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Insulin Resistance as a Key Factor in the Development of Metabolic and Inflammatory Biomarkers in Obese Children

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Abstract

Introduction: Pediatric obesity is a major health problem. Insulin resistance (IR), is one of the early complications related to obesity but its role along that process has not been well investigated in children.

Aim: To investigate the role of insulin resistance in the development of obesity related complications (dyslipidemia, inflammatory markers, adipocyte hormones).

Method: Children who attended the gastroenterology clinic were prospectively recruited. Exclusion criteria included diseases that may affect obesity and metabolic complications. Fasting blood levels for glucose, liver enzymes, insulin, adiponectin, leptin, IL-6, and TNF were measured. Children were divided into groups: obese children with IR (A), obese children without IR (B), and normal weight children without IR (C). The indices were compared among the groups.

Results: A total of 69 children were recruited. 26 were in group A, 15 in group B, and 28 in group C. Group A was significantly different in lipid profiles (TG, HDL), liver enzyme (ALT), leptin, adiponectin, and IL-6 compared to the control groups (C). No significant difference was found between groups B and C but for HDL and adipocyte hormones (leptin, adiponectin).

Conclusion: Insulin resistance is crucial in the development of obesity related complications. We suggest that in order to detect early signs of obesity related complications insulin resistance should be measured in every obese child seen by the primary care physician.

Keywords: Insulin; Obesity; Biomarkers

Introduction

Obesity and its associated metabolic, endocrine, and cardiovascular complications are major health problems in the United States and may result in increased morbidity and mortality later in life [1, 2].

Nonalcoholic fatty liver disease (NAFLD) is a common liver disorder in obese children characterized with lipid accumulation in the liver (steatosis) [3]. NAFLD may progress to nonalcoholic steatohepatitis (NASH), histologically characterized by steatosis and inflammation and later may advance to cirrhosis [3]. The pathological processes that lead from NAFLD to cirrhosis have not been fully revealed but involvement of dyslipidemia, oxidative damage, adipose tissue hormones, and inflammatory mediators have been suggested [3]. Insulin resistance may be one of the earlier metabolic processes in this pathophysiology.

Metabolic syndrome is described as the association of three or more risk factors including abdominal obesity, dyslipidemia, insulin resistance (IR), high blood pressure and pro-inflammatory state [4]. Insulin resistance is considered as an early occurrence in the development of obesity related complications [4].

Accumulation of lipids in the liver associated with elevated aminotransferases is a well-known pathological step in the development of fatty liver disease (NAFLD) and NASH. The pathophysiology of this process has been previously described [5, 6].

Adipose tissue has been considered an active secretory organ modulating various organs including the gastrointestinal tract and the endocrine system [7]. In support of this hypothesis, many endocrine and cytokine receptors were identified in the adipose cells (adipocytes). Some of the proteins secreted by the adipose tissue include adiponectin, resistin, visfatin, leptin as well as few pro-inflammatory cytokines such as: TNF-α and IL-6 [7]. Adiponectin is an anti-inflammatory protein with anti-diabetes properties enhancing insulin sensitization, fatty acid oxidation, and increased glucose uptake in the muscles [7-9]. Adiponectin has negative association with obesity and has emerged as a valuable

biomarker for obesity related complications [8, 10]. Leptin is an adipose tissue hormone that has multiple functions including appetite control and insulin regulation [7, 11] Leptin has pro-inflammatory properties and increases cytokine production such as IL-6 and TNF-α [7].

Obesity has a crucial role in the development of diabetes and metabolic syndrome [12]. Insulin resistance is one of metabolic syndrome components but its significance in the pathophysiological process leading to obesity related complications has not been well investigated in children. In the present study we investigate the relationship between IR, obesity (BMI), adipose tissue hormones (leptin, adiponectin), and inflammatory cytokines (IL-6, TNF-α) in obese children from West Virginia.

Methods

Patient population

Children between 8 and 18 years of age who attended the pediatric gastroenterology clinic at Marshall University School of Medicine were prospectively recruited for the study. Exclusion criteria included children with various systemic diseases that affect the immune system such as: celiac disease, inflammatory bowel disease, or children with endocrine problems including hypothyroidism, hypocalcemia, children with primary metabolic diseases (dyslipidemia, etc.), or drug induced obesity (steroids). After consent forms were signed by the parent(s) and the child, when appropriate, fasting blood samples were obtained for the following indices: glucose, liver aminotransferases (ALT, AST), lipid profile [Total cholesterol, Triglycerides (TG), HDL, LDL], insulin, inflammatory markers (IL-6, TNF-α), and adipose tissue hormones (Adiponectin, Leptin). Insulin resistance is calculated by using the formula of Homeostasis Model Assessment (HOMA) as previously described (10C-D). Insulin resistance was determined by the HOMA model and values >2.0 were considered positive for IR [13-15]. Obesity was defined by the CDC BMI growth charts for both genders (≥ 95%). Children were then divided into 3 groups: obese children with IR (Group A); obese children without IR (Group B); and normal weight children without IR (Group C). The study was approved by the Joan C Edwards School of Medicine, Marshall University IRB committee.

Materials and Method

Blood samples

After overnight fasting, venous blood was drawn from the participants and serum levels of inflammatory cytokines (IL-6, TNF-α), adiponectin, leptin, glucose, insulin, lipid profile and aminotransferases were measured as well as inflammatory cytokines and hormones. The serum was stored at -80°C until analyzed.

Inflammatory cytokines

The serum concentrations of TNF- α (EMD Millipore, Temecula, Ca), and Serum IL-6 concentration (EMD Millipore, Temecula, Ca) were measured by enzyme linked immunosorbent assay kits (ELISA) according to the manufacturer's protocols.

Adiponectin and leptin levels

Serum adiponectin and leptin levels were determined using enzyme immunoassay kits (abcam, San Francisco, USA), according to the manufacturer protocol respectively.

Aminotransferases, Lipid panels, insulin, and glucose levels were measured by the hospital laboratory using a standard methodology.

Statistics

Statistical analyses were performed using GraphPad Prism for Windows version 6.02 (Graph Pad Software, Inc. La Jolla, CA). A non-parametrical test (Mann Whitney test) was used to assess the differences between the groups and column statistics were run to determine the mean and Standard Error of the Mean (SEM) values between the groups.

Results

A total of 69 children participated in the study of whom 26 were obese children with IR (Group A), and 15 were obese children without IR (group B), and 28 were normal weight children without IR (Group C) **(Table 1).**

All participants were Caucasians, and male/female ratio was 2:1, 1.27:1, and 1.3:1 for Group A, B and C, respectively. There were no significant differences among the groups in the mean age or gender ratios.

Overall, obese children with IR showed significant differences in most of the indices examined compared to normal weight children and to obese children without IR **(Table 1).** Lipid profiles of those children showed elevated TG and lower HDL but no difference in the LDL or total cholesterol levels.

In addition, elevated liver enzymes (ALT), as one marker for fatty liver, were elevated in the obese with IR group (Group A) compared to obese children without IR (Group B) or to normal control (Group C).

Adipose tissue secreted hormones, leptin and adiponectin, were also different among the groups. Obese children with IR showed higher leptin and lower adiponectin levels compared to both control groups (obesity without IR and normal weight children).

The inflammatory markers (IL-6, TNF-α) examined in the study showed that obese children with IR have higher levels of

IL-6 compared to the control groups but TNF-α was not statistically different.

Table 1 Obesity and clinical/laboratory indices.

Group	Obese-IR-pos	Obese-IR-neg	Normal Wt. IR-neg	P-value
	(± SEM)	(± SEM)	(± SEM)	(A and B *vs.* C)
# Pts	26	15	28	
M/F ratio	02:01	1.27:1	1.3:1	NS
Mean Age, (years) [+SD]	13 [± 2.3]	12 [± 2.9]	14 [± 2.5]	NS
BMI (Kg/m^2)	32.0* (± 1.1)	29.7* (± 1.3)	21.0 (± 0.5)	<0.001
Insulin (μU/ml)	27.4* (± 1.8)	9.3 (± 1.1)	8.7 (± 0.6)	<0.001
IR (HOMA-2)	3.4* (± 0.2)	1.2 (± 0.1)	1.1 (± 0.7)	<0.001
Aminotransferases				
ALT (unit?)	46.5* (± 16.5)	20.9 (± 4.2)	16.1 (± 0.6)	<0.001
AST (unit?)	32.5 (± 7.3)	22.8 (± 2.0)	23.6 (± 1.3)	NS
Lipid profile				
Cholesterol (mg/dL)	155.1 (± 4.6)	150.5 (± 7.7)	147.1 (± 5.5)	NS
TG (mg/dL)	131.3* (±13.2)	104.9(± 18.9)	70.9 (± 5.1)	<0.001
HDL (mg/dL)	45.2* (± 3.3)	46.1* (± 1.5)	51.1 (± 1.4)	<0.019
LDL (mg/dL)	83.7 (± 5.3)	83.4 (± 5.3)	81.64 (± 4.5)	NS
Adipocyte hormones				
Leptin (pg/ml)	5695* (± 968)	7253* (± 2611)	1378 (± 233)	<0.001
Adiponectin (ng/ml)	44.3 (± 19.4)	62.4* (± 14.6)	66.7 (± 11.5)	< 0.001
Inflammatory mediators				
IL-6 (pg/ml)	2.78* (± 0.30)	1.85 (± 0.24)	2.40 (± 0.57)	<0.022
TNF-α (pg/ml)	3.25 (± 0.28)	2.73 (± 0.29)	3.01 (± 0.27)	NS

Discussion

In the present study we investigated the role of IR in the development of obesity related complications in obese children such as: elevated aminotransferase, adipose tissue hormones, and the status of inflammatory markers. Our results showed that obese children with IR had significantly different levels of aminotransferase (ALT), lipid indices (TG, HDL), and inflammatory markers (IL-6) compared to normal weight children or obese children without IR **(Table 1).** The adipocyte secreted hormone, Leptin, was significantly higher in IR-positive obese children, while the adiponectin hormone levels were significantly lower in obese children who were IR-positive compared to both control groups. Overall, our data suggested that IR is a critical factor in the development of those obesity related complications. Our data may suggest that IR may be used as a possible clinical marker to measure in every obese child in order to prevent future development of those complications.

Leptin hormone has a pro-inflammatory effect and is associated with obesity related complications including IR, elevated aminotransferase and diabetes mellitus however, causal relationships between leptin and those complications have not been documented [10, 11]. In contrast, adiponectin is an anti-inflammatory hormone that is negatively associated with obesity and inflammation [10, 11]. In support of that role, serum adiponectin level decreased in obese children who reduced their weight [16]. In agreement with our study, Lebensztein et al. [17] reported that leptin was higher and adiponectin was lower in obese children with fatty liver compared to control, but only adiponectin correlated with IR [17].

Previous publications reported the relationship between obesity, IR, adipocyte hormones and inflammatory markers. For example, Makni et al. investigated the relationship between resistin (pro-inflammatory hormone), IR, and MS in obese children compared to normal weight children and obese children without MS [18]. The authors showed that there was a significant correlation between resistin, IR, and serum inflammatory markers (IL-6, TNF-α). Similar findings were reported by McFarlin et al. [19]. who showed that obese children had elevated TG, insulin, leptin, and inflammatory

cytokine such as TNF-α, compared to overweight and normal weight children, but no difference was found in other markers including IL-6 [19]. The authors suggested that the difference in inflammatory markers between obese, overweight and normal weight children may represent a difference in the timeline along the development of obesity complications, i.e., TNF-α will increase early in the process of complications compared to IL-6 or other markers. Unfortunately, IR was not measured in that study. Comparable results were obtained in our study except that only one inflammatory marker (IL-6) showed statistical differences *vs.* the control groups. Our data suggest that IR may be one of earliest step in the development of obesity related complications while other will developed later in this pathological process.

Intra-abdominal fat accumulation and dyslipidemia have been recognized as risk factors in the development of cardiovascular disease and fatty liver disease in obese subjects [12, 19-21]. In our study we confirmed the association of hypertriglyceridemia and elevated ALT in obese children. Significant elevation of serum TG and HDL were noted only in obese children with positive IR but not in those who were IR-negative ($p<0.019$). This is consistent with the report by Fu et al. [22] who showed that fatty liver in children was significantly associated with MS including dyslipidemia and IR [22].

We would like to acknowledge few limitations in the study: 1) Ethnicity is a well characterized risk factor for the development of obesity related complications [23]. Unfortunately, due to the lack of ethnic diversity in the State of West Virginia (www.cdc.org), all our participants were Caucasian. Accordingly, our result may be limited to Caucasian ethnicity only. 2) In spite of the prospective study, we could not show a causal correlation between IR and obesity related complications, and were only able to show statistical differences in the indices among the groups. Larger studies will be needed to investigate this important point.

In conclusion, our study compared between 3 groups of children; obese children with IR, obese children without IR, and normal weight children without IR. Our results showed that the major changes in obesity related complications, including Liver aminotransferase (ALT), lipid profile, adipocyte hormones, and inflammatory markers, are mostly increased in children with IR and not in obese children without IR or normal weight children. Our data suggest that calculating the IR value in obese children is crucial in assessing the development of obesity related complications. Moreover, the data suggest that IR should be measured in any obese pediatric patient who visits the primary care physician's office in order to initiate early therapy.

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