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Twinning propensity and offspring *in utero* growth covary in rural African women

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In humans and other mammals, some females are more likely to experience twin pregnancies than others, but the reasons behind such individual variation are poorly understood. One hypothesis invokes variation in the dynamics of the insulin-like growth factor (IGF) system, which also regulates foetal growth. Using data from a rural African population living in a highly seasonal environment, we test a novel prediction generated by this hypothesis, that maternal twinning status predicts offspring birthweight. We found that among singleton offspring who experience a favourable *in utero* environment (born January–June), births before and after twins are, respectively, associated with a 134.07 g and 226.41 g increase in birthweight compared with those born to non-twinning mothers. These results were not mediated by maternal anthropometry. This is consistent with a role for the IGF system in individual variation in twinning propensity, a possibility with implications for understanding mechanisms of life-history variation in humans and other vertebrates.

Keywords: twins; twinning; birthweight; insulin-like growth factor I; insulin-like growth factor; life history

1. INTRODUCTION

The number of offspring produced in a single reproductive event is an important life-history trait [1]. In primates such as humans where one offspring is the norm, the probability of twinning varies between females [2]. One possible reason is that individual endocrine profile affects rates of polyovulation, and thus dizygotic twinning [2]. The insulin-like growth factor (IGF) system regulates follicle growth and apoptosis [3] and may thereby influence the likelihood of polyovulation [4]. Selection experiments with mice and cattle indicate a relationship between circulating IGF-I level and litter size [5,6], with IGF-I level being more than 1.5 times higher in twinning versus non-twinning cattle [6]. Furthermore, human twinning risk covaries with traits associated with IGF-I, such as ethnicity [4] and body mass [7].

As well as promoting polyovulation, maternal or offspring IGF dynamics may play a role in regulating foetal growth [8]. Thus, if variation in the maternal IGF axis is linked to twinning risk, then the probability of a woman producing twins may be associated with her offspring's intrauterine growth. We should thus expect differences in singleton birthweight according to maternal lifetime twinning status. We tested this prediction using a longitudinal population database of rural Gambian women. Because intrauterine growth can be influenced by previous pregnancies [9], we analysed singletons separately that were born before and after their mother produced twins. In addition, we tested whether any effect of maternal twinning status on offspring birthweight was modified by other maternal and environmental factors known to limit intrauterine growth.

2. MATERIAL AND METHODS

Since 1950, residents of the West Kiang district of the Gambia have been treated and studied by the UK Medical Research Council. From 1976, routine data collection included birthweights, and from 1978, estimated gestational age. The population primarily depends on sustenance agriculture. Women typically begin reproducing at about the age of 18, and have on average about seven children in their lifetime [7]. We assembled birthweights (mean: 2.97 kg $\pm$ 0.40 s.d., range: 1.10–4.63 kg) of 1889 singletons born between 1978 and 2009 to 685 mothers. Eight per cent (151) of these offspring were born to 43 women who were recorded as having produced twins by the end of 2009. Owing to sample size constraints, it was not possible to stratify our analysis by mothers of pairs of twins who were definitely dizygotic (i.e. mixed sex pairs) and who were not.

We controlled for environmental and maternal factors associated with offspring birthweight. These were: offspring sex, maternal age, maternal parity, offspring birth cohort, and offspring birth season. Maternal age was analysed both as a linear and quadratic continuous term. Furthermore, twinning risk is associated with maternal age [2]. Maternal parity was analysed as a four-level categorical variable (1; 2; 3–9; 10+). Birth season (January–June versus July–December) was included because of the substantial reduction in the birthweight of babies born between July and December each year (hungry season exposed) relative to those born in the first half of the year (harvest season exposed), a result of seasonal changes in maternal energy balance [10]. There were three birth cohorts (<1990; 1990–1999; 2000–2009). After the initial test of the hypothesis that maternal twinning status predicts singleton offspring growth, we tested the significance of two-way interactions between maternal twinning status and each of the above covariates. Analysis was conducted using the GENMOD procedure in SAS v. 9.1 (SAS Institute, Inc., Cary, NC, USA). This allowed us to analyse multiple measures for mothers without biasing variance estimates, by including a term for maternal ID in the model. Statistical significance of each term was assessed at the $\alpha = 0.05$ level using type 3 generalized estimating equation scores, which follow a χ^2 distribution.

Because maternal body size may be associated with both maternal twinning [7] and offspring birthweight, we conducted a secondary analysis on a subsample of offspring for whom measures of maternal anthropometry were available ($n = 1550$ offspring). We predicted that effects of maternal twinning status would be independent of these individual factors, indicating that size itself does not mediate effects of maternal twinning status. We thus added height (mean 1588 $\pm$ 58 mm s.d.) and body mass index (BMI) (21.42 $\pm$ 2.91 kg m^{-2} s.d.) to the final model described above. These were the oldest measurement available for each woman and were all taken when she was at least 15 years old (mean 26.4 $\pm$ 5.8 years s.d.).

Finally, in order to determine whether or not the above results could be owing to a small number of influential mothers accounting for several offspring in one or more of the twinning categories, we carried out an analysis that included only one offspring per mother (selected at random). We focused on a comparison between the singleton offspring of twinning mothers born before the birth of twins as the crucial category of interest and compared the birthweights of these with those born to mothers who did not produce twins. This analysis contained all variables entered into the initial analysis and used the general linear model procedure in SAS.

3. RESULTS

We found an effect of maternal twinning status on offspring birthweight contingent on offspring birth season. Among those born between January and June

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Table 1. Results of analysis of predictors of singleton birthweight for the full sample ($n = 1889$). (Estimates of effect sizes and χ^2-test statistics for effect of maternal twinning status and other factors potentially associated with offspring *in utero* growth are shown. Results obtained from generalized estimating equations using the GENMOD procedure in SAS. Units are grams.)

term	level	estimate	error	lower limit	upper limit	Z	p	d.f.	χ^2	p
intercept		−477.26	255.91	−1037.63	83.12	−1.67	0.095			
gestational age		67.41	6.43	54.81	80.00	10.49	<0.0001	1	55.50	<0.0001
maternal age		46.72	12.18	22.86	70.59	3.84	0.0001	1	13.89	0.0002
maternal age^2		−0.73	0.19	−1.10	−0.35	−3.83	0.0001	1	13.60	0.0002
parity	2	86.82	27.47	32.98	140.65	3.16	0.0016	3	10.69	0.014
	3–9	71.81	34.96	3.23	140.33	2.05	0.040			
	10+	57.61	43.00	−26.66	141.89	1.34	0.18			
sex	male	121.32	16.92	88.15	154.49	7.17	0.0016	1	46.19	<0.0001
cohort	90–99	−46.49	22.64	−90.85	−2.12	−2.05	0.040	2	12.26	0.0022
	00–09	21.84	28.02	−33.08	76.76	0.78	0.44			
birth season	harvest	23.35	17.53	11.00	57.71	1.33	0.18	1	8.96	0.18
twin status	before	−42.87	54.44	−149.57	63.82	−0.79	0.43	2	4.04	0.13
	after	100.14	83.06	−62.65	262.93	1.21	0.23			
birth season × twin status	harvest: before	176.94	70.13	39.48	314.39	2.52	0.012	2	7.08	0.029
	harvest: after	126.27	58.95	10.71	241.81	2.14	0.032			

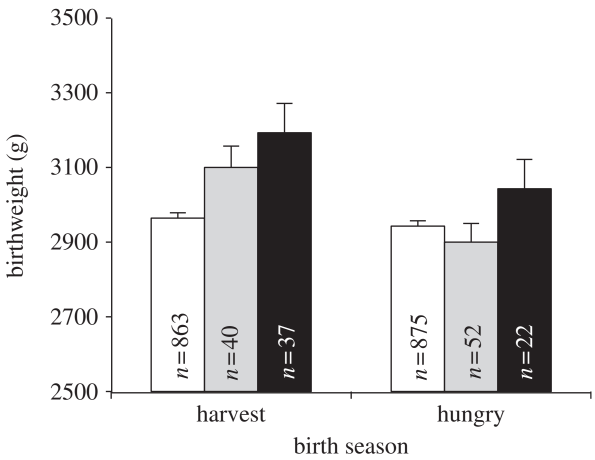

Figure 1. Singleton birthweight according to maternal twinning status and offspring birth season. White bars represent those born to non-twinning women. Grey bars represent those born to twinning women prior to the birth of their first twin pair. Black bars represent those born to twinning women after those women had first produced twins. Values are predicted means from models. Bars are 1 s.e.

(harvest), individuals born before and after twins were 134.07 g and 226.41 g heavier, respectively, than offspring of non-twinning mothers, whereas there was no comparable pattern among those born in the hungry season (table 1 and figure 1). This two-way interaction was statistically significant ($\chi^2_2 = 7.08$, $p = 0.029$), but no others were (all $p > 0.3$).

In the reduced sample of neonates for which maternal anthropometry data were available, we found that the effect of maternal twinning status on birthweight was independent of both maternal BMI and height. After inclusion of these terms in the model (as well as interactions with birth season), only those babies born after twins in the hungry season were significantly heavier than their counterparts born to non-twinning mothers (table 2). However, the differences between maternal twinning status' effects in this instance and the previously reported result (table 1) were entirely owing to the reduced size of the sample of women for whom these variables were available. The statistics pertaining to the effect of maternal twinning status was virtually unchanged between analyses of the reduced sample with and without these terms included in the model.

In our final analysis using only one offspring per mother, we found that individuals born before twins to twinning mothers weighed significantly more than individuals born to non-twinning women ($F_1 = 4.33$, $p = 0.038$). The interaction between maternal twinning status and offspring birth season was non-significant ($F_1 = 0.80$, $p = 0.37$; figure 2).

4. DISCUSSION

Using data from a rural African population, we found support for our prediction that maternal twinning propensity is associated with intrauterine growth of singletons. Further analysis revealed that under environmental conditions favourably associated with *in utero* growth, there was a strong effect of maternal twinning status on birthweight of offspring both before and after twin pregnancies.

Among individuals exposed to the 'hungry' season during their third trimester (born July–December), both groups of singleton offspring born to mothers of twins were greater than 100 g heavier than those born to non-twinning women. One explanation for this could be that any individual differences in potential offspring growth can be realized only when *in utero* growth is not subject to the more influential constraints of external influences on energy balance. Interpreted alternatively, the well-documented association between birth season and *in utero* growth in this population [10] may be restricted to twinning women, with non-twinning women producing smaller offspring regardless of the season.

Table 2. Results of analysis of predictors of singleton birthweight for the reduced sample comprised offspring born to mothers with available maternal anthropometry data ($n = 1889$). (Estimates of effect sizes and χ^2-test statistics for effect of maternal twinning status and other factors potentially associated with offspring *in utero* growth are shown. Results obtained from generalized estimating equations using the GENMOD procedure in SAS. Units are grams.)

term	level	estimate	error	lower limit	upper limit	Z	p	d.f.	χ^2	p
intercept		−2497.63	500.77	−3479.12	−1516.14	−4.99	<0.0001			
gestational age		67.36	6.95	53.74	90.99	9.69	<0.0001	1	46.26	<0.0001
maternal age		29.44	14.96	0.12	58.75	1.97	0.049	1	3.72	0.054
maternal age^2		−0.49	0.23	−0.93	−0.044	−2.15	0.031	1	4.40	0.036
parity	2	115.32	33.44	49.78	180.85	3.45	0.0006	3	12.87	0.0049
	3–9	141.10	42.92	56.98	225.23	3.29	0.0010			
	10+	149.77	54.66	42.64	256.91	2.74	0.0061			
sex	male	121.03	17.92	85.91	156.15	6.75	<0.0001	1	40.22	<0.0001
cohort	90–99	−68.50	23.38	−114.32	−22.68	−2.93	0.0034	2	12.82	0.0016
	00–09	−3.98	30.92	−64.57	56.62	−0.13	0.90			
birth season	harvest	148.97	448.30	−729.69	1027.62	0.33	0.74	1	0.26	0.61
twin status	before	−18.41	66.19	−240.22	19.23	−1.67	0.095	2	3.30	0.19
	after	81.49	75.64	66.76	229.73	1.08	0.28			
maternal height		1.20	0.25	0.71	1.68	4.81	<0.0001	1	20.07	<0.0001
maternal BMI		15.58	5.31	5.18	25.98	2.94	0.0033	1	9.81	0.0017
birth season × twin status	harvest: before	110.49	66.19	−19.23	240.22	1.67	0.095	2	5.34	0.069
	harvest: after	126.56	53.73	21.26	231.86	2.36	0.019			
birth season × maternal height	harvest	0.024	0.27	0.56	0.51	0.09	0.93	1	0.01	0.93
birth season × maternal BMI	harvest	3.50	5.42	14.14	7.13	0.65	0.52	1	0.40	0.53

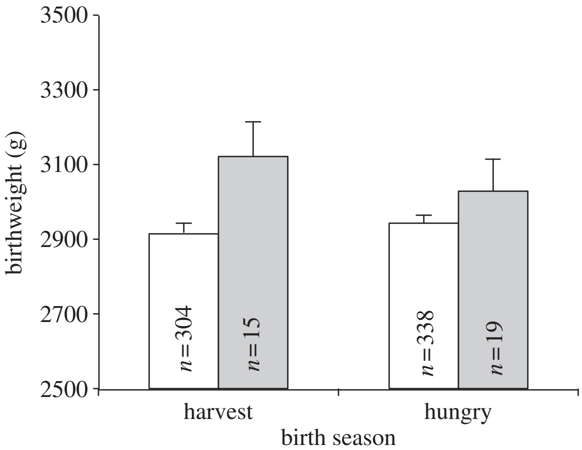

Figure 2. Singleton birthweight according to maternal twinning status and offspring birth season (only one offspring per mother). White bars represent those born to non-twinning women. Grey bars represent those born to twinning women prior to the birth of their first twin pair. Values are predicted means from models. Bars are 1 s.e.

As the effect of maternal twinning status was not restricted to neonates born after twins, it is unlikely that anatomical or physiological changes associated with prior pregnancies are entirely responsible. However, increased vascularization as a result of twin pregnancy relative to a singleton pregnancy may account for the difference in birthweights between the two maternal twinning groups, similar to the parity progression effect on birthweight, whereby firstborns are generally lighter than subsequent offspring [9].

It should also be noted that the reference group will contain some mothers who are prone to twinning but in whom this trait has not emerged within their reproductive span (or within the truncated data available), because even in twinners the odds of a twin pregnancy rarely exceed one in 20. Consequently, the effect we have observed may under-represent the real effect.

Although any individual factor associated with both intrauterine growth and risk of twin pregnancy could underlie the relationships described here, the results of this study are consistent with a role for maternal or offspring IGF dynamics, which has previously been proposed to be associated with twinning rate in humans [4], as has been shown in cattle [6]. In support of this, we found that available measures of maternal anthropometry did not appear to mediate the relationships described, indicating that the link between maternal twinning status and singleton offspring birthweight may not be mediated by variation in anthropometry but by factor(s) further upstream in the chain of causal relationships. The IGF system may have multiple roles across males and females and could play an important role in life-history regulation [11–15]. This study of two hitherto apparently unrelated reproductive outcomes highlights the importance of considering trait covariation when investigating individual variation in life histories, and evaluating assumptions underlying hypotheses of trade-offs between fitness traits.

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