Insulin-Like Growth Factor-I and -II Levels Are Associated with the Progression of Nonalcoholic Fatty Liver Disease in Obese Children

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Objective To correlate circulating levels of insulin-like growth factor (IGF)-I, IGF-II, and IGF binding protein (IGFBP)-3 in a population of obese children with biopsy-proven nonalcoholic fatty liver disease (NAFLD) with clinical, biochemical, and histological features.

Study design We conducted a cross-sectional study at the Hepatometabolic Unit of the Bambino Gesù Children's Hospital, Rome, Italy. Obese children (42 girls and 57 boys) underwent liver biopsy, anthropometry, biochemical assessment, and IGF system evaluation. Serum concentrations of IGF-I, IGF-II, and IGFBP-3 were measured. The liver biopsy features of each case were graded according to the NAFLD Activity Scoring system. The degrees of steatosis, inflammation, ballooning, and fibrosis were calculated.

Results Nonalcoholic steatohepatitis was diagnosed in 14/99 obese subjects. Stepwise regression analysis revealed that IGF-I was the major predictor of ballooning ($\beta = -0.463$; $P < .0001$) and NAFLD activity score ($\beta = -0.457$; $P < .0001$), IGF-I/IGFBP-3 ratio was the major predictor of liver inflammation (β = -0.285; $P = .005$), and IGF-II was the major predictor of liver fibrosis ($\beta = 0.343$; $P < .005$).

Conclusion Circulating levels of IGF-I and IGF-II are associated with the histological stages of NAFLD and may represent novel markers of liver damage progression in obese children. *(J Pediatr 2014;165:92-8)*.

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The insulin-like growth factor (IGF) family comprises ligands (IGF-I, IGF-II, and insulin), 6 well-characterized IGF binding proteins (IGFBPs), and cell surface receptors that mediate the actions of the ligands (IGF-I receptor, insulin receptor, and IGF-II mannose-6-phosphate receptor).[1] The IGFs are involved in growth, development, and differentiation and act primarily through the IGF1R.[1] Over the past 2 decades, it has become apparent that IGFs control not only growth, but also protein, carbohydrate, and lipid metabolism.[2,3]

The incidence of metabolic syndrome in children and adolescents has been growing worldwide along with increases in obesity, type 2 diabetes mellitus, and nonalcoholic fatty liver disease (NAFLD).[4] NAFLD includes a wide spectrum of liver conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis associated with hepatocyte damage (ie, ballooning), inflammation, and eventually fibrosis. NASH is a potentially serious condition associated with increased risk of liver-related morbidity and mortality.[5]

The growth hormone (GH)–IGF-I axis has been implicated in the evolution of NAFLD, which is considered the hepatic manifestation of the metabolic syndrome. The incidence of hepatic steatosis is higher in patients with GH deficiency compared with those without GH deficiency,[6,7] and hypopituitarism may be associated with obesity, insulin resistance, and NAFLD.[8] GH replacement therapy in GH-deficient adults has been shown to reverse NASH[9] and reduce liver fibrosis.[7] Discontinuation of GH therapy in patients with childhood-onset GH deficiency is associated with multiple metabolic comorbidities, including NAFLD.[10] Furthermore, in adults with NAFLD, circulating levels of GH, IGF-I, and IGFBP-3 have been reported to predict liver steatosis, NASH, and fibrosis.[11-13] IGF-I has been proposed to exert an anti-inflammatory action in the liver of adults with NAFLD.[14] In a rat model of GH deficiency, IGF-I administration was found to protect against the development of NASH.[15]

apoB	Apolipoprotein B	IL	Interleukin
BMI	Body mass index	IR	Insulin receptor
CRP	C-reactive protein	ISI	Insulin sensitivity index
CV	Coefficient of variation	NAFLD	Nonalcoholic fatty liver disease
GH	Growth hormone	NAS	Nonalcoholic fatty liver disease activity score
HOMA-IR	Homeostasis model assessment for insulin resistance	NASH	Nonalcoholic steatohepatitis
IGF	Insulin-like growth factor	OGTT	Oral glucose tolerance test
IGFBP	Insulin-like growth factor binding protein	TNF	Tumor necrosis factor
		WC	Waist circumference

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IGF-II is emerging as a potential metabolic regulator. Several studies have reported associations between *IGF2* polymorphisms and body weight, insulin sensitivity, fat-free mass, lipid profile, and blood pressure.[16] Furthermore, the degree of *IGF2* methylation has been related to the development of overweight in early childhood[17] and to the lipid profile in obese children.[18]

The aim of the present study was to correlate circulating levels of IGF-I, IGF-II, and IGFBP-3 in a population of obese children with biopsy-proven NAFLD and with clinical, biochemical, and histological features.

Methods

The study cohort comprised obese Caucasian children referred to the Hepatometabolic Unit of Bambino Gesù Children's Hospital. Children with persistently elevated serum aminotransferase level and/or diffusely hyperechogenic liver on ultrasonography were selected for liver biopsy. Obese children (42 girls and 57 boys) were consecutively enrolled into this cross-sectional study between January 2012 and June 2013. Eligibility criteria included obesity, established using body mass index (BMI) cutoffs of the International Obesity Task Force[19]; absence of underlying diseases; and all 4 grandparents of Italian descent. Maternal diabetes (either preexisting or developed during or after the index pregnancy) was the sole exclusion criterion. After obtaining informed consent, we performed in-person study visits. The mean age of the subjects at the study visit was 8.7 ± 1.9 years. The Institutional Review Board of Bambino Gesù Children's Hospital approved the study protocol.

At the in-person visit, trained research assistants measured height to the nearest 0.1 cm using a calibrated stadiometer and weight to the nearest 0.1 kg using a calibrated scale. Each child's BMI was calculated using the following formula: BMI = weight (in kg)/height (in m^2). The anthropometric data were compared with national standards.[20] Waist circumference (WC) was measured using a tape measure at just above the uppermost lateral border of the right ilium, at the end of a normal expiration, as described by the National Center of Health Statistics.

Biochemical Measurements

Baseline fasting blood samples were obtained to measure plasma glucose, serum insulin, lipid profile (ie, total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B [apoB] levels), alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, bilirubin, albumin, C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α. An oral glucose tolerance test (OGTT) was performed by administering glucose solution at 1.75 g/kg body weight to a maximum of 75 g. Blood samples were obtained at 0, 30, 60, 90, and 120 minutes after administration for glucose and insulin measurements.

The insulinogenic index, computed as the ratio of the increment of plasma insulin to that of plasma glucose during the first 30 minutes of the OGTT, was determined according to the formula $\Delta I\ 30/\Delta G\ 30 = (I\ 30 - I\ 0)/(G\ 30 - G\ 0)$, where I is insulin, expressed in pmol/L, and G is glucose, expressed in mmol/L.[21] The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as (fasting insulin, mU/L) × (fasting glucose, mM)/22.5.[22] The insulin sensitivity index (ISI) was derived from OGTT results using the following formula:

$$ISI = 10\,000/\sqrt{[(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin})\text{during OGTT}]}$$

Liver Histopathology

The clinical indication for biopsy was to assess for the presence of NASH and fibrosis or for other likely independent or competing liver diseases. Liver biopsy was performed in all children, after an overnight fast, using an automatic core biopsy 18-gauge needle (BioPince; Amedic, Kista, Sweden) under general anesthesia and ultrasound guidance. The length of the liver specimen (in millimeters) was recorded. Only samples that were not fragmented, at least 15 mm long, and included at least 6 complete portal tracts were considered adequate for use in this study. Biopsy specimens were processed routinely (ie, formalin-fixed and paraffin-embedded), and 5-mm-thick liver tissue sections were stained with hematoxylin and eosin, van Gieson, periodic acid-Schiff diastase, and Prussian blue stains.

Liver biopsy specimens were evaluated by a single expert pediatric hepatopathologist who established the histopathological diagnosis of NASH.[24,25] To determine the intraobserver agreement, the pathologist scored the biopsy specimens twice while blinded and calculated the weighted κ coefficients for various histological features. Steatosis, inflammation, hepatocyte ballooning, and fibrosis were scored based on the NAFLD Clinical Research Network criteria.[26] Steatosis was graded on a 4-point scale: grade 0, steatosis involving <5% of hepatocytes; grade 1, steatosis involving up to 33% of hepatocytes; grade 2, steatosis involving 33%-66% of hepatocytes; grade 3, steatosis involving >66% of hepatocytes. Lobular inflammation also was graded on a 4-point scale: grade 0, no foci; grade 1, <2 foci per 200 × field; grade 2, 2-4 foci per 200 × field; grade 3, >4 foci per 200 × field. Hepatocyte ballooning was graded from 0 to 2: 0, none; 1, few balloon cells; 2, many/prominent balloon cells. The stage of fibrosis was quantified on a 5-point scale: stage 0, no fibrosis; stage 1, perisinusoidal or periportal (1a, mild, zone 3, perisinusoidal; 1b, moderate, zone 3, perisinusoidal; 1c, portal/periportal); stage 2, perisinusoidal and portal/periportal; stage 3, bridging fibrosis; stage 4, cirrhosis.

Features of steatosis, lobular inflammation, and hepatocyte ballooning were combined to obtain the NAFLD activity score (NAS). As recommended by the NASH Clinical Research Network,[27] a microscopic diagnosis based on overall injury pattern (eg, steatosis, hepatocyte ballooning, inflammation) as well as the presence of additional lesions