

## Insulin-Like Growth Factor II and Binding Proteins 1 and 3 from Second Trimester Human Amniotic Fluid Are Associated with Infant Birth Weight<sup>1,2</sup>

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**ABSTRACT** The developing fetus begins to swallow amniotic fluid (AF) early in gestation, a process that results in ingestion of numerous growth factors. Our objectives were 2-fold: 1) to assess the concentration and distribution of insulin-like growth factor II (IGF II) and its binding proteins (BP) 1 and 3 in 2nd trimester amniotic fluid using ELISA, and 2) to establish whether concentrations of AF IGF II and its binding proteins IGF BP1 and 3, measured early in pregnancy, were associated with and predictive of infant birth weight. Birth weights were categorized using recently developed birth-weight-for-gestational-age percentiles for fetal growth in which infants <10% were classified as SGA (small-for-gestational-age) and those >90% as LGA (large-for-gestational-age). AF samples were collected after routine genetic testing (15.1 ± 0.04 wk, range 12–20 wk) from 543 mother-infant pairs in Montreal, QC, Canada. Maternal and fetal characteristics were obtained from questionnaires and medical chart review. Multivariate regression analysis that controlled for maternal height, prepregnancy weight, smoking behavior, infant gender, gestational age, parity, as well as amniocentesis week showed that higher AF IGF BP1 was associated with lower birth weight (partial  $r^2 = 0.0062$ ). Regression analyses revealed that AF IGF BP3 was positively associated with birth weight within LGA and macrosomia subpopulations (partial  $r^2 = 0.0283$  and 0.0404, respectively). These results show that 2nd trimester AF IGF BP1, BP3, and IGF II may emerge as early indicators of fetal growth. *J. Nutr.* 135: 1667–1672, 2005.

**KEY WORDS:** • birth weight • insulin-like growth factor • fetal growth • somatomedins • amniotic fluid

Amniotic fluid (AF)<sup>4</sup> is crucial to fetal health. Aside from protecting the fetus from mechanical and thermal shock, possessing antimicrobial action, and assisting in acid-base balance, AF contains nutritional factors including proteins (1,2). These proteins enter the AF from both maternal and fetal sources (3,4). Fetal swallowing accounts for 80% of AF protein clearance in late gestation (5). Previous studies identified several proteins in human AF (3,6), including a range of growth factors such as insulin-like growth factor (IGF) II and 2 of its binding proteins, IGF BP1 and BP 3 (7,8).

IGF II is considered to be a major modulator of early embryonic and fetal growth (9–11). IGF II promotes differentiation of cells by endocrine (11,12) and autocrine (13) means. IGF II is synthesized by the developing fetal lung in the first

trimester (9,14). IGF II has a 3.2-fold greater concentration in early 2nd trimester AF than IGF I (15), which supports the general belief that IGF II predominates over IGF I in human AF from 9 to 12 wk (16); thereafter, it increases rapidly and peaks at 19 wk (17) of gestation. Although less potent than IGF I, IGF II with its later emerging endocrine function can increase glucose uptake (11). The role of IGF II as a key fetal growth factor is further supported by the creation of a growth-deficiency phenotype produced after disruption of IGF II gene expression in mice (18). Human AF IGF II has been less extensively examined, but one study showed that IGF II was not correlated with infant birth weight if gestational age was corrected for, but was correlated with placental weight and the birth weight:placental weight ratio (19).

Evidence also exists demonstrating important regulatory roles for IGF BPs during fetal growth and development. IGF BPs are established regulators of the action of IGFs and can both augment (20) and inhibit (21) IGF effects. IGF BP1, produced primarily by maternal deciduas (15) as well as fetal liver and kidney (21), is considered the most abundant IGF BP in human AF (21); it increases 20-fold from 9 to 12 wk (16). IGF BP1 binds to and modulates the activity of both IGF I and IGF II (13). Interestingly, a relation with birth weight was established for AF IGF BP1 in which high 2nd trimester AF IGF BP1 concentrations were associated with lower infant birth weight (21,22). However, the existence of this relation

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<sup>4</sup> Abbreviations used: AF, amniotic fluid; AGA, appropriate-for-gestational-age; ANCOVA, analysis of covariance; IGF insulin-like growth factor; IGF BP, IGF binding protein; LBW, low birth weight; LGA, large-for-gestational-age; SGA, small-for-gestational-age.

was disputed in another human study that showed that once gestational age was corrected for, AF IGF BP1 remained strongly correlated with amniotic fluid IGF I and IGF II levels, but not with infant birth weight (19). These authors also concluded that high IGF BP1 did not predict small-for-gestational-age (SGA), but was associated with lower placental weights (19).

In sharp contrast to the possible negative relation of IGF BP1 with fetal growth, there is the suggestion in the literature that IGF BP3, which increases 20-fold from 9 to 12 wk in human amniotic fluid (16), may promote IGF 1 action and be positively associated with fetal growth. Through in utero catheterization and infusion, AF IGF BP3 was also correlated in vivo with late gestational IGF I binding in fetal sheep and the regulated delivery of IGF 1 to the developing fetus late in gestation (23). Recently, lower AF IGF BP3 was associated with intrauterine growth retardation in humans (24).

The literature suggests that these 2 AF IGF BPs, present in high concentrations in 2nd trimester AF (17,19), could have opposite effects on fetal growth and development in humans. In a recent publication (25) we demonstrated that AF total protein was inversely associated with infant birth weight. In the current study, we hypothesize that AF IGF BP1, the binding protein found in greatest abundance in amniotic fluid (7,19), would demonstrate this same inverse relation. We also investigated whether IGF BP3 or IGF II was associated with infant birth weight. We studied a large population of pregnant women ( $n = 543$  mother-infant pairs) undergoing age-related amniocentesis (12–20 wk) for genetic testing to establish whether these AF growth factors were associated with infant birth weight. With our large cohort, we provided a more comprehensive multivariate regression analysis model than previously published studies.

## SUBJECTS AND METHODS

**Design, recruitment and consent.** From 1998–2002, pregnant women undergoing age-related amniocentesis at St Mary's Hospital Center in Montreal, Canada were approached to participate in this prospective study; <50% of the population overlapped with that of our previously published study (25). Ethics approval was obtained from Institutional Review Boards of McGill, Montreal Children's Hospital, and St Mary's Hospital Centre. Signed consents allowed researchers to obtain amniotic fluid after genetic testing and to access medical charts. Exclusion criteria included genetic abnormalities, multiple pregnancy, subsequent diagnosis of gestational diabetes mellitus, and prematurity (<37 wk). Analyzed AF samples were matched to medical record information including prepregnancy weight, height, BMI, smoking behavior, age, amniocentesis week, parity and ethnicity, infant gestational age, gender, and birth weight. Infant gender, gestational age, and birth weight were then used to calculate birth-weight-for-gestational-age (26). Gestational age was based on physicians' estimates using last menstrual period and uniform hospital protocols. Completeness of each data subset depended on the availability of information in medical charts and on questionnaires and a sufficient quantity of AF after genetic testing.

**Biochemical analysis.** Amniotic fluid samples, stored at  $-80^{\circ}\text{C}$ , were analyzed for IGF II ( $n = 388$ ), IGF BP3 ( $n = 401$ ), and IGF BP1 ( $n = 543$ ) by ELISA using Diagnostics Systems Laboratories (DSL) kits 10–9100, 10–6600, and 10–7800, respectively. These kits were chosen for the following reasons: the IGF BP1 kit is unaffected by phosphorylation state of IGF BP1, which can result in underestimation of total IGF BP1 (27,28). The IGF II methodology, which uses a modified acidification followed by ethanol precipitation to extract IGF II from its binding proteins where <5% is free, extracts as effectively as size-exclusion gel chromatography in acid (29). Extraction of IGF II ensures that the binding proteins are not permitted to sequester IGF II in the reaction mixture (30,31), thus allowing for

more accurate quantification. The IGF BP3 kit is able to detect IGF BP3 despite glycosylation state and whether it has been proteolyzed (32); both of these can complicate detection and quantification (33–36).

**Statistical analysis.** All analyses were done using SAS Version 8.02. ANOVA and analysis of covariance (ANCOVA) were performed by dividing the study population into subgroups according to clinical classifications of infant gender-corrected birth-weight-for-gestational-age (26) including SGA (<10%), appropriate-for gestational age (AGA; 10–90%), and large-for-gestational-age (LGA; >90%) followed by post-hoc testing using Scheffé's test ( $P < 0.05$ ). Data were reported as means  $\pm$  SEM unless otherwise indicated. Nonlinear data (prepregnancy week, BMI, amniocentesis week, IGF II, and IGF BP3) were square-root transformed. Covariates for analysis included maternal height, prepregnancy weight, ethnicity, parity, and infant gender, due to previously established associations with birth weight (26,37) and amniocentesis week due to its effect on 2nd trimester AF IGF concentrations (16,38,39). Multivariate regression analysis for birth weight was performed on the whole population and within the clinical classifications of fetal growth including those categories based on infant birth weight in grams: low birth weight (LBW; <2500 g), normal birth weight (2500–4000 g) and macrosomia (>4000 g) and newer percentile categories that correct for infant gender and gestational age (SGA, AGA, and LGA). Significance was established at  $P < 0.05$ .

## RESULTS

The study population consisted of 543 multiethnic (69% Caucasian, 16% Asian, and 15% Black, Middle-Eastern, and Hispanic), nonsmoking (88%), mother-infant pairs; the majority (54%) were normal weight, with only 28% overweight/obese. Maternal prepregnancy BMI ranged from 23 to 25  $\text{kg}/\text{m}^2$ . Birth weight was  $3510 \text{ g} \pm 20$ . Based on gender-corrected birth weight percentiles, our population consisted of 5, 83, and 11% SGA, AGA, and LGA, respectively; if classified by traditional gram weight, 1, 83, and 16% were LBW, normal, and macrosomic infants, respectively. As expected, maternal height and prepregnancy weight were greater in mothers of LGA infants compared with those of SGA and AGA infants (Table 1). Population distributions showed a wide range of concentrations for each of the AF growth factors (Fig. 1). IGF II did not differ across amniocentesis week, whereas both IGF BP1 and BP3 increased as gestation progressed (data not shown).

Comparisons of birth weight across gender-corrected birth-weight-for-gestational-age categories showed that IGF BP1 differed when the following covariates were included: maternal height, prepregnancy weight, ethnicity, parity, infant gender and amniocentesis week (Table 1). Concentrations of IGF-BP 1 were lower in LGA compared with AGA and in macrosomic compared with LBW infants. Multiple regression analyses revealed that IGF BP1 was negatively associated with infant birth weight in our study population (Table 2). Moreover, IGF BP3 was a significant predictor of birth weight in both LGA and macrosomic infants (Table 2). Interestingly, gestational age was not associated with infant birth weight using macrosomia as a classification variable, but it was associated with infant birth weight when the gender-corrected LGA classification was used.

## DISCUSSION

Our goal was to establish whether AF IGF II and its 2 binding proteins, AF IGF BP1 and AF IGF BP3, measured

TABLE 1

Differences in maternal, infant and human amniotic fluid characteristics across gender-corrected birth-weight-for-gestational-ages SGA (<10%), AGA (10–90%), and LGA (>90%)<sup>1</sup>

Characteristic	SGA	AGA	LGA	F	P
<b>Infant</b>					
<i>n</i>	20–28	323–451	46–65		
Birth weight, <i>g</i>	2744 ± 48 <sup>a</sup>	3440 ± 16 <sup>b</sup>	4330 ± 41 <sup>c</sup>	275	<0.0001
Gender, % female	44.7	51.0	44.9	2.03	NS
Gestational age, <i>wk</i>	39.8 ± 0.2	39.7 ± 0.05	39.9 ± 0.1	1.25	NS
<b>Maternal</b>					
Height, <i>m</i>	1.60 ± 0.01 <sup>a</sup>	1.62 ± 0.003 <sup>a</sup>	1.66 ± 0.01 <sup>b</sup>	8.86	0.0002
Prepregnancy weight, <i>kg</i>	60.1 ± 2.5 <sup>a</sup>	61.3 ± 0.5 <sup>a</sup>	70.4 ± 1.9 <sup>b</sup>	17.12	<0.0001
BMI, <i>kg/m</i> <sup>2</sup>	23.5 ± 0.9 <sup>ab</sup>	23.3 ± 0.2 <sup>a</sup>	25.5 ± 0.6 <sup>b</sup>	7.80	0.0005
Ethnicity, %				1.03	NS
Caucasian	52.6	58.1	63.3		
Asian	28.9	22.4	14.3		
Other <sup>2</sup>	18.4	19.5	22.5		
Nonsmoking, %	84.2	83.4	93.8	1.32	NS
Parity	1.03 ± 0.3	1.1 ± 0.05	1.3 ± 0.1	1.17	NS
<b>Amniotic fluid</b>					
Amniocentesis week, <i>wk</i>	15.6 ± 0.2 <sup>b</sup>	15.1 ± 0.05 <sup>a</sup>	15.3 ± 0.1 <sup>ab</sup>	4.22	0.0152
<b>IGF, <math>\mu\text{g/L}</math></b>					
IGF II	89.5 ± 7.5	87.8 ± 1.5	90.0 ± 3.3	0.22	NS
ANCOVA <sup>3</sup>				0.15	NS
BP 1	43,935 ± 8152	36,498 ± 1458	28,220 ± 3582	2.93	NS
ANCOVA <sup>3</sup>	— <sup>ab</sup>	— <sup>b</sup>	— <sup>a</sup>	3.94	0.0202
BP 3	2685 ± 504	2415 ± 101	2849 ± 301	1.32	NS
ANCOVA <sup>3</sup>				0.50	NS

<sup>1</sup> Values are means ± SEM. Means in a row with superscripts without a common letter differ,  $P < 0.05$ . NS = not significant ( $P > 0.05$ ).

<sup>2</sup> Other includes Black, Middle-Eastern, and Hispanic.

<sup>3</sup> Covariates included maternal height, prepregnancy weight, ethnicity, parity, infant gender, amniotic fluid pH, and amniocentesis week.

early in gestation, were associated with infant birth weight. Five important points emerged: 1) AF IGF BP1, as previously shown for total protein (25), was inversely associated with birth weight; 2) AF IGF II was negatively associated with birth weight in macrosomic and LGA infants only; 3) there was a direct link between IGF BP3, macrosomic, and LGA infants; 4) AF IGF BP1 and BP3, but not AF IGF II, increased with gestational age (16); and 5) previously established predictors for birth weight (37) may not be applicable in conditions of

excessive fetal growth. Taken together, our findings describe for the first time in vivo a positive and a negative predictive relation for birth weight between the 2 IGF binding proteins, suggesting that both of these binding proteins may emerge as important early prognosticators of fetal growth. Moreover, biological mechanisms for these opposing BP relations can be supported, but the underlying mechanism for the negative association between AF IGF II and birth weight for infants classified as LGA and macrosomic at term warrants further investigation.

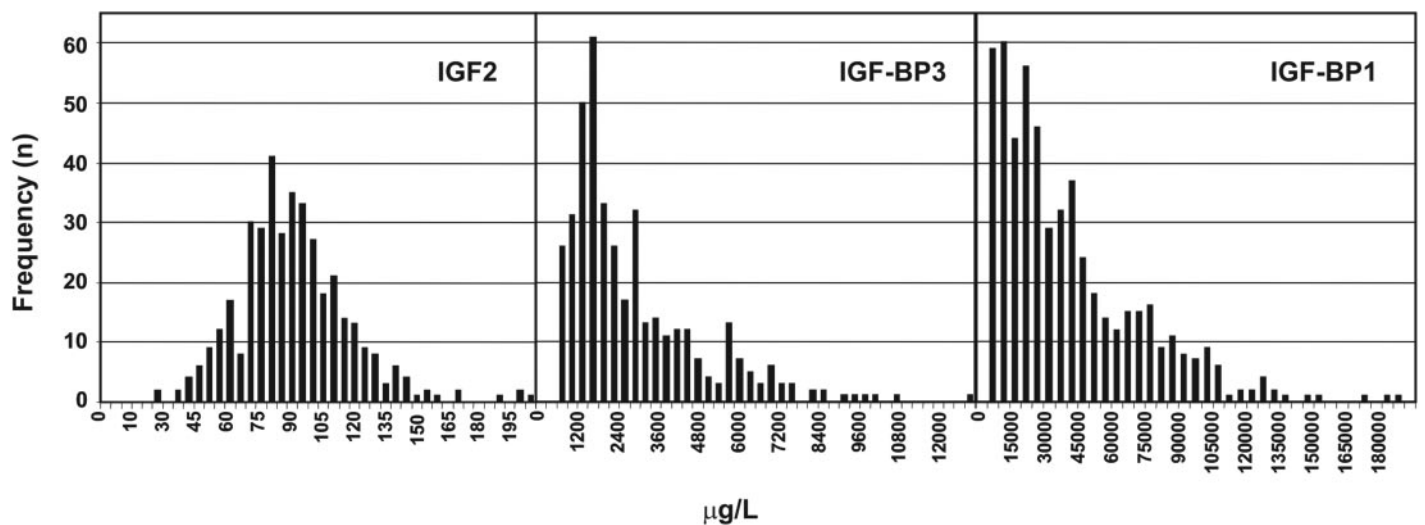


FIGURE 1 Population distributions of amniotic fluid IGF II, IGF BP3, and IGF BP1 in our mother-infant pairs after routine genetic testing.

TABLE 2

Regression analyses for infant birth weight in study population and macrosomia and LGA subpopulations<sup>1</sup>

Indicator	Multiple regression <sup>2</sup> Study population (n = 523)		Step-wise models <sup>3,4</sup>			
	$\beta$ -Coefficient	P	Macrosomia (n = 57)		LGA subgroup (n = 40)	
			$\beta$ -Coefficient	P	$\beta$ -Coefficient	P
Maternal height, m	694	0.0135	—	—	—	—
Maternal prepregnancy weight, kg	7.62	<0.0001	—	—	—	—
Amniocentesis week, wk	29	NS	—	—	—	—
Smoking: 0 = smoker, 1 = non	-63	NS	—	—	—	—
Gestational age (GA), wk	144	<0.0001	17.9	NS	88.9	0.0157
Infant gender, 0 = female, 1 = male	131	0.0005	—	—	—	—
IGF BP1, $\mu\text{g/L}$	-0.0015	0.0166	-0.0017	NS	-0.003	NS
IGF II, $\mu\text{g/L}$	0.16	NS	-2.33	0.0276	-4.90	0.0246
IGF BP3, $\mu\text{g/L}$	0.0004	NS	0.047	0.0067	0.07	0.0212
Variability captured ( $R^2 \times 100$ ), %	22.16		16.55		30.55	

<sup>1</sup> NS = not significant ( $P > 0.05$ ).

<sup>2</sup> IGF variables were included one-at-a-time with all other model variables, and the overall model variability capture and  $\beta$ -coefficients reported are from the model including IGF BP1 (partial  $r^2 = 0.0062$ ).

<sup>3</sup> Dash (—) indicates that variable was originally entered in model, but not chosen by computer selected regression model (backwards/forwards) regression analysis. Results are reported for step 5 of 6-step backwards multiple regressions.

<sup>4</sup> Partial  $r^2$  values for the macrosomia model were 0.0095, 0.0712, 0.0404, and 0.0341 for GA, IGF2, BP3, and BP1 respectively, whereas in LGA, these values were 0.1654, 0.0520, 0.0283, and 0.0379 for GA, IGF2, BP3, and BP1, respectively.

Our study population consisted of mothers undergoing age-related amniocentesis (age =  $37.8 \pm 0.1$  y), and it was interesting to note that our older maternal population differed only slightly from the Canadian population at large. Canadian statistics show that birth weight is  $3420 \pm 466$ , with 9.7% of the population SGA and 10.0% LGA (40). We had a shift to fewer SGA (5%) because we eliminated prematurity but reported a comparable prevalence of LGA (11%). Maternal smoking behavior showed the correct directional effect (41) but did not enter significantly as a predictor of birth weight due to the low incidence of smoking in our slightly older women (42). Birth weight was only slightly higher than the Canadian norm. We also observed that previously established (37) maternal and fetal predictors of birth weight including maternal height, prepregnancy weight, fetal gestational age, and gender were similar positive predictors in our older population (43).

Second trimester AF IGF BP1 was reported previously to be negatively correlated with birth weight (19,21,22). Our findings strengthen this observation because we controlled for established predictors of birth weight (37). Given that IGF BP1 is one of the most abundant binding proteins in AF (21), it is likely that IGF BP1 contributes to the negative association reported previously for AF total protein and birth weight (25). However, the failure of our findings to replicate the previously reported relation between high IGF BP1 and fetal growth retardation (22) may be explained by the absence of prematurity and the low incidence of LBW in our study population. A recent study (44) indicated that prematurity rather than intrauterine growth retardation drives the inverse relation between IGF BP1 and birth weight. Usually prematurity dominates in SGA infant populations; our study population included no premature infants, supporting the absence of this relation. Moreover, a previous study using 209 mother-infant pairs found that once gestational age was corrected for, AF IGF BP1 remained strongly correlated with AF IGF I and IGF II concentrations, but not with birth weight (19). Like us, they concluded that high AF IGF BP1 did not predict SGA; they

associated higher concentrations with lower placental weights (19).

AF IGF BP1 was also not associated with fetal birth weight in our LGA or macrosomic subpopulations. Previous findings showed elevations in plasma IGF BP1 in food-deprived or malnourished rats (45). Our mothers had prepregnancy BMIs  $> 20$  (82%); 28% were overweight/obese and likely were not fasting or malnourished. Previous studies showed that elevated plasma insulin inhibits hepatic IGF BP1 production early in gestation (46), and that lowered growth hormone results in overproduction of IGF BP1 in humans (47). Although growth hormone was not measured in our study, we did observe that AF insulin concentrations were greater in LGA compared with AGA infants (data not shown). Moreover, considering the lower concentrations of AF IGF BP1 in LGA compared with AGA, we suggest that, as with plasma, a similar inverse relation between higher insulin and lower IGF BP1 exists in AF.

In our study, we demonstrated a positive predictive relation in vivo between AF IGF BP3 and fetal growth in LGA and macrosomic infants. Previous in vitro findings using animal and human cells hinted that IGF BP3 may potentiate IGF action (20,48). Our finding represents the first in vivo report of an association of AF IGF BP3 (12–20 wk) with birth weight. Previous studies in plasma established that growth hormone levels are inversely proportional to IGF BP1 (47) and directly proportional to IGF BP3 (49,50). It was shown that IGF BP3 synthesis is growth hormone dependent (51). Although we did not observe higher AF IGF BP3 across our 3 birth weight categories, literature findings suggest that our positive association between AF IGF BP3 and birth weight in our stepwise regression might be linked with elevated AF concentrations of growth hormone (49). Thus, only larger fetuses would be exposed to both decreased AF IGF BP1 and increased AF IGF BP3. This relation requires further investigation, and measurement of AF growth hormone is proposed.

Last, we showed that AF IGF II was negatively associated with infant birth weight in LGA and macrosomic infants. The

previous literature showed that IGF II is the primary growth factor during early fetal development (10,11,24); plasma levels are normally positively associated with fetal growth (10). It is known that IGF II binds to both IGF BP1 and IGF BP3 (52); its greatest association is for IGF BP3 and its lowest dissociation is for IGF BP1 (52). Because total AF IGF II concentrations were essentially the same across the birth weight and gender-corrected birth-weight-for-gestational-age categories, it would seem that IGF BP1 in these groups might play an intervening role in the observed inverse relation between AF IGF II and infant birth weight in macrosomic and LGA infants. Specifically, decreased IGF BP1 occurs with elevated AF insulin (46), which was observed in AF from the macrosomic and LGA infants. The increased levels of insulin in AF at this stage in development may lead to increased competitive binding with IGF II receptors, thus leading to generalized tissue downregulation of receptors. This would subsequently lead to increased levels of IGF II remaining in AF. Until results of an assay that can distinguish between IGF II bound to one protein or another are reported, no conclusive mechanism can be described. However, the underlying mechanism for the negative association with early concentrations of AF IGF II and birth weight for infants classified as LGA and macrosomic at term does warrant further investigation.

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