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# IGF-1R mRNA expression is increased in obese children

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ABSTRACT

*Objectives:* Obese children are often taller than age-matched subjects. Reports on GH and IGF-I levels in obese individuals are controversial, with normal and reduced GH-IGF-I levels having been reported in this group of patients. Thus, the aim of this study was to analyse insulin-like growth factor type 1 receptor (IGF-IR) mRNA expression in obese children.

*Methods:* Forty-seven pre-pubertal children were included in this study: 29 were obese and taller than their target height, and 18 were normal eutrophic controls. Fasting blood samples were collected for IGF-IR mRNA expression in isolated lymphocytes and serum IGF-I, ALS, IGFBP-3, and IGFBP-1 concentration analysis.

*Results:* Relative IGF-IR gene expression ($2^{-\Delta\Delta CT}$) was significantly ($P = 0.025$) higher in obese children (median 1.87) than in controls (1.15). Fourteen of the 29 obese subjects showed $2^{-\Delta\Delta CT}$ values greater than or equal to 2, while only 2 individuals in the control group showed values above 2 ($P = 0.01$). Obese children showed significantly ($P = 0.01$) higher IGF-I concentrations than the control group (237 ng/ml and 144 ng/ml, respectively). Among obese patients, 65.5% had IGF-I values above the 75 percentile of the control group ($P = 0.02$). ALS concentration was significantly ($P = 0.04$) higher in the obese group, while IGFBP-3 levels were similar in obese and control children. IGFBP-1 concentration was lower in obese children, while insulin levels and HOMA-IR index were higher than in controls.

*Conclusions:* The higher IGF-IR mRNA expression observed in obese children, associated with the higher IGF-I and ALS and the lower IGFBP-1 levels, suggest that the higher stature observed in these children may be due to increased IGF-I bioactivity.

## 1. Introduction

Childhood obesity is associated with faster maturation and growth acceleration [1]; however, the final height achieved corresponds to the one determined genetically. Obese children have been reported to be 0.6 standard deviation score (SDS) taller than normal weight children [2]. Studies on the GH-IGF axis in obese subjects have generated conflicting results. Normal and reduced GH secretion have been reported in association with normal, reduced, and high levels of IGF-I [3]. The majority of IGF-I actions are mediated by its interaction with the IGF type 1 receptor (IGF-IR), which is modified by the IGF-binding proteins (IGFBP-1 to -6). GH is the main regulator of the ternary complex composed by IGF-I, IGFBP-3, and ALS; which contains 90% of circulating IGF-I and regulates its availability to the tissues [4,5].

Reduced IGFBP-1 concentrations have been described in some obese subjects and could be associated to greater IGF-I bioactivity [6]. IGF-IR is expressed on all osteogenic cells [5] and increased IGF-IR expression could be associated to greater cellular proliferation and high stature, as observed in individuals carrying 3 copies of the IGF-IR gene [7,8]. Nevertheless, the reasons for the higher stature observed in obese children remain unclear.

There are currently no reports on IGF-IR expression in obese children. Thus, the aim of this study was to analyse IGF-IR mRNA expression in obese children with height above the mid-parental height percentile in order to elucidate the growth physiology in these individuals.

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**Table 1**
Gender distribution, age (years), height-SDS (mean ± SD) and BMI-SDS (mean ± SD) in the obese and control groups.

| | Obese | Control | *P* |
|---|---|---|---|
| Gender | 15 M/14F | 9 M/9F | N.S. |
| Age (yrs) | 7.0 ± 1.4 | 7.3 ± 1.4 | N.S. |
| Height-SDS | 1.61 ± 0.63 | − 0.27 ± 0.61 | < 0.0001 |
| BMI-SDS | 3.21 ± 1.26 | 0.09 ± 0.77 | < 0.0001 |

SD: standard deviation, M: male, F: female, N.S.: not significant ($P > 0.05$).

## 2. Subjects and methods

### 2.1. Study population

A convenience sample of 47 pre-pubertal children, aged 5 to 10 years, was included in this study. Twenty-nine obese children (15 boys and 14 girls), with height above the mid-parental height percentile, composed the obese group while 18 eutrophic individuals, with normal height and similar age, were used as controls (Table 1). None of the children studied showed any signs or symptoms of systemic disorders nor reached puberty in the 12 months that followed the study.

This study was approved by the Ethics Research Committee of the Clinical Hospital of the School of Medicine of Ribeirao Preto–USP (HCFMRPUSP) and a written consent obtained from all participants and guardians prior to being included in the study.

### 2.2. Study design

Detailed anamnesis and physical examination were performed by the same observer. The height, body weight, and BMI of all participants were recorded and information on paternal height obtained.

At the time of clinical evaluation, fasting peripheral blood samples were collected for IGF-I, IGFBP-1, IGFBP-3, ALS, glycaemia, insulin, and IGF-IR mRNA expression analysis. Serum samples were stored at − 20 °C until analysis. Glycaemia was determined on the day of sampling.

### 2.3. Assays

IGF-I and IGFBP-3 (Immulite 2000, Siemens, Los Angeles, CA, USA), ALS (DIAsource ImmunoAssays, Belgium), IGFBP-1 (Raybiotech - Norcross, Georgia, USA), and insulin (Beckman-Coulter – Prague, Czech Republic) were determined by specific immunoassays. All samples were analysed in duplicate within the same assay. The intra-assay coefficient of variation for IGF-I was 2.4%, IGFBP-1 3.3%, IGFBP-3 2.3%, ALS 10%, and insulin 6.6%. Assay sensitivity for IGF-I was 5 ng/ml, IGFBP-1 5 ng/ml, IGFBP-3100 ng/ml, ALS 2 µg/ml, and insulin 0.5 µIU/ml. Glycaemia was determined by the glucose-oxidase method.

### 2.4. IGF-IR mRNA analysis

Lymphocyte IGF-IR mRNA expression has been considered as a possible marker of IGF-IR expression in the whole body [9]. Therefore, peripheral lymphocyte mRNA was extracted using the TRISOL technique for IGF-IR mRNA expression analysis. The extracted mRNA was converted into cDNA and quantified by quantitative polymerase chain reaction (PCR) using specific probes (TaqMan®), as previously reported [10]. The sample from a normal height eutrophic 7.3 years old boy was used as normalizer. PCR was performed using the 7500 Real-time PCR System® (Applied Biosystems, USA).

### 2.5. Statistical analysis

The continuous variables are expressed as medians and interquartile ranges or means and standard deviation (SD). The Mann-Whitney test and the Student *t*-test were used for comparison between the groups, when appropriate. The Fisher test was used in the distribution analysis of the variables within each group. Spearman's rank correlation coefficient was used when appropriate. Significance was considered at 5% ($P \leq 0.05$).

Nutritional diagnosis was determined through BMI calculations [BMI = weight in kg / (height in meters)$^2$] adjusted for gender and age using the World Health Organization's (WHO) curves from 2007 (http://www.who.int/growthref/en/) as reference for children older than 5 years. Children above + 2 DP were considered obese. IGF-I and IGFBP-3 levels were adjusted for gender and age by subtracting the value corresponding to the 50th Percentile (P50) from the value obtained and dividing the result by the same P50, as follows: [value-P50]/P50 [11]. The HOMA-IR index was calculated from fasting glycaemia and insulin levels, according to the following equation: [glycaemia (mmol/l) × insulin (µIU/ml)] / 22.5 [12]. Insulin resistance was considered if HOMA-IR ≥ 2.0 [13]. Relative IGF-IR expression was determined by the $2^{-\Delta\Delta CT}$ method. Statistical analysis was performed using the software GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA).

## 3. Results

### 3.1. IGF-IR mRNA

IGF-IR mRNA expression ($2^{-\Delta\Delta CT}$) (median, interquartile range) was significantly ($P = 0.03$) greater in obese (1.87, 098–2.85) than in eutrophic children (1.15, 0.7–1.72). Fourteen of the 29 obese subjects showed $2^{-\Delta\Delta CT}$ values greater than or equal to 2, while only 2 individuals from the control group showed values above 2 ($P = 0.01$) (Fig. 1).

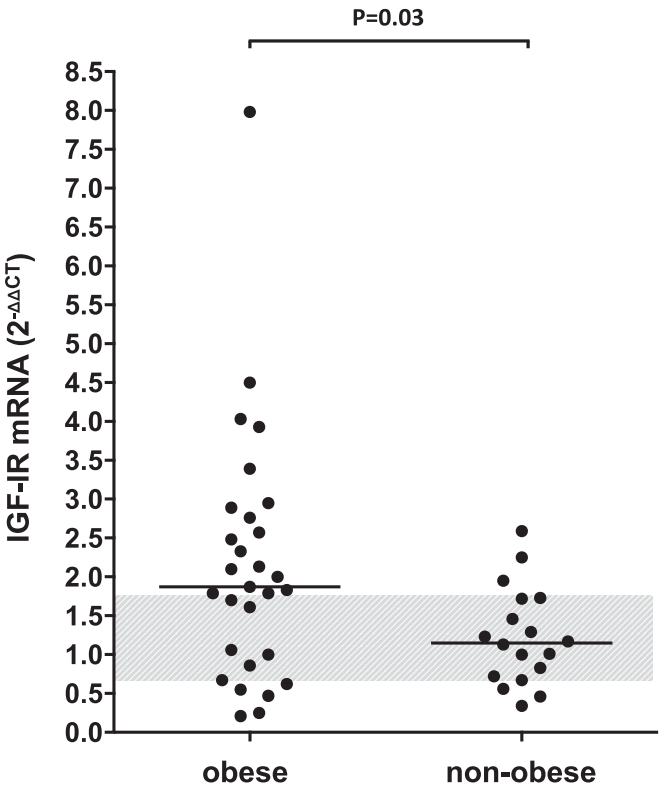


**Fig. 1.** IGF-IR mRNA expression ($2^{-\Delta\Delta CT}$) in obese and non-obese children. Bars represent medians. Shaded area represents the interval between the 25th and 75th percentile values of non-obese children. IGF-IR mRNA expression was significantly greater in obese than in eutrophic children ($P = 0.03$).