**Growth factors, adiponectin, leptin and body mass index in pre-pubertal children born large for gestational age**

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**ABSTRACT.** Objective: To examine whether the IGF axis in pre-pubertal children born large for gestational age (LGA) differs from that of those born appropriate for gestational age (AGA). Research design and methods: The study population consisted of 98 non-obese children aged 5.5-8 yr, of whom 37 were LGA, with birth weight (BW) >90th percentile, and 61 AGA. The LGA children were subdivided into two subgroups, with BW 90th-97th percentile (no.=24) and BW >97th percentile (no.=13), respectively. Total and free IGF-I, their binding proteins 1 and 3 (IGFBP-1 and IGFBP-3), leptin, adiponectin, fasting glucose (GF) and insulin (IF) were measured, and the homeostasis model assessment for insulin resistance (HOMA-IR index) was determined. Results: IGF-I, free IGF-I and IGFBP-1 were similar in both groups. Both LGA subgroups had lower IGFBP-3 levels than the AGA group (2.34±0.61 and 2.70±0.90, respectively, vs 3.92±1.1 μg/ml, p<0.01). Adiponectin was higher in the 90th-97th percentile LGA subgroup than the AGA group (p<0.01). GF and IF were higher in the LGA group (86.5±5.6 mg/dl, p<0.01, and 5.84±2.13 μU/ml, respectively, p<0.05) than in the AGA group (82.6±7.7 mg/dl and 4.62±1.9 μU/ml, respectively), as was the HOMA-IR index (1.27±0.60 vs 0.94±0.43, p<0.01). These three parameters were also found higher in the >97th percentile LGA subgroup. Conclusion: The IGF axis was not different in pre-pubertal children born LGA or AGA, with the exception of IGFBP-3, which was lower in the LGA children. In LGA pre-pubertal children the severity of intrauterine overgrowth was associated with the insulin resistance indices. (J. Endocrinol. Invest. 34: 411-416, 2011)

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**INTRODUCTION**

Fetal macrosomia occurs more often (in the range of 25-42%) in pregnancies of women with diabetes mellitus (DM), but is also a feature of a considerable percentage of non-diabetic pregnancies (8-14%) (1). A variety of factors appear to be implicated in abnormal fetal growth, including fetal insulin (2), fetal and maternal IGF (3) and leptin (4). It has been suggested that IGF-I is an important regulator in infants with macrosomia (5). The IGF-binding protein-3 (IGFBP-3) has also been associated with excessive fetal growth, in coordination with IGF-I (6). IGFBP-3 is the major binding protein identified so far and about 80% of the IGF are bound to it. As other binding proteins, IGFBP-3 interacts with the IGF or their receptors, modifying their actions by either inhibiting or enhancing their effects on target cells (7), and specifically by binding to IGF-I, IGFBP-3 appears to control its bioavailability and thus to regulate somatic growth (7).

There are indications that macrosomic babies are at risk of developing obesity and DM in later life (8). Large for gestational age (LGA) infants are born after a pregnancy complicated by a hyperinsulinemic state, even in the case of non-diabetic pregnancy (9). In-utero hyperinsulinemia is associated with fetal macrosomia and increased fat accumulation, and it may also cause alterations in metabolic programming, which can have long-term effects, such as impaired glucose homeostasis during childhood (8). There is evidence that even minor alterations in glucose tolerance can result in disturbed fetal growth (10). It is not known whether this prenatal complication also causes disturbances in the IGF axis later in life, similar to those reported in children and adults with in-utero growth restriction (IUGR) (11). Adiponectin is a plasma protein that appears to play a role in the regulation of insulin resistance (IR) and glucose homeostasis (12).

This study was designed to investigate the hypothesis that children born LGA may have a pattern in the IGF axis at the pre-pubertal age different from that of age-matched children born appropriate for gestational age (AGA), and to examine its association with indices of glucose metabolism, the adipokines leptin and adiponectin, and the body mass index (BMI).

**MATERIALS AND METHODS**

**Study groups**

Two groups of pre-pubertal non-obese children were examined at the age of 5.5-8 yr. All the children were born between the 37th and 41st weeks of gestation to non-diabetic mothers during a period of 18 months at the regional University Hospital. This hospital hosts the majority of deliveries (>85%) in a well-defined geographical area. The LGA group (no.=37) comprised children born with birth weight (BW) >90th percentile and the AGA group (no.=61) children who were born with BW between the 10th and the 90th percentiles, based on growth charts for Greek children. The LGA group was subdivided into children with BW between the 90th and the 97th percentiles (no.=24) and those with BW>97th percentile (no.=13). All the births were singletons and no child had evidence of congenital malformation.

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Key-words: IGF, adiponectin, leptin, insulin resistance, large for gestational age.

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or genetic disorder. Exclusion criteria were the following: the presence of obesity during the study period, i.e. BMI > 85th percentile based on charts for Greek children, the intake of drugs known to affect glucose metabolism before or during the study period, and a family history of DM. The mothers had all been given a glucose challenge test at 24-28 weeks of gestation, which is a routine procedure at this hospital for all pregnant women.

The Research Ethics Committee of Ioannina University Hospital approved the experimental protocol and informed written consent was obtained from the parents of the participating children.

**Hormone and biochemical assays**

Venous blood was collected from each child in the morning after an overnight fast. The parameters determined in the serum were: total IGF-I and free IGF-I, IGFBP-1 and IGFBP-3, adiponectin, leptin, fasting glucose (G_f) and fasting insulin (I_f). The ratio on a molar basis between IGF-I and IGFBP-3 (the predominant IGFBP) was estimated.

The growth factors were determined by enzyme-linked immunosorbent assays (ELISA) using the kits from Diagnostic Systems Laboratories, Inc., USA. The sensitivities for IGF-I, free IGF-I, IGFBP-1 and IGFBP-3 were 0.01, 0.02, 0.25 and 0.04 ng/ml, respectively. The respective intra- and inter-assay coefficients of variation (CV) were: for IGF-I 4.5 and 6.0%, for free IGF-I 3.6 and 10.1%, for IGFBP-1 2.5 and 6.8%, and for IGFBP-3 9.5 and 10.4%.

Serum adiponectin levels were measured by the ELISA method using the kit of Phoenix Pharmaceuticals Inc (EK-AD1-01; Belmont, CA, USA). The sensitivity of the assay was 0.40 μg/ml and the intra- and inter-assay CV were <10 and 15%, respectively. Serum leptin was determined by ELISA, using a kit purchased from BioVandor Laboratories Medicine Inc, Czech Republic, which had a sensitivity of 0.50 ng/ml and intra- and inter-assay CV of 5 and 10%, respectively.

I_f was measured on an AXSYM analyzer (Abbott) by an immunoenzymatic method, and G_f by the glucose oxidase method. I_f and the homeostasis model assessment for IR (HOMA-IR = I_f (mU/l) × G_f (mmol/l)/22.5) were chosen as indices of IR.

The BW and the crown to heel length (CHL) at birth were retrieved from the birth records, and the body weight (BoW), height (BH) and waist circumference (WC) at the time of study were recorded for each child. Skinfold thickness (SFT) in the bi-ces, triceps, subscapular and suprailiac areas, and BMI were used as obesity parameters. BMI was calculated according to the formula: weight (kg)/height (m)^2, and the specific for age and sex BMI standard deviation scores (SDS) were calculated based on Greek growth charts.

**Statistics**

The results are reported as mean±SD. The data were analyzed by one-way analysis of variance (ANOVA). Simple regression analyses were used to correlate growth factors with IR indices, BW and current anthropometric measurements. Multiple regression analysis was used to define relationships between BW, IR indices and IGF, independently of current anthropometric measurements. Statistical analyses were performed using the StatView software package of SAS Institute Inc (Carey, NC, USA). Differences were considered statistically significant at p < 0.05.

The overall sample size of 98 children was calculated to be adequate for detecting a difference of 20% in the levels of blood parameters between the AGA group and the LGA groups, and the subgroups (i.e. AGA vs LGA > 97th percentile and AGA vs LGA 90th-97th percentile) with power of 80% at a significance level of 5% (13).

**RESULTS**

Of the 62 LGA children born during a 20-month period at the hospital who were eligible, parental consent for participation in the study was given for 45. Eight of the 45 children were excluded because they were found to be obese or overweight, five in the 90-97th range and three in the >97th range. The remaining 37 (15 female, 22 male) were included in the study, and were matched according to age, gender, BoW, BH and BMI with 61 AGA children (28 female, 33 male) born during the same period in the same hospital. Maternal risk factors that predispose to fetal macrosomia were detected for 30/37 LGA born children. These were excessive weight gain during pregnancy for 14/37, overweight at pre-pregnancy (BMI > 25 kg/m^2) for 10/37 and age (>35 yr) for 6/37. The AGA children were also matched with the LGA group with regard to maternal characteristics, and specifically maternal height, ethnic origin and socio-economic status. Apart from excessive weight gain during pregnancy, which was observed in a higher percentage of the mothers of LGA babies compared to those of AGA babies (14/37 vs 6/61, p < 0.01), no differences were found in any of the identified maternal clinical characteristics between the AGA children and the two subgroups of LGA children.

At the time of the study no differences were found between the groups in SFT in the different parts of the body (Table 1). The concentrations of IGF-I, free IGF-I and IGFBP-1 were similar in both groups (Table 2). No differences were found between the LGA subgroups and the AGA group, or between the two LGA subgroups. The IGFBP-3 concentration was significantly higher in the AGA than in the LGA group (p < 0.0001), as was the IGF-I/IGFBP-3 molar ratio (Table 2).

Serum G_f and I_f concentrations were significantly higher in the LGA group than in the AGA group (Table 2), due to the higher levels of G_f and I_f in the >97th percentile LGA subgroup (Table 2). The HOMA-IR index was higher in the LGA group than in the AGA group, again due to higher values in the >97th percentile LGA subgroup (Table 2).

Adiponectin concentrations were significantly higher in the LGA group than in the AGA group (Table 2) and in the 90th-97th percentile LGA subgroup compared to the AGA group (Table 2). Circulating leptin levels did not differ significantly between the AGA group and LGA group (Table 2), although there was a tendency for them to be higher in the >97th percentile LGA subgroup than in the AGA group (p = 0.07), and significantly higher than in the 90th-97th percentile LGA subgroup (p = 0.05) (Table 2).

No significant difference in adiponectin levels was found between males and females. Leptin levels were higher in females than males in both the LGA and AGA groups. No gender differences were observed in any of the growth factors.