Correlation of Insulin-Like Growth Factor-I and -II Concentrations at Birth Measured by Mass Spectrometry and Growth from Birth to Two Months

Colin P. Hawkes^{a,c} Deirdre M. Murray^{c,d} Louise C. Kenny^{d,e} Mairead Kiely^{d,f}
Jonathan O'B Hourihane^{c,d} Alan D. Irvine^{b,g} Zengru Wu^h Yair Argonⁱ
Richard E. Reitz^h Michael J. McPhaul^h Adda Grimberg^{a,j}

^aDivision of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; ^bThe National Children's Research Centre, Dublin, Ireland; ^cDepartment of Paediatrics and Child Health, University College Cork, Cork, Ireland; ^dThe Irish Centre for Fetal and Neonatal Translational Research, Cork, Ireland; ^eDepartment of Obstetrics and Gynaecology, University College Cork, Cork, Ireland; ^fSchool of Food and Nutritional Science, University College Cork, Cork, Ireland; ^gDepartment of Clinical Medicine, Trinity College Dublin, Dublin, Ireland; ^hQuest Diagnostics Nichols Institute, San Juan Capistrano, CA, USA; ⁱDepartment of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA; ^jDepartment of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Keywords

IGF-I · IGF-II · Assay · Mass spectrometry · Growth hormone

Abstract

Background: Immunoassays used to measure insulin-like growth factor (IGF)-I and -II concentrations are susceptible to interference from IGF-binding proteins. The aim of this study was to investigate the association of IGF-I and -II concentrations at birth with neonatal anthropometry using a liquid chromatography/mass spectrometry (LCMS) assay. **Methods:** LCMS was used to measure IGF-I and -II concentrations in umbilical cord blood of term, healthy infants enrolled in the Cork BASELINE Birth Cohort Study. Weight, length, and occipitofrontal head circumference (OFC) were measured at birth and 2 months. **Results:** Cord blood IGF-I and -II concentrations were measured in 1,100 infants. Mean (SD) IGF-I and -II concentrations were 52.5 (23.9) ng/mL and 424.3 (98.2) ng/mL, respectively. IGF-I and -II concentrations at birth

were associated ($p < 0.05$) with weight ($R^2 = 0.19$, $R^2 = 0.01$), length ($R^2 = 0.07$, $R^2 = 0.004$), and OFC ($R^2 = 0.03$, $R^2 = 0.04$) at birth. Low IGF-I concentrations at birth were associated with increases in weight ($p < 0.001$) and OFC ($p < 0.01$) Z-scores in the first 2 months. **Conclusion:** Using an LCMS assay, we have shown that anthropometric parameters at birth are associated with IGF-I and weakly with IGF-II concentrations. This indicates that, at the time of birth, IGF-I is the more important growth factor for regulating infant growth.

© 2018 S. Karger AG, Basel

Background

The growth hormone (GH)/insulin-like growth factor (IGF) axis is a key regulator of postnatal human growth. Although IGF-I and -II are under the influence of GH secretion in the older child [1], prenatal regulation of these factors is less dependent on GH production [2]. Conse-

quently, congenital GH deficiency is associated with normal size at birth [3], while mutations affecting IGF-I [4, 5] and IGF-II [6, 7] signaling affect birth size in humans and mouse models, with up to 60% reductions [8, 9].

Numerous factors outside of the GH/IGF axis may influence prenatal IGF-I and -II production. Umbilical cord blood samples from female infants have higher IGF-I concentrations than from males [10]. Maternal factors such as cigarette smoking [11], obesity [12], and preeclampsia [13, 14] may also affect IGF concentrations, although most studies to date have focused on IGF-I and not IGF-II measurements. Furthermore, small studies have demonstrated a correlation between cord blood IGF-I concentration and birth size [15–17], but the association for IGF-II is reported to be weak or absent [16, 18, 19].

Over 90% of circulating IGF-I and -II is bound to IGF-binding proteins (IGFBPs), prolonging their serum half-life and regulating their bioavailability. Prior to measurement, IGF-I and -II must be separated from these IGFBPs, and extraction methods such as acid ethanol precipitation are commonly used [20]. This approach recovers only 75–80% of the IGF-I complexed to IGFBPs [21], and may affect the reliability of the reported measurements. This interference is likely to be more problematic in infants, who have lower IGF concentrations and higher concentrations of some of the IGFBPs than older children [22]. For example, IGFBP-1 concentrations can be increased by the stress of labor [23, 24], or in pregnancies complicated by preeclampsia [14], and it is likely that interference will significantly affect IGF measurement accuracy when susceptible assays are used. Differences in IGF-I measurements also have been attributed to their reliance on different reference populations, resulting in assay-specific reference ranges, but a study of six IGF-I immunoassays using the same healthy population-based reference samples still yielded different assay-specific reference ranges with variations most noticeable at higher IGF-I concentrations [25].

Liquid chromatography/mass spectrometry (LCMS) assays are now available to measure IGF-I and -II concentrations [26], a method that is less susceptible to IGFBP interference even in the presence of supraphysiological levels of IGFBP-3 [27]. Isotopically labeled IGF-I can be used with the LCMS assay to adjust for the IGF-I and -II lost in acid ethanol extraction and chromatography, further improving the accuracy of this method. We hypothesized removing IGFBP interference from IGF-I and -II measurement at birth would allow us to study the relationship between IGF-I and -II concentrations with size

at birth and early infant growth. The aim of this study was to explore the relationships of age- and gestational age-corrected umbilical cord IGF-I and -II concentrations measured at birth by LCMS with weight, length, and occipitofrontal head circumference (OFC) at birth and 2 months.

Methods

The Cork BASELINE birth cohort includes healthy Irish children born between August 2008 and August 2011 (ClinicalTrials.gov NCT: 01498965). A detailed description of this cohort has been reported previously [28]. Firstborn children of singleton pregnancies born to low-risk mothers were included in this study. Exclusion criteria were maternal prepregnancy hypertension, prepregnancy or gestational diabetes, renal disease, systemic lupus erythematosus, antiphospholipid syndrome, major uterine anomalies, major fetal anomalies, cervical cone biopsy, and ≥ 3 miscarriages. The Clinical Research Ethics Committee of the Cork Teaching Hospitals approved this study procedures and protocol, and parental consent was provided for participation.

Subjects

For this study, all children born between 37 and 42 weeks' gestation were included. Gestational age was determined by the first day of the last menstrual period. If fetal ultrasound was performed before 16 weeks' gestation and a discrepancy of greater than 6 days was noted, ultrasonographic gestational age was used. Similarly, gestational age was determined by ultrasound if performed up to 21 weeks' gestation and a discrepancy of 10 days was noted. Smoking status during the first trimester was ascertained by self-report during pregnancy.

Anthropometry

Weight, length, and OFC were measured on the first day of life using standardized protocols. A Harpenden neonatometer (Harlow Healthcare, London, UK) was used to measure supine length, and naked weight was measured using the Seca 384 Baby Scales (Seca, Hamburg, Germany). Sex- and gestational age-specific Z-scores for weight, length, and OFC were generated from the UK reference charts [29] using Stata 12.0 (StataCorp, TX, USA) [30]. Subjects were measured again at 2 months of age using the same standardized protocols.

Sample Collection and Storage

Umbilical cord blood samples were collected at birth and processed to serum within 3 h of collection. They were stored at -80°C until analysis.

Mass Spectrometry

IGF-I and -II concentrations were measured by Quest Diagnostics (San Juan Capistrano, CA, USA) using LCMS, in an approach described in detail elsewhere [27, 31]. Isotopically labeled IGF-I was added to the sample as an internal standard for IGF-I and -II. IGF-I and -II were released from their binding proteins by an acid ethanol extraction followed by automated online extraction and analytical chromatography using an Aria TX-4 (Thermo-

Table 1. Characteristics of the infants and pregnancies included in this cohort

	Male	Female	All
Infants, <i>n</i>	563	537	1,100
Caucasian, <i>n</i>	555	527	1,082
Gestational age, weeks	40.1 (1.1)	40.2 (1.1)	40.2 (1.1)
Weight at birth, kg	3.55 (0.47)	3.48 (0.44)	3.52 (0.45)
Length at birth, cm	50.7 (2.1)	50.2 (1.9)	50.4 (2)
Head circumference at birth, cm	35.1 (1.4)	34.6 (1.3)	34.9 (1.4)
Umbilical cord IGF-I, ng/mL	48.5 (23.2)	56.7 (23.9)	52.5 (23.9)
Umbilical cord IGF-II, ng/mL	420.8 (95)	428 (101.4)	424.3 (98.2)
Maternal age, years	30.1 (4.6)	30 (4.2)	30.1 (4.4)
Maternal BMI at 15 weeks	24.8 (4.1)	24.9 (4.2)	24.9 (4.1)
Maternal smoking during first trimester, <i>n</i>	145	132	277

Values of all continuous variables are presented as mean (SD).

Fisher, San Jose, CA, USA). IGF-I and -II were quantitated using a time-of-flight mass spectrometer using narrow mass extraction of full-scan spectra. The internal standard was used to adjust for any procedural losses during extraction.

Performance characteristics of this assay have been previously described [27, 31]. For IGF-I using quality control samples, the interassay coefficient of variation and percent recovery were 5 and 104% at 100 ng/mL, 5.2 and 103% at 400 ng/mL, and 3.5 and 103% at 700 ng/mL. For IGF-II, the interassay coefficient of variation and percent recovery were 6.1 and 102% at 200 ng/mL, 3.2 and 99% at 500 ng/mL, and 5.3 and 99% at 1,200 ng/mL. Similar performance was seen with serum pools [27, 31].

Statistical Analysis

The lower limit of detection of IGF-I using this assay is 16 ng/mL; samples with concentrations below this limit were assigned a value of 15 ng/mL. Similarly, the lower limit of detection of IGF-II was 32 ng/mL; samples with concentrations below 32 ng/mL were assigned a value of 31 ng/mL.

Sex-specific reference curves for IGF-I and IGF-II concentrations were generated using LMS Chartmaker Pro (Harlow Printing Ltd., South Shields, UK). The LMS method [32, 33] uses a Box-Cox transformation to obtain normality. Three distinct curves were generated for skewness, median, and variability. These were combined in one graph, with smoothening of changes over time. They were then adjusted until fit of the curve was visually optimized as per software recommendations. IGF-I and IGF-II concentrations were then converted to age- and sex-specific Z-scores from this population for subsequent analysis.

Data analyses were performed using SPSS 22.0 (IBM, New York, NY, USA). Normally distributed data were described as mean (SD) and compared using independent-sample *t* tests. Linear regression analysis was used to determine the relationship between continuous variables. Multiple linear regression analysis was performed to determine the relationship of IGF-I or -II Z-scores at birth with the infant's weight, length, head circumference, and gestational age at birth and maternal BMI.

Results

A total of 1,100 term infants (563 male) met the inclusion criteria for this study. Characteristics of the population are shown in Table 1. The mean (SD) IGF-I concentration was 52.5 (23.9) ng/mL (males 48.5 [23.3] ng/mL, females 56.7 [23.9] ng/mL). The mean (SD) IGF-II concentration was 424.3 (98.2) ng/mL (males 420.8 [95] ng/mL, females 428 [101.4] ng/mL). There was a significant difference between males and females in IGF-I ($p < 0.001$) but not in IGF-II concentrations ($p = 0.2$).

Linear regression analysis showed a slight reduction in IGF-I concentrations with increasing gestational age in both males (regression coefficient [SEM] -0.005 [0.002], $p = 0.01$) and females (regression coefficient [SEM] -0.004 [0.002], $p = 0.03$). Gestational age was not associated with IGF-II concentrations in either males (regression coefficient [SEM] 0 [0], $p = 0.4$) or females (regression coefficient [SEM] 0.001 [0], $p = 0.06$). IGF-I and -II concentrations in term male and female infants according to gestational age are presented in Figure 1.

Correlation with Anthropometry and Early Growth

Z-scores for gestational age- and sex-specific IGF-I concentrations at birth correlated with weight, length, and OFC Z-scores at birth. The strongest association was with birth weight ($R^2 = 0.19$). Length and weight Z-scores at age 2 months correlated significantly with IGF-I concentrations at birth, but the strength of the association was lower than at birth. Over 2 months, weight R^2 decreased from 0.19 to 0.02 and length R^2 decreased from 0.07 to 0.04. Although IGF-II concentrations correlated

Fig. 1. Sex-specific centile charts for IGF-I (a, b) and IGF-II (c, d) concentrations in term infants in a healthy population-based cohort, according to gestational age from 37 to 42 weeks' gestation.

Table 2. The relationship between sex- and gestational age-corrected IGF-I and IGF-II Z-scores and growth parameters at birth and 2 months, and growth trajectory from birth to 2 months

	IGF-I			IGF-II		
	<i>R</i> ²	regression coefficient (SEM)	<i>p</i>	<i>R</i> ²	regression coefficient (SEM)	<i>p</i>
<i>Z-scores at birth</i>						
Length	0.07	0.28 (0.031)	<0.001	0.004	0.071 (0.033)	0.03
Weight	0.19	0.51 (0.032)	<0.001	0.005	0.088 (0.036)	0.01
Head circumference	0.028	0.174 (0.03)	<0.001	0.004	0.066 (0.032)	0.04
<i>Z-scores at 2 months</i>						
Length	0.04	0.206 (0.032)	<0.001	0.004	0.069 (0.033)	0.04
Weight	0.022	0.152 (0.033)	<0.001	0.002	0.048 (0.034)	0.16
Head circumference	0.001	0.04 (0.035)	0.24	0.003	0.063 (0.035)	0.06
<i>Delta Z-score from birth to 2 months</i>						
Length	0.002	−0.05 (0.04)	0.14	0.001	0.01 (0.04)	0.8
Weight	0.07	−0.31 (0.04)	<0.001	0.001	−0.03 (0.04)	0.4
Head circumference	0.02	−0.17 (0.04)	<0.001	0.001	−0.02 (0.04)	0.5

Length, weight, and head circumference are presented as sex-, age-, and gestation age-corrected Z-scores. For this analysis, IGF-I and -II are the dependent variables and anthropometric measures are independent variables.

Table 3. Multiple linear regression analysis of IGF-I and -II Z-scores at birth with birth weight, length, and head circumference, gestational age, and maternal BMI at 15 weeks

	IGF-I Z-score (<i>R</i> ² = 0.21)			IGF-II Z-score (<i>R</i> ² = 0.01)		
	B	SE	<i>p</i>	B	SE	<i>p</i>
Weight Z-score at birth	0.66	0.05	<0.001	0.04	0.06	0.5
Length Z-score at birth	−0.04	0.38	0.3	0.03	0.04	0.5
Head circumference Z-score at birth	−0.15	0.03	<0.001	0.03	0.04	0.4
Maternal BMI at 15 weeks	−0.02	0.01	0.01	0.01	0.01	0.1
Gestational age	0.12	0.03	0.6	0.01	0.03	0.7

significantly with weight, length, and OFC at birth, this accounted for <1% of the variance seen in each of these parameters (Table 2). Multiple linear regression analysis including these measurements at birth with maternal BMI at 15 weeks' gestation and gestational age increased the *R*² for IGF-I to 0.21 and for IGF-II Z-score to 0.01 (Table 3).

Both IGF-I and -II concentrations at birth were associated with length at age 2 months (*p* < 0.001 and *p* = 0.04, respectively), while only IGF-I concentration at birth was associated with weight at 2 months (*p* < 0.001). The rates of change in OFC and weight Z-scores over the first 2 months of life were negatively associated with IGF-I con-

centrations at birth. IGF-II concentrations at birth were not associated with growth trajectories in weight, length, or OFC over the first 2 months (Fig. 2).

An increase in weight, OFC, and length Z-score of 1 or more over the first 2 months was seen in 198, 250, and 343 infants, respectively. Those with an increase in weight Z-score of 1 or more had significantly lower mean IGF-I Z-scores at birth (−0.6 vs. 0.1, *p* < 0.001), but not IGF-II Z-scores (−0.06 vs. 0.02, *p* = 0.4). Similarly, infants with an increase in OFC Z-score during the first 2 months tended to have lower IGF-I Z-scores (−0.2 vs. 0.05, *p* = 0.003) but not IGF-II Z-scores (−0.08 vs. 0.02, *p* = 0.2). No difference was seen in IGF-I (*p* = 0.9) or IGF-II (*p* = 0.9) Z-scores at

Table 4. Comparison of gestational age- and sex-corrected mean (SD) Z-scores for IGF-I and -II, according to history of maternal smoking, maternal obesity, preeclampsia, or length, weight or head circumference at birth

	IGF-I Z-score			IGF-II Z-score		
	yes	no	<i>p</i>	yes	no	<i>p</i>
Smoking during first trimester	−0.2 (1) <i>n</i> = 277	0.07 (0.99) <i>n</i> = 823	<0.001	−0.07 (0.97) <i>n</i> = 277	0.01 (1.04) <i>n</i> = 823	0.2
Maternal BMI >30 at 15 weeks' gestation	−0.07 (1.1) <i>n</i> = 137	0.01 (0.99) <i>n</i> = 963	0.4	0.05 (1.2) <i>n</i> = 137	−0.02 (1) <i>n</i> = 963	0.5
Preeclampsia	−0.31 (0.85) <i>n</i> = 34	0.01 (1) <i>n</i> = 1,066	0.04	−0.13 (1.01) <i>n</i> = 34	−0.01 (0.01) <i>n</i> = 1,066	0.5
Birth length <10th percentile	−0.79 (0.87) <i>n</i> = 48	0.04 (1) <i>n</i> = 1,052	<0.001	−0.33 (0.99) <i>n</i> = 48	0 (1.02) <i>n</i> = 1,052	0.03
Birth weight <10th percentile	−1.17 (0.77) <i>n</i> = 59	0.07 (0.97) <i>n</i> = 1,041	<0.001	−0.35 (0.94) <i>n</i> = 59	0.01 (1.03) <i>n</i> = 1,041	0.01
Head circumference <10th percentile	−0.21 (1.01) <i>n</i> = 139	0.03 (1) <i>n</i> = 961	0.01	−0.31 (1.2) <i>n</i> = 139	0.03 (1) <i>n</i> = 961	0.001

Fig. 2. The relationship between IGF-I and -II Z-scores at birth with head circumference, weight, and length Z-scores at birth.

birth in infants who had an increase in length Z-score of 1 or more. Lower mean (SD) birth weight Z-scores were seen in infants with increases in weight (−0.5 vs. 0.1, $p < 0.001$), length (−0.08 vs. 0.09, $p < 0.001$), and OFC (−0.3 vs. 0.1, $p < 0.001$) Z-scores of 1 or more in the first 2 months.

Factors Influencing IGF Concentrations

Of the 1,100 infants included in this study, 40 had birth length and 57 had birth weight below the 10th percentile. In those with birth length below the 10th percentile, IGF-I Z-scores (−0.7 vs. 0.1, $p < 0.001$) and IGF-II Z-scores (−0.3 vs. 0, $p = 0.03$) at birth were lower (Fig. 3d). This was

(For legend see next page.)

also seen in infants with birthweight below the 10th percentile (IGF-I Z-scores -1.2 vs. 0.07 , $p < 0.001$; IGF-II Z-scores -0.4 vs. 0 , $p = 0.01$) (Fig. 3e).

Maternal cigarette smoking during the first trimester and preeclampsia were associated with lower mean (SD) cord IGF-I concentrations ($p < 0.001$ and $p = 0.04$, respectively) but not IGF-II concentrations ($p = 0.2$ and $p = 0.5$, respectively). Maternal BMI >30 ($n = 137$) at 15 weeks' gestation was not associated with increased umbilical cord IGF-I or -II concentrations ($p = 0.4$ and 0.5 , respectively) (Table 4).

Discussion

We have described the largest reported population of infants with IGF-I and -II concentrations measured at birth using LCMS. We have used an LCMS assay that is less susceptible to IGFBP interference [27], thus generating accurate measurements at an age where IGF-I concentrations tend to be low. Our large cohort of well-characterized term infants allowed us to investigate the relationship between these measurements and birth size and early infant growth. Consistent with previous studies using other assays, IGF-I and -II concentrations at birth were associated with weight, length, and OFC at birth. At age 2 months, weight and length were associated with birth IGF-I concentrations whereas only length was associated with birth IGF-II concentrations. Low IGF-I concentrations at birth were associated with accelerated increases in weight and OFC Z-scores over the first 2 months. Maternal smoking and preeclampsia were associated with lower cord IGF-I but not IGF-II concentrations.

The correlation of an infant's birth weight and length with IGF-I concentration has been shown previously in smaller studies using radioimmunoassays [11, 16, 19, 34, 35]. The relationship between birth weight and IGF-II levels are less well defined, with some studies showing no correlation [35, 36]. Our results support the findings of other studies, where a weak association has been shown [16, 37]. This weak association between cord IGF-II and neonatal anthropometry is surprising given the signifi-

cant effect of mutations affecting IGF-II signaling on mouse [8] and human [6, 7] size at birth. Possible explanations for this include a potential neutralizing effect of circulating IGF-II receptors on the bioavailability of circulating IGF-II [16]. This interference may be of clinical significance but it does not affect IGF-II measurement by LCMS. Alternatively, IGF-II may be more important in regulating growth prior to the third trimester [38, 39], and IGF-II concentrations at birth may be clinically less significant.

Infants with lower IGF-I concentrations at birth had a greater change in weight and OFC over the first 2 months of life. The mechanism of this effect is unclear, but this finding may be related to low IGF-I concentrations being a marker of nutritional status [40]. Umbilical cord leptin concentration is a marker of nutritional status at birth and an inverse relationship also is seen between early growth and cord leptin [41]. IGF-I concentrations at birth correlate with adiposity and cord leptin concentrations [42]. Thus, this may represent "catch-up" growth in infants born small for gestational age who initially have lower IGF-I concentrations.

Birth IGF-II concentrations do not correlate with early infant growth in any of these parameters, further supporting the interpretation that the role of IGF-II in fetal growth is less clinically significant by the time of birth.

Maternal smoking has been shown previously to be associated with reduced cord IGF-I concentrations in females [11]. However, we have shown that this effect is seen both in male and female infants. The interplay between IGF-I and -II and placental function may provide insight into this relationship. Placental vasculature is affected by maternal smoking, with reduced length of villous capillaries and decreased trophoblast volume [43]. This may affect nutrient availability to the fetus and subsequently reduce growth parameters and IGF-I concentrations. IGF-I and -II are both expressed in chorionic and basal plates of the placenta [44], and thus may play a key role in regulating placentation.

The main strengths of this study in evaluating the relationship between IGFs and infant size are the large number of healthy term infants included, and the use of an LCMS assay. This assay has been shown previously to be free from IGFBP interference when measuring IGF-I and -II concentrations [27]. A limitation of this study is that the Cork BASELINE birth cohort was a relatively homogenous population of Caucasian Irish healthy term infants. Consequently, it is not known if the reference data can be applied directly to other patient populations. This may be particularly important when considering the sig-

Fig. 3. The effect of smoking (a), preeclampsia (b), and maternal obesity (c) on umbilical cord IGF-I and -II age- and-sex specific Z-scores. The association of birth length (d), birth weight (e), and head circumference (f) below the 10th centile with cord IGF-I and -II concentrations are also shown.

nificant effect of smoking during the first trimester on cord IGF-I concentrations, as a maternal cigarette use occurred during a quarter of pregnancies included here. IGF-I concentrations may be marginally higher in populations with lower rates of cigarette smoking, such as America, where 13% of all females smoke [45]. In addition, our findings are only applicable to term infants and it is not known if the associations are also seen in preterm infants. Not all infants in this cohort had cord blood available for analysis, and it is not known if this introduced bias to our study, although this is unlikely as our findings are generally consistent with previous smaller studies. A small number of subjects had IGF-I and -II concentrations below the detectable limits of the assay, and this may also have influenced our results.

In conclusion, this study has provided reference data for IGF-I and IGF-II concentrations at birth in term infants, as measured by LCMS. These data will be useful clinically, where prenatal defects of IGF production are suspected, and may support future research in evaluating the prenatal role of these growth factors. We recommend using the LCMS assay when measuring IGF-I and -II in conditions where IGFBP interference may significantly affect interpretation of results, particularly in young infants where IGF concentrations are generally low [22]. Using this assay, we have corroborated many of the previously known associations between IGF-I concentrations and anthropometry at birth, demonstrated a weaker but significant association between IGF-II and anthropometry at birth, and shown a negative association between IGF-I measurements at birth and change in weight or OFC over the first 2 months of life.

Acknowledgement

The authors would like to acknowledge Julia Larsen at Quest Diagnostics and Klaudia Sikora at University College Cork for sample handling and data retrieval.

Disclosure Statement

Financial Disclosure

A.G. serves on the Steering Committee of the Pfizer International Growth Study Database. Z.W., M.J.M., and R.E.R. are employees and stockholders of Quest Diagnostics. All other authors have no financial relationships relevant to this article to disclose.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

Funding Sources

Dr. Hawkes is supported by a PhD grant by the National Children's Research Centre, Dublin, Ireland, and Dr. Grimberg was supported by grant 1R01 HD57037 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The SCOPE Ireland Study was funded by the Health Research Board of Ireland (CSA 02/2007). L.C.K. is a Science Foundation Ireland (SFI) principal investigator (PI) (08/IN.1/B2083). L.C.K., D.M.M. J.O'B H., A.D.I. and M.K. are PIs in the SFI-funded Research Centre, INFANT (12/RC/2272). The Cork BASELINE birth cohort is funded by the National Children's Research Centre.

References

- 1 Aguiar-Oliveira MH, Gill MS, de ABES, Alcantara MR, Miraki-Moud F, Menezes CA, Souza AH, Martinelli CE, Pereira FA, Salvatore R, Levine MA, Shalet SM, Camacho-Hubner C, Clayton PE: Effect of severe growth hormone (GH) deficiency due to a mutation in the GH-releasing hormone receptor on insulin-like growth factors (IGFs), IGF-binding proteins, and ternary complex formation throughout life. *J Clin Endocrinol Metab* 1999;84:4118–4126.
- 2 Hawkes CP, Levitt Katz LE: Growth factor regulation of fetal growth; in Polin RA, Fox WW, Abman SH (eds): *Fetal and Neonatal Physiology*. Amsterdam, Elsevier/Saunders, 2016, pp 1461–1470.
- 3 Savage MO, Blum WF, Ranke MB, Postel-Vinay MC, Cotterill AM, Hall K, Chatelain PG, Preece MA, Rosenfeld RG: Clinical features and endocrine status in patients with growth hormone insensitivity (Laron syndrome). *J Clin Endocrinol Metab* 1993;77:1465–1471.
- 4 Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfaffle R, Raile K, Seidel B, Smith RJ, Chernausk SD; Intrauterine Growth Retardation Study Group: IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003;349:2211–2222.
- 5 Klammt J, Kiess W, Pfaffle R: IGF1R mutations as cause of SGA. *Best Pract Res Clin Endocrinol Metab* 2011;25:191–206.
- 6 St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D, Bouchard L: IGF2 DNA methylation is a modulator of newborn's fetal growth and development. *Epigenetics* 2012;7:1125–1132.
- 7 Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Buttel HM, Schweizer R, van Wolkum W, Binder G, Eggermann T: Paternally inherited IGF2 mutation and growth restriction. *N Engl J Med* 2015;373:349–356.
- 8 Baker J, Liu JP, Robertson EJ, Efstratiadis A: Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 1993;75:73–82.
- 9 DeChiara TM, Robertson EJ, Efstratiadis A: Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 1991;64:849–859.

- 10 Vatten LJ, Nilsen ST, Odegard RA, Romundstad PR, Austgulen R: Insulin-like growth factor I and leptin in umbilical cord plasma and infant birth size at term. *Pediatrics* 2002;109:1131–1135.
- 11 Clifton VL, Hodyl NA, Murphy VE, Giles WB, Baxter RC, Smith R: Effect of maternal asthma, inhaled glucocorticoids and cigarette use during pregnancy on the newborn insulin-like growth factor axis. *Growth Horm IGF Res* 2010;20:39–48.
- 12 Vidal AC, Murtha AP, Murphy SK, Fortner K, Overcash F, Henry N, Schildkraut JM, Forman MR, Demark-Wahnefried W, Kurtzberg J, Jirtle R, Hoyo C: Maternal BMI, IGF-I levels, and birth weight in African American and white infants. *Int J Pediatr* 2013;2013:191472.
- 13 Christians JK, Gruslin A: Altered levels of insulin-like growth factor binding protein proteases in preeclampsia and intrauterine growth restriction. *Prenat Diagn* 2010;30:815–820.
- 14 Vatten LJ, Odegard RA, Nilsen ST, Salvesen KA, Austgulen R: Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight. *Obstet Gynecol* 2002;99:85–90.
- 15 Rohrmann S, Sutcliffe CG, Bienstock JL, Monsegue D, Akereyeni F, Bradwin G, Rifai N, Pollak MN, Agurs-Collins T, Platz EA: Racial variation in sex steroid hormones and the insulin-like growth factor axis in umbilical cord blood of male neonates. *Cancer Epidemiol Biomarkers Prev* 2009;18:1484–1491.
- 16 Ong K, Kratzsch J, Kiess W, Costello M, Scott C, Dunger D: Size at birth and cord blood levels of insulin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-3, and the soluble IGF-II/mannose-6-phosphate receptor in term human infants. The ALSPAC Study Team. *Avon Longitudinal Study of Pregnancy and Childhood. J Clin Endocrinol Metab* 2000;85:4266–4269.
- 17 Wiznitzer A, Reece EA, Homko C, Furman B, Mazor M, Levy J: Insulin-like growth factors, their binding proteins, and fetal macrosomia in offspring of nondiabetic pregnant women. *Am J Perinatol* 1998;15:23–28.
- 18 Shibata A, Harris DT, Billings PR: Concentrations of estrogens and IGFs in umbilical cord blood plasma: a comparison among Caucasian, Hispanic, and Asian-American females. *J Clin Endocrinol Metab* 2002;87:810–815.
- 19 Klauwer D, Blum WF, Hanitsch S, Rascher W, Lee PD, Kiess W: IGF-I, IGF-II, free IGF-I and IGFBP-1, -2 and -3 levels in venous cord blood: relationship to birthweight, length and gestational age in healthy newborns. *Acta Paediatr* 1997;86:826–833.
- 20 Daughaday WH, Parker KA, Borowsky S, Trivedi B, Kapadia M: Measurement of somatomedin-related peptides in fetal, neonatal, and maternal rat serum by insulin-like growth factor (IGF) I radioimmunoassay, IGF-II radioreceptor assay (RRA), and multiplication-stimulating activity RRA after acid-ethanol extraction. *Endocrinology* 1982;110:575–581.
- 21 Blum WF, Breier BH: Radioimmunoassays for IGFs and IGFBPs. *Growth Regul* 1994;4(suppl 1):11–19.
- 22 Hawkes CP, Grimberg A: Measuring growth hormone and insulin-like growth factor-I in infants: what is normal? *Pediatr Endocrinol Rev* 2013;11:126–146.
- 23 Wang HS, Lee JD, Soong YK: Effects of labor on serum levels of insulin and insulin-like growth factor-binding proteins at the time of delivery. *Acta Obstet Gynecol Scand* 1995;74:186–193.
- 24 Hills FA, Crawford R, Harding S, Farkas A, Chard T: The effects of labor on maternal and fetal levels of insulin-like growth factor binding protein-1. *Am J Obstet Gynecol* 1994;171:1292–1295.
- 25 Chanson P, Arnoux A, Mavromati M, Brailly-Tabard S, Massart C, Young J, Piketty ML, Souberbielle JC: VARIETE Investigators: Reference values for IGF-I serum concentrations: comparison of six immunoassays. *J Clin Endocrinol Metab* 2016;101:3450–3458.
- 26 Bredehoft M, Schanzer W, Thevis M: Quantification of human insulin-like growth factor-1 and qualitative detection of its analogues in plasma using liquid chromatography/electrospray ionisation tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2008;22:477–485.
- 27 Bystrom C, Sheng S, Zhang K, Caulfield M, Clarke NJ, Reitz R: Clinical utility of insulin-like growth factor 1 and 2; determination by high resolution mass spectrometry. *PLoS One* 2012;7:e43457.
- 28 O'Donovan SM, Murray DM, Hourihane JO, Kenny LC, Irvine AD, Kiely M: Cohort profile: The Cork BASELINE Birth Cohort Study: Babies after SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints. *Int J Epidemiol* 2014;44:764–775.
- 29 Cole TJ, Freeman JV, Preece MA: British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 1998;17:407–429.
- 30 Vidmar SI, Cole TJ, Pan H: Standardizing anthropometric measures in children and adolescents with functions for egen: update. *Stata J* 2013;13:366–378.
- 31 Bystrom CE, Sheng S, Clarke NJ: Narrow mass extraction of time-of-flight data for quantitative analysis of proteins: determination of insulin-like growth factor-1. *Anal Chem* 2011;83:9005–9010.
- 32 Cole TJ: The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990;44:45–60.
- 33 Cole TJ, Green PJ: Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992;11:1305–1319.
- 34 Simmons D: Interrelation between umbilical cord serum sex hormones, sex hormone-binding globulin, insulin-like growth factor I, and insulin in neonates from normal pregnancies and pregnancies complicated by diabetes. *J Clin Endocrinol Metab* 1995;80:2217–2221.
- 35 Osorio M, Torres J, Moya F, Pezzullo J, Salafia C, Baxter R, Schwander J, Fant M: Insulin-like growth factors (IGFs) and IGF binding proteins-1, -2, and -3 in newborn serum: relationships to fetoplacental growth at term. *Early Hum Dev* 1996;46:15–26.
- 36 Lassarre C, Hardouin S, Daffos F, Forestier F, Franken F, Binoux M: Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. *Pediatr Res* 1991;29:219–225.
- 37 Samaan NA, Schultz PN, Pham FK: Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns. *Am J Obstet Gynecol* 1990;163:1836–1839.
- 38 Langford K, Nicolaides K, Miell JP: Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. *Hum Reprod* 1998;13:1389–1393.
- 39 Langford K, Blum W, Nicolaides K, Jones J, McGregor A, Miell J: The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? *Eur J Clin Invest* 1994;24:851–856.
- 40 Hawkes CP, Grimberg A: Insulin-like growth factor-I is a marker for the nutritional state. *Pediatr Endocrinol Rev* 2015;13:465–477.
- 41 Chaoimh CN, Murray DM, Kenny LC, Irvine AD, Hourihane JO, Kiely M: Cord blood leptin and gains in body weight and fat mass during infancy. *Eur J Endocrinol* 2016;175:403–410.
- 42 Kadakia R, Ma M, Josefson JL: Neonatal adiposity increases with rising cord blood IGF-1 levels. *Clin Endocrinol (Oxf)* 2016;85:70–75.
- 43 Larsen LG, Clausen HV, Jonsson L: Stereologic examination of placentas from mothers who smoke during pregnancy. *Am J Obstet Gynecol* 2002;186:531–537.
- 44 Iniguez G, Gonzalez CA, Argandona F, Karkieka E, Johnson MC, Cassorla F: Expression and protein content of IGF-I and IGF-I receptor in placentas from small, adequate and large for gestational age newborns. *Horm Res Paediatr* 2010;73:320–327.
- 45 World Health Organization: Tobacco: Data and Statistics. Copenhagen, WHO Regional Office for Europe, 2017.