World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:15, No:8, 2021

Relationship between Hepatokines and Insulin Resistance in Childhood Obesity

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***Abstract*—** Childhood obesity is an important clinical problem, because it may lead to chronic diseases during the adulthood period of the individual. Obesity is a metabolic disease associated with low-grade inflammation. The liver occurs at the center of metabolic pathways. Adropin, fibroblast growth factor-21 (FGF-21) and fetuin A are hepatokines. Due to the immense participation of the liver in glucose metabolism, these liver derived factors may be associated with insulin resistance (IR), which is a phenomenon discussed within the scope of obesity problems. The aim of this study is to determine the concentrations of adropin, FGF-21 and fetuin A in childhood obesity, to point out possible differences between the obesity groups and to investigate possible associations among these three hepatokines in obese and morbid obese children. A total of 132 children were included in the study. Two obese groups were constituted. The groups were matched in terms of mean±SD values of ages. Body mass index values of the obese and morbid obese groups were 25.0±3.5 kg/m^2 and 29.8±5.7 kg/m^2, respectively. Anthropometric measurements including waist circumference, hip circumference, head circumference, and neck circumference were recorded. Informed consent forms were taken from the parents of the participants and the Ethics Committee of the institution approved the study protocol. Blood samples were obtained after an overnight fasting. Routine biochemical tests including glucose- and lipid-related parameters were performed. Concentrations of the hepatokines (adropin, FGF-21, fetuin A) were determined by enzyme-linked immunosorbent assay. Insulin resistance indices such as homeostasis model assessment for IR (HOMA-IR), alanine transaminase-to aspartate transaminase ratio (ALT/AST), diagnostic obesity notation model assessment laboratory index, diagnostic obesity notation model assessment metabolic syndrome index as well as obesity indices such as diagnostic obesity notation model assessment-II index, and fat mass index were calculated using the previously derived formulas. Statistical evaluation of the study data as well as findings of the study were performed by SPSS for Windows. Statistical difference was accepted significant when $p < 0.05$. Statistically significant differences were found for insulin, triglyceride, high density lipoprotein cholesterol levels of the groups. A significant increase was observed for FGF-21 concentrations in the morbid obese group. Higher adropin and fetuin A concentrations were observed in the same group in comparison with the values detected in the obese group ($p > 0.05$). There was no statistically significant difference between the ALT/AST values of the groups. In all of the remaining IR and obesity indices, significantly increased values were calculated for morbid obese children. Significant correlations were detected between HOMA-IR and each of the hepatokines. The highest one was the association with fetuin A ($r = 0.373$, $p = 0.001$). In conclusion, increased levels observed in adropin, FGF-21 and fetuin A have shown that these hepatokines possess increasing potential going from the obese to morbid obese state. Out of the correlations found with IR index, the most affected hepatokine was fetuin A, the parameter possibly used as the indicator of the advanced obesity stage.

***Keywords*—**Adropin, fetuin A, fibroblast growth factor-21, insulin resistance, pediatric obesity.

I. Introduction

THE liver is the major regulator of energy homeostasis. It is the center for the regulation of glucose homeostasis. Many pathways related to carbohydrate metabolism are located in this organ. Dysregulation of these pathways may result in metabolic dysfunctions that can contribute to the development of insulin resistance (IR). The liver is an endocrine organ, which secretes factors called hepatokines. In recent years several hepatokines have been introduced. Their participation in obesity and IR are being investigated [1], [2].

Fetuin A is one of the most important hepatokines regulating human metabolism. It was suggested as an alternative marker for IR in children [3]. Therefore it may serve as a target for obesity and IR, establishing a link between complications of obesity and inflammation [1].

The beneficial effects of FGF-21 suggest that this factor or its agonist could be a potential therapeutic agent for diabetes [4]. However, FGF-21 concentrations increase with obesity and in diabetes with metabolic syndrome [5], [6]. In a report, a novel clinical evidence linking FGF-21 resistance and diabetes pathogenesis was suggested [4]. These findings make FGF-21 as a potential marker for metabolic disorders [7]. Fibroblast growth factor 21 analogs and FGF-21 receptor agonists constitute the new "FGF21-class" of anti-obesity and anti-diabetic molecules [8], [9]. However, the controversies related to FGF-21 metabolism and the possible existence of FGF-21 resistance may postpone the potential use of FGF-21 for therapeutic purposes in metabolic diseases [10].

Adropin is a protein closely associated with the regulation of energy metabolism and IR. Its potential anti-inflammatory effects were suggested. Increased or decreased levels were reported in various inflammatory diseases [11]-[13].

Obesity, a low-grade inflammatory disease, is associated with IR. So far, concentrations of various hepatokines were of great concern both in childhood and adulthood obesity as well as related chronic diseases. Levels obtained in overweight/obese patients were compared to those determined in individuals with normal body mass index (N-BMI) [3], [5], [6], [13]-[17].

In this study, the aim was to determine the concentrations of three hepatokines (fetuin A, FGF-21, adropin) in obese (OB) and morbid obese (MO), the advanced form of obesity, children. The association of each hepatokine with IR as well as obesity indices was also investigated.

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II. Patients and Methods

A. Patients

Thirty-three OB and 99 MO children participated in the study. Written informed consent forms were taken from the parents of the participants. The study design was approved by Namik Kemal University, Medical Faculty, Institutional Ethics Committee.

B. Measurements

Anthropometric measurements including weight (kg), height (cm), waist circumference, hip circumference, head circumference and neck circumference of the study population were performed. Systolic blood pressure and diastolic blood pressure values were measured. Body mass index values were calculated from weight and height values of the children.

C. Obesity Classification

Tables containing age- and sex-adjusted BMI percentile values prepared by World Health Organization are used to constitute OB and MO groups [18]. The age- and sex-adjusted BMI percentile values of OB children were between 95 and 99. The children with values above 99 were included into MO group.

D. Laboratory Analysis

Routine laboratory tests including fasting blood glucose (FBG), insulin (INS), alanine transaminase (ALT), aspartate transaminase (AST), triglycerides (TRG), high density lipoprotein cholesterol (HDL-C) were performed. Fibroblast growth factor-21, fetuin A and adropin levels were determined by enzyme-linked immunosorbent assay.

E. Insulin Resistance and Obesity Indices

Homeostatic model assessment for insulin resistance (HOMA-IR) index, ALT/AST ratio, diagnostic obesity notation model assessment index-II (D2I), diagnostic obesity notation model assessment laboratory index (D_{LAB}), diagnostic obesity notation model assessment metabolic syndrome index (D_{METS}) and fat mass index (FMI) were calculated.

Formulas used to calculate indices were FBG (mg/dl) *INS (μIU/ml)/22.5 for HOMA-IR, (total body fat mass (kg)*100/height(cm)) for D2I, ln (TRG (mg/dl)/HDL-C (mg/dl))*INS for D_{LAB}, (TRG (mg/dl)/HDL-C (mg/dl))*INS for D_{METS}, (total body fat mass (kg)/(height(cm)^2)) for FMI.

F. Statistical Analysis

The study data and the findings were statistically evaluated by SPSSx for Windows. Mean±standard deviation or median values were calculated. T-test or Mann-Whitney U test was used depending on the type of the distribution of the data to determine possible differences between the groups. Bivariate correlations were calculated. $P<0.05$ was accepted as the statistical significance degree.

III. Results

There was no statistically significant difference between the ages of the groups. Mean age±SD values were 12.0±3.1 years and 11.4±3.3 years for OB and MO groups, respectively.

Body mass index values were 25.0±3.5 kg/m^2 in OB and 29.8±5.7 kg/m^2 in MO groups. Corresponding values for waist circumference were 83.1±12.3 cm. and 92.3±15.5 cm.

Fasting blood glucose, INS, TRG, HDL-C values were tabulated in Table I.

TABLE I
SOME GLUCOSE AND LIPID PARAMETERS IN GROUPS

Groups	OB (x±SD)	MO (x±SD)	p
FBG (mg/dl)	93.7±8.9	94.2±9.8	NS
INS (μIU/mL) [m]	16.1	23.7	0.05
TRG (mg/dL)	100.2±56.7	138.5±80.9	0.01
HDL-C (mg/dL)	54.5±11.5	46.5±10.7	0.001

OB=obese, MO=morbid obese, FBG=fasting blood glucose, INS=insulin, TRG=triglycerides, HDL-C=high density lipoprotein cholesterol, [m] median

There was not any difference between FBG values of the groups. Significantly increased INS ($p<0.05$) and TRG ($p<0.01$) concentrations, decreased HDL-C concentrations ($p<0.001$) were detected in MO group.

Concentrations of FGF-21, adropin and fetuin A were shown in Table II.

TABLE II
CONCENTRATIONS OF HEPATOKINES IN GROUPS

Groups	OB (m)	MO (m)	P
FGF-21 (pg/ml)	152.2	183.9	0.05
Adropin (ng/L)	96.8	109.1	NS
Fetuin-A (mg/L)	325.0	389.2	NS

OB=obese, MO=morbid obese, FGF-21=fibroblast growth factor-21, m= median

Of hepatokines studied, significantly higher FGF-21 levels were found in MO group ($p<0.05$). In the same group, elevated values of adropin and fetuin A were detected. These elevations were statistically insignificant.

Values related to IR and obesity indices were listed in Table III.

TABLE III
SOME INSULIN RESISTANCE AND OBESITY INDICES IN GROUPS

Groups	OB (x±SD)	MO (x±SD)	P
HOMA-IR [m]	3.8	5.2	0.05
ALT/AST	0.93±0.29	0.97±0.32	NS
D2I	12.9±4.7	16.4±6.9	0.05
D_{LAB}	3.3±1.1	4.1±1.1	0.001
D_{METS} [m]	27.3	71.1	0.001
FMI	8.3±2.5	10.8±3.9	0.001

OB=obese, MO=morbid obese, HOMA-IR=homeostatic model assessment of insulin resistance, ALT=alanine transaminase, AST=aspartate transaminase, D2I=diagnostic obesity notation model assessment 2 index, D_{LAB}=diagnostic obesity notation model assessment laboratory index, D_{METS}=diagnostic obesity notation model assessment metabolic syndrome index, FMI=fat mass index, [m] median.

All IR and obesity indices except ALT/AST were significantly increased in MO children.

Body mass index and waist circumference values, waist circumference and HOMA-IR values as well as BMI and HOMA-IR values were significantly correlated in both OB and MO groups. However, the correlation between BMI and HOMA-IR values calculated for MO group ($p<0.001$) was much stronger than the correlation detected in OB group ($p<0.05$). Fig. 1 shows the association between BMI and log HOMA-IR.

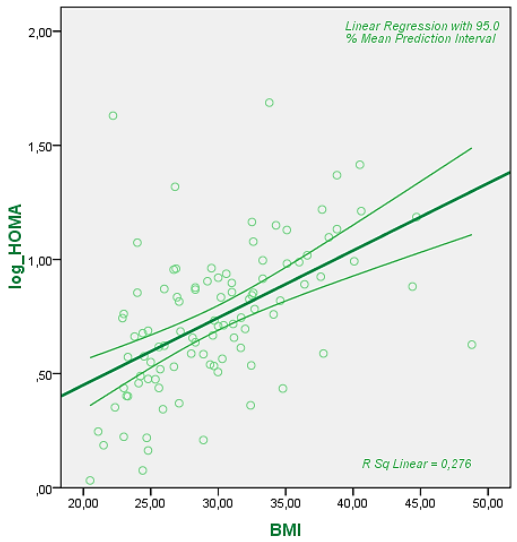

Fig. 1 Graph for correlation between log HOMA-IR and BMI

Correlations between HOMA-IR and hepatokines in MO group were shown in Table IV.

TABLE IV
CORRELATIONS BETWEEN HOMA-IR AND HEPATOKINES IN MO GROUP

HOMA-IR	r	p
FGF-21	0.215	0.05
Adropin	0.223	0.05
Fetuin-A	0.373	0.001

MO=morbid obese, HOMA-IR=homeostatic model assessment of insulin resistance, FGF-21=fibroblast growth factor-21, r=correlation coefficient

Fibroblast growth factor 21, adropin and fetuin A were significantly correlated with HOMA-IR. Among all, the most powerful association was observed between fetuin A and HOMA-IR. Such correlations were not detected in the OB group.

IV. DISCUSSION

Interpretation of the mechanisms concerning hepatokine expression may be helpful for the development of regimens to treat metabolic diseases [1], [4], [19]. Their involvement on diabetes, obesity, cardiovascular diseases makes the matter even more important.

Correlations between fetuin A and severity of coronary artery disease have been shown [17]. Levels are increased and associated with IR in polycystic ovary syndrome [20]. Significant reductions observed on IR and fetuin-A levels following oral supplementation of ellagic acid in diabetic patients points out one of the integrative medicine applications related to the matter [21]. Acute high-fat overfeeding leads to increases in both FGF-21 and fetuin A concentrations [22].

In this study, insignificantly elevated fetuin A levels were determined in MO children. Upon evaluation of the associations between fetuin A and indices of IR or obesity, the only correlation found was the one that exists between HOMA-IR and fetuin A. This was the highest correlation compared to correlations detected between HOMA-IR and FGF-21 as well as adropin in the MO group.

Fibroblast growth factor-21 is a metabolic regulator with beneficial effects. However, FGF-21 levels in circulation are associated with IR and increased in obesity and diabetes. This suggests a phenomenon called FGF-21 resistance [4].

In this study, significantly higher FGF-21 concentrations were observed in MO children than OB group. In MO group, also a correlation was found between HOMA-IR and this parameter.

Studies on adropin are still under investigation. The association of this hepatokine with some inflammatory diseases are being reported. The potential anti-inflammatory effects of adropin were also reported [12]-[14], [16].

We have obtained lower levels of adropin in OB children in comparison with the values obtained in children with N-BMI [23]. However, an insignificant increasing tendency was noted when we compared the OB and MO groups. This finding is transferred to the IR index in an interesting manner. In spite of the fact that no correlation existed between adropin and HOMA-IR in the OB group, a statistically significant correlation was obtained between adropin and HOMA-IR in the MO group. This may be the indicator of the existence of some different mechanisms during the transition from the OB to MO stage.

In conclusion, it may not be expected a straightforward, consistent increase or decrease in obesity-related parameters as going from the step with N-BMI towards the stages of obesity. This is confirmed by adropin in this study. Also, fetuin A was the most remarkable hepatokine when the association of IR with hepatokines was considered.

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