Determinants and Consequences of Major Insulin-like Growth Factor Components among Full-Term Healthy Neonates

Alkistis Skalkidou,1 Eleni Petridou, Evgenia Papatheoma, Heraklis Salvanos, Simos Kedikoglou, Georgios Chrousos, and Dimitrios Trichopoulos

Department of Hygiene and Epidemiology, Athens University Medical School [A. S., E. P., S. K., D. T.] and First Department of Pediatrics, ‘Ag. Sofia’ Children’s Hospital [G. C.], Athens University Medical School, Athens; Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts 02115 [E. P., D. T.]; Department of Neonatology, ‘Alexandra’ Maternity Hospital, Athens [E. P.]; and Department of Neonatology, ‘Marika Iliadi’ Maternity Hospital, Athens, Greece [H. S.]

Abstract
The purpose of this research was to investigate determinants of the insulin-like growth factor (IGF) system among healthy full-term newborns and explore their relation with anthropometric variables at birth. Components of the IGF system have been implicated in the pathogenesis of several forms of cancer, and perinatal events have been linked to chronic diseases in later life. Measurements of weight and length, as well as blood samples, were obtained from 331 healthy full-term newborns delivered during 1999 in Athens, Greece. Because the liver is important for IGF production, newborns were chosen to have bilirubin levels either ≤8 mg/dl or ≥12 mg/dl to operationally distinguish them according to the liver function. IGF-I, IGF-II, and IGF binding protein-3 were inversely associated with the presence of neonatal jaundice and blood creatinine, after controlling for blood protein levels. IGF-I increased rapidly and significantly over a period of a few days and was strongly positively associated with both birth weight and ponderal index. Newborn levels of IGF-I declined with maternal age. In comparison with first-born newborns, later-born ones had significantly higher blood IGF-I levels. We conclude that IGF-I plays a dominant role in growth during the perinatal period and that all three studied components of the IGF system are sensitive to liver and kidney function. These findings provide an insight into the processes involved in perinatal growth.

Introduction
The IGF system plays an important role in the regulation of cellular proliferation, differentiation, apoptosis, and transcription (1, 2). Components of the IGF system include the polypeptide ligands IGF-I and IGF-II, which exert their actions through the interaction with two types of cell membrane receptors, i.e., IGF-IR and IGF-IIIR (3), under the regulation of six binding proteins, i.e., IGFBP-1 through IGFBP-6 (4). IGF-I is a peptidic growth factor implicated in the proliferation of a wide variety of cell types (5); IGF-II has similar physiological properties to those of IGF-I but plays a crucial role during fetal life (6).

Major components of the IGF system in blood have been associated with cancers of the prostate (7–9), breast (10), colon (11, 12), lung (13), and with leukemia (14), and a role for IGF-I in the etiology of these diseases has been postulated. Accumulating epidemiological evidence suggests that individuals with IGF-I levels in the “high normal” range have increased risk of common cancers relative to individuals with levels in the “low normal” range (15). Evidence from in vitro and animal studies suggests that overexpression of IGF components by cancer cells may play a significant role in establishing a transformed phenotype in an increasing number of malignancies (16–20). More specifically, components of the IGF system may promote cell cycle progression and inhibition of apoptosis either by directly associating with other growth factors or indirectly by interacting with other molecular systems, which have an established role in carcinogenesis and cancer promotion, such as the steroid hormones and integrins (18). Dietary and other factors may influence cancer risk via their effects on serum insulin concentrations and on the bioavailability of IGF-I (19). IGF-I has also been associated with increased cancer cell survival after chemotherapeutic treatment, through inhibition of apoptosis (21). At the same time, evidence has been accumulated over the role of intrauterine and perinatal events and conditions in the etiology of such malignancies as childhood leukemia (22, 23), breast cancer (24, 25), and even prostate cancer (26).

The purpose of this study was to establish the role of IGF-I and IGF-II in pre- and perinatal growth of full-term newborns and to examine their relation to indices of hepatic and renal function. We determined the serum levels of IGF-I, IGF-II, and IGFBP-3 in a large group of well-characterized full-term newborns delivered in the Greater Athens region in Greece. Because in pilot studies we observed that IGF-I was inversely associated with serum bilirubin levels, we chose full-term newborns without clinical jaundice and blood bilirubin levels ≤8 mg/dl, or others with clinical jaundice and serum bilirubin levels ≥12 mg/dl. We also measured serum creatinine concentrations in all of the newborns as an index of renal function.

Materials and Methods
During the 1999 calendar year, ~10,000 newborns were delivered in the two departments of Obstetrics and Gynecology of the University of Athens teaching hospitals. These hospitals mostly admit lower income women from Greece, as well as from Albania, Poland, Bulgaria, and other eastern European countries.
To be eligible for the study, a newborn had to be full term (gestation period ≥37 weeks), of Caucasian origin, with a birth weight of ≥2500 g, and apparently healthy, i.e., without serious signs of disease, need for a neonatal Intensive Care Unit, or need of a blood transfusion. Newborns were distinguished to those who were clearly jaundiced (bilirubin ≥12 mg/dl) and clearly nonjaundiced (bilirubin ≤8 mg/dl); newborns with intermediate bilirubin levels were not included in this study to avoid misclassification. In addition, the mother should not suffer from any chronic disease, such as malignancy, connective tissue disorder, diabetes mellitus (unless it was gestation related), anemia (unless it was gestation related), major neuropsychiatric disorders (e.g., epilepsy and psychoses), chronic renal failure, peptic ulcer, ulcerative colitis, bronchial asthma requiring treatment, or chronic infectious diseases, including hepatitis B and C. Finally, to secure proper informed consent, the mother had to be able to communicate adequately in Greek. The study protocol has been approved by the University of Athens Medical School Ethical Committee.

All of the eligible newborns that were delivered while two of the authors (E. P. and H. S.) were the neonatologists on call, and whose mothers consented to study participation, were enrolled in the study. A total of 60 newborns or their mothers failed to satisfy the inclusion criteria and were excluded from the analyses (32 for biomedical exclusion criteria and 28 because they were unable to communicate in Greek or English and/or explicitly indicate their consent). An additional 243 newborns with bilirubin levels between 8 and 12 mg/dl were not included in the study to reduce misclassification. Thus, completed maternal questionnaires as well as infant blood samples were eventually obtained for a total of 331 newborns. Although it seems to be no apparent circadian or diurnal variation of IGF levels (27), all of the samples were received in conjunction with routine morning blood taking and no later than the fifth day of life for bilirubin measurements and/or the nationally mandated screening for hypothyroidism, phenylketonuria, and G6PD deficiency.

Blood samples were obtained from all of the eligible newborns for masked measurements of the major IGF system components examined in this study (IGF-I, IGF-II, and IGFBP-3), as well as of renal and liver function variables. IGF-I was run on the Nichols Advantage Automated Specially System (Nichols Institute, San Juan Capistrano, CA). The sensitivity of the assay is 6 ng/ml. IGF-II was determined by using the DSL-2600 ACTIVE Non-Extraction Insulin-Like Growth Factor-II Coated-Tube IRMA kit. The procedure uses a two-site IRMA. The DSL-2600 ACTIVE IGF-II IRMA includes a simple extraction step in which IGF-II is separated from its binding protein in serum. The IRMA is a noncompetitive assay in which the analytic to be measured is “sandwiched” between two antibodies. The sensitivity of the procedure is 12 ng/ml. IGFBP-3 concentrations (in μg/ml) were measured using a commercially available radioimmunoassay kit (IGFBP-3100T kit; Nichols Institute, San Juan Capistrano, CA). During a single incubation period radiolabeled IGFBP-3 competes with unlabeled IGFBP-3 in the test sample, the standards, and the controls for a limited number of specific antibody binding sites. A standard curve is prepared using this dose-response relationship, and test sample and control concentrations are read from the curve. The sensitivity of the assay is 0.0625 μg/ml. No cross-reactivity with IGF-II, proinsulin, insulin, thyrotropin, or luteinizing hormone was detected.

The data were analyzed through multiple regression. Log transformation of IGF-I, IGF-II, and IGFBP-3 was necessary when these measurements were used as dependent variables to assure approximate symmetry and, thus, applicability of parametric techniques. A consequence of the log transformation is that the adjusted regression coefficients can be expressed in proportional (percentage) changes of the dependent variable for a specified increment in each independent variable.

### Results

Table 1 shows the distribution of 331 full-term newborns by selected demographic and biosocial variables, and presence of
jaundice. These results mostly serve descriptive purposes. The high proportion of newborns with clinical jaundice (63.1%) is the result of intentional selection, whereas the relatively high prevalence of maternal smoking during pregnancy (21.5%) is an unfortunate but well-known phenomenon among Greek women. Among the jaundiced children, 87 were subsequently submitted to phototherapy. Table 2 shows mean values and SEs of some maternal and neonatal variables, as well as of the IGF hormones by gender and presence of jaundice in the newborn. There is a statistically significant inverse association between blood bilirubin levels and the levels of both IGF-I and IGF-II in all infants (boys: $P = 0.05$ for IGF-I and $P = 0.001$ for IGF-II; girls: $P = 0.01$ for IGF-I and $P = 0.0001$ for IGF-II). In addition, length and total blood protein levels are inversely associated with the existence of jaundice and bilirubin levels (boys: $P = 0.002$ for length and $P = 0.0001$ for blood protein levels; girls: $P = 0.02$ for length and $P = 0.0001$ for blood protein levels).

Tables 3–5 show mutually adjusted proportional changes of IGF-I (Table 3), IGF-II (Table 4), and IGFBP-3 (Table 5) per indicated increments of the independent variables. Because changes in IGF-I and IGF-II are controlled for the IGFBP-3 values, the findings of the regression analyses reasonably approximate the corresponding changes of the unbound, biologically active, free compounds of these hormones. As expected, there are strong positive associations of both IGF-I and IGF-II with IGFBP-3 ($P < 0.0001$ and $P = 0.0004$, respectively).

The data in Tables 3–5 show that the presence of jaundice is inversely associated with the values of both IGF-I and IGF-II ($P = 0.06$ and $P = 0.002$, respectively). There is also a statistically significant positive association of birth weight and ponderal index (birth weight over the cube of the birth length) with the value of IGF-I ($P = 0.0001$ and $P = 0.04$, respectively). The respective associations for IGF-II are not statistically significant. In addition, high levels of creatinine are strongly associated with low levels of IGF-I and IGF-II ($P = 0.0001$ and $P = 0.003$, respectively). With respect to IGFBP-3, the observed pattern in Table 5 reflects the positive association of this compound with both IGF-I and IGF-II ($P = 0.0001$ and $P = 0.0004$, respectively). Of the 331 women included in the study, 8 have developed gestational diabetes, and among their newborns 6 were jaundiced and 2 nonjaundiced. Our primary intention was not to exclude these women, because of the uncertainty surrounding the role of gestational diabetes in the processes underlying the studied associations. Nevertheless, inclusion of gestational diabetes in the models presented in Tables 3–5 did not reveal any significant or suggestive association of this condition with IGF-I, IGF-II, or IGFBP-3, nor did it affect to a noticeable extent the other regression coefficients in the respective models. We have also added in the models indicated in Tables 3–5 weight gain during pregnancy. A 5-kg increment in weight gain was associated with an increase of 3.7% of IGF-I, 1.2% of IGF-II, and 6.7% for IGFBP-3; however, none of these differences was statistically significant nor did the introduction of weight gain into the models depicted in Tables 3–5 substantially affect the other regression coefficients in the respective models.
similar trend is evident with respect to IGF-II and IGFBP-3 (31). As it has been reported in earlier studies (32, 33), boys have lower levels of IGF-I and IGFBP-3 than girls, although in our investigation the differences have not reached statistical significance.

The positive association between total blood proteins and the three studied IGF components, which was significant for IGF-II and IGFBP-3, may be a reflection of variable hemocentration. In contrast, the strong inverse associations between the three studied components on the one hand and bilirubin and creatinine levels on the other, are likely to reflect underlying pathophysiologic processes, impaired liver and kidney function, respectively. Because IGF-I is mainly produced in the liver, its reduction may signal transient or permanent hepatic malfunction as it has also been reported in the adult (34). With respect to the kidney, the fact that IGF-I is not only produced in this organ, but also contributes to its development, provides biological plausibility to our findings (35). It is of interest that the total serum protein was significantly lower in the jaundiced group, as has been reported previously (36).

The IGF system is crucial for growth and this is reflected in the strong association of IGF-I with birth weight, ponderal index and birth length. The pattern of associations, presented in Tables 3–5, confirm that IGF-I is more important than IGF-II or IGFBP-3 with respect to growth during the perinatal period. The effect of IGF-I concerns both length and ponderal index. Among the variables that are likely to affect the levels of IGF-I and IGFBP-3, maternal smoking, maternal age, gestational age, birth order, birth weight, and sex show the most consistent and significant associations, whereas other variables, such as birth length, maternal BMI, and jaundice, show no consistent association with these components.

Among the variables that are likely to affect the levels of the studied IGF components, maternal age is inversely associated with the levels of IGF-I. There are several reports indicating that the levels of IGF-I among adults decline with age (28, 29), and it is likely that the lower values of IGF-I among newborns with older mothers is a reflection of this phenomenon. It is of interest that no pattern of decline with maternal age is evident for IGF-II, whereas the nonsignificant decline with maternal age for IGFBP-3 is brought about by the corresponding reduction of IGF-I, because these two biochemical variables are strongly correlated.

There is a suggestion that firstborn children, in comparison to later born children, have lower levels of IGF-I, but similar levels of IGF-II and IGFBP-3. In a previously reported study, correlations between serum IGF components and size at birth were stronger in nonprimiparous than in primiparous pregnancies (30). Moreover, even within a few days after birth, IGF-I is increasing sharply with age, ∼10% per day, whereas no

**Discussion**

We have evaluated levels of IGF-I, IGF-II, and IGFBP-3 in relation to physiological parameters of the newborn. In some of these relationships, the studied factors are likely to affect the levels of the IGF components, whereas in others the IGF system may be responsible for changes in the variables under investigation. Although an attempt is made to assess the direction of causality, the emphasis is in the interpretability of the findings from a clinical point of view.

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**Table 5** Multiple regression-derived estimates of change, percent, of IGFBP-3 per indicated increment of predictor variables and corresponding 95% CI among 331 studied newborns

<table>
<thead>
<tr>
<th>Variable Category or increment</th>
<th>Proportional change (%)</th>
<th>95% CI (%)</th>
<th>P (two tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age &lt;25 years</td>
<td>−3.8</td>
<td>7.9</td>
<td>0.68</td>
</tr>
<tr>
<td>25–34 years Baseline</td>
<td>1.3</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Maternal BMI (before pregnancy)</td>
<td>35+ g/kg/m²</td>
<td>−0.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Maternal smoking (during pregnancy) No</td>
<td>Baseline</td>
<td>4.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Gender Female Baseline</td>
<td>−0.4</td>
<td>6.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Male</td>
<td>0.5</td>
<td>4.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Gestational age 1 week Baseline</td>
<td>1.7</td>
<td>0.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Birth order First Baseline</td>
<td>−3.5</td>
<td>9.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Other</td>
<td>4.9</td>
<td>2.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Total blood proteins 1 g/dl</td>
<td>−30.0</td>
<td>−44.6</td>
<td>−11.5</td>
</tr>
<tr>
<td>Creatinine 1 mg/dl</td>
<td>−9.3</td>
<td>−14.7</td>
<td>−3.6</td>
</tr>
<tr>
<td>Jaundice Yes Baseline</td>
<td>−0.8</td>
<td>−18.6</td>
<td>−30.8</td>
</tr>
<tr>
<td>Days from birth to blood draw 1 day</td>
<td>2.5</td>
<td>−0.9</td>
<td>6.1</td>
</tr>
<tr>
<td>IGF BP-3 SD Baseline</td>
<td>7.4</td>
<td>2.3</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Additionally introduced variables

Model 1
- Birth weight 250 g 1.65 −0.31 3.65 0.10
Model 2
- Length 1 cm 0.4 −0.83 1.65 0.52
Model 3
- Birth ponderal index 1 kg/m³ 0.5 −0.42 1.39 0.29
Model 4
- Length 1 cm 1.1 −0.38 2.59 0.15
- Birth ponderal index 1 kg/m³ 0.9 −0.16 2.02 0.09

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**Table 4** Multiple regression-derived estimates of change, percent, of IGF-II per indicated increment of predictor variables and corresponding 95% CI among 331 studied newborns

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Additionally introduced variables

Model 1
- Birth weight 250 g 5.98 0.65 11.6 0.03
Model 2
- Length 1 cm 3.5 0.1 6.9 0.04
Model 3
- Birth ponderal index 1 kg/m³ −0.03 −2.4 2.4 0.98
Model 4
- Length 1 cm 5.0 0.9 9.2 0.02
- Birth ponderal index 1 kg/m³ 1.9 −0.9 4.9 0.19
evaluated in fewer investigations and was generally reported as weaker than the relation between IGF-1 and birth weight (40, 41).

Among the advantages of the present investigation are the use of standardized methods for both biochemical and clinical measurements, and restriction of the study to healthy full-term newborns, a process that minimizes the influence of confounding factors that may be involved in prematurity or prenatal pathology. The study was moderately large because of cost constraints, but earlier investigations of similar objectives were generally of smaller size (38, 42). The study subjects were Greek women of lower income or migrant women from eastern European countries, but results were virtually identical among Greek and other women and did not change when educational status was controlled for.

In conclusion, our study design in a relatively large sample of healthy full-term newborns allowed us to document a dominant role of IGF-1 in pre- and perinatal growth. Moreover, our study points out that liver dysfunction and kidney immaturity are reflected in reduced levels of all three of the studied components of the IGF system. These findings provide an insight into the processes involved in perinatal growth and point out a possible pathophysiological link between perinatal events and conditions, and adult life diseases, notably cancer.

References


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