Firstborn sex defines early childhood growth of subsequent siblings

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Animal studies have shown that maternal resource allocation can be sex-biased in order to maximize reproductive success, yet this basic concept has not been investigated in humans. In this study, we explored relationships between maternal factors, offspring sex and prenatal and postnatal weight gain. Sex-specific regression models not only indicated that maternal ethnicity impacted male (n = 2456) and female (n = 1871) childrens postnatal weight gain differently but also that parity and mode of feeding influenced weight velocity of female (β ± s.e. = −0.31 ± 0.11 kg, p = 0.005; β ± s.e. = −0.37 ± 0.11 kg, p < 0.001) but not male offspring. Collectively, our findings imply that maternal resource allocation to consecutive offspring increases after a male firstborn. The absence of this finding in formula fed children suggests that this observation could be mediated by breast milk. Our results warrant further mechanistic and epidemiological studies to elucidate the role of breastfeeding on the programming of infant growth as well as of metabolic and cardiovascular diseases, with potential implications for tailoring infant formulae according to sex and birth order.

1. Introduction

From an evolutionary perspective, reproductive success represents an individual's number of offspring reaching sexual maturity [1,2]. This implies that beyond the transmission of heritable characteristics to successive generations, offspring quality in terms of survival and fecundity is shaped by a multitude of environmental factors. Among these factors the parental nutritional contribution towards offspring reproductive success is probably best studied across the animal kingdom. It has been argued that parental resource allocation can be effectively manipulated, a concept coined by Trivers as parent-offspring conflict [3]. The idea here is that while the offspring genetically is completely related to itself, but only partly to future siblings, it aims to maximize its own reproductive success by increased consumption of parental resources. By contrast, a parent is equally related to all her offspring and must, therefore, consider saving resources for future offspring. The parent-offspring conflict theory [3] has been further developed by Haig [4], focusing on the concept of maternal-fetal conflict. The concept of maternal–fetal conflict forms the evolutionary explanation for common pregnancy-related complications such as pre-eclampsia and gestational diabetes, where in the former the mother is perceived to hold back her energy stores for future offspring by compromising placental nutrient transfer and in the latter, the fetus tries to maximize maternal nutritional allocation for itself by increasing maternal blood sugar levels [4].

The early maternal nutritional allocation has been shown to influence the most important early determinants of offspring fitness, namely growth as a prerequisite of survival. Continuous and age-appropriate prenatal and postnatal growth are strongly associated with protection against infant morbidity and mortality [5]. Furthermore, growth propensity appears to be an important
determinant of life-time reproductive fitness. This has been well described in animal models [6] and does to some extent hold true in humans, in that, for example, taller men reportedly have more reproductive success [7].

Consequently, the early maternal nutritional allocation has been deemed to enhance the reproductive performance of offspring in a sex-dependent way in many species. This phenomenon of sex-biased parental investment has been thoroughly examined in the field of evolutionary biology and a variety of hypotheses have been proposed, explaining the adaptive advantages of sex-biased parental resource allocation [8]. Trivers & Willard [9] proposed in their resource competition model, that if fitness gains from parental resource allocation are greater in one sex, parents will allocate more resources towards that sex. However, several other factors have been hypothesized to influence parental resource allocation. For example, competition for local resources might affect parental resource allocation as relatives might need to compete for the same resource [10–12]. In this context, resource allocation towards the sex that is more likely to geographically disperse into food abundant areas outside of the family group would reduce competition between relatives and be favourable for the reproductive success of that sex [12].

Just as in other mammals, remarkable sex differences in growth rates have been recognized in humans. Size differences can be seen as early as during the first trimester of pregnancy [13]. Even postnatally the importance of infant sex has been emphasized, together with other maternal factors and feeding pattern [14]. While some human studies have independently associated factors such as infant sex and breastfeeding with infant weight [14], the underlying mechanisms behind the observed weight differences remain unclear. Based on the Trivers & Willard model [9] and results from prior non-human primate studies [15–19], we hypothesize that differences in infant weight between sexes are an expression of evolutionary resource allocation patterns. In order to advance our understanding of human infant growth we, therefore, aim to investigate the influence of infant sex and maternal factors (such as parity) on maternal energy transfer and a variety of hypotheses have been proposed, explaining the adaptive advantages of sex-biased parental resource allocation [8]. Trivers & Willard [9] proposed in their resource competition model, that if fitness gains from parental resource allocation are greater in one sex, parents will allocate more resources towards that sex. However, several other factors have been hypothesized to influence parental resource allocation. For example, competition for local resources might affect parental resource allocation as relatives might need to compete for the same resource [10–12]. In this context, resource allocation towards the sex that is more likely to geographically disperse into food abundant areas outside of the family group would reduce competition between relatives and be favourable for the reproductive success of that sex [12].

2. Results

Paediatric medical entries of patients admitted between January 2007 and February 2018 were matched with entries from the state-wide mandatory electronic obstetric database (n = 11 848). This allowed exploration of the effects of several perinatal and maternal factors on perinatal and postnatal growth. Children with suspected or ultrasound confirmed fetal growth compromise were excluded (n = 50).

(a) Maternal characteristics

Data were successfully obtained for 11 798 mother–infant dyads (55.4% male infants). In general, mothers were 28.80 ± 5.75 years old at the time of birth. Naturally, primiparous mothers were younger than multiparous mothers (26.64 ± 5.58 years versus 30.40 ± 5.34 years; p < 10–77). Primiparous mothers were also found to weigh less (73.44 ± 19.65 kg versus 77.58 ± 20.90 kg; p < 10–15) and consequently had a lower body mass index (BMI) (26.21 ± 6.49 kg m–2 versus 27.92 ± 7.15 kg m–2; p < 10–41). Interestingly, offspring birth weight constituted a slightly higher percentage of the pre-pregnancy maternal weight in primiparous mothers compared to multiparous mothers (4.84 ± 1.37% versus 4.67 ± 1.32%; p < 10–36). As there was no offspring sex-bias between parity groups (firstborns: 55.8% male, consecutive children: 55.1% male; p = 0.43) this might indicate that age-accumulated body fat does not contribute to perinatal weight development.

(b) Birth interval

The birth interval before the subsequent child was calculated for all children for which subsequent siblings could be identified using the maternal medical record number. Birth interval data was available for 2330 primiparous mothers and 1864 multiparous mothers. Primiparous mothers were of comparable age at parturition of a male (26.72 ± 5.48 years) compared to female offspring (26.53 ± 5.71 years; p = 0.24). Birth intervals after male (median [interquartile range] = 2.33 [1.72–3.38] years) and female offspring (2.41 [1.66–3.47] years; p = 0.91) were not different. However, primiparous mothers had longer birth intervals (2.36 [1.69–3.41] years) than multiparous mothers (2.22 [1.47–3.54] years; p < 10–3). Because birth intervals were long, they were unlikely to be affected by the feeding method. Birth intervals were excluded from further analysis owing to the rather homogeneous distribution and the amount of missing data for this variable (64.5%).

(c) Fetal growth as a surrogate for antenatal maternal nutritional allocation

First, we explored fetal growth, using a backward eliminated multiple regression model for birth weight, birth length and birth head circumference, including all children with complete entries (table 1). As expected, the gestational age had the largest effect on all anthropometric measurements at birth. Maternal BMI, maternal height, maternal diabetes, multiparity as well as male infant sex were also positively associated with in utero growth. In addition, maternal age was linearly related, although weakly, with birth parameters (table 1, electronic supplementary material, figure S1). Furthermore, maternal factors such as being born in Asia or Africa, smoking during pregnancy, illegal drug use, multiple birth and assisted conception were associated with impaired prenatal growth affecting all parameters at birth.

Sex-specific multiple regression models for birth parameters (electronic supplementary material, tables S1–S3) revealed that maternal continent of origin actually affected males and females differently. While the growth of female infants was mostly decreased depending on the continent of origin, the growth of males was either enhanced or decreased to a lesser extent.

The sex-specific models also shed light on the relationship of alcohol consumption during pregnancy and birth weight, which had not been detected in the general model. While male (1.3%, n = 84) and female infants (1.5%, n = 77) were similarly exposed to alcohol during pregnancy (p = 0.41),
alcohol only seemed to inhibit the growth of females (electronic supplementary material, table S1).

(d) Postnatal offspring growth as a surrogate for antenatal maternal nutritional allocation

To analyse the effect of maternal and offspring factors on postnatal growth, we used paediatric medical entries. To ensure that children have had time for postnatal growth we used paediatric medical entries. To analyse the effect of maternal and offspring factors on postnatal growth, we chose to only include children that were more than one week old. Characteristics of the included children (total n = 10904 children) were as follows: n = 1871. Several factors important for late postnatal growth rather than the birth weight gain, however, this difference was not observed in males. This can be seen as the interaction variable male x breastfeeding, infant sex and firstborn sex, we adjusted postnatal weight for all variables that were entertained in the general regression model of postnatal weight, except for sex, feeding method, male x breastfeeding and parity category. In this adjustment, all children’s postnatal weights were adjusted (table 3), though they initially showed enhanced in utero growth compared to children of non-Australian born mothers (table 1).

When examining the sex-specific models it became apparent that a complex relationship between feeding pattern, infant sex and firstborn sex existed. It seemed that breast milk compared to infant formula generally led to a slower postnatal weight gain, however, this difference was not observed in males. This can be seen as the interaction variable male x breastfeeding cancelled out the negative impact of feeding: breastfed and also in the sex-specific model of males, in which feeding method was not a detectable predictor. The sex-specific effect of feeding pattern on females on the other hand reveals that breastfed females that are born consecutive to another female might experience a greater growth compared to females that are born consecutive to a male might experience a greater growth compared to females that are born consecutive to another female. To explore factors important for late postnatal growth rather than the birth weight we also created regression models similar to the ones above (table 3) for children admitted after the age of four weeks (electronic supplementary material, table S5).

To further investigate the complex relationship between breastfeeding, infant sex and firstborn sex, we adjusted postnatal weight for all variables that were entertained in the general regression model of postnatal weight, except for sex, feeding method, male x breastfeeding and parity category. In this adjustment, all children’s postnatal weights were adjusted...
towards the age of a 1 year old, for information regarding the other adjustment parameters see table 4. Next we compared adjusted postnatal weight based on sex, feeding method and parity group (figure 1a–c). Expectedly, female offspring tended to grow slower than male offspring (12.1 ± 1.9 kg versus 12.7 ± 2.1 kg; p < 10^{-26}). Firstborn generally grew slightly faster than consecutive children (12.6 ± 2.1 kg versus 12.4 ± 2.0 kg; p < 10^{-3}). On a group level, however, only a trend for a difference in postnatal weight gain occurred between breastfed children and children fed with infant formula (12.4 ± 2.0 kg versus 12.6 ± 2.1 kg; p = 0.069).

Next, we grouped children by firstborn sex (if applicable), feeding method and their own sex and calculated an ANOVA for the 12 groups (figure 1d,e, table 4). Post hoc testing was conducted using the Bonferroni test. As can be seen, the most marked difference is found between firstborn, breastfed males and females. The most striking finding, however, is that there is a difference between breastfed males and females born consecutive to a female firstborn, while there is no detectable difference between breastfed males and females born consecutive to a male firstborn.

This analysis indicated that breastfed consecutive males and females were not heavier at admission than their breastfed firstborn counterparts, which indicated that parity might not have a general effect on postnatal growth rate. Interestingly enough, it seemed that the general difference in postnatal weight between breastfed males and females was of comparable magnitude between firstborn children (male versus female: 12.8 ± 2.2 kg versus 12.2 ± 1.9 kg; p < 10^{-5}) and children born consecutive to a female firstborn (male versus female: 12.9 ± 1.9 kg versus 12.0 ± 1.8 kg; p < 10^{-5}), though seemed to decrease in children born consecutive to a male firstborn (male versus female: 12.6 ± 1.9 kg versus 12.2 ± 1.9 kg; p = 0.425). Importantly, no differences in postnatal weight were seen between formula fed children.

We then hypothesized that postnatal growth rates of a female offspring preceded by a male offspring are similar to growth rates of male offspring, while the postnatal growth
rate of a female offspring preceded by female offspring would be close to that of a firstborn female. Indeed we found that the difference in adjusted weight was less marked between a breastfed firstborn male and a breastfed female born consecutive to a male (12.8 ± 2.2 versus 12.2 ± 1.9; p < 10^{-5}) than the difference between a breastfed firstborn male and a breastfed female born consecutive to a female (12.8 ± 2.2 versus 12.0 ± 1.8; p < 10^{-5}).

3. Discussion

Using infant weight development as an indirect measure of postnatal maternal resource allocation, our results suggest that mothers generally allocate more in male than in female offspring. Interestingly, we found that weight differences between male and female firstborns only persisted in consecutive children after a firstborn female but not a firstborn male. Similar sex-dependent growth patterns have been described previously in mammal studies [20,21]. Because these observations exclusively pertained to breastfed but not formula fed children it seems plausible that this process might be mediated by alterations in breast milk composition. However, the small sample size of formula fed infants in our study and the absence of fetal energy intake measurements limits the confidence in this assertion.

Investigating human breast milk from women in northern Kenya, Fujita et al. [22] found a higher lipid content in breast milk from high socioeconomic status women nursing sons.

Table 3. Multiple regression of maternal and offspring factors that affect postnatal growth in children older than one week.

| predictors                      | ped. weight (kg) |          |          |          | ped. weight (kg)^a |          |          |          | ped. weight (kg)^b |          |          |
|--------------------------------|------------------|----------|----------|----------|-------------------|----------|----------|----------|-------------------|----------|----------|
|                                | β                | s.e.     | p        | β        | s.e.     | p        | β        | s.e.     | p        | β        | s.e.     | p        |
| intercept                      | −3.69            | 1.03     | <10^{-3} | −4.43    | 1.39     | 0.001    | −3.84    | 1.41     | 0.007    |
| mother born in:                |                  |          |          |          |          |          |          |          |          |          |          |          |
| Australia                      | −0.36            | 0.11     | 0.001    | −0.25    | 0.12     | 0.036    |          |          |          |          |          |
| Asia                           | −0.36            | 0.17     | 0.029    |          |          |          |          |          |          |          |          |
| Europe                         |                  |          |          |          |          |          |          |          |          |          |          |
| North America                  | −0.97            | 0.52     | 0.064    |          |          |          |          |          |          |          |
| South America                  |                  |          |          |          |          |          |          |          |          |          |          |
| Africa                         |                  |          |          |          |          |          |          |          |          |          |          |
| maternal age (years)           | −0.01            | 0.01     | 0.084    | −0.02    | 0.01     | 0.050    |          |          |          |          |          |
| maternal BMI (kg m^{-2})       | 0.03             | 0.00     | <10^{-7} | 0.03     | 0.01     | <10^{-5} | 0.02     | 0.01     | 0.001    |          |          |
| maternal height (cm)           | 0.03             | 0.00     | <10^{-10}| 0.04     | 0.01     | <10^{-8} | 0.03     | 0.01     | <10^{-4} |          |          |
| maternal diabetes: yes         |                  |          |          |          |          |          |          |          |          |          |          |
| maternal smoking: yes          |                  |          |          |          |          |          |          |          |          |          |          |
| alcohol when pregnant: yes     |                  |          |          |          |          |          |          |          |          |          |          |
| illegal drug use: yes          | −0.40            | 0.19     | 0.033    | −0.64    | 0.26     | 0.015    |          |          |          |          |          |
| multiple birth: yes            |                  |          |          |          |          |          |          |          |          |          |          |
| conception type: assisted      |                  |          |          |          |          |          |          |          |          |          |          |
| parity: multiparous            | −0.14            | 0.07     | 0.046    | −0.11    | 0.11     | 0.003    | −0.31    | 0.11     | 0.005    |          |          |
| feeding: breastfed             | −0.28            | 0.10     | 0.007    |          |          |          | −0.37    | 0.11     | <10^{-3} |          |          |
| infant sex: male               | 0.34             | 0.11     | 0.003    |          |          |          |          |          |          |          |          |
| gestational age (week)^*        | 0.04             | 0.02     | 0.012    | 0.04     | 0.02     | 0.073    | 0.05     | 0.02     | 0.026    |          |          |
| birth weight (kg)^*             | 0.59             | 0.06     | <10^{-21}| 0.60     | 0.08     | <10^{-11}| 0.58     | 0.09     | <10^{-10}   |          |          |
| admission age (years)           | 3.19             | 0.02     | <10^{-100}| 3.23    | 0.03     | <10^{-100}| 3.15    | 0.03     | <10^{-100}  |          |          |
| n of prior admissions           |                  |          |          |          |          |          |          |          |          |          |          |
| male × breastfed^a              | 0.29             | 0.14     | 0.031    |          |          |          | 0.29     | 0.16     | 0.067    |          |          |
| multiparous × male firstborn × breastfed^a |          |          |          |          |          |          |          |          |          |          |          |
| male × admission age            |                  |          |          |          |          |          |          |          |          |          |          |
| model                          | Adj. R^2 = 0.813; p < 10^{-300}; F = 1344.796 | Adj. R^2 = 0.802; p < 10^{-300}; F = 1241.396 | Adj. R^2 = 0.827; p < 10^{-300}; F = 1115.907 |          |          |          |

^a Male offspring only.

^b Female offspring only.

*Correlation coefficient between birth weight and gestational age is 0.44 and therefore does not violate the assumptions of multiple regression.

Attempts have been made to combine these into multiparous × male firstborn × male × breast milk, but the complexity of this four-way interaction was not fully captured by the multiple regression model and hence did not achieve statistical significance.
Sex differences have also been reported by Powe et al. [23], showing that human breast milk had a 25% higher energy content when mothers were nursing sons, compared to daughters. This difference was found sufficient to explain the growth rate difference between male and female offspring [23]. Furthermore, Thakkar et al. [24] reported that both breast milk energy and lipid composition were higher when nursing sons compared to daughters. These findings explain the general weight differences between breastfed male and female offspring observed in our study (figure 1c) and align to animal studies of polygynous species such as wallabies (Macropus eugenii) [25], sheep (Ovis aries) [26], apes (Macaca mulatta) [20,27] and deer (Cervus elaphus hispanicus, Dama dama) [28]. The novel finding in the present study, however, is that firstborn sex seems to influence the weight of future siblings in humans (figure 1d,e). In this context, Hinde et al. [20], investigating breast milk composition in apes, suggested that male offspring might alter breast milk composition for consecutive offspring through hormone-stimulated epigenetic changes in the mammary gland. Mothers with immature mammary gland tissue might be especially sensitive to changes. In agreement with this finding, we find that the weight difference between breastfed males and females decreases after a male firstborn. Our findings are mirrored by findings in 2.39 million Holstein dairy cows (Bos taurus) [21,29], in which the effects of birth order and sex on milk production was investigated. Additionally, investigation in Holstein cattle found that milk yield was permanently higher after a firstborn female compared to male calves, while milk energy content remained comparable [21]. A cow that had given birth to a firstborn and a consecutive male calf had the lowest milk yield. The results from this analysis and similar results from a latter study on Holstein cattle [29], although sex-biased toward daughters and focusing on milk production rather than offspring growth, are in concurrence with observations made in our study.

Table 4. Adjusted postnatal weight for all children grouped by firstborn sex, feeding pattern and their own sex. (Admission weight has been adjusted according to β-values detained from the general regression model for the following parameters: mother born: Australia = no, Asia = no, North America = no; maternal age = 28 years; maternal BMI = 25; maternal height = 160 cm; multiple birth = no; gestational age = 40; birth weight = 3 kg; admission age = 1 year. Adj., adjusted.)

| firstborn sex | firstborn | female | male |
|---------------|-----------|--------|------|
| infant sex    |           |        |      |
|               | female    |        |      |
| n             | 212       | 266    | 83   |
| Adj. weight (kg) | 12.7 ± 2.1 | 12.9 ± 2.3 | 12.4 ± 1.9 |
| children fed with infant formula | | | |
| n             | 781       | 1127   | 247  |
| Adj. weight (kg) | 12.2 ± 2.1 | 12.8 ± 2.2 | 12.0 ± 1.8 |
| children fed with breast milk | | | |
| n             | 289       | 255    | 320  |
| Adj. weight (kg) | 12.9 ± 2.0 | 12.2 ± 1.9 | 12.6 ± 1.9 |

But why would human males have this growth advantage? Following the Trivers & Willard model, maternal resource allocation is biased towards the sex that exhibits more variation in reproductive success, given that the mother is in better condition than the average mother [9]. The Trivers & Willard model relies on three assumptions: (i) parental condition correlates with offspring condition, (ii) offspring condition persists into adulthood and hence has reproductive value, and (iii) that offspring condition affects the reproductive success of each sex differentially [9]. The maternal condition has in many previous studies been associated with offspring condition [30,31] and the correlation of parental investment with protection against infant morbidity and mortality [5] is well known in humans. Especially, it has long been recognized that male infants have a higher risk of neonatal mortality, something known as the ‘male disadvantage’ [32]. It is imaginable that increased maternal resource allocation is required to overcome this disadvantage and enable male reproductive success. Apart from substantial human sexual dimorphism in weight and muscle mass [33], there are several indicators that humans (at least historically) have been a polygynous species in which maternal investment might have contributed more to male reproductive success by increasing male survival during early life and by optimizing male offsprings physique for the male–male competition concerning reproductive access. Examination of the second-to-fourth digit ratio of early modern humans and Neanderthals indicates a historically higher incidence of polygyny [34]. Even today, women across cultures appear to value cues to resource acquisition, such as physical work or earning capacity [7,35,36], whereas men value characteristics indicating reproductive capability, such as physical attractiveness, youth and health [35,36]. Testosterone levels have previously been positively correlated with maternal allocation in the form of birth weight [37]. A growing body of literature links testosterone levels to physical fitness, defined by the authors as an individuals performance in a standardized, gym-based, physical performance exam, [38] and life-time mating success [38,39] in males. Historically, men have been tasked with protecting the family [40] and acquiring rare resources, which was physically demanding and involved health-threatening hunting manoeuvers and ubiquitous male–male violence in the competition for resources [41,42]. Hence, sexual dimorphism might be a result of both sexual and natural selection, where males have greater benefits from early maternal resource allocation.

A further explanation for sexual dimorphism might be provided by the metabolic efficiency model, which hypothesizes that a certain sex will be more capable of converting maternal resources into growth, because natural/sexual selection might have resulted in the generalization of this phenotype [18,43–45]. While this is an appealing theory, we find no detectable weight differences between males and females fed with infant formula in our study. Owing to the fixed energy concentration of infant formula we, therefore, would not expect differences in metabolic efficiency between male and female.
offspring, assuming they consumed similar quantities. Even though previous publications have found no differences in energy balance (kcal kg$^{-1}$ d$^{-1}$) between male and female infants [46], the absence of energy intake measurements in this study makes distinction between metabolic efficiency and maternal resource allocation impossible and hence such measurements should be included in future studies.

It is also imaginable that a sex-bias in offspring energy allocation towards growth, maintenance or activity might be explanatory for the perceived sexual dimorphism; though previous studies on infants have found no fundamental differences in resting energy expenditure (REE; measured in kcal kg$^{-1}$ d$^{-1}$) [46] that could be indicative of such allocation differences. Similarly, studies on preschool children did not detect differences in body composition in terms of body fat measured by bio-impedance [47], BMI (kg m$^{-2}$) [48], fat mass index (kg m$^{-2}$) [48], fat-free mass index (kg m$^{-2}$) [48], nor was a difference in endurance, measured by shuttle run test (laps) [48], levels of moderate-to-vigorous physical activity (min d$^{-1}$) [48] or sedentary behaviour (min d$^{-1}$) [48] found. One study, investigating a slightly older population of children aged 4–10 years, found that REE tended to be higher among males while no difference in activity-related energy expenditure was found between sexes, resulting in a greater total energy expenditure among males [49]. While it is uncertain to what extent the results from Goran et al. [49]

Figure 1. The complex impact of firstborn sex, sex and feeding method on postnatal growth. Here, we compare mean adjusted postnatal weight (kg) by (a) sex, (b) parity category, and (c) feeding method. Error bars represent the 95% confidence interval for the mean. In (d) children are grouped by firstborn sex (if applicable), sex and feeding method. Row names are compiled of abbreviations for firstborn sex (M = male, F = female, NA = not applicable as this is a firstborn), sex (M = male, F = female) and feeding method (B = breast milk, I = infant formula). p-values from Bonferroni test conducted for comparison of the groups in (d) are shown in (e). As can be seen, the most significant difference is found between firstborn, breastfed males and females. (Online version in colour.)
are applicable to our population (median age 1.16 years), they would rather suggest that males experience faster postnatal growth besides having a greater energy expenditure.

The local resource competition model, as mentioned in the introduction, is also a prominent theory explaining the evolutionary advantage of sexual dimorphism. In this study, we, however, find the vast majority of mothers to be normal or overweight and therefore conclude that resource shortage is not apparent in our population.

Another interesting question is how such a sex-biased resource allocation might be mediated. The ‘parent-offspring conflict’ described by Trivers is a trade-off between parents aiming to produce several offspring to maximize biological fitness and the offspring aiming to maximize its own chances to forward its genome to future generations [3]. In that context, the deployment of fetal hormones to manipulate maternal resource allocation could play a role. It has previously been shown that, for example, fetal testosterone can cross the placental barrier, enter the mother’s blood stream, where it is converted to oestrogen and then stimulates accumulation of adipose tissue during pregnancy [50]. This has been hypothesized to be a strategy deployed by male infants to refill maternal energy reservoirs enabling higher levels of energy transfer during lactation [51]. It is imaginable that fetal hormones might also affect the differentiation of the mammary gland, resulting in epigenetic changes that increase available milk energy for this and consecutive offspring, as previously suggested by Hinde [20]. While speculative, this could explain the observed closing of the weight difference between male and female offspring born consecutive to a male firstborn.

In accordance with previous research [14], our findings also suggest that parity is associated with both prenatal and postnatal growth velocity. In particular, we report that prenatal growth for both sexes was enhanced by parity. Furthermore, we find that parity was negatively correlated with postnatal weight in females but not in males. The finding of enhanced prenatal weight is in agreement with the literature, where parity frequently has been shown to increase the quality of the intrauterine environment.

In concurrence with previous reports of fetal growth restraints [14,52,53], we also found that maternal smoking during pregnancy impaired intrauterine growth. Earlier studies have discussed if growth deficits persist into childhood [54–56] and while maternal smoking affected birth weight, length and head circumference, we did not observe an effect on paediatric growth propensity (adjusting for birth weight). A possible mechanism behind the prenatal growth restriction could be that the offspring in utero is affected by both higher inflammatory stress and less nutritional supply owing to placental vascular changes. The analysis of hospital records allowed a sample size that is considerably larger than that of most other studies in this field (n = 11,798) and is, therefore, a major strength of this study. The routine documentation of obstetric outcomes minimized missing data and allowed adjustment for the investigation of a high number of confounders, resulting robustness of our regression models for postnatal weight (adj. \( R^2 \approx 0.813 \)). By exclusion of all children admitted to the paediatric ward within a week after birth we ensured that children in this study had no pregnancy or birth-related complications, maximizing generalizability of our results. Furthermore, we ensured that sex-specific weight gain was not unevenly affected by disease. While the large sample size allowed establishment that infant weight is sex-biased and affected by firstborn sex, the retrospective design as well as the absence of energy intake or expenditure measurements does not allow us to determine whether this bias is mediated by differences in maternal resource allocation or metabolic efficiency. While the interaction of firstborn sex and feeding method on consecutive children’s growth were notable and are of similar proportions compared to studies on three to four months old primates (rhesus macaque) [20], it is important to distinguish their biomedical and evolutionary significance. In biomedicine, large effect sizes are important, because of the practical implications for public healthcare policies and decisions [57]. In evolution, small effect sizes can be very important [58]. This is all more likely to be the case in studies that aim to detect the signature of past evolutionary processes against a background of anthropic influences, such as the present study. It is, nonetheless, interesting to speculate on factors that might account for the small effect sizes in the present study. Firstly, it should be noted that the feeding method recorded in the obstetric database is based on a combination of feeding intention and observed feeding during the perinatal period and postnatal hospital stay and does not necessarily correspond to the feeding method that was applied at home. In addition, the lack of information on breastfeeding duration and breastfed volumes is likely to result in an understating of the effect of breastfeeding, as mothers commonly exclusively breastfeed for only short periods of time [59]. Additionally, many children included in this study are above 1 year of age and might hence have been introduced to solid food. This unlimited access to solid nutrition might allow children to compensate for differences in maternal allocation, which also could lead to an under-estimation of the effect of breastfeeding.

4. Conclusion

Here, we report that sex of the firstborn can affect the growth patterns of consecutive children, through birth order and offspring sex dependent on breast feeding. Furthermore, this study has identified several factors influencing pre- and postnatal weight and resulted in the creation of a highly predictive model for postnatal weight. In part, our results are consistent with previous animal data, adding to the literature by examining humans, using solid statistical methods, a large sample size and adjusting for a high number of factors. Our findings warrant further mechanistic and epidemiological studies to elucidate birth order dependent breast milk composition and could prove relevant for identification of children that are at-risk for impaired postnatal weight gain, as well as for tailoring infant formula composition. Furthermore, our results highlight the importance of birth order and offspring sex as major confounders for early weight development and warrants studies to show whether this programmes weight and metabolic health for later stages of life.

5. Methods

(a) Study population

Between January 2007 and February 2018, data were obtained from electronic medical records from 33,874 admissions to the Paediatric ward of a metropolitan teaching hospital in Western Sydney, Australia. Entries of children that had been transferred from the obstetric ward to another ward, within a week after birth were excluded (n = 5111), as were entries of children older than 5 years

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of age at admission \((n = 5611)\). Several children \((n = 5762)\) had been admitted multiple times, to ensure that every child only entered the analysis once, the latest medical entry with anthropometric measures was chosen and the remaining entries for that child were excluded, leaving medical records for 16,355 children.

Obstetrix [60], a state-wide mandatory database collecting obstetric data, included information on the mother, pregnancy-related information and infant related information such as anthropometric measurements for 67,268 infants delivered at the hospital between 2000 and 2017. Obstetrix was matched with the paediatric database using the children’s individual medical record number.

This study complies with the Helsinki Declaration and relevant national guidelines and was approved by the Human Research Ethics Committee of the Nepean Blue Mountains Local Health District (ethics number: 10/16). As this is a retrospective study conducted on databases, obtaining of patient’s informed consent prior to inclusion was not applicable.

(b) Statistical information
Descriptive statistical analyses were conducted for maternal and infant characteristics. Two-sided Mann Whitney U-tests were used for comparison of maternal age, maternal weight and maternal BMI. t-tests (two-sided) were used for comparison of offspring birth weight per maternal weight between groups and the \(\chi^2\)-test (two-sided) was generally used for comparison of sex ratio between groups.

The included variables were screened for two-way variable interaction using generalized linear models. If several interactions included the same variable the possibility of three-way interactions was investigated. Entering variables and variable interactions into a stepwise backward eliminated multiple regression model ensured that only the best sets of predictive variables were included in the final model. Variables with a \(p\)-value of \(p \geq 0.1\) were removed through backward elimination. The parsimonious regression models were then followed up with groupwise comparisons, using \(\beta\)-values for all statistically significant variables in the regression model (except the investigated grouping variables) to adjust postnatal weight. Grouping children by firstborn sex (if applicable), infant sex and feeding method a two-sided ANOVA was conducted. ANOVA was followed up with post hoc Bonferroni tests to investigate the specific effects of feeding method, firstborn sex and infant sex on postnatal growth.

During analysis maternal weight, maternal BMI, maternal age, gestational age (in weeks), birth weight, birth head circumference, birth length, admission age and admission weight were used as continuous variables, while maternal diabetes, multiple birth, maternal smoking during pregnancy, illegal drug use, alcohol during pregnancy as well as all dummy variables describing the maternal continent of birth were binary variables with the categories yes and no. Feeding pattern was categorized into either breast milk (including infants that had been fed with both breast milk and formula) or infant formula, and infant sex was either male or female. The variable parity was used as a binary variable (primiparous or multiparous). Significance was set at \(p < 0.05\) and statistical analysis was conducted in SPSS statistics (v.24, IBM Corporation, Chicago, Illinois, USA).

Ethics. This study complies with the Helsinki Declaration and relevant national guidelines and was approved by the Human Research Ethics Committee of the Nepean Blue Mountains Local Health District (ethics number: 10/16). As this is a retrospective study conducted on databases, obtaining of patient’s informed consent prior to inclusion was not applicable.

Data accessibility. The data analysed in this study was obtained from electronic hospital records. The data for this manuscript will not be made publicly available because of patient integrity and confidentiality. Requests to access the data should be directed to the Nepean Blue Mountains Local Health District.

Author contributions. R.N., A.L., and S.S. designed research; S.S. and F.S. collected data. D.R. and S.S. extracted and prepared data. S.S. performed research, analysed data and took the lead in writing the manuscript. R.N., A.L., D.R. provided critical feedback and helped shape the manuscript. The authors declare no conflict of interest.

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Author contributions. R.N., A.L., and S.S. designed research; S.S. and F.S. collected data. D.R. and S.S. extracted and prepared data. S.S. performed research, analysed data and took the lead in writing the manuscript. R.N., A.L., D.R. provided critical feedback and helped shape the manuscript. The authors declare no conflict of interest.

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