diseases, as well as systemic endocrine, neurological, and chronic pathologies were excluded from the study.

This was a case-controlled and prospective study and its protocol was approved by the Non-interventional Ethics Committee of the Faculty of Medicine, Namık Kemal University.

Anthropometric Measurements

Each child was anthropometrically measured following the physical examination, and a detailed history was taken from the parents. Waist circumference (C), hip C, head C, and the neck C of each child were measured in addition to their weight and height. Children standing *barefoot* with thin issued clothing were measured regarding their weights by an electronic weighing instrument sensitive to 0.1 kg intervals. Similarly, they were evaluated in terms of their heights by a portable stadiometer designed in 0.1 cm intervals, in a position that child completely looked at in the horizontal plane and a position that his/her occiput, back, hip, and heels were in contact with the vertical posterior plane. Additionally, the circumference of body

parts was identified as follows.

- Waist C: As a horizontal line at the midpoint of the upper limit of the iliac crest and the lower rib, followed by a normal expiration;
- Hip C: As a horizontal line passing through supra-pubically on the anterior aspect and the largest area of the gluteus on the posterior aspect;
- Head C: As a line passing through the glabella on the anterior aspect and the external occipital protuberance on the posterior aspect;
- Neck C: As the horizontal measurement passing through the most prominent part of the thyroid cartilage while the child was looking forward with their neck in an upright position.

In addition, measurements were performed by a flexible and non-elastic tape. Each measurement was taken twice and the mean was recorded as well. Finally, BMI values were calculated using weight and height values, followed by computing waist-to-hip and head-to-neck ratios.

Obesity and Metabolic Syndrome Criteria

The study population included the control group and MO children. Children with age- and sex-dependent BMI values greater than 99th percentiles were accepted as MO. Further, tables prepared by WHO and approved by the Ministry of Health of the Republic of Turkey were used to determine obesity evaluation criteria (20) and 2 groups of only MO and MO with MetS were selected for the study.

MetS was diagnosed based on the criteria suggested by the International Diabetes Federation. (21).

In MO children having BMI values greater than the 99th percentile

1. Systolic and diastolic blood pressures were above 130 and 85 mm Hg, respectively;
2. Triacylglycerol and high-density lipoprotein cholesterol concentrations were above 150 mg/dL and/or below 40 mg/dL, respectively;
3. Fasting blood glucose (FBG) levels above 100 mg/dL were considered as pathological values in terms of MetS.

Furthermore, children having 2 pathological values of the above three-criteria-list were evaluated as MO+MetS in addition to being MO while those with only one or no pathological value were included in the MO group.

Evaluation of Insulin Resistance and Biochemical Analyses

The peripheral venous blood samples of children were obtained after overnight fasting. Following a-2-hour-period, the blood samples were centrifuged at +4°C 3000 rpm for 15 minutes and then the serum samples were stored in Eppendorf tubes at -80°C until further analyses.

Insulin resistance was determined by the homeostatic model assessment of insulin resistance in all three groups as well.

Then, the obtained peripheral blood samples were used for biochemical analyses and parameters, supposed to be within the network of iron metabolism, were determined in Medical Biochemistry Laboratory of Faculty of Medicine, Tekirdag Namik Kemal University. Within this context, complete blood count, serum iron, total-iron binding capacity, FBG, insulin, ferritin were estimated in autoanalyzer. Eventually, hepcidin, irisin, ferroportin, transferrin, brain-derived neurotrophic factor, Wisp1, and fortilin were analyzed using the enzyme-linked immunosorbent assay.

Statistical Analyses

Data were recorded and then SPSS, version 16.0 was utilized for statistical analyses. The mean, standard deviation and error, and median values were calculated for each variable, followed by applying the Shapiro-Wilk test for determining the type of the distribution related to the values of the parameters. Likewise, the ANOVA test was used to evaluate the differences between the variables which exhibited normal distribution among the three groups. Moreover, the subgroups were compared by the post-hoc Tukey test and differences among the groups were evaluated by the Kruskal-Wallis test for non-normal distribution. Finally, the Mann-Whitney U test was employed to compare the subgroups and the correlation analyses were performed using bivariate and partial correlation tests. The $P<0.05$ was considered as statistically significant.

Results

Thirty MO children, as well as 28 MO children with MetS, and 30 non-obese children were included in the study. The age range of the groups was not statistically different while the BMI, hip C, neck C, and waist C-to-hip C values of MO and MO+MetS groups were higher than the values for the control group ($P<0.001$). However, highly reduced values were obtained for head-to-neck ratios ($P<0.001$) whereas the head C values of the MO group showed an increase compared to those obtained for control group ($P<0.05$).

Moreover, the basal metabolic rate exhibited an increasing direction from the control to MO and MO+MetS groups and the difference between the control and MO+MetS groups was statistically significant ($P<0.001$), the details of which are provided in Table 1.

Similarly, statistically higher systolic and diastolic blood pressure values were measured in MO and MO+MetS groups compared to the control group, as well as MO+MetS group compared to MO group (Table 2).

Table 3 summarizes the values related to fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR). As shown, these related values significantly increased in both MO and MO+MetS groups in comparison with the values of the control group ($P<0.05$).

Concerning lipid parameters, significantly increased triacylglycerol concentrations were found in the MetS

Table 1. Demographic and Anthropometric Profiles in the Study Population

Parameters	Group			*P*
	Control Mean ± SD (SE)	MO Mean ± SD (SE)	Metabolic Syndrome Mean ± SD (SE)	
Age (month)	120.4 ± 35.8 (6.5)	120.0 ± 10.7 (2.0)	119.0 ± 30.2 (5.7)	NS
BMI (kg/m^2)	17.0 ± 2.3 (0.4)	26.9 ± 2.3 (0.4)	28.8 ± 5.5 (1.1)	C-MO <0.001, C-MetS <0.001
BMR (kcal)	1276 ± 235 (48)	1422 ± 285(55)	1597 ± 361 (71)	C-MetS <0.001
Waist C (cm)	61.2 ± 8.9 (1.6)	86.9 ± 6.9 (1.3)	96.0 ± 22.5 (4.3)	C-MO <0.001, C-MetS <0.001, MO-MetS <0.05
Hip C (cm)	75.1 ± 12.2 (2.2)	95.5 ± 6.2 (1.1)	100.2 ± 14.2 (2.7)	C-MO <0.001, C-MetS <0.001
Head C (cm)	51.8 ± 3.5 (0.7)	54.2 ± 2.1 (0.4)	53.8 ± 4.7 (0.9)	C-MO <0.05
Neck C (cm)	28.9 ± 4.4 (0.8)	33.1 ± 2.1 (0.4)	35.0 ± 5.1 (1.0)	C-MO <0.001, C-MetS <0.001
Waist C/Hip C	0.82 ± 0.06 (0.01)	0.91 ± 0.05 (0.01)	0.96 ± 0.19 (0.04)	C-MO <0.01, C-MetS <0.001
Head C/Neck C	1.82 ± 0.16 (0.03)	1.64 ± 0.10 (0.02)	1.57 ± 0.22 (0.04)	C-MO <0.001, C-MetS <0.001

C: Control; MO: Morbid obese; MetS: MO with metabolic syndrome; NS: Not significant; BMI: Body mass index; BMR: Basal metabolic rate; MO: Morbid obese.

Table 2. Blood Pressure Values of the Study Groups

Parameters	Groups			*P*
	Control Mean ± SD (SE)	Morbid Obese Mean ± SD (SE)	Metabolic Syndrome Mean ± SD (SE)	
Systolic pressure (mm Hg)	107.6 ± 10.7 (2.0)	117.1 ± 14.9 (2.8)	130.3 ± 13.5 (2.6)	C-MO <0.05, C-MetS <0.001, MO-MetS <0.001
Diastolic pressure (mm Hg)	66.3 ± 11.4 (2.1)	74.2 ± 10.3 (1.9)	82.8 ± 9.0 (1.7)	C-MO <0.05, C-MetS <0.001, MO-MetS <0.01

C: Control; MO: Morbid obese; MetS; MO with metabolic syndrome.

Table 3. Fasting Blood Glucose (FBG), Insulin and HOMA-IR Values of the Groups

Parameters	Group			*P*
	Control Median	MO Median	Metabolic Syndrome Median	
FBG (mg/dL)	88.5	90.5	93.0	NS
Insulin (μIU/mL)	9.1	16.6	21.4	C-MO <0.05, C-MetS <0.05
HOMA-IR	2.0	3.7	4.8	C-MO <0.05, C-MetS < 0.05

C: Control; MO: Morbid obese; MetS: MO with metabolic syndrome; NS: Not significant; HOMA-IR: Homeostatic model assessment of insulin resistance.

group compared to control ($P<0.001$) and MO ($P<0.01$) groups. However, high-density lipoprotein cholesterol values represented a significant decrease in both MO and MO+MetS groups compared to the control group ($P<0.001$).

Based on the results, no iron deficiency anaemia was detected in our study population. Iron and ferritin levels among our subjects were within the reference range while hemoglobin ($P<0.01$) and mean corpuscular haemoglobin concentration ($P<0.05$) levels significantly differed between MO children and those with MO+MetS.

Upon the evaluation of iron and iron-related parameters, it was revealed that serum iron levels reduced in both MO and MO+MetS children compared to the control group although this reduction was not statistically significant. Contrarily, transferrin, total iron-binding capacity (TIBC), and ferritin concentrations significantly increased in MO+MetS group in comparison with the control group whereas the saturation percent showed a significant reduction in MO+MetS group ($P<0.05$). Finally, ferroportin and hepcidin levels were compatible with serum iron levels and there was no difference among the groups in this regard ($P>0.05$). Table 4 provides data concerning iron and its metabolism-related components.

As regards BDNF levels, no significant difference was observed among the groups as well. Although statistically insignificant, increasing irisin and decreasing fortilin levels were noted from control to MO and MO+MetS groups. However, Wisp1 levels indicated a significant decrease in both MO and MO+MetS groups when compared to the control group ($P<0.05$), the related results are presented in Table 5.

The correlation between hepcidin and HOMA-IR was only found in children with normal BMI ($r=0.372$, $P<0.05$) although correlations of Wisp1 received importance by controlling the HOMA-IR in partial correlations. However, a negative correlation existed between Wisp1 and hepcidin ($r=-0.407$, $P<0.05$) in

Table 4. Iron and its Metabolism-Related Parameters

Parameters	Groups			*P*
	Control Mean ± SD (SE)	MO Mean ± SD (SE)	Metabolic Syndrome Mean ± SD (SE)	
Iron (µg/dL)	88.8 ± 43.8 (8.0)	73.9 ± 31.2 (5.7)	69.7 ± 31.2 (5.9)	NS
TIBC (µg/dL)	370.5 ± 39.4 (7.2)	388.2 ± 39.5 (7.2)	398.2 ± 38.2 (7.2)	C-MetS <0.05
Saturation (%)	24.3 ± 12.7 (2.3)	19.3 ± 8.3 (1.5)	17.6 ± 7.9 (1.5)	C-MetS <0.05
Ferritin (µg/dL)	35.2 ± 2.9 (4.0)	47.4 ± 24.5 (4.5)	55.5 ± 32.4 (6.1)	C-MetS <0.05
Transferrin (mg/dL)	272.9 ± 26.8 (4.9)	285.0 ± 26.9 (4.9)	291.8 ± 26.0 (4.9)	C-MetS <0,05
Ferroportin (ng/mL)	18.0 ± 2.0 (0.4)	18.1 ± 3.3 (0.6)	18.5 ± 2.2 (0.4)	NS
Hepcidin (ng/mL)	21.5 ± 12.0 (2.2)	27.8 ± 11.1 (2.0)	24.0 ± 10.3 (2.1)	NS

C: Control; MO: Morbid obese; MetS: MO with metabolic syndrome; NS: Not significant; TIBC: total iron-binding capacity.

Table 5. Values of BDNF, Irisin, Fortilin, and Wisp1 in the Study Groups

Parameters	Group			*P*
	Control Median	MO Median	Metabolic Syndrome Median	
BDNF (pg/mL)	7.12	6.31	7.51	NS
Irisin (ng/mL)	18.87	29.46	30.71	NS
Fortilin (ng/mL)	5.63	3.83	3.35	NS
Wisp1 (ng/mL)	4381.3	2957.9	3331.1	C-MO <0.005, C-MetS < 0.005

C: Control; MO: Morbid obese; MetS: MO with metabolic syndrome; NS: Not significant; HOMA-IR: Homeostatic model assessment of insulin resistance.

MO+MetS group since no correlation was observed in children with normal BMI and those in the MO group.

Considering bivariate correlations (Figure 1), only TIBC and Wisp1 demonstrated a positive relationship in children with MO+MetS (r = 0.396, $P < 0.05$). Partial correlation analysis revealed hepcidin was related to Wisp1 (r = - 0.443, $P < 0.05$) by controlling the serum iron in MO+MetS group.

Given the partial relationship between hepcidin and irisin, the strongest correlation (Figure 2) was observed in the case of controlling the iron and MetS parameters (i.e., waist C, systolic blood pressure, diastolic blood pressure, FBG, triacylglycerols, high-density lipoprotein cholesterol) in MO+MetS group (r = 0.667, $P < 0.001$ (normal-BMI); r = 0.633, $P < 0.01$ (MO); r = 0.816, $P < 0.001$ (MO+MetS).

Interestingly, the correlations between iron and Wisp1 (r = 0.440, $P < 0.05$) in normal-BMI group, as well as iron and BDNF (r = 0.452, $P < 0.05$) in MO group were lost in MO+MetS group by controlling the MetS parameters.

Discussion

Inflammation might induce changes in iron metabolism (22). Since obesity is described as a low-grade inflammation, the status of iron-related parameters such as iron, TIBC, transferrin saturation, transferrin, ferritin, ferroportin, and hepcidin was investigated as well (18,19,22).

Irisin, as an anti-inflammatory cytokine and a myokine that increases energy expenditure, is regarded as a physiologically protective factor against obesity. In children

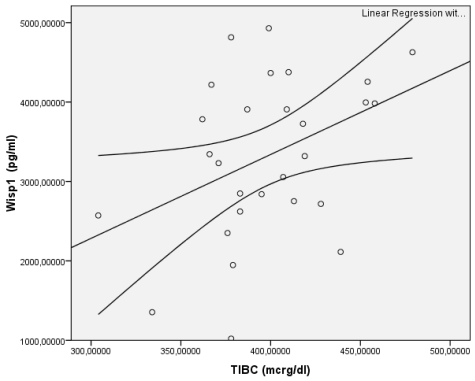

Figure 1. The Scatterplot of Individual Measurements for Wisp1 vs. TIBC With a Linear Regression Line in MO+MetS Group

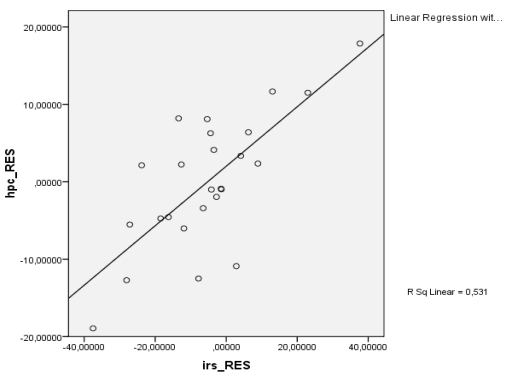

Figure 2. Scatterplot Obtained for the Partial Correlation Between Hepcidin (Hpc) and Irisin (Irs) in MO Children With MetS Controlling for Iron and MetS Parameters

with obesity and MetS, it is suggested that increased irisin either increases energy expenditure through thermogenesis or represents a resistance state (23-25). Conflicting results were reported regarding *type 1 diabetes* mellitus (T1DM) and T2DM and the lack of association between DM and irisin was also observed despite the increasing and decreasing values (26-28).

In our study, almost the same values were obtained in MO and MO+MetS groups. These concentrations were higher ($P>0.05$) than those observed in the group with normal BMI. This is consistent with the results of another study in which the overweight and obese groups showed no difference compared with the normal weight group (29).

Another study reported that the rise in blood irisin was related to fat tissue amount after cryostimulation, suggesting that the subcutaneous fat tissue was the main source of irisin in response to extreme coldness instead of the skeletal muscle. This was supported by the fact that an increase in irisin was inversely proportional to the muscle mass and changes in hepcidin were probably fat-tissue dependent (30).

This previously reported data confirms our unexpected findings concerning the strong relationship between irisin and hepcidin. In addition, considering a partial relationship between hepcidin and irisin, the strongest correlation was observed in MO+MetS group in the case of controlling for iron and MetS parameters.

The loss of correlations between iron and Wisp1, as well as iron and BDNF in MO+MetS group may imply that metabolic derangements in this group of children severely interfered with these associations.

Studies performed on fortilin are scarce (10,31,32). Fortilin plays a role in facilitating atherosclerosis development, contributing to hypertension, participating in the development of different cancers. Further, fortilin is a potent anti-apoptotic molecule and thus it prevents cancer cells from undergoing apoptosis (10,31,32). In our study, fortilin levels exhibited a pattern quite similar to that of Wisp1 although it demonstrated a slight significance. Considering the emerging role of Wisp1 proteins in tumorigenesis, and given that fortilin is known as translationally controlled tumour protein, this similarity is probably predictable.

Furthermore, a decreased BDNF level is expected in obesity and MetS and its functions in the CNS may be affected as well. Similarly, cognitive failure may be observed, including a decline in memory, neuroprotection and the control of inflammation (33,34). Iron is also related to these parameters. In our study, the serum BDNF levels of healthy non-obese, MO children, and those with MetS failed to differ from one another. This pattern was consistent with the serum iron profile of the groups involved in the study. Since the study population demonstrated no iron deficiency anaemia, BDNF levels displayed parallelism with this picture.

In this study, some unique correlations confined to MO children with MetS were highlighted as well. The only relation between TIBC and Wisp1 existed in MO children with MetS. Partial correlation analysis performed by controlling for serum iron indicated that hepcidin was related to Wisp1 in MO+MetS group. The interesting point was the association between hepcidin and Wisp1 in MO+MetS group. However, it was not a direct association but the one by way of TIBC (irisin *vs.* TIBC ($r=-0.432$, $P=0.035$) and Wisp1 *vs.* TIBC ($r=0.409$, $P=0.047$ and hepcidin *vs.* TIBC ($r=-0.395$, $P=0.056$).

Based on some previous reports, significantly higher serum Wisp1 levels were found in obese adults and children (35,36). In addition, Wisp proteins could demonstrate oncogenic or tumour suppressive functions in different tumour types (14). However, it was interesting to observe that Wisp1 levels in the primary prostate cancer stroma and in the serum of patients represented a decrease by increasing the severity of cancer (37). In another study, low levels of Wisp1 in breast cancer patients with poor prognosis indicated that Wisp1 seems to act as a tumour suppressor in breast cancer (38). The decreased Wisp 1 expression was also detected in higher-grade chondrosarcomas (39).

Similarly, a recent multidisciplinary and multicenter clinical trial (40) reported decreased Wisp1 concentrations in individuals whose BMI values were above 30 kg/m^2 in comparison with concentrations found for those with BMI values below 25 kg/m^2. The results of the above-mentioned study further revealed that the differences in Wisp1 levels between normal weight, overweight, and obese subjects failed to reach a statistical significance (40). In our study, although there was no difference between the Wisp1 concentrations of children with morbid obesity and those with MetS, the values were significantly lower in these 2 groups when compared to those measured in children with normal-BMI. Wisp1 may be an important marker for distinguishing between MO children and those with normal BMI.

Overall, obesity is associated with low serum iron concentrations and most obesity-related studies were generally performed on subjects with iron deficiency anaemia. The number of studies conducted on people with subclinical iron deficiency (deficiency without anaemia) is extremely less and those studies regarding paediatric obesity with normal iron concentrations are scarce. Our study was performed on the children whose serum iron and ferritin levels were within the reference ranges. To the best of our knowledge, considering the evaluated parameters, the current study is the first to assess the associations among serum irisin, hepcidin, BDNF, fortilin, and Wisp1.

Conflict of Interest Disclosures

The authors declare no potential conflicts of interest relevant to this article.

Authors' Contributions

MMD is the principal investigator and head of the research project supported by Namik Kemal University Scientific Research Fund Coordination Unit. MMD supervised and coordinated the clinical study. MMD and SG designed the study. MMD and ZEG oversaw patient recruitment, collected clinical samples, finalized the dataset, performed data analysis, analyzed clinical data, and maintained participants' records. SG and AY performed laboratory experiments. SG and OD supervised clinical chemistry. OD conducted the statistical analyses. MMD and OD drafted the paper, which was reviewed by all authors. All authors have read and approved the final version of the manuscript.

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