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Features of placental morphology, fetal growth, and adverse cognitive outcomes in childhood

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Features of placental morphology, fetal growth, and adverse cognitive outcomes in childhood

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An abstract of
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Abstract

Features of placental morphology, fetal growth, and adverse cognitive outcomes in childhood

By Alexa A. Freedman

Background: The placenta plays a critical role in regulating healthy fetal development by mediating oxygen, nutrient, and waste transfer between the mother and the fetus. Poor placental development may result in suboptimal fetal growth due to failure of the placenta to meet the needs of the fetus. Neonates who experienced suboptimal fetal growth are at greater risk of perinatal morbidity and mortality, as well as cognitive delays in childhood. There may also be sex-specific differences in these associations due to potential differences in placental and fetal development.

Methods: We used data from four completed studies to investigate relationships between placental morphology (thickness, surface area, shape, and umbilical cord insertion), birthweight, and intelligence quotient (IQ). First, we examined the validity of using placental diameters to calculate surface area using 491 placentas that underwent a standardized examination. Next, we evaluated the associations between placental morphology and birthweight separately in 1,229 singletons and 208 sets of dichorionic twins. Finally, we assessed the relationships between placental morphology and IQ in 514 singletons at age five and 82 sets of twins at age seven.

Results: We found that diameter-based measures slightly under estimated placental surface area but were a good proxy for use in subsequent analyses. In investigating relationships between placental morphology and birthweight, we found that thickness and surface area were independently associated with birthweight among singletons and twins. Further, estimates of the associations diverged for same-sex male and same-sex female twin pairs, with stronger associations observed among same-sex male pairs. Finally, we found that features of placental morphology were not associated with IQ in childhood among singletons or twins. However, when we considered interaction between sex and placental morphology, estimates were stronger among both male singletons and same-sex male twin pairs as compared to female singletons and same-sex female twin pairs, respectively.

Conclusions: Our results suggest that features of placental morphology are associated with birthweight but not with IQ. Further, results from analyses in twins support sex-specific differences in these associations. Given the critical role of the placenta in regulating fetal growth, future studies should consider the role of the placenta in the developmental programming of long-term health outcomes. Promoting healthy pregnancy may be an important form of primary prevention for many adverse health outcomes.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The placenta plays a critical role in regulating fetal growth and development

Characterized as the "diplomat of maternal fetal relations," the placenta regulates fetal development by enabling communication between mother and fetus across all critical physiologic systems.[1] In particular, the placenta acts as a mediator of oxygen, nutrient, and waste exchange, regulates interaction with the maternal immune system, and produces hormones and growth factors necessary for fetal development.[2] Placental size and shape are factors that affect the capacity of the placenta to support the fetus through these processes, supported by associations between these factors and placental function and efficiency.[2-4] Additionally, one study reported that approximately 40% of the variation in birthweight can be explained by features of placental morphology.[5] This dissertation is motivated by the critical and functional role of the placenta as the regulator of fetal growth and development.

The placenta grows and develops during pregnancy as fetal demands for nutrients and oxygen increase.[6] Throughout pregnancy, the placenta covers 15% to 30% of the uterine surface.[6] The main components of the placenta responsible for exchange include the chorionic villi and the maternal spiral arteries. Remodeling and erosion of maternal spiral arteries by trophoblasts allow maternal blood to flood the intervillous space around the chorionic villi to facilitate exchange of oxygen, nutrients, and waste.[7-9] Approximately 80 to 100 spiral arteries supply the fetus and placenta.[6] The chorionic villi expand to increase exchange capacity and can have a total surface area of between 4 and 14 m^2.[6, 7] Gases, glucose, water, and waste products are exchanged through diffusion, and placental efficiency for gas exchange is similar to that of the lungs.[7] A mature placenta's intervillous space can hold approximately 150 mL of blood, which is replenished every three to four minutes.[6] At term, the typical placenta is round or oval,

weighs an average of 470 grams, has a diameter of 15 – 25 cm, and is approximately 2.5 cm thick.[6, 10]

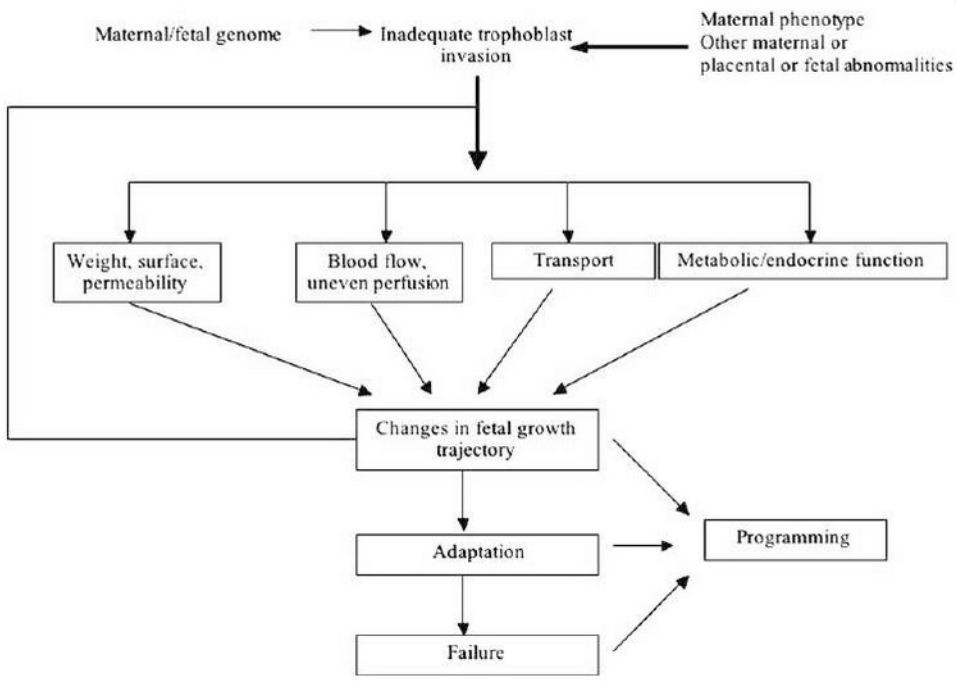

Figure 1.1. Potential mechanisms for suboptimal fetal growth and developmental programming (Burton et al., 2011, p. 117, reproduced in alignment with publisher's permission policy).[11]

As the placenta regulates fetal growth, one proposed mechanism for suboptimal fetal growth suggests that poor placental development results in failure of the placenta to meet the oxygen and nutrient needs of the fetus to support optimal development (Figure 1.1 and Figure 1.2, relationship 1).[11, 12] This results primarily from poor vascularization, including abnormal spiral arteries, inadequate invasion of spiral arteries, and restricted migration of trophoblast cells (cells from which placental tissues are derived), which prevents the placenta from adequately supplying the fetus.[6, 13] Poor perfusion may result in hypoxia, which can also result in changes to placental vascular structure.[13] Studies of both women living at high altitudes and women with

anemia have demonstrated that placental development is hindered in a hypoxic environment.[14] Additionally, a prominent hypothesis for preeclampsia suggests that inadequacy in spiral artery remodeling results in a hypoxic environment that further damages placental development.[2] A hypoxic environment has also been associated with brain injury, although the pathologies are different in preterm and term infants.[15] In animal studies, a hypoxic environment generally results in compensatory increased fetal cerebral blood flow, although abnormal cerebral structure and function still develop and the overall growth rate of the fetus tends to slow.[13, 15] However, these animal experiments are based on restricting blood flow to the uterus rather than directly affecting placental structure or function to create an adverse in utero environment in a manner similar to the proposed mechanisms.[13, 15]

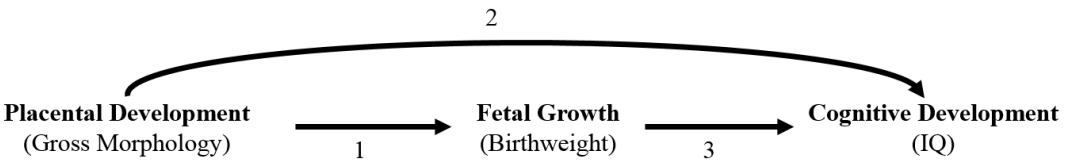

Figure 1.2. Simplified diagram of relationships between placental morphology, birthweight, and IQ.

Another mechanism related to placental development and oxygenation is based on timing of oxygenation. The placenta initially develops in a low-oxygen environment; the oxygen concentration does not increase until the onset of maternal circulation at the end of the first trimester.[10] Failure of trophoblast invasion and remodeling of spiral arteries can result in an increase in blood flow velocity and oxygenation.[16] Premature oxygenation of the placenta may result in oxidative stress and damage to placental tissue.[10] In extreme cases, this may result in miscarriage.[10] In less extreme cases, this may result in a smaller placenta, which may lead to an

abnormal growth trajectory for the placenta, and subsequently, the fetus.[10] This mechanism may be reflected in an abnormal placental shape or eccentric umbilical cord insertion due to tissue loss or adaptive growth.[10]

Oxidative stress may also be related to a proposed mechanism that involves inflammatory processes. In the presence of stress, the placenta may release pro-inflammatory cytokines. Cytokines detected in umbilical cord blood, likely originating in the placenta, have been associated with fetal brain injury.[13] Inflammation related to maternal infection has also been associated with brain injury through exposure via amniotic fluid. Additionally, perinatal brain injury has been associated with inflammatory markers in the placenta.[13] Inflammation may affect placental structure and/or function, although the pathway is not well understood.[15] Inflammation may also result due to hypoxia, which makes these proposed mechanisms difficult to disentangle.

The morphology of the placenta may reflect these mechanisms. Placental characteristics associated with a growth-restricted fetus include reduced two-dimensional surface area and size, lower density of trophoblasts, and reduced chorionic villi.[2, 13] Additionally, various types of lesions are associated with poor perfusion and inflammatory processes.[13, 17] However, the placenta has the ability to adapt and respond to stressors through altered blood flow and changes in cell structure, vascularization, endocrinology, and metabolism.[8, 15] This plasticity makes it difficult to determine if features of placental morphology dictate placental function, or if reduced function results in adaptive placental changes that appear in the morphology.

In addition to placental-based explanations for suboptimal fetal growth, fetal and maternal characteristics may also be responsible (Figure 1.1).[11] Fetal characteristics include genetic and structural disorders, such as congenital heart disease, trisomy 13, and trisomy 18.[12] Maternal

conditions include pre-gestational diabetes mellitus, autoimmune disease, hypertension, infectious diseases, and substance abuse.[12] However, maternal hypertension-related conditions may have placental origins. While chronic maternal hypertension may affect placental development leading to suboptimal fetal growth, poor placental development may result in the development of preeclampsia and eclampsia.[18] In both of these scenarios, the placenta is affected but the direction of the effect differs.

Suboptimal fetal growth is much more common in twin than in singleton gestations, with an estimated prevalence of 15 to 25%.[19] The placental mechanisms in twins depends on placentation. Monozygotic twins can be either monochorionic (shared placenta) or dichorionic (separate placentas), depending on when in development division occurs (division generally must occur within two days of fertilization for separate placentas to develop).[20] Dizygotic twins are always dichorionic, however placentas in close proximity may fuse, giving the appearance of a monochorionic placenta.[20] In monochorionic twins, suboptimal fetal growth may occur in one twin as a result of unequal placental sharing or twin-to-twin transfusion syndrome.[10]

In dichorionic twins, placental development and the fetal-placental environment are similar to singletons; however, the uterine environment is different, as dichorionic twins may compete with each other for space and resources.[21] If implantation occurs in close proximity, then the individual placentas may grow into each other, impeding further growth and resulting in a fused placenta.[21] Fused placentas reflect the most extreme outcome of placental proximity and one study has demonstrated that there is greater birthweight discordance in twins with fused placentas as compared to separate placentas.[22] Similarly, another study reported that twins with fused placentas are lighter than twins with separate placentas.[23] Although monochorionic and fused dichorionic twins may have additional mechanisms for suboptimal fetal growth, these

mechanisms are less of a concern in dichorionic twins with separate placentas. Additionally, dichorionic twins with separate placentas are reported to have the same allometric scaling factor as singletons, which indicates that the relationship between placental weight and birthweight is similar in both groups.[24]

The placenta is the least understood organ

The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) recently allocated $41.5 million to study the placenta as part of the Human Placenta Project (HPP). Given the dynamic nature of the placenta over the course of pregnancy, one of the priorities of the HPP is real-time assessment through advances in imaging and biotechnology.[25] While an extremely important initiative, it may be years before the technologies and methods developed as a part of this initiative are affordable and available for use in the general population.

Several studies have evaluated the use of placental imaging in identifying suboptimal fetal growth with mixed results.[26-28] One study assessed vascularization using 3-dimensional power Doppler (3DPD) and found no difference in the first trimester between growth-restricted fetuses and those who develop normally.[26] Similarly, another study found that 3DPD in the first trimester could not be used to predict growth restriction.[27] A study using specialized magnetic resonance imaging (MRI) found that growth-restricted fetuses had up to a 33% reduction in functional placental tissue in the second and third trimesters as compared to normally developing fetuses. Although a promising tool, the specialized MRI is difficult to use; 19 of the 54 women in the study were excluded due to poor image quality.[28] As the placenta grows and develops with the fetus, there has also been a push to evaluate serial placental measures. One study with

repeated placental thickness measures reported correlations between thickness in the second and third trimesters and birthweight.[29]

Although imaging technology is widely regarded as safe, there is concern that increased use of existing technology, as well as the introduction of new technology, could result in harmful exposure.[30] Further, guidelines issued following a joint workshop of the NICHD, the Society for Maternal-Fetal Medicine, the American College of Obstetricians and Gynecologists (ACOG), and several radiology and ultrasound organizations state that use should be limited to pregnancies in which clinical indications are present and that unnecessary use should be avoided.[31] Additionally, specialized imaging technology may have limited applicability in epidemiologic studies due to potential cost, availability, and utility.[29]

Studies have also evaluated placental structure and function through methods other than imaging. Several studies have evaluated histologic characteristics of postnatal placental specimens, including chronic villitis, villous maturation, placental abruption, and infarction, although there are conflicting results.[32] Biomarkers, some of which are produced by the placenta, have also been evaluated as possible indicators of placental function. Commonly evaluated biomarkers include placental growth factor, pregnancy-associated plasma protein a, placental growth hormone, C-reactive protein, and soluble fms-like tyrosine kinase-1, which has been used to identify preeclampsia. However, a meta-analysis of studies exploring these biomarkers found that none of the 37 biomarkers evaluated are sufficient on their own to use as predictors of suboptimal fetal growth.[33] Time between sample obtainment and biomarker assessment was not consistent across studies and this may have affected the results.[33] Due to the limited findings of studies evaluating placental structure and function using imaging, postnatal

histology, and biomarkers, evaluating these potential markers in combination may be more useful and informative than evaluating them individually.[8]

One challenge in both imaging-based and biotechnology-based assessments is the timing at which the placental characteristics or biomarkers are evaluated. Mixed results may be due to differences in timing of assessment with respect to gestational age.[33] Additionally, one concern with novel measures that rely on postnatal placental specimens is the ability to collect and store placental samples, as quality is highly dependent on numerous factors, including timing of sample collection and method of preservation.[34] Given these limitations and guidelines, findings from imaging and biotechnology-based advances may not be applicable and/or generalizable to women with low risk pregnancies. This supports the need for easily obtainable, population-based measures of placental function. These types of measures may also offer guidance to etiologic researchers pursuing technological advancement by identifying a more homogenous population of affected neonates to study.

Suboptimal fetal growth has short and long-term health consequences

Suboptimal fetal growth is a strong risk factor for perinatal mortality.[12, 35] A meta-analysis found that fetuses identified as having a weight < 10th percentile for gestational age have four times the risk of stillbirth.[36] In addition to perinatal mortality, suboptimal fetal growth is also a risk factor for perinatal morbidity.[12] One study reported an increase in preterm delivery, caesarean section, and neonatal intensive care unit admission in cases of severe growth restriction identified before 24 weeks gestation.[37] Additionally, another study reported that respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis are more common among growth-restricted neonates born between 30 and 40 weeks gestation.[38]

Suboptimal fetal growth also increases the risk for adverse health outcomes later in life, including cognitive impairment and cardiovascular disease (Figure 1.2, relationship 3).[12, 39] This reflects the concept of the Developmental Origins of Health and Disease, which postulates that adult health outcomes can be traced back to early fetal development.[40] Low birthweight, defined as a birthweight < 2,500 grams, is associated with coronary heart disease, hypertension, and stroke.[41] One suggested mechanism for cardiovascular outcomes is that increased placental vascular resistance places a burden on the fetal heart.[2] Animal studies indicate that this may result in a variety of physiological adaptations in the offspring, including redistribution of cardiac output, impaired adrenaline response, and increased cortisol concentration.[42] These physiologic changes may lead to several pathways for adverse adult health outcomes, including reduced functional capacity of affected organs due to altered structure, different hormone responses and metabolism, and increased vulnerability to exposures later in life, including oxidative stress.[41, 43] Cardiovascular outcomes are also related to the idea of 'brain-sparing,' where in the case of an adverse environment, the fetus prioritizes resources to the nervous system at the expense of other organ systems, including the cardiovascular system.[44] However, studies have demonstrated that cognitive impairment is also associated with suboptimal fetal growth, indicating that the brain is not entirely spared from adverse effects.[45] In addition to cardiovascular and cognitive outcomes, studies have reported that measures of fetal growth are associated with diabetes, osteoporosis, and certain cancers.[41]

Since the placenta regulates growth and suboptimal fetal growth is associated with adverse health outcomes, it is not surprising that features of placental morphology are also associated with adverse childhood and adult health outcomes (Figure 1.2, relationship 2). Studies have reported that placental characteristics, including a thin placenta and a small surface area, are

associated with adverse adult health outcomes including sudden cardiac death and chronic heart failure.[46, 47] In children, one study reported that increasing placental weight relative to birthweight is associated with high systolic blood pressure and another study found that placental weight and diameters are positively associated with body mass index (BMI).[48, 49]

Additionally, features of placental morphology, including weight, surface area, and diameters, have been associated with both reduced intelligence quotient (IQ) and adverse mental health outcomes in childhood, although the associations differed by sex.[50, 51] This may result from the previously described mechanisms having a functional impact on the placenta's ability to produce neuropeptides, which may affect brain development.[52] The brain also grows rapidly during late gestation, which makes it particularly sensitive to inadequate oxygen and nutrient supply.[53] An alternative hypothesis suggests that the relationship between placental morphology and cognitive development may be confounded by vascular endothelial growth factor (VEGF). VEGF regulates angiogenesis in both the placenta and developing brain and has also been shown to have neurotrophic effects.[54] Features of placental morphology that are dependent on angiogenesis, such as thickness and surface area, may reflect levels of VEGF.[50, 55] This may also account for sex-specific differences in reported associations, as there may be sex-specific differences in levels of VEGF.[56]

Similarly, studies have found evidence of sex-specific differences in placental development. One study reported that placentas of males tend to be thicker while placentas of females tend to have a larger surface area.[57] The authors hypothesize that this may reflect differing growth strategies, with males thought to invade spiral arteries deeper and females thought to expand the surface area of the placenta. Another study reported that male and female fetuses may respond and develop differently in the presence of stressors.[58] This is supported by

results suggesting that placentas of males are smaller than placentas of females when controlling for birthweight, which indicates that placentas of males may be more efficient than placentas of females.[59] However, as a trade-off for efficiency, placentas of males are hypothesized to have less reserve capacity, which may result in a higher risk of becoming undernourished.[59, 60] Placentas of males are also thought to be more sensitive to factors that influence the intrauterine environment, including maternal stress.[60, 61]

Sex-specific differences in placental development may also have implications for developmental programming. One study reported differences in associations between placental morphology and adult hypertension in males and females.[59] Hypertension in males is associated with an increase in the shortest placental diameter in relation to birthweight and hypertension in females is associated with a small surface area. The authors postulate that differing growth strategies may explain the increased risk of hypertension and reduced life expectancy among males.[59] Additionally, males tend to have a larger head circumference and thinner body size than females, which may suggest that males may prioritize brain development at the expense of body size.[8] This prioritization has potential implications for cardiovascular and cognitive development later in childhood. However, males are also at higher risk for adverse neurodevelopmental outcomes and learning disabilities.[13] A study on features of placental morphology and mental health outcomes in childhood, including symptoms of Attention Deficit Hyperactivity Disorder, reported significant associations among males only.[51] Further, a study evaluating placental characteristics and cognitive outcomes in childhood found sex-specific differences in associations, reporting that placental diameters are only associated with IQ in females while increased placental thickness is associated with higher IQ in both males and females.[50] The long-term implications of placental development and suboptimal fetal growth on both cardiovascular

disease and cognitive impairment have a lasting impact on quality of life.[62] Enhancing our understanding of these relationships may improve our ability to develop prevention and early intervention strategies.

LITERATURE REVIEW

Evaluating error in estimated placental surface area

Only two studies, summarized in Table 1.1, have considered the validity of recorded placental measures as compared to image analysis. One study reported that image-based measures explain 14% more of the variation in birthweight as compared to diameter-based measures.[63] However, no direct comparison between image-based surface area and diameter-based surface area was made. Regression results from image analysis of a cohort study conducted in the early 2000s were compared to regression results from diameter-based analysis of a cohort study conducted in the 1950s.[63] Another study compared maximum and minimum diameters obtained from images to those obtained from manual measurements in the same study population and reported that they are highly correlated.[64] However, this study did not take the additional step of comparing diameter-based surface area to image-based surface area, which presumably has more variation since this comparison requires the additional assumption that placentas have a uniform, elliptical shape.

To our knowledge, no studies have directly examined the correlation between image-based and diameter-based measures for constructs such as surface area, where the potential for error is increased. Quantifying the difference in the two methods is informative for sensitivity analyses, which can then be applied to results from diameter-based measures, which are available in some historical cohorts, including the Collaborative Perinatal Project, the Helsinki Birth Cohort, the Northern Finland Birth Cohort, and the Dutch Famine Birth Cohort.[55, 65-67] Additionally, measures of placental diameters are relatively easy to obtain following delivery and can also be evaluated during routine ultrasound scans.[68]

Table 1.1. Studies evaluating the validity of placental measures.			
Study	**Sample**	**Measures**	**Notes**
Salafia et al., 2005[63]	628 placentas, North Carolina, United States, 2002-2004	• Surface area • Major axis • Minor axis • Eccentricity • Umbilical cord insertion	• 35% of birthweight variance explained by image-based measures, as compared to 21% in a different study of diameter-based measures (measures reflect R^2 values)
Coall et al., 2009[64]	513 placentas, Perth, Australia, 2001-2003	• Major axis • Minor axis • Umbilical cord insertion	• Manual and digital measures of major axis, minor axis, and umbilical cord insertion were highly correlated (range of correlation coefficients: 0.71, 0.87)

Features of placental morphology and fetal growth

Studies that have evaluated associations between placental morphology and birthweight have limitations that merit further study. Most studies evaluate placental weight and/or fetal-placental weight ratio, as placental weight is the most commonly collected placental variable. One study based on an analysis of siblings reported that a small fetal-placental weight ratio (high placental weight in relation to neonate size) is associated with a diagnosis of small for gestational age (SGA; birthweight <10th percentile) in term births.[69] However, one limitation in using placental weight is its dependence on several factors, including if the cord and membranes are trimmed, timing of cord clamping, time between delivery and weighing, and how the placenta is stored prior to weighing, which make consistent assessment challenging.[10, 70] Additionally, while weight is an important characteristic, weight and efficiency are determined by the underlying shape and structure of a placenta.[71] Further, specific features of placental morphology have different critical periods and reflect the growth and function of different parts of the placenta.[5] For example, thickness may reflect the vascularization and branching of the chorionic villi, which is the key aspect of placental development during the third trimester.[63, 72] Similarly, surface area is generally established prior to third trimester.[5] These characteristics of features of

placental morphology may provide insight into etiology of conditions of interest, including suboptimal fetal growth. Studies evaluating the associations between postnatal placental measures (other than placental weight and fetal-placental weight ratio) and birthweight are discussed below and summarized in Table 1.2.

There is conflicting literature on the relationship between placental thickness and birthweight. Chisholm and Folkins (2016) reported that placentas of SGA neonates were thinner than the placentas of appropriate for gestational age (AGA; birthweight in the 10^{th}-90^{th} percentile) neonates, while a similar study by Vedmedovska et al. (2011) found no association.[32, 73] Further, Salafia et al. (2007) reported that for every 1 cm increase in placental thickness, birthweight increases by 213.7 grams (95% confidence interval (CI): 202.5, 224.8) when adjusting for other placental measures, gestational age, parity, and sex.[71] However, Grandi et al. (2016) did not find an association between placental thickness and birthweight when controlling for maternal, neonatal, and other placental characteristics.[74] Similarly, a study of pregnancies with reduced fetal movement found no difference in thickness between pregnancies with an adverse outcome as compared to a normal outcome (of the 23 neonates with an adverse outcome, 21 were SGA).[75]

Several studies have evaluated longest and shortest placental diameters. Three studies found that both diameters are smaller in SGA neonates as compared to AGA neonates.[32, 76, 77] However, other studies have found associations with only one of the two diameters. Grandi et al. (2016) reported that for every 1 cm increase in longest diameter, birthweight increases by 16.4 grams (95% CI: 2.7, 30.9) but reported no association with shortest diameter when controlling for maternal, neonatal, and other placental characteristics.[74] Conversely, in unadjusted models,

Alwasel et al. (2012) found no association with longest diameter but reported that for every 1 cm increase in shortest diameter, birthweight increases by 125 grams (95% CI: 88, 162).[78]

Measures of placental shape and surface area are commonly derived from longest and shortest diameter, although some studies have also used placental images to evaluate these measures.[63, 64] In studies that use diameters, two found reduced surface area in placentas of SGA neonates as compared to placentas of AGA neonates.[32, 79] Similarly, Balihallimath et al. (2013) reported that birthweight and surface area are positively correlated and Salafia et al. (2007) reported a similar association in a model adjusted for other placental characteristics, gestational age, parity, and sex.[71, 80] However, Coall et al. (2009) reported only a weak correlation between surface area calculated from placental images and birthweight (Spearman's correlation: 0.37; p-value <0.01).[64]

There may also be sex-specific differences; Misra et al. (2009) reported that the relationship between surface area and birthweight is stronger in females than in males.[58] Further, Salafia et al. (2005) reported that when measures of lateral growth, including surface area and shape from placental images, are added to a model containing umbilical cord insertion site to predict birthweight, the adjusted R^2 increases by 0.32 and 0.11 in preterm and term births, respectively.[63] Two studies evaluated the association between shape parameters and birthweight and found no association.[71, 81] These studies had different measurement protocols; Salafia et al. (2007) relied on diameters to determine shape while Haeussner et al. (2013) used placental images.

Centrality of umbilical cord insertion has been evaluated in two ways in the literature: based on a categorical description of insertion site (central, paracentral, marginal, and velamentous) or based on a ratio of the distance from the insertion site to the edge of the placenta

compared to the average radius of the placenta (continuous variable). Biswas and Ghosh (2008) (based on categorical description) reported that 29% of SGA neonates had a central umbilical cord insertion, as compared to 55% of AGA neonates.[76] Salafia et al. (2005) calculated the continuous measure using chorionic plate images and reported that umbilical cord insertion accounted for 34% and 10% of the variation in birthweight of preterm and term births, respectively.[63] However, three studies found no correlation between birthweight and umbilical cord insertion.[64 75, 82] Similarly, two studies found no difference in umbilical cord insertion between SGA and AGA neonates.[32, 73] These five studies did not adjust for any covariates.

One explanation for the overall conflicting results is the variety in measurement and definition of the features of placental morphology. For example, some studies use equations based on diameters to determine shape and surface area, while others use chorionic plate images. Additionally, some studies characterize variables like umbilical cord insertion site while others use a ratio comparing the radius of the placenta to the distance between the umbilical cord and edge of the placenta. Another explanation is the variety in analytic methods applied and covariates included. Some studies report only unadjusted correlations or t-tests, some studies report analyses adjusted for other placental variables, and some studies report models additionally adjusted for fetal and maternal characteristics. Conflicting results may also be due to the variety in populations studied: the fifteen studies described in Table 1.2 represent ten countries spanning six continents.

A limitation of several of the studies that are most similar to this proposed research project is their small sample size.[32, 73, 75-77, 79, 80] The studies with large sample sizes used data from Collaborative Perinatal Project (CPP),[5, 58, 71] a large cohort study conducted in the United States. The CPP enrolled pregnant women in 1959-1966 and gestational age is based on reported last

menstrual period, as this was before ubiquitous use of ultrasound dating. Due to the known measurement error, gestational age has been restricted in these studies to minimize misclassification, thus the results are only applicable to neonates born at or near term. As accurate gestational age is an important confounder, examining these results in cohorts with better data on gestational age is an important addition to the literature.

One challenge in synthesizing the results of these studies is the variety in analytic methods used. Eleven of the sixteen studies described in Table 1.2 are based on statistical tests and correlations, which do not account for important covariates like gestational age, parity, pre-pregnancy BMI, and infant sex.[32, 58, 64, 73, 75-77, 79-82] Three of the five studies reporting adjusted models account only for other placental characteristics.[63, 78] In addition to placental characteristics, two studies adjust for gestational age, parity, and infant sex, with one study further adjusting for preeclampsia and smoking status.[71, 74] These two studies reported significant associations between features of placental morphology and birthweight, although they only had one placental feature in common. Both studies evaluated placental thickness at the center and reported conflicting results. For every 1 cm increase in thickness, Salafia et al. (2007) found that birthweight increases by 213.7 grams (95% CI: 202.5, 224.8) while Grandi et al. (2016) reported that birthweight increases by 17.1 grams (95% CI: -30.7, 65.1).[71, 74] Further study is needed to explore the associations of features of placental morphology with birthweight, adjusting for important confounders. Additionally, only one study reported results stratified by sex to evaluate potential interaction, which has been reported by studies evaluating placental development and long-term adverse outcomes.[58] We utilized a dataset that oversampled preterm births and determined gestational age from ultrasound measures, which provided a valuable opportunity to evaluate these associations in models adjusted for important covariates, such as gestational age.

Table 1.2. Studies of placental morphology (excluding placental weight) and fetal growth in singletons.

Study	Sample	Measures	Notes
Woods et al., 1982[79]	80 placentas, South Africa, no years provided	• Surface area	• Surface area smaller in infants with birthweight <10th percentile as compared to normal birthweight infants • Unadjusted (t-test)
Salafia et al., 2005[63]	628 placentas, United States, 2002 – 2004 (Pregnancy, Infection, and Nutrition Study)	• Placental weight • Umbilical cord length • Digital image of chorionic plate o Umbilical cord insertion o Surface area o Radius o Shape (eccentricity) o Diameters	• Chorionic plate measures account for 17% of variation in gestational age and 35% of variation in birthweight • Adjusted for placental variables (only reported adjusted r^2 from models)
Salafia et al., 2007[71]	23,313 neonates, United States, 1959 – 1965 (Collaborative Perinatal Project)	• Thickness • Chorionic plate area • Shape (eccentricity)	In models adjusted for other placental measures, gestational age, parity, and infant gender: • Increase in eccentricity associated with increased placental weight and decreased fetal-placental weight ratio • Increase in chorionic plate area associated with increase in birthweight and placental weight, decrease in fetal-placental weight ratio • Increase in thickness associated with increase in birthweight and placental weight, decrease in fetal-placental weight ratio
Biswas & Ghosh, 2008[76]	50 placentas (28 growth restricted, 28 normal), India, no years provided	• Umbilical cord insertion • Weight • Volume • Diameters	• IUGR placentas more likely to have non-central umbilical cord insertion, smaller diameters, lower weight, and smaller volume as compared to non-IUGR placentas • Fetal-placental weight ratio higher in IUGR placentas as compared to non-IUGR placentas • Unadjusted (t-test)
Salafia et al., 2008[5]	24,061 neonates, United States, 1959 – 1965 (Collaborative Perinatal Project)	• Shape (round or oval vs. other) • Umbilical cord insertion • Diameters • Thickness • Umbilical cord length	• Placental shape, diameters, thickness, and umbilical cord insertion accounted for nearly 40% of variation in birthweight • No multicollinearity problems resulting from interdependence of placental measures

		• Placental weight	• Adjusted for placental characteristics
Misra et al., 2009[58]	24,061 neonates, United States, 1959 – 1965 (Collaborative Perinatal Project)	• Chorionic plate area (calculated from diameters) • Thickness • Umbilical cord length • Placental weight	• Relationships between thickness and umbilical cord length with birthweight differed between males and females • Fetal-placental weight ratio was larger for males as compared to females • Unadjusted models, stratified by sex
Coall et al., 2009[64]	513 placentas, Australia, 2001 – 2003	• Surface area • Thickness • Umbilical cord insertion	• Birthweight correlated with surface area and thickness, not eccentricity of umbilical cord insertion • Analyses based on correlations, restricted to term births of first-time mothers
Pathak et al., 2010[82]	861 placentas, United Kingdom, 2007 – 2008	• Umbilical cord insertion • Shape (eccentricity)	• No difference in placental characteristics between normal pregnancies and pregnancies with adverse outcomes • Unadjusted (Mann-Whitney test)
Tomas et al., 2010[77]	121 placentas (52 growth-restricted, 69 normal), Croatia, no years provided	• Diameters	• Smaller diameters in growth-restricted placentas as compared to normal placentas • Unadjusted (t-test)
Vedmedovska et al., 2011[73]	100 placentas (50 growth restricted, 50 normal), Latvia, 2007 – 2008	• Thickness • Umbilical cord insertion	• No difference in thickness or umbilical cord insertion between placentas of growth-restricted and normal neonates • Unadjusted (t-test)
Alwasel et al., 2012[78]	401 placentas, Saudi Arabia, 2009 – 2010	• Diameters (length (longest) and breadth (shortest))	• Placental breadth related to fetal size, but not length when modeled together
Haeussner et al., 2013[81]	418 placentas, Germany, 2011 – 2012	• Diameters • Thickness • Roundness • Umbilical cord insertion • Shape variability (geometric morphometry)	• Shape variability is not correlated with birthweight • Unadjusted (correlations)
Balihallimath et al., 2013[80]	164 placentas, India, no years provided	• Placental weight • Surface area • Volume • Thickness	• Placental weight, surface area, and volume have a significant, positive correlation with birthweight

Higgins et al., 2015[75]	100 placentas of neonates with reduced fetal movement (23 with adverse outcomes, of which, 21 were SGA), United Kingdom, 2012 – 2014	• Diameters • Volume • Thickness • Umbilical cord insertion	• Placentas from pregnancies with adverse outcomes had smaller diameters • No difference in thickness, umbilical cord insertion, or fetal-placental weight ratio • Unadjusted (Mann-Whitney, χ^2)
Chisholm & Folkins, 2016[32]	134 neonates (67 SGA, 67 AGA), United States, 2009 – 2011	• Weight • Diameters • Thickness • Shape (difference between longest and shortest diameters) • Surface area • Umbilical cord insertion	• Smaller weight, diameters, thickness, and surface area in SGA neonates as compared to AGA neonates • No difference in umbilical cord insertion or shape between SGA and AGA neonates • Unadjusted (t-test)
Grandi et al., 2016[74]	875 neonates, Argentina, 2011 – 2012	• Weight • Diameters • Thickness	• Placental weight and longest diameter associated with birthweight, smallest diameter and thickness not associated with birthweight when controlling for placental variables and maternal and neonatal characteristics (gestational age, parity, preeclampsia, tobacco, gender)

Suboptimal fetal growth is much more common in twin gestations than in singleton gestations, with an estimated prevalence of 15 to 25%, which makes this a population of interest for understanding the inter-relationships between fetal growth, placental morphology, and long-term outcomes.[19] Evaluating placental morphology and fetal growth in dichorionic twins presents a unique opportunity to better understand these relationships by improving the ability to control for certain in utero confounders such as gestational age, fetal-placental environment, and maternal characteristics. In the twins literature, dichorionic twins are understudied, as most placental research in twins focuses on unequal placental sharing or twin-to-twin transfusion syndrome in monochorionic twins. Furthermore, a recent study found that dichorionic twins have the same allometric scaling factor as singletons, suggesting that the relationship between

placental weight and birthweight is similar in singletons and twins.[24] Twins are also advantageous because placental measures can be assessed in a relative manner, which does not require developing norms.

The majority of studies that evaluate postnatal features of placental morphology in relation to birthweight in dichorionic twins evaluate umbilical cord insertion. Several studies have found that non-central umbilical cord insertion is associated with lower birthweight.[83-85] However, Hanley et al. (2002) and DePaepe et al. (2015) reported that umbilical cord insertion is not a risk factor for birthweight discordance.[86, 87] Placental lesions have also been studied in dichorionic twins, with three studies reporting that placental lesions are more common in the lighter twin.[86, 88, 89] In a study of placental proximity in dichorionic twins, Blickstein et al. (2006) found that twins with fused placentas are lighter than twins with separate placentas.[23] While studies in dichorionic twins have evaluated a few features of placental morphology, to our knowledge, no studies have examined other features of placental morphology, including thickness, surface area, and shape. The studies briefly described in this section are summarized in the table below (Table 1.3).

Table 1.3. Studies of placental morphology (excluding placental weight) and fetal growth in twins.

Study	Sample	Measures	Notes
Eberle et al., 1993[88]	164 twin pairs, United States, 1986 – 1992	• Placental lesions	• In dichorionic twins, lighter twin placenta more likely to have lesions than heavier twin placenta
Loos et al., 2001[83]	4529 twin pairs, Belgium, 1964 – 1997 (East Flanders Prospective Twin Survey)	• Umbilical cord insertion	• Peripheral umbilical cord insertion associated with lower birthweight
Victoria et al., 2001[84]	382 twin pairs, United States, 1993 – 1995	• Umbilical cord insertion	• Velamentous umbilical cord insertion more likely in smaller twin than larger twin

Hanley et al., 2002[86]	447 twin pairs, United States, 1993 – 1996	• Umbilical cord insertion	• Umbilical cord insertion not a risk factor for discordant birthweight in dichorionic twins • Advanced villous maturation lesions associated with discordant birthweight in dichorionic twins
Blickstein et al., 2006[23]	1155 twin placentas, United States, 1988 – 1995 (Northwestern twin chorionicity study)	• Chorionicity (monochorionic, dichorionic, dichorionic fused)	• Dichorionic twins heavier than dichorionic fused and monochorionic twins
Kent et al., 2011[85]	816 twin pairs, Ireland, 2007 – 2009 (Evaluation of Sonographic Predictors of Restricted Growth in Twins (ESPRiT) Study)	• Umbilical cord insertion	• Non-central umbilical cord insertion site more common in smaller twin of birthweight discordant pairs as compared to larger twin
Kent et al., 2012[89]	668 twin pairs, Ireland, 2007 – 2009 (Evaluation of Sonographic Predictors of Restricted Growth in Twins (ESPRiT) Study)	• Placental abnormalities (various lesions)	• In dichorionic twins, smaller twin more likely to have placental abnormalities as compared to larger twin
DePaepe et al., 2015[87]	198 dichorionic placentas, United States, 2010 – 2013	• Umbilical cord insertion	• Birthweights of twins with paracentral cords similar to birthweights of twins with peripheral cords

Features of placental morphology and cognitive outcomes

While many studies have reported associations between suboptimal fetal growth and adverse cognitive outcomes in childhood, few studies have considered the role of the placenta in this relationship.[12, 90, 91] Only three studies have evaluated macroscopic features of placental morphology in relation to cognitive outcomes in childhood, two of which are based on CPP data (Table 1.4). Niswander and Gordon (1972) reported that placental lesions are associated with neurologic abnormalities at one year of age.[67] However, this study did not adjust for gestational age or birthweight and did not consider sex-specific differences in fetal or cognitive development. Additionally, the authors used a broad definition of neurologic abnormality. Misra

et al. (2012) reported a positive association between placental thickness and higher IQ at age seven in both males and females.[50] They also found positive associations between both longest and shortest diameters and higher IQ in females but not in males. Other features of placental morphology were not associated with IQ in males or females. In contrast, Khalife et al. (2012) reported that increased placental weight and surface area were associated with antisocial behavior, inattention, and hyperactivity at age eight among males, but not among females.[51] However, the magnitudes of the associations were small (odds ratio range: 1.03 to 1.19). Further study on the relationships between features of placental morphology and cognitive outcomes using other datasets are necessary to evaluate reproducibility.

Table 1.4. Studies of placental morphology and cognitive outcomes.

Study	Sample	Measures	Notes
Niswander and Gordon, 1972[67]	31,785 infants, United States, 1959 – 1965 (Collaborative Perinatal Project)	• Placental weight • Umbilical cord length • Various placental lesions and markers of inflammation	• Outcome was neurologic abnormality at one year of age • Found associations with short umbilical cord, placental weight, presence of macrophages, and neutrophilic infiltration • Did not adjust for gestational age or birthweight
Misra et al., 2012[50]	24,061 children, United States, 1959 – 1965 (Collaborative Perinatal Project)	• Placental weight • Diameters • Surface area • Thickness • Shape • Umbilical cord length • Umbilical cord insertion	• Outcome was IQ at seven years of age • Small association between placental weight and IQ in boys • Longest and shortest surface diameters associated with IQ in girls • Thickness associated with IQ in both boys and girls
Khalife et al., 2012[51]	8,101 children, Finland, 1986 (The Northern Finland Birth Cohort)	• Placental weight • Surface area • Placenta weight/birthweight ratio	• Increased placental weight and surface area associated with antisocial disorder, inattention, and hyperactivity at age 8 and ADHD and inattention at age 16 among males • No associations between placental size and mental health/behavior among females at age 8 or 16

Twins present a unique opportunity to evaluate cognitive outcomes in childhood. Genetics and the postnatal environment heavily influence cognitive development but can be challenging to quantify and control for in epidemiologic studies. Twins have similar genetics, as well as postnatal environment and experiences, which allows for improved control of these characteristics. To our knowledge, only one study has evaluated the relationship between features of placental morphology and cognitive outcomes in dichorionic twins.[92] Antoniou et al. (2013) reported that umbilical cord insertion and the total weight of the placental mass were not associated with IQ among children aged 7 to 15 years old.

Table 1.5. Studies of placental morphology and cognitive outcomes in twins.

Study	Sample	Measures	Notes
Antoniou et al., 2013[92]	663 twin pairs, Belgium, 1964 – 1997 (East Flanders Prospective Twin Survey)	• Umbilical cord insertion • Placental weight • Chorionicity	• IQ from Wechsler Intelligence Scale for Children – Revised at 7-15 years old • Umbilical cord insertion and placental weight not related to IQ

Gaps in our knowledge

No one has quantified the relationship between exact placental surface area and surface area derived from diameters, despite researchers acknowledging the need for this type of study.[55] A better understanding of this relationship is important for studies using diameters, which are often included in placental pathology reports and are available in some historic cohorts. Further, diameters can be obtained during routine ultrasounds[68], and understanding the utility and error associated with using these measures is important for studies evaluating longitudinal measures of placental development.

The aims in singleton populations have been evaluated in the literature, although further study is needed. Only two of the fifteen studies evaluating placental morphology and birthweight

considered important covariates, like gestational age. Further, there is evidence of sex-specific differences in placental development and developmental programming, but only one study reported results stratified by sex and this study did not consider other covariates. Additional research is needed to evaluate interaction by sex while controlling for important covariates, which is what this dissertation adds to the literature. Two studies have evaluated features of placental morphology in relation to cognitive and mental health outcomes, but further study in a more recent population is helpful to determine consistency of reported results. Investigating these associations in dichorionic twins is a novel approach to improve control of potential confounders. To our knowledge, no studies have evaluated placental characteristics other than umbilical cord insertion, lesions, and placental proximity in dichorionic twins. Most placental research on twins focuses on monochorionic twins and is related to unequal placental sharing and twin-to-twin transfusion syndrome. Studying dichorionic twins will allow us to control for confounders that are otherwise uncontrolled, such as postnatal environment and genetics, which will improve our ability to evaluate the research questions. This dissertation addresses gaps in our knowledge by improving upon the modeling strategy of current findings in singletons and by using a novel approach of evaluating similar associations in dichorionic twins.

SPECIFIC AIMS

The placenta plays a crucial role in regulating healthy fetal development, primarily through mediating oxygen, nutrient, and waste transfer between the mother and the fetus. Poor placental development may result in failure of the placenta to meet the oxygen and nutrient needs of the fetus, which is believed to result in poor growth in utero. Neonates who experienced suboptimal fetal growth are at greater risk of perinatal morbidity and mortality, as well as cognitive delays in childhood and cardiovascular disease in adulthood. Features of placental morphology, including thickness, shape, surface area, and umbilical cord insertion, are associated with placental function and efficiency. We will evaluate the use of features of placental morphology as indicators of fetal growth and cognitive development through the following three aims:

Aim 1. Evaluate the validity of using placental diameters to estimate surface area (Chapter 2).

Placental surface area reflects the number of spiral arteries supplying the placenta, which is indicative of the placenta's ability to facilitate oxygen, nutrient, and waste transfer. Surface area is commonly estimated by incorporating placental diameters, which are easy to obtain and frequently available, into the formula for the area of an ellipse. The measurement error that results from assuming a perfect elliptical shape has not been evaluated. Using 491 placentas with stored images and recorded diameters from the Stillbirth Collaborative Research Network (SCRN) Study, we will quantify this error by comparing estimated surface area to surface area determined from images of the placental surface. Findings from this analysis will inform analyses in subsequent aims and will benefit researchers with data limited to diameters.

Aim 2. Determine the features of placental morphology that are associated with suboptimal fetal growth, as approximated by birth weight, separately in singletons and dichorionic twins (Chapters 3 & 4).

Many studies that have evaluated the relationship between placental morphology and birthweight have relied on statistical tests and do not consider potential confounders. Additionally, only one study has evaluated sex-specific differences; however, this study did not adjust for important confounders. We will add to existing literature by estimating sex-specific associations between features of placental morphology and birthweight using models adjusted for important confounders in a 1,229 singletons from the SCRN Study. Further, we will investigate a similar relationship in dichorionic twins. Dichorionic twins have a higher prevalence of suboptimal fetal growth than singletons and present a unique opportunity to control for key confounders such as in utero environment, gestational age, and maternal characteristics. Results from this analysis will improve our understanding of the relationship between placental morphology and fetal growth by providing improved control of confounding. We will evaluate this aim using data on 208 sets of dichorionic twins from three completed studies (SCRN, Collaborative Perinatal Project [CPP], and the NICHD Fetal Growth Study: Twin Gestations). We hypothesize that features of placental morphology will be independently and positively associated with birthweight.

Aim 3. Evaluate the features of placental morphology that are associated with adverse cognitive outcomes separately in singletons and dichorionic twins (Chapter 5).

The only study to evaluate the relationship between placental morphology and cognitive outcomes determined gestational age from last menstrual period, which is less accurate

than other measures. As gestational age is an important confounder, we will assess the reproducibility of these results using data on 514 children who completed a cognitive assessment at age five from the Alabama Fetal Growth Study, which used ultrasound dating to estimate gestational age. In a separate analysis, we will leverage the unique characteristics of dichorionic twins to assess the relationship between within-pair differences in placental features and within-pair differences in cognitive development using 82 sets of twins with cognitive assessment data at age seven in the CPP. We hypothesize that larger features of placental morphology will be associated with increased IQ.

By evaluating error in a common measure, taking advantage of the features of dichorionic twins, and leveraging data on cognitive development, the results of this dissertation will be a unique addition to the literature. With these aims, we hope to add to the growing body of literature aimed at identifying features of placental morphology that may be indicators of fetal and childhood growth and development.

CHAPTER 2: COMPARISON OF DIAMETER-BASED AND IMAGE-BASED MEASURES OF PLACENTAL SURFACE AREA FOR USE IN EPIDEMIOLOGIC STUDIES

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ABSTRACT

Introduction: Some studies of developmental programming consider features of gross placental morphology, including surface area. Surface area is often determined from placental diameters, which are available in several large cohort studies, but this calculation assumes that all placentas are elliptical. We evaluated this assumption by comparing diameter-based surface area to surface area calculated from images of the fetal surface.

Methods: We used placental examination data from 491 participants in the Stillbirth Collaborative Research Network Study (416 live births, 75 stillbirths). We obtained image-based surface area by manually tracing the fetal surface of the placenta and we determined the area of the outline using ImageJ software. Three reviewers evaluated each placental image and we calculated the intra-class correlation coefficient to assess inter-rater reliability. We obtained diameters from placental pathology reports, completed by trained pathologists using a standardized protocol, and surface area was estimated from these diameters using the formula for an ellipse. We used the Bland-Altman method to quantify the difference between image-based and diameter-based surface area.

Results: The intra-class correlation coefficient for the total sample was 0.94, indicating excellent inter-rater reliability. On average, diameter-based measures underestimated placental surface area by -5.58% (95% confidence interval: -30.23, 19.07). Differences between image-based and diameter-based surface area were similar for placentas of normal and abnormal shape.

Conclusion: Our results indicate that diameter-based surface area is a good proxy for image-based surface area for placentas of both normal and abnormal shape. This supports the utility of existing cohort studies with data on gross placental morphology.

INTRODUCTION

Measures of gross placental morphology, including placental surface area, are often used as indicators of placental function.[8] Surface area reflects the placenta's growth and size, which may indicate the placenta's ability to facilitate nutrient, oxygen, and waste exchange.[8] Placental surface area may also reflect the number of spiral arteries supplying the placenta.[5] Some studies of developmental programming consider the role of the placenta, given its importance in regulating growth, and this area of research has been aptly called 'placental programming.'[41] Studies have reported associations of placental surface area with mental health in childhood and hypertension and lung cancer in adulthood.[51, 55, 93]

Studies of placental programming require lengthy follow-up to assess long-term health outcomes, which makes existing cohort studies ideal samples to investigate these research questions. Some large cohort studies, such as the Collaborative Perinatal Project, the Helsinki Birth Cohort, the Northern Finland Birth Cohort, and the Dutch Famine Birth Cohort, contain information on gross placental morphology, including placental diameters.[55, 65-67] Calculating surface area from diameters requires the assumption that all placentas are elliptical. This may introduce a larger degree of error for abnormally shaped placentas. Imaging technology offers the capability to calculate exact placental surface area without assuming an elliptical shape.[4]

As modern technology facilitates the use of routine placental imaging and software allows for automated calculation of placental measures, it is important to understand the utility of existing diameter measures. To our knowledge, no one has investigated the accuracy of estimating placental surface area from diameters. The purpose of our study is to evaluate the validity of using diameter-based measures of surface area as compared to image-based measures of surface area.

METHODS

The Stillbirth Collaborative Research Network (SCRN) Study was a population-based case-control study of stillbirth. Participants were enrolled post-partum from deliveries occurring between March 2006 and September 2008 at 59 hospitals representing five catchment areas in the United States: Rhode Island and counties in Massachusetts, Georgia, Texas, and Utah. The SCRN Study enrolled 663 women with stillbirths and 1,932 women with live births. Details on the study design, including sampling methods, have been published elsewhere.[94] The study was approved by the Institutional Review Board at each study site and all participants provided written informed consent.

This analysis included placentas from stillbirths and live births that underwent a standardized placental examination and had stored placental images. Three of the five study sites were able to provide access to some of their placental images. Of the 912 un-fragmented placentas of singletons evaluated at these sites, we obtained images for 491 participants (416 live births, 75 stillbirths). During the standardized placental examination, trained pathologists measured the longest and shortest placental diameters. Diameter-based surface area was calculated based on the formula for the area of an ellipse using the longest (a) and shortest (b) diameters (area = $ab\pi/4$). Pathologists also categorized placental shape as round, ellipse, bilobed, membranacea, or other. In this analysis, shape was dichotomized by grouping round and ellipse to indicate normal shapes and grouping bilobed, membranacea, and other to indicate abnormal shapes.[95] Additional details on the placental examination protocol have been reported elsewhere.[96]

Pathologists captured images of the fetal surface of the placenta during the placental examination using a digital camera with a minimum resolution of 3 megapixels. Peripheral

membranes were trimmed prior to imaging, and each image included a label with the study identification number and a metric ruler. We determined surface area from the placental images using ImageJ software.[97] Due to the potential variability in manually evaluating the placental images, three reviewers with basic understanding of placental morphology analyzed each image. Reviewers were instructed to set the scale of each image by measuring the number of pixels in 1 cm on the included ruler. Following this, reviewers traced the outline of the placental disc to determine the area of the placental surface.

We calculated the intra-class correlation coefficient (ICC) based on a single measure, two-way random effects model to compare agreement in estimates of placental surface area across the three reviewers.[98-101] We compared the average measure across the three reviewers to the diameter-based measure using the Bland-Altman method. This method typically results in a plot of the difference between the two measures versus the average of the two measures.[102] We used the percent difference rather than the absolute difference to account for the increased variability associated with increased placental size.[103] We also considered the image-based measure the gold standard and calculated the percent difference by dividing the difference (diameter-based – image-based) by the image-based measure and multiplying by 100.[103] We plotted the percent differences against the image-based measure. The mean percent difference reflects the potential measurement error. This method is preferred to evaluating the correlation because two measures of the same object are expected to be correlated and thus a significant correlation is uninformative.[103] In addition to evaluating the total sample, we evaluated the sample stratified by stillbirth or live birth, term live birth ($\geq$ 37 weeks' gestation) or preterm live birth ($<$ 37 weeks' gestation), and normal or abnormal shape, since these classifications may affect the agreement.

To evaluate the impact of measurement type, we estimated the associations between each measure of surface area with birthweight. We adjusted models for important covariates, including stillbirth/live birth status, gestational age, race/ethnicity, maternal education, maternal smoking, maternal height, maternal weight, preeclampsia, gestational diabetes, parity, sex, placental shape, and placental thickness. We conducted all analyses using SAS version 9.4 (SAS Institute INC., Cary, North Carolina).

RESULTS

Among term live births eligible for inclusion in our analysis, estimates of placental diameters and diameter-based surface area were similar for those with and without available placental images (Table 2.1). The ICC for inter-rater reliability in the estimates of placental surface area were 0.94 among the total sample and 0.91 among the live birth sample, which indicate a high degree of consistency across the three reviewers (Table 2.2).[101] The average surface area from image-based measures across the three reviewers was 253.30 cm^2 (standard error [SE]: 3.80; Table 2.3) and the average surface area from the diameter-based measure was 237.05 cm^2 (SE: 3.60).

The mean percent difference in the measures was within ±5% for 29.2% of placentas and within ±10% for 51.4% of placentas. On average, diameter-based measures underestimated placental surface area by -5.58% (95% confidence interval [CI]: -30.23, 19.07; Table 2.4, Figure 2.1). Similarly, among term live births, diameter-based measures underestimated surface area by -5.83% (95% CI: -29.67, 18.01). Diameter and image-based measures were closest among stillbirths (-1.77%, 95% CI: -29.85, 26.31). The difference in the measures was most pronounced among preterm live births (-8.42%, 95% CI: -31.48, 14.64) and among those with abnormal shapes (-7.93%, 95% CI: -36.04, 20.18).

Estimates of the association between surface area and birthweight, adjusted for important covariates, were similar for both image-based and diameter-based measures (image-based β: 3.65, 95% CI: 3.00, 4.31; diameter-based β: 3.81; 95% CI: 3.13, 4.49; Table 2.5).

DISCUSSION

Our results suggest that while diameter-based measures slightly underestimate placental surface area, they are an adequate proxy for actual surface area. The percent difference in the measures was within ±10% for over half of the placentas. Additionally, estimates restricted to abnormally shaped placentas were not meaningfully different from normally shaped placentas, which indicates that the elliptical assumption required when using diameter-based measures does not introduce a large degree of error. Further, estimates of the association between birthweight and surface area were similar when each area measure was modeled separately.

Similar measures of error for normal and abnormal shapes may be due to the heterogeneity among abnormal shapes, defined as non-round or oval shapes. Some abnormal shapes, such as triangular and heart-shaped placentas, may be better approximated by placental diameters than other abnormal shapes, such as bilobed placentas or placentas with accessory lobes. Shape classification may also vary by pathologist, although examinations were conducted using a standardized protocol. Unfortunately, we were unable to evaluate the error among specific types of abnormal shapes. However, approximately 10% of placentas are thought to be abnormally shaped, so assuming an elliptical shape may not introduce a large degree of error overall.[104]

The similarity between diameter-based and image-based surface area is consistent with studies exploring similar relationships. One study compared maximum and minimum placental diameters obtained from images to those obtained from manual measurements and reported that

they were highly correlated.[64] Another study reported that image based measures of placental morphology explained 14% more of the variation in birth weight as compared to diameter-based measures.[63] However, the image-based measures and diameter-based measures were obtained from two different samples, which makes direct comparisons of the two methods challenging.

We obtained the placental images in this study from a convenience sample of the SCRN Study, and thus the results may not be generalizable to the rest of the SCRN Study or to the general population. While this represents a convenience sample of the images for which the sites were able to provide access, we have no reason to believe that findings from this subset are not generalizable to the results that would have been obtained using all of the images from these three sites. This is supported by the consistency of the diameter estimates in those with and without available images (Table 2.1). Additionally, the SCRN Study included stillbirths and oversampled live births < 32 weeks' gestation and as a result, 26.5% of our total sample was preterm (< 37 weeks' gestation, Table S2.1). However, our results for the total sample were consistent with the results restricted to term live births.

Another limitation of our study is the inherent measurement error in manually outlining the placental surface. Due to the characteristics of the placental images, we were unable to automate this process. However, we did have three reviewers evaluate each image to account for this and inter-rater reliability was excellent. Another limitation of using measures derived from images is time. Manually tracing each image took about 1-2 minutes per image, although images can be taken in such a way to automate the analysis, which would reduce the time burden.

Strengths of our study include a large sample size of 491 images and the use of three reviewers to measure placental surface area in each image. Placental examinations were also conducted by trained pathologists using a standardized protocol, which ensures consistent

measures. Further, the diversity of the SCRN Study allowed us to estimate agreement in samples restricted to term live births, preterm live births, and stillbirths.

Our findings support the validity of using diameters to determine placental surface area. This is important given the growing body of research evaluating relationships between placental size and long-term health outcomes.[72] Diameters are also readily available in some completed cohort studies with long-term follow up, including the Collaborative Perinatal Project.[67] Measures of placental diameters are relatively easy to obtain following delivery and can also be evaluated during routine ultrasound scans.[68, 105] Future studies should consider the ease of obtaining placental diameters and the validity of using these measures to determine surface area when establishing protocols for placental examination.

FIGURES AND TABLES

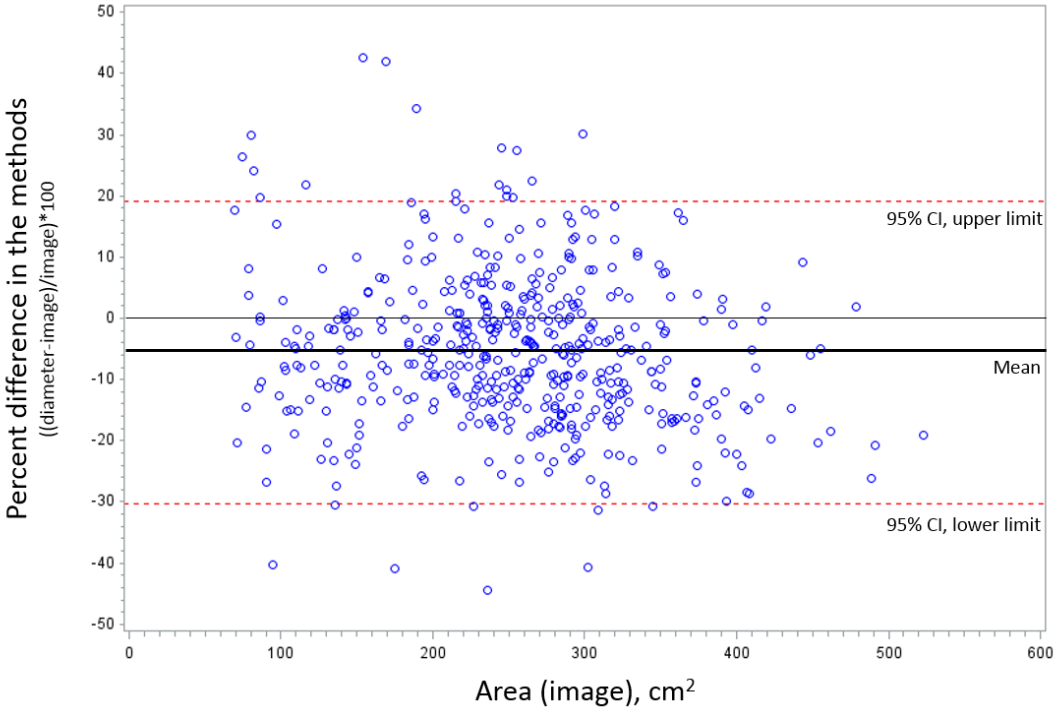

Figure 2.1. Results for agreement between image-based and diameter-based measures for the total sample (n=491).

Abbreviations: CI – confidence interval

Table 2.1. Placental characteristics for eligible term live births (n=556), stratified by whether or not the placental image was available.

Characteristic Mean (SE)	**Image Available** (n=344)	**Image Unavailable** (n=212)
Minimum diameter, cm	17.02 (0.11)	16.56 (0.16)
Maximum diameter, cm	20.04 (0.15)	20.86 (0.24)
Diameter-based area, cm^2	269.85 (3.24)	273.38 (4.70)

Abbreviation: SE – standard error

Table 2.2. Intra-class correlation coefficients (ICC) for agreement in image-based surface area across the three reviewers for the total sample and subsets of the sample.

	ICC
Total sample (n=491)	0.94
Stillbirths (n=75)	0.97
Live births (n=416)	0.91
Term live births (n=344)	0.87
Preterm live births (n=72)	0.93
Normal shapes (n=418)	0.93
Abnormal shapes (n=73)	0.95

Abbreviation: ICC – intra-class correlation coefficient

Table 2.3. Means and standard errors for placental surface area from the image-based and diameter-based measures.

	Mean (SE)
Image-based area (average), cm^2	253.30 (3.80)
Image-based area, reviewer 1, cm^2	255.07 (3.83)
Image-based area, reviewer 2, cm^2	255.47 (4.06)
Image-based area, reviewer 3, cm^2	249.35 (3.74)
Diameter-based area, cm^2	237.05 (3.60)

Abbreviations: SE – standard error

Table 2.4. Results for agreement between image-based and diameter-based measures of placental surface area using the Bland-Altman Method.

	Percent Difference[a]	95% CI
Total sample (n=491)	-5.58	-30.23, 19.07
Stillbirths (n=75)	-1.77	-29.85, 26.31
Live births (n=416)	-6.28	-30.04, 17.48
Term live births (n=344)	-5.83	-29.67, 18.01
Preterm live births (n=72)	-8.42	-31.48, 14.64
Normal shapes (n=418)	-5.18	-29.14, 18.77
Abnormal shapes (n=73)	-7.93	-36.04, 20.18

Abbreviations: CI – confidence interval

[a] Percent difference calculated as ((diameter-based – image-based)/image-based)*100

Table 2.5. Estimates of the association between birthweight and placental surface area modeling image-based and diameter-based measures separately.

	β[a]	95% CI
Image-based area[b]	3.65	3.00, 4.31
Diameter-based area[b]	3.81	3.13, 4.49

[a] Estimates reflect a 1 cm^2 increase in surface area

[b] Both models adjust for stillbirth/live birth status, gestational age, race/ethnicity, maternal education, maternal smoking, maternal height, maternal weight, preeclampsia, gestational diabetes, parity, sex, placental shape, and placental thickness

SUPPLEMENTAL MATERIAL

Table S2.1. Descriptive characteristics for the total sample and stratified by live birth or stillbirth status.

Characteristic	Total N (%) N=491	Live Births N (%) N=416	Stillbirths N (%) N=75
Neonatal/Placental Characteristics			
Adjusted birth weight percentile[a]			
<5th percentile	25 (5.2)	14 (3.4)	11 (14.9)
5th-10th percentile	22 (4.6)	12 (3.0)	10 (13.5)
10th-90th percentile	400 (83.2)	351 (86.2)	49 (66.2)
90th-95th percentile	11 (2.3)	11 (2.7)	0 (0.0)
95th-100th percentile	23 (4.8)	19 (4.7)	4 (5.4)
Gestational Age			
20-23 completed weeks	41 (8.4)	26 (6.3)	15 (20.0)
24-27 completed weeks	27 (5.5)	15 (3.6)	12 (16.0)
28-31 completed weeks	29 (5.9)	13 (3.1)	16 (21.3)
32-36 completed weeks	33 (6.7)	18 (4.3)	15 (20.0)
>37 completed weeks	361 (73.5)	344 (82.7)	17 (22.7)
Infant sex, male	248 (50.5)	209 (50.2)	39 (52.0)
Placental shape, abnormal	73 (14.9)	62 (14.9)	11 (14.7)
Maternal/Pregnancy Characteristics			
Maternal Age			
<20	69 (14.1)	57 (13.7)	12 (16.0)
20 – 34	360 (73.3)	307 (73.8)	53 (70.7)
35 – 39	52 (10.6)	45 (10.8)	7 (9.3)
40+	10 (2.0)	7 (1.7)	3 (4.0)
Maternal Race/Ethnicity			
Non-Hispanic white	143 (29.1)	113 (27.2)	30 (40.0)
Non-Hispanic black	277 (56.4)	244 (58.7)	33 (44.0)
Hispanic	35 (7.1)	30 (7.2)	5 (6.7)
Other	36 (7.3)	29 (7.0)	7 (9.3)
Maternal Education			
0 – 11 (none/primary/some secondary)	138 (29.8)	117 (29.6)	21 (30.4)
12 (completed secondary)	131 (28.2)	107 (27.1)	24 (34.8)
13+ (college)	195 (42.0)	171 (43.3)	24 (34.8)
Maternal BMI, kg/m^2			
<18.5	10 (2.1)	7 (1.7)	3 (4.1)
18.5 – 24.9	230 (47.9)	196 (48.3)	34 (46.0)
25 – 29.9	103 (21.4)	86 (21.2)	17 (23.0)
30 – 34.9	70 (14.6)	60 (14.8)	10 (13.5)

≥35	67 (14.0)	57 (14.0)	10 (13.5)
Nulliparous	160 (32.9)	121 (29.4)	39 (52.7)
Preeclampsia/hypertension	58 (12.1)	51 (12.6)	7 (9.3)
Gestational Diabetes	40 (8.3)	0 (0.0)	40 (9.8)
Maternal Smoking Status[b]			
Did not smoke	392 (84.7)	335 (85.0)	57 (82.6)
< 10	39 (8.4)	34 (8.6)	5 (7.3)
≥ 10	32 (6.9)	25 (6.4)	7 (10.1)
Alcohol Use[c]			
Did not drink	274 (59.4)	234 (59.5)	40 (58.8)
Drank, no binging	88 (19.1)	76 (19.3)	12 (17.7)
Binged	99 (21.5)	83 (21.1)	16 (23.5)

Abbreviations: BMI – body mass index

[a] Based on Alexander percentiles

[b] Average number of cigarettes during 3 months prior to pregnancy

[c] Alcohol consumption during 3 months prior to pregnancy

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CHAPTER 3: ASSOCIATIONS BETWEEN FEATURES OF GROSS PLACENTAL MORPHOLOGY AND BIRTHWEIGHT

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ABSTRACT

The placenta plays a critical role in regulating fetal growth. Recent studies suggest there may be sex-specific differences in placental development. Our purpose was to evaluate the associations between birthweight and placental morphology in models adjusted for covariates and to assess sex-specific differences in these associations. We analyzed data from the Stillbirth Collaborative Research Network's population-based case-control study conducted in 2006-2008, which recruited cases of stillbirth and population-based controls in five states. Our analysis was restricted to singleton live births with a placental examination (n=1,229). Characteristics of placental morphology evaluated include thickness, surface area, difference in diameters, shape, and umbilical cord insertion site. We used linear regression to model birthweight as a function of placental morphology and covariates. Surface area had the greatest association with birthweight; a reduction in surface area of 83 cm^2, which reflects the interquartile range, is associated with a 260.2-gram reduction in birthweight (95% confidence interval: -299.9, -220.6), after adjustment for other features of placental morphology and covariates. Reduced placental thickness was also associated with lower birthweight. These associations did not differ between males and females. Our results suggest that reduced placental thickness and surface area are independently associated with lower birthweight and that these relationships are not related to sex.

INTRODUCTION

The placenta plays a critical role in regulating fetal growth and development. In particular, the placenta acts as a mediator of oxygen, nutrient, and waste exchange; is immunologically active; and produces hormones and growth factors necessary for fetal development and maintenance of pregnancy.[106] Reduced placental function can prevent the placenta from meeting the oxygen and nutrient needs of the fetus.[6] This may result in suboptimal fetal growth, which has both short and long-term health consequences and is a strong risk factor for perinatal morbidity and mortality.[12, 35]

Gross features of placental morphology reflect its function and efficiency.[3, 4] Placental thickness may reflect the vascularization and branching of the chorionic villi and is the main dimension of placental growth during the third trimester.[63, 72] Surface area of the chorionic plate is mostly established prior to the third trimester and may reflect the number of spiral arteries supplying the placenta.[5, 63, 72, 107] Placentas are typically round or oval, but abnormal shapes may result from uterine abnormalities or as an adaptive response to stress during early placental development.[21, 95] Similarly, the difference in the diameters of the placenta reflects how circular or oval the placenta is, which may indicate adaptive growth.[55, 72] Non-central umbilical cord insertion may reflect poor placental development in early pregnancy or adaptive growth of the placenta and has been associated with reduced placental efficiency.[10, 21, 108]

Several studies have identified sex-specific differences in placental development and morphology. One found that placentas of males tend to be thicker while placentas of females tend to have a larger surface area.[57] Another group reported that placentas of males are smaller than those of females when controlling for birthweight, which indicates that placentas of males may be more efficient than placentas of females.[59] However, as a trade-off for efficiency,

placentas of males are hypothesized to have less reserve capacity, which may result in a higher risk of the fetus becoming undernourished.[59] While studies have reported differences in placental development, few have evaluated the functional impact of these differences in relation to fetal development.

Previous studies of placental morphology and fetal size have limitations that warrant additional investigation. Most studies report correlations or statistical tests for differences and do not quantify the magnitude of the relationship between placental morphology and fetal size.[32, 64, 73, 75, 77, 81, 82] Additionally, many studies have not adjusted for covariates like gestational age, parity, maternal body size, and fetal sex.[32, 58, 63, 64, 73, 75, 77, 78, 81, 82] These maternal and pregnancy characteristics may influence both birthweight and placental size and may confound reported associations.[109, 110] Two studies have estimated associations in models adjusted for some covariates; however, they report inconsistent results.[71, 74] Further, only one study has evaluated sex-specific differences in placental development in relation to birthweight and this study did not adjust for many important covariates.[58]

The purpose of our study was to evaluate the associations between placental morphology and birthweight while adjusting for key confounders, including evaluating interaction by sex. We hypothesized that features of placental morphology would be associated with birthweight, and that these associations would differ by sex.

METHODS

Study Sample

The Stillbirth Collaborative Research Network (SCRN) conducted a population-based case-control study of stillbirth. Participants were enrolled around the time of delivery between March 2006 and September 2008 at 59 hospitals representing five catchment areas of the United

States: the state of Rhode Island and counties in Massachusetts, Georgia, Texas, and Utah. Cases included stillbirths in the catchment areas and controls were sampled from all live births in the same catchment areas. The SCRN Study enrolled 663 women with a stillbirth and 1,932 women with a live birth. Details on the study design, including sampling methods, have been published.[94] The study was approved by the Institutional Review Board at each study site and all participants gave written informed consent.

We restricted our analysis to the 1,760 singleton live births with complete chart abstraction and maternal interview. Of these, 93.8% consented to a placental examination and the examination was conducted in 1,229 (Figure 3.1). Some women were enrolled postpartum and the most common reason for an incomplete examination was that the placenta was discarded and could not be examined.[96] Characteristics related to the completion of a placental examination included clinic site, induction, mode of delivery, year of study, time of day of delivery, and weekend delivery. Data weights were calculated and used in the analysis to account for differences in characteristics related to completion of the placental examination, differential consent for enrollment, and sampling methods.[94]

Information on maternal, pregnancy, and neonatal characteristics, including birthweight, were obtained from medical chart abstraction and maternal interview. The features of placental morphology of interest were determined from the placental examination, which was performed by perinatal pathologists using a standardized, published protocol.[96]

Thickness – Measured at the thickest point and rounded to the nearest 0.5 cm.

Surface Area – Determined based on the formula for the area of an ellipse using the recorded maximum (a) and minimum (b) diameters (area = $ab\pi/4$).

Difference in Diameters – Measured by subtracting minimum diameter from maximum diameter. Differences of zero indicate circular placentas while larger differences indicate increasingly oval placentas.

Shape – Determined based on pathologist classification. Options included round, ellipse, bilobed, membranacea, or other. We dichotomized shape by grouping round and ellipse to indicate normal shapes and grouping bilobed, membranacea, and other to indicate abnormal shapes.[95]

Umbilical Cord Insertion – Pathologists measured the distance from the insertion site to the nearest placental edge and recorded if the insertion was velamentous. We divided the distance from the insertion site to the nearest edge by the average radius of the placenta (velamentous insertions were coded as a negative distance). Ratios of zero indicate marginal insertions, ratios close to zero indicate peripheral insertions, and ratios close to one indicate central insertions.[50, 111]

Statistical Analysis

We conducted linear regression to evaluate the relationship between birthweight and the placental characteristics. Linear regression allows for quantifying the association between birthweight and placental characteristics while controlling for other variables that may affect the observed association.[112] We incorporated the data weights in the analysis to account for study design, characteristics related to consent, and characteristics related to the availability of the placental examination. We controlled for maternal age, maternal education, maternal race/ethnicity, smoking, maternal height, maternal weight, gestational hypertension, gestational diabetes, parity (primiparae vs. multiparae), gestational age, and sex, as these characteristics may influence both placental morphology and birthweight and confound the association. We also

evaluated potential interaction between the features of placental morphology and sex. In addition to modeling the total sample of live births, we evaluated models restricted to term births, defined as ≥37 weeks' gestation.

In supplemental models, we further adjusted for presence of one or more indicators of placental developmental disorders (umbilical cord: single umbilical artery, velamentous insertion, furcate insertion; placental membranes: circummarginate insertion, circumvallate insertion; fetal villous capillaries: terminal villous immaturity (diffuse), terminal villous hypoplasia (diffuse)), inflammatory disorders (maternal inflammatory response: acute chorioamnionitis – placental membranes, acute chorioamnionitis – chorionic plate; fetal inflammatory response: acute funisitis, acute umbilical cord arteritis, acute umbilical cord phlebitis, chorionic plate acute vasculitis, chorionic plate vascular degenerative changes), maternal vascular malperfusion (retroplacental hematoma, parenchymal infarction, intraparenchymal thrombus, perivillous/intervillous fibrin/fibrinoid deposition (diffuse), and fetal vascular malperfusion (fetal vascular thrombi in the chorionic plate, avascular villi, placental edema) in order to evaluate the independence of the features of gross morphology from these characteristics.[113] Contributions of the specific placental diameters were also evaluated by modeling birthweight as a function of maximum diameter, minimum diameter, and thickness.[78]

We conducted a secondary analysis using polytomous logistic regression to evaluate the placental variables associated with small for gestational age (SGA; birthweight <10^{th} percentile for gestational age) or large for gestational age (LGA; >90^{th} percentile) as compared to appropriate for gestational age (AGA; 10^{th}-90^{th} percentile). Categories of birthweight for gestational age were determined from adapting individualized norms using estimated fetal weight developed by Bukowski, et al., which adjust for pregnancy characteristics.[114] Details on

the modifications made to the individualized norms in this study have been published.[35] As this birthweight percentile is adjusted for many of the potential confounders, only maternal age, gestational hypertension, and gestational diabetes were included as confounders in the logistic models.

To facilitate comparison of the magnitude of the association across placental measures, reported measures of association for the continuous variables reflect a comparison of the 25th and 75th percentiles of the characteristic of interest within the specified sample (total or term). Estimates reflect a comparison of 25th vs. 75th for thickness (thin vs. thick), surface area (small vs. large), and umbilical cord insertion (eccentric insertion vs. more central insertion). For difference in diameters, estimates reflect a comparison of 75th to 25th (oval vs. circular). The values used for each comparison are reported in Table S3.1. Shape was analyzed as a dichotomous variable and measures of association reflect the comparison of abnormal to normal.

A p-value of < 0.05 was used to determine statistical significance. Analyses were performed using SAS version 9.4 (SAS Institute INC., Cary, North Carolina) and SUDAAN version 11.0 (Research Triangle Institute, Research Triangle Park, North Carolina).[115]

RESULTS

Descriptive Characteristics

The majority of our sample (71.1%) had a birthweight that was considered to be between the 10th and 90th percentiles for gestational age and 50.0% of the neonates were male (Table 3.1). Additionally, 43.9% of the women were non-Hispanic white, 12.1% were non-Hispanic black, and 36.8% were Hispanic. A majority of the women did not smoke (87.1%) or report consuming alcohol (58.0%) in the three months prior to pregnancy.

In the total sample, males had a mean birthweight of 3,309 grams and females had a mean birthweight of 3,224 grams (Table 3.2). On average, males had larger minimum placental diameters (males: 16.63, standard error [SE]: 0.12; females: 16.29, SE: 0.11; p-value < 0.05) while females had larger differences in diameters (males: 3.14, SE: 0.13; females: 3.61, SE: 0.13; p-value < 0.05). There were no differences in the placentas of males and females with respect to the other features of placental morphology. When SGA, AGA, and LGA births were compared, there were statistically significant differences in placental weight, maximum diameter, minimum diameter, and surface area, with these characteristics increasing with increasing birthweight for gestational age category. Results stratified by both sex and fetal size for gestational age category were similar when restricted to term births (Table S3.2).

Birthweight Analysis

In a model including all placental variables of interest (model 2), reduced thickness and smaller surface area were associated with reduced birthweight in the total (Figure 3.2) and term (Figure 3.3) samples (Table S3.3). After adjustment for covariates (model 3), reduced thickness and smaller surface area remained associated with lower birthweight, although estimates of association were attenuated. Among term births, abnormal shape was additionally associated with lower birthweight (Figure 3.3, Table S3.3). Difference in diameters and umbilical cord insertion site were not associated with birthweight. Surface area had the largest impact on birthweight. In the total sample, a neonate with a placenta in the 25th percentile for surface area is expected to have a birthweight 260.2 grams lower than a neonate with a placenta in the 75th percentile (95% confidence interval [CI]: -299.9, -220.6). In comparison, a neonate with a placenta in the 25th percentile for thickness is expected to have a birthweight 82.8 grams lower than a neonate with a placenta in the 75th percentile (95% CI: -117.9, -47.7). There was no

statistically significant interaction between sex and any of the features of placental morphology. When placental thickness and surface area were evaluated in a model additionally containing placental findings indicative of developmental disorders, inflammatory disorders, maternal vascular malperfusion, and fetal vascular malperfusion, estimates of association for thickness and surface area were consistent with estimates obtained in models not taking these abnormalities into account (Table S3.4).

In a model containing maximum diameter, minimum diameter, and thickness, all three placental characteristics were significantly associated with birthweight (Figure S3.1, Table S3.5). In the total sample, a thin placenta (25^{th} vs. 75^{th}) is associated with a birthweight 90.0 grams lower than a thick placenta (95% CI: -123.6, -56.3). Similarly, a smaller maximum diameter is associated with a reduction in birthweight (-103.4 grams; 95% CI: -136.5, -70.2). Minimum diameter exhibited a larger magnitude of association; a smaller minimum diameter was associated with a reduction in birthweight of 174.5 grams (95% CI: -217.2, -131.9). These associations were not significantly different for males and females.

SGA Analysis

The observed associations in the linear models of birthweight were mirrored by changes in the risk of SGA (Figure 3.4, Table S3.6). In the total sample, reduced surface area (25^{th} vs. 75^{th}) was associated with increased risk of SGA as compared to AGA in a model adjusted for covariates (odds ratio [OR]: 3.1; 95% CI: 2.2, 4.5). Additionally, reduced surface area was associated with lower risk of LGA as compared to AGA (OR: 0.5; 95% CI: 0.4, 0.7). A similar relationship was observed with placental thickness for SGA and LGA compared to AGA; however, the magnitude of the association was smaller. The other placental variables were not associated with risk of SGA or LGA as compared to AGA.

DISCUSSION

We found that gross placental features, particularly thickness and surface area, were associated with birthweight. Our results indicate that these features of placental morphology may independently contribute to birthweight. Adjustment for confounders, including gestational age and maternal body size, reduced observed associations by nearly half. Additionally, adjustment for characteristics indicative of placental developmental disorders, inflammatory disorders, maternal vascular malperfusion, and fetal vascular malperfusion did not affect the observed associations for placental thickness and surface area. Additionally, we did not observe evidence of interaction between placental morphology and sex. The results of the linear and logistic models were similar, which supports the robustness of the observed relationships.

Only one other study explored sex-specific differences in the relationships between features of placental morphology and birthweight. These authors used multivariate adaptive regression splines and reported that the relationships of surface area and thickness with birthweight are different for males and females and that the differences depend on the size of the placental characteristic.[58] Specifically, the authors reported that the relationships differed for higher z-scores of thickness and surface area. Higher z-scores may reflect greater gestational ages, which is inconsistent with our null findings of interaction among term births. Our null findings may be due the use of linear models, which do not allow the interaction between males and females to differ based on the size of the placental characteristic. However, our null results may also be explained by adjustment for additional confounders, including gestational age.

Our results for the overall associations between placental morphology and birthweight were consistent with available data, although differences in samples studied and analytic methods used make direct comparisons challenging. To our knowledge, only two studies have

evaluated the independent contributions of features of gross placental morphology in relation to birthweight in models adjusted for covariates. Grandi, 2016 #822;Salafia, 2007 #505} Grandi et al. (2016) found no association between a 1 cm increase in thickness and birthweight (β: 17.1; 95% CI: -30.7, 65.1).[74] However, the data from Grandi et al. (2016) were based on a study conducted in Argentina and the placentas in this sample appear to be thicker (mean: 3.0 cm) with a smaller surface area (mean: 164.8 cm^2), which may explain the conflicting results. In contrast, Salafia et al. (2007) reported that for every 1 cm increase in thickness, birthweight increases by 213.7 grams (95% CI: 202.5, 224.8) in a model adjusted for gestational age, parity, and sex, which is double the magnitude of our findings (equivalent comparison in our study: 97.4 grams; 95% CI: 56.1, 138.7).[71] Salafia et al. (2007) also reported that for every 10 cm^2 increase in surface area, birthweight increases by 41.9 grams (95% CI: 40.8, 42.9; equivalent comparison in our study: 31.3 grams; 95% CI: 26.5, 36.0).[71] The smaller associations that we observed may be due to our ability to adjust for additional confounders, including maternal body size and gestational diabetes, which are related to both birthweight and placental size.[109, 110] Several groups have reported no correlation between umbilical cord insertion and birthweight, which is consistent with our results.[64, 75, 82] To our knowledge, no studies have evaluated difference in diameters and abnormal shape in relation to birthweight in models adjusted for confounders. However, in evaluating placental shape as the ratio of longest and shortest diameters, Salafia et al. (2007) reported no association with birthweight, which is consistent with our results.[71]

Our findings that surface area and thickness have the largest association with birthweight are consistent with other studies reporting a positive relationship between placental weight and birthweight.[81] Surface area and thickness both contribute to placental weight, which is commonly evaluated because it is easy to measure and readily available in existing datasets. However, a

limitation of using placental weight is that it is dependent on several factors, including if the cord and membranes are trimmed, timing of cord clamping, time between delivery and weighing, and how the placenta is stored prior to weighing.[70, 104] Evaluating specific components of size, like surface area and thickness, may be more informative, as these components develop during different periods of gestation and may reflect different mechanisms that have independent effects on birthweight.[21, 74]

One concern with modeling multiple measures from the same placenta is interdependence of the measures. Although the correlation coefficients between some placental variables, such as thickness and surface area, were statistically significant (p-value < 0.05), the magnitudes of the correlations were small, with none exceeding 0.2, suggesting collinearity is not a concern. Weak correlations between placental characteristics are consistent with available data.[5]

Another concern with this analysis is how the placental variables were defined. Dichotomization of placental shape may not adequately reflect the variation in this measure and is subject to pathologist classification. Abnormally shaped placentas likely represent a heterogeneous group with different etiologies. Further, our measures of surface area, difference in diameters, and umbilical cord insertion site assume an elliptical shape and may not be appropriate for use in abnormal shapes. However, excluding placentas with abnormal shapes did not modify the results or our conclusions (results not shown). Similarly, we modeled thickness as a continuous variable, which may not capture the adverse associations that have been reported with both thick and thin placentas, or with placentas of variable thickness. In modeling and graphical evaluations, thickness did not demonstrate a gross violation of the linearity assumption and modeling non-linear relationships did not meaningfully improve model fit (Figure S2).

Further, our adjusted models control for factors that may influence the potential non-linear relationship, such as preeclampsia.

Another limitation is that the placental measures were obtained at delivery. The placenta is a dynamic organ and studies have shown that it has the ability to adapt and compensate in the presence of stressors.[72] Thus, we cannot determine if the placental measures reflect an adaptive response to a poor intrauterine environment, if they influence the development of a poor intrauterine environment, or a combination of the two. Ongoing longitudinal studies as part of the Human Placenta Project should further inform the relationship between placental function and fetal growth.

Strengths of this analysis include the use of a large dataset with detailed information on maternal and pregnancy characteristics. This facilitated control for covariates affecting both placental and fetal development and allowed us to investigate sex-specific differences in the associations. Gestational age was also determined from ultrasound measures, which allows for improved control of this important confounder in comparison to studies using last menstrual period.[116] Additionally, placental examinations were conducted by trained pathologists using a standard protocol to ensure consistent data collection. We also accounted for selection bias related to differences in those who did not consent to a placental examination and for whom the placental examination was incomplete. A focus on gross placental morphology is also a strength, as these features can be ascertained during routine ultrasound scans.[68]

While our results do not suggest sex-specific differences in the associations between birthweight and placental measures at delivery, we found that reduced thickness and surface area were associated with lower birthweight. Further, these results were independent of placental characteristics indicative of developmental and inflammatory disorders, and of maternal and fetal

vascular malperfusion. Specifically, surface area had the strongest association with birthweight. This suggests that the number of spiral arteries supplying the placenta may be a key contributor to fetal growth and development.[107] Future studies should adjust for potential confounders when evaluating relationships between placental size and fetal size, as adjustment for confounding related to maternal and pregnancy characteristics reduced the magnitudes of the reported associations. Future studies should also consider the adaptability of the placenta and focus on determining the directionality of these associations. This includes evaluating serial placental images throughout pregnancy to track placental development and adaptation, which may allow for improved detection of sex-specific differences in these processes.

FIGURES AND TABLES

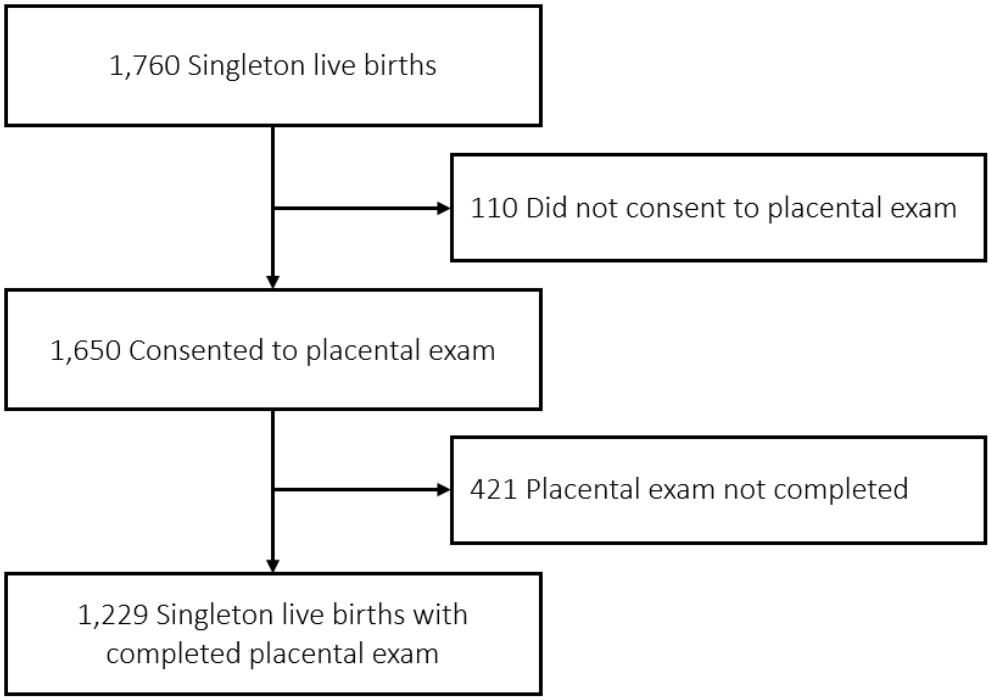

Figure 3.1. Study enrollment and inclusion.

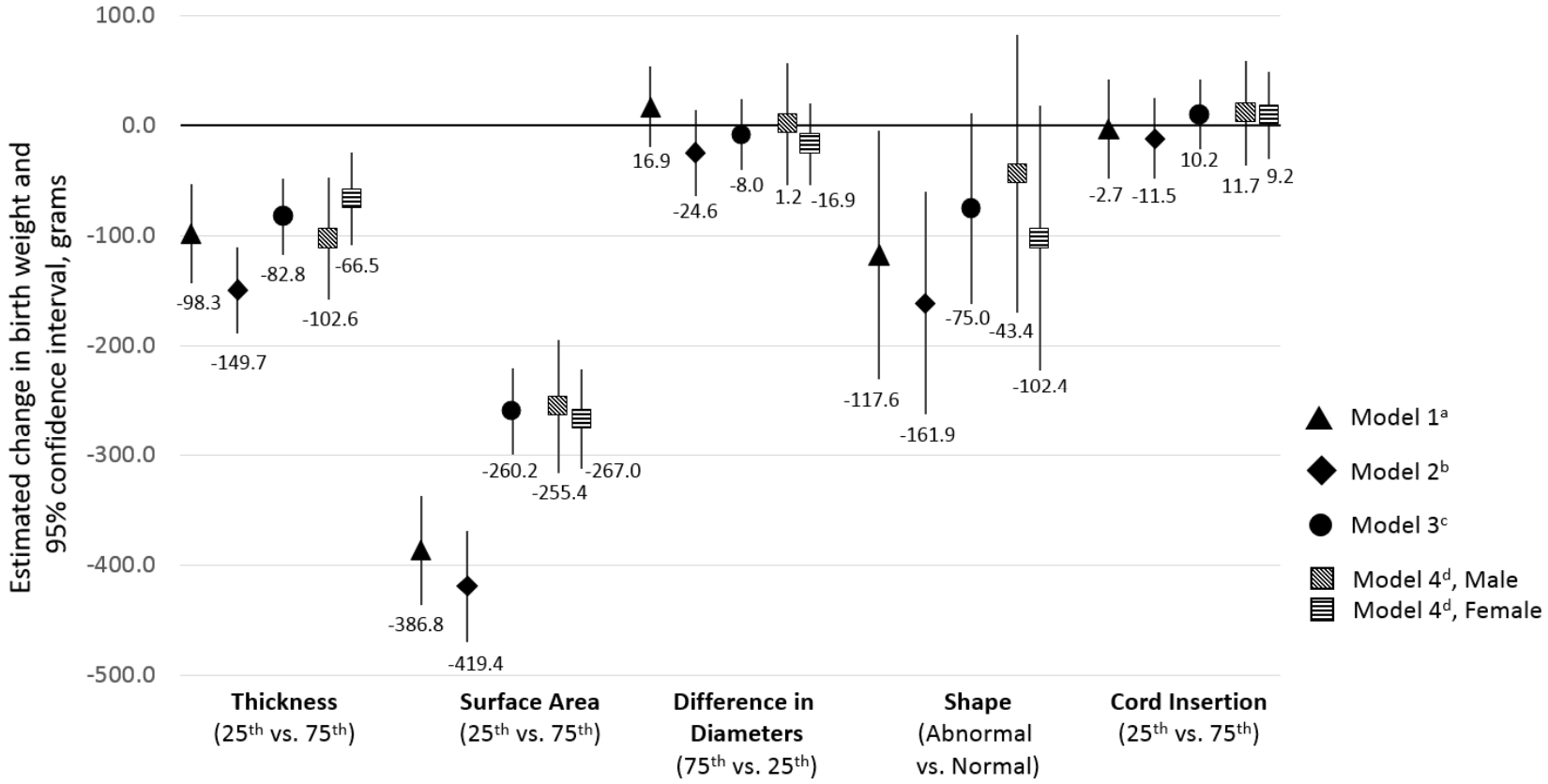

Figure 3.2. Estimated change in birthweight associated with change in feature of placental morphology of interest using weighted linear regression in the total sample.

[a] Model 1 contains each placental variable modeled individually (four separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains placental variables and covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex)
[d] Model 4 contains placental variables, covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex), and interaction between placental variables and sex

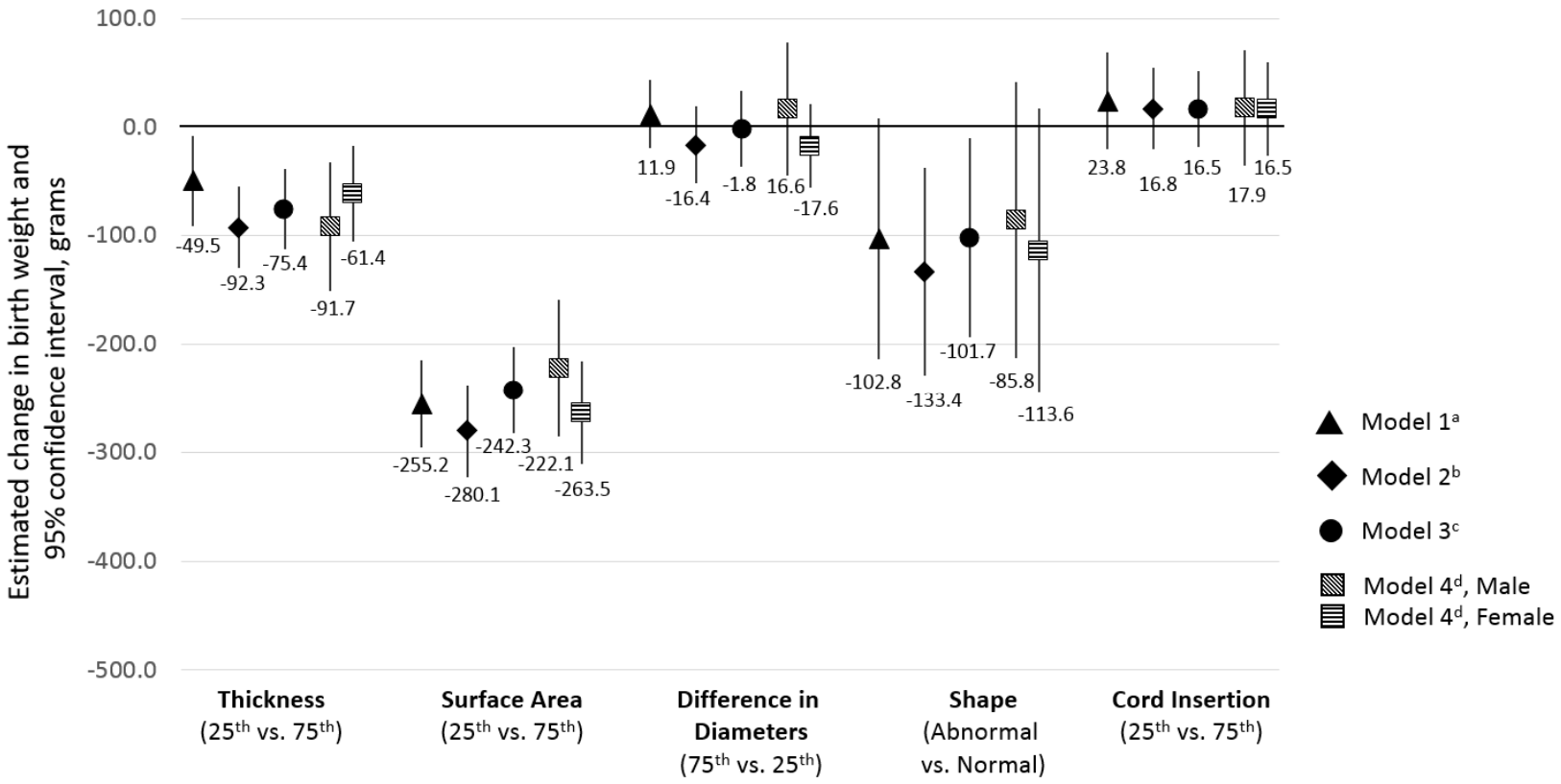

Figure 3.3. Estimated change in birthweight associated with change in feature of placental morphology of interest using weighted linear regression, restricted to term births.

[a] Model 1 contains each placental variable modeled individually (four separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains placental variables and covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex)
[d] Model 4 contains placental variables, covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex), and interaction between placental variables and sex

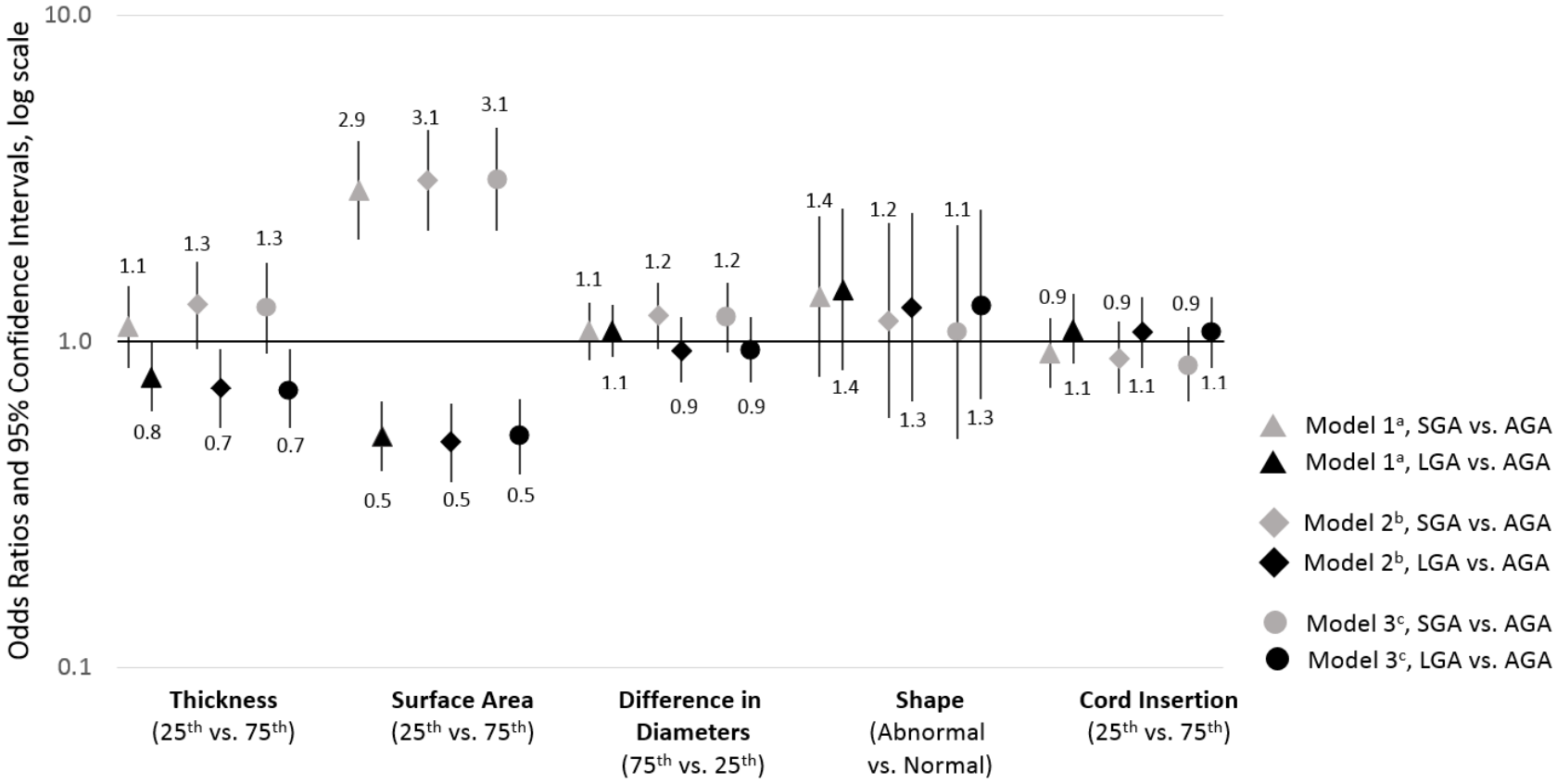

Figure 3.4. Estimated odds ratio for small for gestational age (<10th percentile) and large for gestational age (>90th percentile) as compared to appropriate for gestational age (10th-90th percentile) for the features of placental morphology of interest using weighted logistic regression in the total sample.

Abbreviations: SGA – small for gestational age; AGA – appropriate for gestational age; LGA – large for gestational age

[a] Model 1 contains each placental variable modeled individually (five separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains placental variables and covariates (maternal age, gestational hypertension, gestational diabetes

Table 3.1. Descriptive characteristics of singleton live births with a completed placental examination for the total sample and term births.

Characteristic	Total	Term
	N_w ($\%_w$) **N=1229** **N_w=955**	**N_w ($\%_w$)** **N=953** **N_w=864**
Adjusted birthweight percentile[a]		
<5th percentile	91 (9.6)	75 (8.7)
5th-10th percentile	61 (6.4)	54 (6.3)
10th-90th percentile	676 (71.1)	627 (72.9)
90th-95th percentile	50 (5.2)	44 (5.1)
95th-100th percentile	73 (7.7)	61 (7.1)
Gestational Age		
20-23 completed weeks	3 (0.4)	0 (0.0)
24-27 completed weeks	6 (0.6)	0 (0.0)
28-31 completed weeks	9 (0.9)	0 (0.0)
32-36 completed weeks	73 (7.7)	0 (0.0)
≥37 completed weeks	864 (90.4)	864 (100.0)
Neonate sex, male	478 (50.0)	422 (48.9)
Primiparae	332 (34.8)	303 (35.1)
Maternal Age		
<20	100 (10.5)	89 (10.3)
20 – 34	720 (75.4)	652 (75.5)
35 – 39	110 (11.6)	101 (11.7)
40+	24 (2.5)	21 (2.5)
Maternal Race/Ethnicity		
Non-Hispanic white	419 (43.9)	396 (45.8)
Non-Hispanic black	116 (12.1)	98 (11.3)
Hispanic	351 (36.8)	306 (35.5)
Other	69 (7.2)	64 (7.4)
Maternal Education		
0 – 11 (none/primary/some secondary)	172 (18.1)	154 (17.9)
12 (completed secondary)	257 (27.0)	224 (26.0)
13+ (college)	521 (54.8)	483 (56.1)
Marital Status		
Not married or cohabitating	146 (15.4)	127 (14.7)
Cohabitating	223 (23.4)	202 (23.4)
Married	584 (61.2)	535 (61.9)
Maternal BMI, kg/m^2		
<18.5	26 (2.7)	25 (2.9)
18.5 – 24.9	479 (50.5)	439 (50.8)
25 – 29.9	218 (23.0)	199 (23.0)

30 – 34.9	117 (12.4)	101 (11.7)
≥35	108 (11.4)	100 (11.6)
Pre-gestational Hypertension	58 (6.1)	47 (5.5)
Preeclampsia/hypertension	88 (9.4)	68 (7.9)
Diabetes (Type 1 or 2)	16 (1.7)	12 (1.4)
Gestational Diabetes	79 (8.3)	73 (8.4)
Insurance		
No insurance	41 (4.3)	38 (4.4)
Any public/private insurance	475 (49.8)	410 (47.5)
VA/commercial health ins/HMO	439 (45.9)	416 (48.1)
Periconceptional Maternal Smoking[b]		
Did not smoke	830 (87.1)	752 (87.2)
< 10 cigarettes/day	61 (6.4)	54 (6.2)
≥ 10 cigarettes/day	61 (6.5)	57 (6.6)
Periconceptional Alcohol Use[c]		
Did not drink	551 (58.0)	497 (57.8)
Drank, no binging	224 (23.6)	202 (23.5)
Binged	174 (18.3)	160 (18.6)
Illicit Drug Use[d]		
Never used drugs	670 (71.5)	615 (72.0)
Ever used drugs w/o addiction	247 (26.3)	221 (25.9)
Ever used drugs w/ addiction	20 (2.1)	18 (2.1)

Abbreviations: N_w – Sample size weighted to reflect differential consent, enrollment, and placental examination (rounded to the nearest whole number)

[a] Based on Bukowski et al. [35, 114]
[b] Average number of cigarettes per day during 3 months prior to pregnancy
[c] Alcohol consumption during 3 months prior to pregnancy (Drank, no binging defined as 0-6 drinks in a typical week and no binge (four or more drinks consumed in a single time period); Binged defined as at least one binge and/or seven or more drinks in a typical week)
[d] Lifetime drug use

Table 3.2. Descriptive characteristics for birthweight and placental morphology stratified by neonate sex and birthweight for gestational age category for the total sample.

Characteristic Mean (SE) or %w	**Sex**		**Birthweight for gestational age category**		
	Male	**Female**	**SGA**	**AGA**	**LGA**
	N=619 N_w=481	N=610 N_w=478	N=217 N_w=152	N=841 N_w=676	N=161 N_w=123
Birthweight, grams	3308.55 (27.13)	3224.02 (22.80)*	2634.13 (37.67)	3304.82 (15.25)	3834.78 (43.55)*
Placental weight, grams	442.51 (4.83)	443.71 (4.74)	367.02 (7.93)	445.42 (3.57)	525.85 (9.08)*
Fetal-Placental weight ratio	7.63 (0.07)	7.48 (0.07)	7.45 (0.14)	7.59 (0.05)	7.48 (0.12)
Maximum diameter, cm	19.77 (0.15)	19.90 (0.14)	18.48 (0.29)	19.87 (0.12)	21.21 (0.24)*
Minimum diameter, cm	16.63 (0.12)	16.29 (0.11)*	14.97 (0.18)	16.55 (0.09)	17.68 (0.20)*
Thickness, cm	2.38 (0.03)	2.43 (0.03)	2.35 (0.06)	2.40 (0.02)	2.52 (0.06)
Surface area, cm^2	261.10 (3.40)	257.26 (3.06)	219.42 (5.08)	260.58 (2.60)	296.79 (5.58)*
Difference in diameters, cm	3.14 (0.13)	3.61 (0.13)*	3.51 (0.28)	3.32 (0.11)	3.54 (0.24)
Shape, abnormal	44 (9.4%)	51 (10.7%)	18 (12.1%)	61 (9.1%)	16 (12.7%)
Cord insertion, abnormal	0.54 (0.01)	0.52 (0.01)	0.54 (0.02)	0.53 (0.01)	0.51 (0.02)

Abbreviations: N_w – weighted sample size; SGA – small for gestational age; AGA – appropriate for gestational age; LGA – large for gestational age; SE – standard error

* Indicates statistically significant ($p<0.05$) difference in the characteristic between males and females or SGA, AGA, and LGA (p-value from Wald chi square or Wald F test)

SUPPLEMENTAL MATERIAL

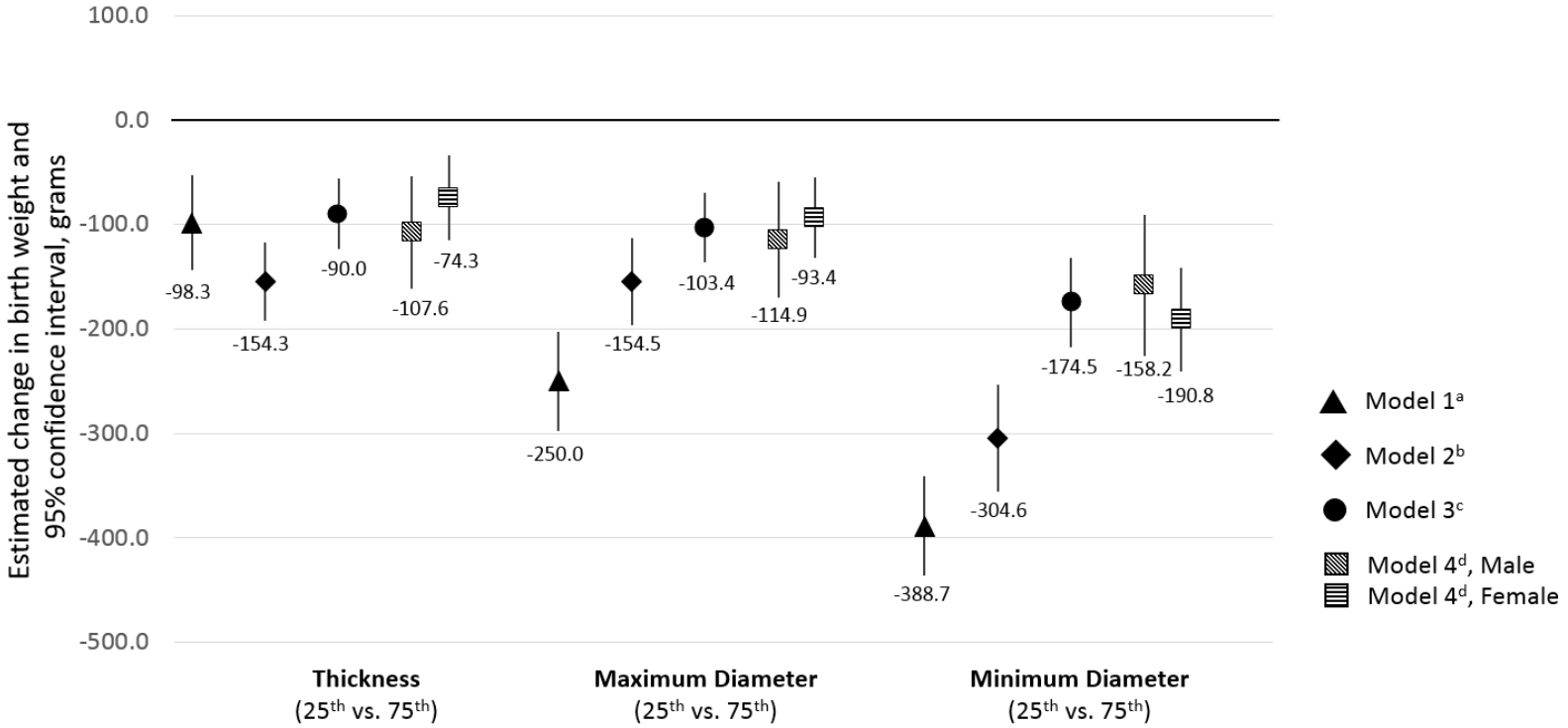

Figure S3.1. Estimated change in birthweight associated with change in feature of placental morphology of interest using weighted linear regression in the total sample.

[a] Model 1 contains each placental variable modeled individually (three separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains placental variables and covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex)
[d] Model 4 contains placental variables, covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy weight, height, gestational hypertension, gestational diabetes, parity, gestational age, sex), and interaction between placental variables and sex

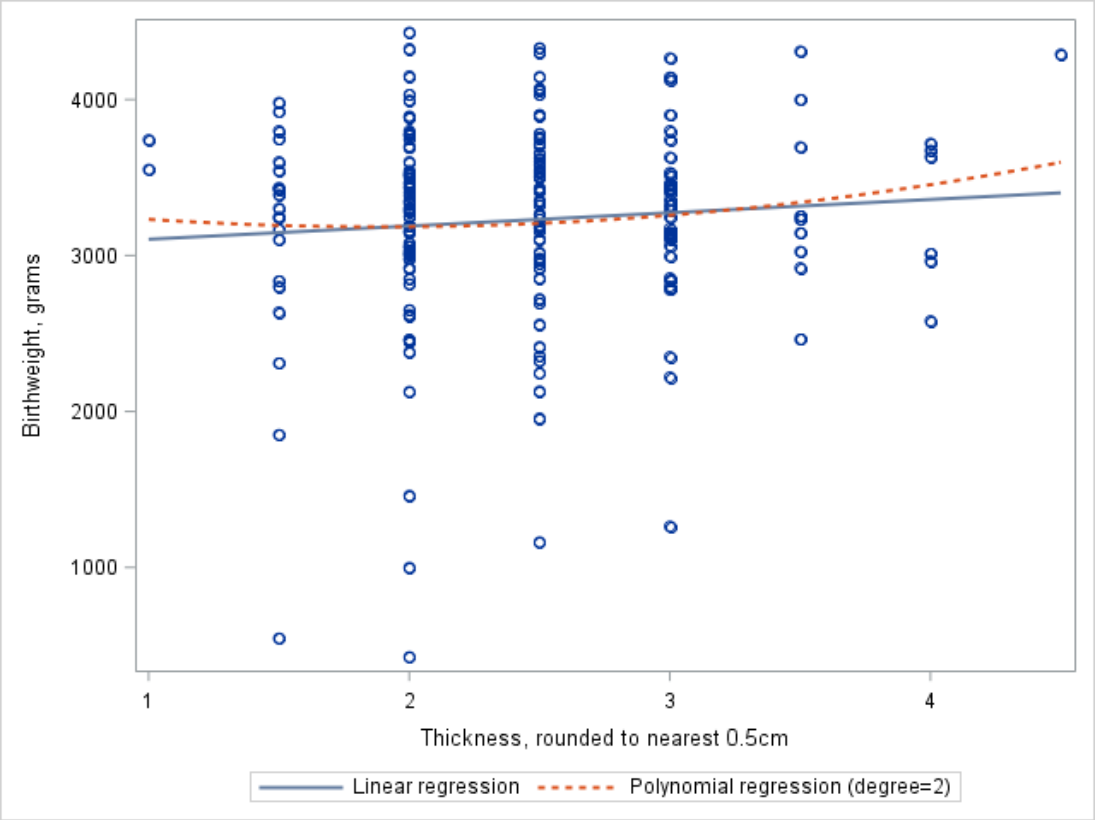

Figure S3.2. Scatterplot of thickness versus birthweight for a subset of the total sample (probability proportional to size sampling, n=200). Plot includes trend lines for linear regression (solid blue line) and polynomial regression (dashed red line), which are similar to each other.

Table S3.1. Descriptive statistics for continuous placental variables.

Placental Variable	25th percentile	75th percentile	Difference used for estimates
Total Sample			
Thickness, cm	1.70	2.55	-0.85
Surface area, cm^2	213.63	296.82	-83.19
Difference in diameters, cm	1.45	4.45	3.00
Umbilical cord insertion	0.39	0.68	-0.29
Maximum diameter, cm	17.95	21.00	-3.05
Minimum diameter, cm	14.89	17.97	-3.08
Term Sample			
Thickness, cm	1.71	2.55	-0.84
Surface area, cm^2	219.09	298.39	-79.30
Difference in diameters, cm	1.45	4.46	3.01
Umbilical cord insertion	0.39	0.69	-0.30
Maximum diameter, cm	18.00	21.44	-3.44
Minimum diameter, cm	14.95	17.98	-3.03

Note: For most variables, estimate reflect a comparison of the 25th percentile to the 75th percentile; however, for difference in diameters, estimates reflect a comparison of the 75th percentile to the 25th percentile.

Table S3.2. Descriptive characteristics for birthweight and placental morphology stratified by neonate sex and birthweight for gestational age category, restricted to term births.

Characteristic Mean (SE) or %w	**Sex**		**Birthweight for gestational age category**		
	Male	**Female**	**SGA**	**AGA**	**LGA**
	N=463 N_w=422	N=490 N_w=442	N=143 N_w=129	N=687 N_w=627	N=120 N_w=105
Birthweight, grams	3439.70 (22.14)	3301.56 (21.54)*	2797.13 (26.46)	3384.66 (13.35)	3976.93 (34.28)*
Placental weight, grams	453.68 (4.93)	450.54 (4.88)	382.58 (8.71)	451.84 (3.68)	540.45 (8.92)*
Fetal-Placental weight ratio	7.79 (0.07)	7.57 (0.07)*	7.67 (0.15)	7.70 (0.06)	7.55 (0.12)
Maximum diameter, cm	20.03 (0.15)	20.07 (0.15)	18.76 (0.28)	20.04 (0.12)	21.57 (0.23)*
Minimum diameter, cm	16.88 (0.12)	16.43 (0.11)*	15.27 (0.18)	16.71 (0.09)	17.86 (0.21)*
Thickness, cm	2.39 (0.03)	2.44 (0.03)	2.37 (0.07)	2.40 (0.02)	2.52 (0.07)
Surface area, cm^2	267.87 (3.45)	261.24 (3.16)	227.01 (5.24)	265.00 (2.69)	303.94 (5.58)*
Difference in diameters, cm	3.15 (0.13)	3.64 (0.14)*	3.48 (0.26)	3.33 (0.11)	3.71 (0.27)
Shape, abnormal	35 (8.4%)	47 (10.8%)	15 (11.9%)	55 (8.8%)	13 (12.0%)
Cord insertion, abnormal	0.54 (0.01)	0.52 (0.01)	0.55 (0.02)	0.53 (0.01)	0.50 (0.03)

Abbreviations: N_w – weighted sample size; SGA – small for gestational age; AGA – appropriate for gestational age; LGA – large for gestational age; SE – standard error

* Indicates statistically significant ($p<0.05$) difference in the characteristic between males and females or SGA, AGA, and LGA (p-value from Wald chi square or Wald F test)

Table S3.3. Estimated change in birthweight associated with change in feature of placental morphology of interest using weighted linear regression for the total and term (≥ 37 weeks' gestation) samples.

		Thickness[a]		Surface Area[a]		Difference in Diameters[b]		Shape, Abnormal		Cord Insertion[a]	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Total											
Model 1[b]		-98.3	-143.5, -53.0	-386.8	-435.9, -337.7	16.9	-19.7, 53.6	-117.6	-230.6, -4.7	-2.7	-47.8, 42.4
Model 2[c]		-149.7	-188.4, -110.9	-419.4	-470.1, -368.6	-24.6	-63.7, 14.4	-161.9	-263.5, -60.4	-11.5	-48.5, 25.5
Model 3[d]		-82.8	-117.9, -47.7	-260.2	-299.9, -220.6	-8.0	-40.3, 24.3	-75.0	-161.6, 11.5	10.2	-21.5, 41.8
Model 4[f]	Males	-102.6	-158.2, -47.1	-255.4	-316.1, -194.7	1.2	-54.0, 56.4	-43.4	-169.8, 83.0	11.7	-35.8, 59.2
	Females	-66.5	-108.9, -24.1	-267.0	-312.6, -221.5	-16.9	-53.7, 19.8	-102.4	-222.6, 17.8	9.2	-30.4, 48.8
Term											
Model 1[b]		-49.5	-91.0, -8.1	-255.2	-295.7, -214.8	11.9	-19.2, 43.0	-102.8	-213.6, 8.1	23.8	-20.7, 68.3
Model 2[c]		-92.3	-129.7, -54.9	-280.1	-322.5, -237.7	-16.4	-52.0, 19.1	-133.4	-228.9, -37.9	16.8	-20.8, 54.4
Model 3[d]		-75.4	-111.9, -38.9	-242.3	-282.3, -202.2	-1.8	-36.6, 32.9	-101.7	-193.0, -10.3	16.5	-18.5, 51.4
Model 4[f]	Males	-91.7	-151.2, -32.1	-222.1	-285.3, -158.9	16.6	-45.0, 78.2	-85.8	-212.8, 41.3	17.9	-35.1, 71.0
	Females	-61.4	-105.3, -17.6	-263.5	-310.8, -216.1	-17.6	-55.9, 20.8	-113.6	-244.1, 16.9	16.5	-26.6, 59.7

Abbreviations: CI – confidence interval

[a] Estimate comparing 25th percentile to 75th percentile
[b] Estimate comparing 75th percentile to 25th percentile
[c] Model 1 contains each placental variable modeled individually (five separate models)
[d] Model 2 contains all placental variables modeled together
[e] Model 3 contains placental variables and covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy body mass index, gestational hypertension, gestational diabetes, parity, gestational age, sex)
[f] Model 4 contains placental variables, covariates (see model 3 detail), and interaction between placental variables and sex

* Indicates statistically significant (p-value < 0.05) interaction between the characteristic and sex

Table S3.4. Estimated change in birthweight associated with placental thickness, placental surface area, and indicators of placental developmental disorders, inflammatory disorders, maternal vascular malperfusion, and fetal vascular malperfusion.

	Thickness[a]		Surface Area[a]		Developmental Disorders[b]		Inflammatory Disorders[c]		Maternal Vascular Malperfusion[d]		Fetal Vascular Malperfusion[e]	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Model 1[f]					-26.4	-113.8, 61.0	40.8	-74.3, 155.9	-24.1	-98.8, 50.6	-60.8	-157.0, 35.5
Model 2[g]	-151.7	-190.9, -112.5	-394.0	-445.8, -342.1	30.8	-44.2, 105.7	-3.3	-95.0, 88.4	-17.8	-84.3, 48.7	-37.3	-118.0, 43.5
Model 3[h]	-90.8	-125.8, -55.9	-249.8	-288.9, -210.7	2.2	-58.7, 63.0	28.9	-43.1, 100.9	-26.8	-81.7, 28.1	-4.5	-81.0, 72.1

[a] Estimate comparing 25th percentile to 75th percentile
[b] Developmental disorders include presence of one or more abnormalities of the umbilical cord (single umbilical cord artery, velamentous insertion, furcate insertion), placental membranes (circummarginate insertion, circumvallate insertion), and/or fetal villous capillaries (terminal villous immaturity (diffuse), terminal villous hypoplasia (diffuse)
[c] Inflammatory disorders include one or more indicators of the maternal inflammatory response (acute chorioamnionitis – placental membranes, acute chorioamnionitis – chorionic plate), the fetal inflammatory response (acute funisitis, acute umbilical cord arteritis (one or more arteries), acute umbilical cord phlebitis, chorionic plate acute vasculitis, chorionic plate vascular degenerative changes), and/or villitis (acute diffuse villitis, chronic diffuse villitis)
[d] Indicators of maternal vascular malperfusion include presence of one or more of the following: retroplacental hematoma, parenchymal infarction, intraparenchymal thrombus, and/or perivillous, intervillous fibrin, fibrinoid deposition (diffuse)
[e] Indicators of fetal vascular malperfusion include one or more of the following: fetal vascular thrombi in the chorionic plate, avascular villi, and/or placental edema
[f] Model 1 includes only presence of placental developmental disorders, inflammatory disorders, maternal vascular malperfusion, and fetal vascular malperfusion
[g] Model 2 includes the variables in Model 1, as well as placental thickness and surface area
[h] Model 3 additionally includes maternal and pregnancy characteristics (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy body mass index, gestational hypertension, gestational diabetes, parity, gestational age, sex)

Table S3.5. Estimated change in birthweight associated with change in feature of placental morphology of interest using weighted linear regression for the total sample.

		Thickness[a]		Maximum Diameter[a]		Minimum Diameter[a]	
		β	95% CI	β	95% CI	β	95% CI
Total							
Model 1[b]		-98.3	-143.5, -53.0	-250.0	-297.4, -202.7	-388.7	-436.6, -340.8
Model 2[c]		-154.3	-191.9, -116.8	-154.5	-196.1, -112.9	-304.6	-355.8, -253.4
Model 3[d]		-90.0	-123.6, -56.3	-103.4	-136.5, -70.2	-174.5	-217.2, -131.9
Model 4[e]	Males	-107.6	-161.6, -53.5	-114.9	-170.4, -59.5	-158.2	-226.2, -90.3
	Females	-74.3	-114.7, -34.0	-93.4	-132.2, -54.7	-190.8	-240.5, -141.2

Abbreviations: CI – confidence interval

[a] Estimate comparing 25th percentile to 75th percentile
[b] Model 1 contains each placental variable modeled individually (three separate models)
[c] Model 2 contains all placental variables modeled together
[d] Model 3 contains placental variables and covariates (maternal age, education, maternal race/ethnicity, smoking, pre-pregnancy body mass index, gestational hypertension, gestational diabetes, parity, gestational age, sex)
[e] Model 4 contains placental variables, covariates (see model 3 detail), and interaction between placental variables and sex

Table S3.6. Estimated odds ratios and 95% confidence intervals for small for gestational age (<10th percentile) and large for gestational age (>90th percentile) as compared to appropriate for gestational age (10th-90th percentile) for the features of placental morphology of interest using weighted logistic regression in the total sample.

		Thickness[a]		Surface Area[a]		Difference in Diameters[b]		Shape, Abnormal		Cord Insertion[a]	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Total											
Model 1[c]	SGA	1.1	0.8, 1.5	2.9	2.1, 4.1	1.1	0.9, 1.3	1.4	0.8, 2.4	0.9	0.7, 1.2
	LGA	0.8	0.6, 1.0	0.5	0.4, 0.7	1.1	0.9, 1.3	1.4	0.8, 2.6	1.1	0.9, 1.4
Model 2[d]	SGA	1.3	1.0, 1.8	3.1	2.2, 4.4	1.2	1.0, 1.5	1.2	0.6, 2.3	0.9	0.7, 1.2
	LGA	0.7	0.5, 1.0	0.5	0.4, 0.6	0.9	0.8, 1.2	1.3	0.7, 2.5	1.1	0.8, 1.4
Model 3[e]	SGA	1.3	0.9, 1.8	3.1	2.2, 4.5	1.2	0.9, 1.5	1.1	0.5, 2.3	0.9	0.7, 1.1
	LGA	0.7	0.5, 1.0	0.5	0.4, 0.7	0.9	0.8, 1.2	1.3	0.7, 2.5	1.1	0.8, 1.4

Abbreviations: OR – odds ratio; CI – confidence interval; SGA – small for gestational age; LGA – large for gestational age

[a] Estimate comparing 25th percentile to 75th percentile
[b] Estimate comparing 75th percentile to 25th percentile
[c] Model 1 contains each placental variable modeled individually (five separate models)
[d] Model 2 contains all placental variables modeled together
[e] Model 3 contains placental variables and covariates (maternal age, gestational hypertension, gestational diabetes)

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CHAPTER 4: ASSOCIATIONS BETWEEN FEATURES OF PLACENTAL MORPHOLOGY AND BIRTHWEIGHT IN DICHORIONIC TWINS

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ABSTRACT

Associations between features of placental morphology and birthweight have not been thoroughly investigated in twins. Evaluating differences within dichorionic twin pairs offers a unique opportunity to estimate associations between placental morphology and birthweight while controlling for key confounders shared within a twin pair, including gestational age. This analysis utilized 208 sets of dichorionic twins with unfused placentas from three studies. We used linear regression to model the difference in birthweight within a twin pair as a function of their differences in placental characteristics. Placental characteristics included thickness, surface area, and difference in diameters. After controlling for sex discordance, a 100cm^2 difference in placental surface area was associated with a difference in birthweight of 188.7 grams (95% confidence interval [CI]: 83.5, 293.9). When stratified by sex, the magnitude of the association was larger for same-sex male pairs than same-sex female pairs (males: 353.0 grams, 95% CI: 80.7, 625.2; females: 176.6 grams, 95% CI: 20.8, 332.4). Other placental characteristics were not associated with birthweight differences in the total sample. Strong associations between placental surface area and birthweight are consistent with reported results for singleton pregnancies. Further, our results support reported differences in placental development between males and females.

INTRODUCTION

The placenta plays a critical role in regulating fetal growth and development, including producing hormones and transporting oxygen, nutrients, and waste.[117] Placental dysfunction has been implicated in suboptimal fetal growth, which is a strong risk factor for perinatal morbidity and mortality.[12, 35, 39] Several studies reported associations between gross placental morphology and fetal growth among singleton pregnancies.[5, 32, 71, 74, 76] A limitation of these studies is inadequate consideration for some maternal and pregnancy characteristics, such as gestational age, which are related to both placental and fetal development.[5, 118, 119]

The unique features of twins, such as (often) shared genetics, gestational age, and intrauterine environment, facilitate control for these potential confounders of the relationship between placental morphology and birthweight. Further, twins have been suggested as a potential means to elucidate the developmental programming of health outcomes due to their shared characteristics.[21, 120] While dichorionic twins may compete for space and resources, the underlying relationship between each twin and their respective placenta is comparable to that of a singleton pregnancy.[21] Suboptimal fetal growth is also more common in twin pregnancies than in singleton pregnancies, with a prevalence of 15-25%, which makes this an ideal population to study the association between placental morphology and birthweight.[19]

Further, evaluating relationships in twins may help elucidate reported sex-specific differences in placental development.[57, 59, 121] In particular, placentas of females are hypothesized to have more reserve capacity and improved ability to adapt and respond to insults and stressors as compared to placentas of males.[59] Additionally, a recent review article of both animal and human studies indicated that placentas of males and females may be sensitive to stressors at different periods of gestation.[122] Evaluating relationships between placental morphology and

birthweight within same-sex twin pairs may help elucidate these associations by improving control for shared insults affecting both placental development and fetal growth, which may be difficult to measure.

While previous studies of dichorionic twins evaluated fetal-placental weight ratio, umbilical cord insertion, and placental proximity in relation to birthweight[23, 85, 87], few studies have examined other features of gross placental morphology. The purpose of our study was to evaluate the associations of differences in placental morphology within twin pairs, including differences in thickness, surface area, and difference in diameters, with difference in birthweight. We hypothesized that greater discordance in features of placental morphology within a twin pair would be associated with a greater difference in birthweight, and that these associations would differ by sex.

METHODS

Study sample

To obtain a sufficiently large sample size, data on dichorionic twins from three completed studies were compiled for this analysis. The analysis was restricted to 208 sets of live born dichorionic twins with unfused placentas (Figure 4.1). Twin pregnancies with one or more stillbirths were excluded due to difficulty determining gestational age at the time of fetal death and potential differences in gestational age within the twin pair.[123] Twins with fused placentas were excluded because they may have lower birthweights as a result of placental crowding leading to restricted placental growth, rather than as a result of intrinsic individual placental development.[23] Each study was approved by appropriate Institutional Review Boards and each participant provided written informed consent.

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies: The twin component of the NICHD study was a longitudinal study of fetal growth patterns in dichorionic twins. Women with dichorionic twin pregnancies were enrolled during the first trimester from 2012-2013 at eight U.S. sites. Details of the study design have been published.[124, 125] Of the 171 sets of dichorionic twins enrolled in the NICHD study, our analysis included the 60 sets of live born twins with unfused placentas.

Stillbirth Collaborative Research Network (SCRN): The SCRN Study was a population-based case-control study of stillbirth. Enrollment occurred from 2006-2008 at 59 hospitals representing residents in five catchment areas of the U.S. Details of the study design and sampling methods have been published elsewhere.[94] Of the 93 sets of twins enrolled in the SCRN Study, our analysis included the 19 sets of live born, dichorionic twins with unfused placentas.

Collaborative Perinatal Project (CPP): The CPP was a longitudinal cohort study that enrolled women from 1959-1966 at twelve study sites in the U.S. Details of the study design have been published.[67] Of the 615 sets of twins enrolled in the CPP, our analysis included the 129 sets of live born, dichorionic twins with unfused placentas.

In all three studies, information on maternal, pregnancy, and neonatal characteristics were obtained by maternal interview and medical chart abstraction. In the NICHD study, genetic testing was conducted on placental samples, or buccal swabs if the placenta was not available, to determine the zygosity of same-sex twins.[124] Zygosity was not evaluated in the SCRN Study. In the CPP, zygosity of same-sex twins was determined based on comparison of blood type, placental characteristics, and finger and palm prints (zygosity was never determined solely based

on finger and palm prints).[126] In the NICHD study, placental data were abstracted from clinical pathology reports. In both the SCRN Study and the CPP, placental examinations were conducted by trained pathologists using standardized protocols.[96, 127] Examination protocols for gross placental morphology were fairly consistent across the two studies.

Thickness – In the NICHD study, the location of the thickness measurement was at the discretion of the pathologist; in the SCRN Study, placental thickness was measured at the thickest point; and in the CPP, placental thickness was measured at the center of the placenta. In all three studies, placental thickness was recorded to the nearest decimal place in centimeters. Due to evidence of digit preference in all three studies, measures of placental thickness were rounded to the nearest 0.5 cm.

Surface area – Surface area was determined based on the mathematical formula for the area of an ellipse using the recorded maximum (a) and minimum (b) diameters (area = $ab\pi/4$).

Difference in diameters – This measure was calculated by subtracting minimum diameter (b) from maximum diameter (a) within the same placenta for an individual twin (b-a). Differences of zero indicate circular placentas while larger differences indicate increasingly oval placentas.

Statistical analysis

To evaluate within-pair associations, we used linear regression to model difference in birthweight within a twin pair as a function of differences in the placental characteristics within a twin pair (model 3 in Carlin et al.[128]). Consistent with suggested techniques, the intercept was constrained to pass through the origin, which results in consistent estimates regardless of how the

differences are calculated.[128] We determined differences for continuous variables by subtracting the second born twin (twin B) from the firstborn twin (twin A), regardless of which was the larger of the twins. Thus, each variable was approximately normally distributed with an expected difference of zero. This method inherently controls for all shared confounders, such as study, maternal, and pregnancy characteristics, including gestational age. We considered sex discordance as a potential confounder. Since our exposure and outcome measures did not account for overall size, and the magnitude of the potential difference in both placental characteristics and birthweight increases with increasing gestational age, we conducted a secondary analysis stratified by gestational age group (<32 weeks, 32-36 weeks, >36 weeks). We also conducted a secondary analysis restricted to same-sex pairs to evaluate differences in the associations by sex. In general, we evaluated three models:

Model 1: Each difference in placental characteristic modeled individually with difference in birthweight. Interpreted as difference in placental characteristic associated with difference in birthweight within a twin pair.

Model 2: All differences in placental characteristics modeled together with difference in birthweight. Interpreted as difference in placental characteristic associated with difference in birthweight within a twin pair, controlling for the other placental characteristics.

Model 3: All differences in placental characteristics modeled together with difference in birthweight, controlling for sex discordance. Interpreted as difference in placental characteristic associated with difference in birthweight within a twin pair, controlling for the other placental characteristics and sex discordance. This model was not evaluated in sub-analysis restricted to same-sex pairs.

For these models, estimates of association reflect a 1 cm difference in thickness, 100 cm^2 difference in surface area, and a 1 cm difference in difference in diameters within a twin pair. Statistical analyses were performed using SAS version 9.4 (SAS Institute INC., Cary, North Carolina) and SUDAAN version 11.0 (Research Triangle Institute, Research Triangle Park, North Carolina).[115] All tests used a *P*-value of <0.05 to determine significance.

RESULTS

Descriptive characteristics

In the overall sample, 26.0% of twins were same-sex male, 27.4% were same-sex female, and 46.6% were opposite sex (Table 4.1). Among the same-sex twins evaluated for zygosity in the NICHD study and the CPP, 18 pairs (9.5%) were identified as monozygotic. Among the overall sample, 55.8% were born at term (≥37 weeks) and 71.2% of mothers were between 20-34 years old. There were significant differences in the distributions of gestational age, maternal age, education, race/ethnicity, smoking, and parity across the three studies per differences in study design.

The average birthweight among all twins was 2,353 grams (standard error: 46 grams) (Table 4.1). Among the overall sample, the average placenta weighed 337.8 grams, was 2.0 cm thick, had a surface area of 214.3 cm^2, and had a 3.8 cm difference in maximum and minimum diameters. Within a twin pair, the difference in birthweight (twin A – twin B, regardless of size) was 0.4 grams (Table 4.2). Differences in placental characteristics were also close to zero (thickness: 0.1 cm, area: 0.0 cm^2, difference in diameters: 0.3 cm). Differences in birthweight and continuous placental variables were normally distributed. The average absolute difference in birthweight within a twin pair (larger twin – smaller twin) was 282.5 grams. Average absolute

differences in placental characteristics within a twin pair were 0.3 cm for thickness, 40.5 cm^2 for surface area, and 2.4 cm for difference in difference in diameters.

Analytic results

In modeling and graphical evaluations, associations between difference in birthweight and differences in the placental characteristics did not demonstrate gross violations of the linearity assumption. Among all twins, a difference in surface area of 100 cm^2 was positively associated with a difference in birthweight of 188.7 grams (95% confidence interval [CI]: 83.5, 293.9) within a twin pair, controlling for sex discordance (Figure 4.2, Table S4.1). Differences in thickness and difference in diameters were not associated with a difference in birthweight within a twin pair in the total sample.

When stratified by gestational age group (<32 weeks, 32-36 weeks, and >36 weeks), the magnitude of the estimates diverged. A 100 cm^2 difference in surface area was not associated with a difference in birthweight among births <32 weeks' gestation (101.2 grams, 95% CI: -59.8, 262.2) or births 32-36 weeks' gestation (45.5 grams, 95% CI: -115.5, 206.6) when controlling for sex discordance (Table S4.1). However, among term births, a 100 cm^2 difference in surface area was associated with a 237.0 gram increase in birthweight (95% CI: 77.5, 396.4) when controlling for sex discordance.

When restricted to same-sex twin pairs, differences in surface area and thickness were both significantly and independently associated with a difference in birthweight (Figure 4.3, Table S4.2). Further, when same-sex male and female pairs were evaluated separately, the estimates of association diverged. Among same-sex male pairs, a 100 cm^2 difference in surface area was associated with a 353.0 gram difference in birthweight within a twin pair (95% CI:

80.7, 625.2). Whereas among same-sex female pairs, the magnitude of the association for a 100 cm^2 difference in surface area was only 176.6 grams (95% CI: 20.8, 332.4). Similarly, there was a significant positive association for a 1 cm difference in placental thickness among same-sex male pairs (283.7 grams, 95% CI: 44.0, 523.3) and no evidence for an association among same-sex female pairs (-9.2 grams, 95% CI: -213.0, 194.7).

DISCUSSION

Our results suggest that a 100 cm^2 difference in placental surface area within a twin pair is positively associated with a 188.7 gram difference in birthweight. Further, our results indicate that associations with surface area and thickness may differ by gestational age and may be stronger for males than females. The positive associations between difference in birthweight and difference in placental surface area were strongest among term births. In addition, differences in placental thickness and surface area were associated with larger differences in birthweight among same-sex male pairs as compared to same-sex female pairs.

The reported associations of placental thickness and surface area with birthweight were largest among term births. The magnitude of these associations may be attributable to our difference calculation (twin A – twin B, regardless of size), which does not incorporate the size of the larger twin to create a relative measure. Thus, larger differences in term births as compared to preterm births are expected due to increasing placental and fetal size across gestation.[118, 119]

The differences in the magnitude of the associations between male pairs and female pairs for placental thickness and surface area provides support for the hypothesized differences in male and female placental development.[57, 59, 121] We found that among same-sex male twins, differences in placental thickness and surface area were positively associated with a difference in

birthweight; however, among same-sex female twins, the associations were attenuated. These differences indicate that growth of males may be more dependent on placental size than growth of females, which is consistent with data suggesting that placentas of males have less reserve capacity or fewer adaptive changes to placental limitations.[59, 121]

It is unclear whether our results can be generalized to singleton pregnancies given differences in fetal growth between singleton and twin pregnancies.[124] To evaluate this, we examined the associations between features of placental morphology and birthweight among all twins using marginal models to account for correlation within a twin pair (Table S3). Our results are within the range of reported results for singleton pregnancies[71, 74], which is consistent with the similarities in the individual fetal-placental relationship between singleton and dichorionic twin pregnancies.[21] These similar relationships suggest that our findings, particularly the sex-specific differences, may be relevant to singleton pregnancies. Further, the use of twins provides improved control of confounding related to the type and timing of insults that potentially affect placental and fetal growth.

A limitation of our analysis is the relatively small sample size of 208 sets of dichorionic twins with unfused placentas. When stratified by gestational age group or sex composition, we may have been unable to detect small differences. Low variability in the features of placental morphology within a twin pair is also a limitation of our analysis. Low variability is of particular concern for placental thickness since 58.9% of twin pairs had no difference in placental thickness. Low variation within a twin pair may explain the observed null association between difference in placental thickness and difference in birthweight in the total sample.

Our results may also be affected by residual confounding. We were unable to control for zygosity as a potential confounder due to the small number of twins identified as monozygotic.

Additionally, we were underpowered to evaluate associations in monozygotic twins, which would allow for near perfect control of confounding by genetics. However, apart from the distribution of sex discordance, there were no significant differences in maternal or pregnancy characteristics between monozygotic and dizygotic twins (Table S4.4). While confounding by zygosity is a potential limitation, confounding by other variables shared within a twin pair, such as temporal trends and pregnancy characteristics, is not a concern with this analysis.

Strengths of this analysis include the use of dichorionic twins. This allowed for efficient control of all shared factors within a twin pair, including gestational age and the intrauterine environment, and improved the ability to isolate specific contributions of placental size to birthweight. Analyzing differences within a twin pair also facilitated the use of data from three completed studies, as differences in the characteristics of the study populations and the quality of the measures across the three studies were controlled for by design. Similarly, characteristics of gross placental morphology of interest, and the methods used to evaluate these characteristics, have remained relatively consistent over time. Additionally, evaluating associations within same-sex twin pairs allowed for improved estimation of sex-specific differences in the relationships between features of placental morphology and birthweight.

Overall, our results suggest that placental surface area, and potentially placental thickness, are positively associated with birthweight, and that these relationships may differ by gestational age or sex. Our findings in dichorionic twins may provide insight into the physiology of the relationship between placental development and fetal growth. Specifically, surface area may reflect the number of spiral arteries supplying the placenta and contributes to the area for exchange of oxygen, nutrients, and waste.[55, 107] Further, the placenta is adaptive and may be able to expand the surface area to promote growth.[55] Future studies should evaluate longitudinal

measures of placental development and fetal growth to better understand these relationships and evaluate the timing of critical aspects of placental development.

FIGURES AND TABLES

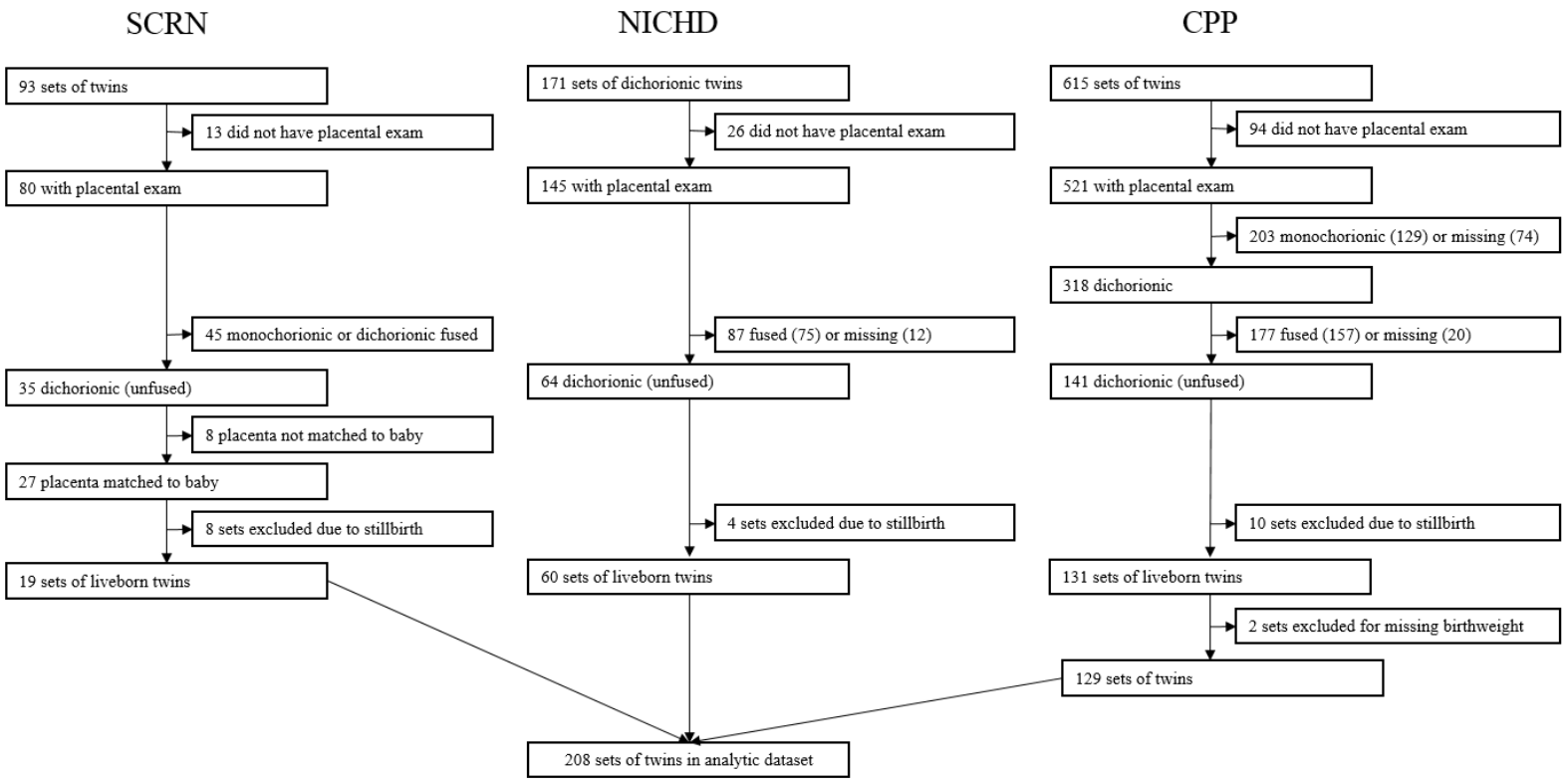

Figure 4.1. Study enrollment and inclusion.

Abbreviations: SCRN – Stillbirth Collaborative Research Network Study; NICHD – *Eunice Kennedy Shriver* National Institute of Child Health and Human Development study; CPP – Collaborative Perinatal Project

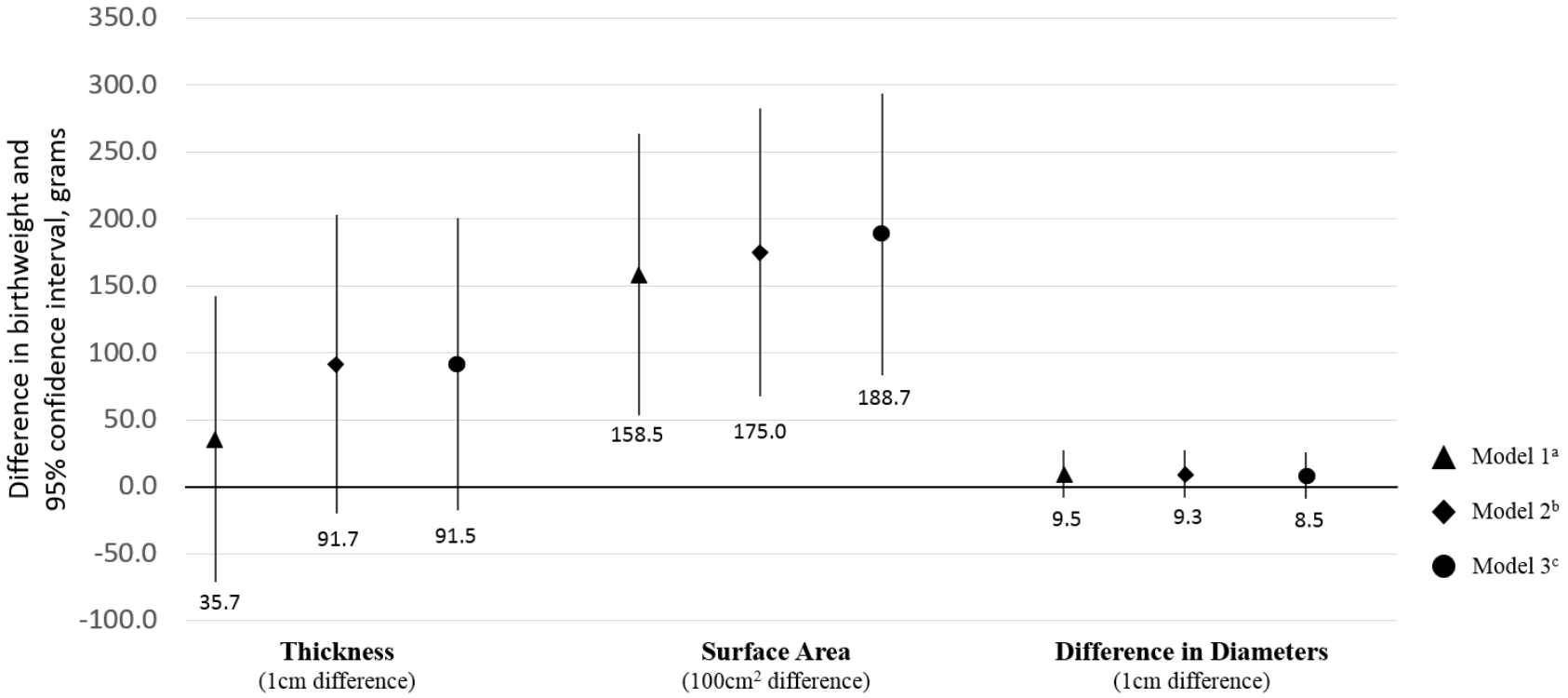

Figure 4.2. Difference in birthweight as a function of difference in the placental characteristics of interest within a twin pair (N=208).

[a] Model 1 contains each placental variable modeled individually (three separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains all placental variables and sex discordance

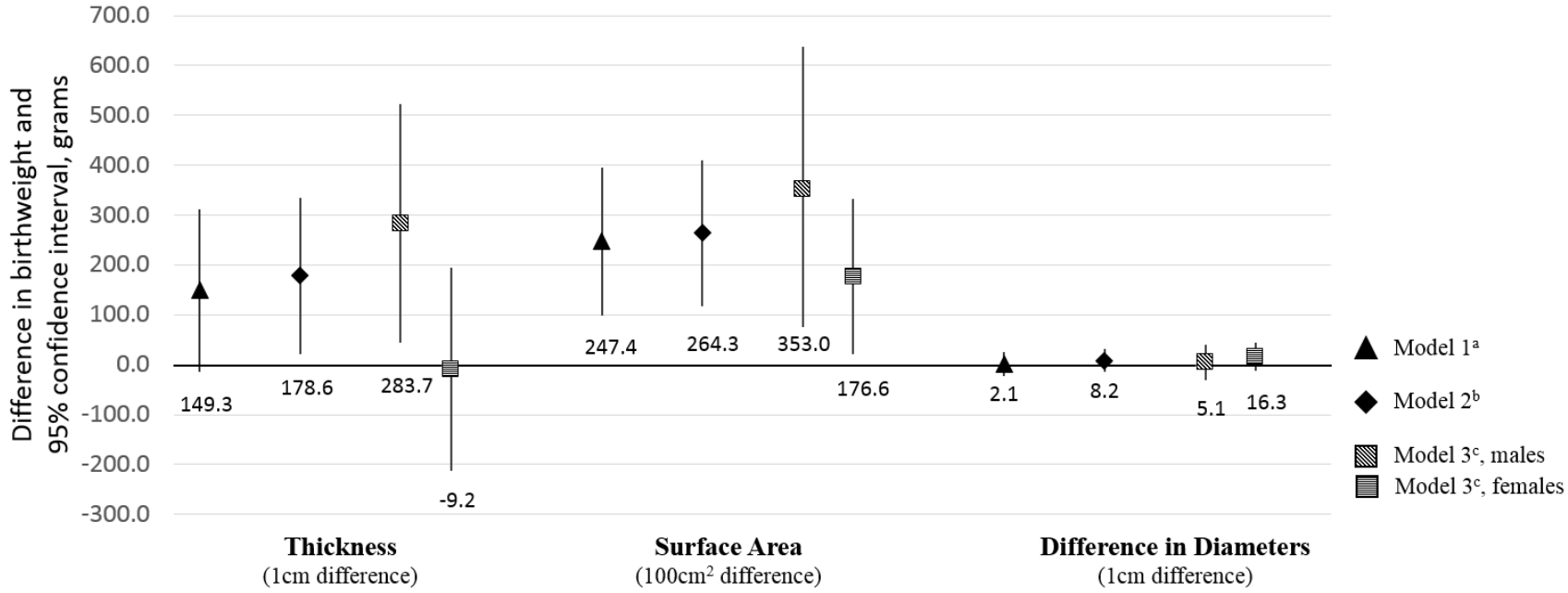

Figure 4.3. Difference in birthweight as a function of difference in the placental characteristics of interest within same-sex twin pairs (N=111).

[a] Model 1 contains each placental variable modeled individually (three separate models)
[b] Model 2 contains all placental variables modeled together
[c] Model 3 contains all placental variables, stratified by sex (same-sex male or same-sex female)

Table 4.1. Descriptive Characteristics for the Total Sample and Stratified by Study (N=208).

Characteristic N (%) or mean (SE)	Overall (N=208)	NICHD (N=60)	SCRN (N=19)	CPP (N=129)	*P*-value[a]
Sex					0.19
Male/Male	54 (26.0)	18 (30.0)	9 (47.4)	27 (20.9)	
Female/Female	57 (27.4)	16 (26.7)	3 (15.8)	38 (29.5)	
Male/Female	97 (46.6)	26 (43.3)	7 (36.8)	64 (49.6)	
Zygosity[b]					0.05
Monozygous	18 (9.5)	8 (13.3)		10 (7.8)	
Dizygous	151 (79.9)	50 (83.3)		101 (78.3)	
Unknown	20 (10.6)	2 (3.3)		18 (13.9)	
Gestational age					<0.01
<28 weeks	13 (6.3)	4 (6.7)	6 (31.6)	3 (2.3)	
28-31 weeks	17 (8.2)	2 (3.3)	5 (26.3)	10 (7.8)	
32-36 weeks	62 (29.8)	21 (35.0)	1 (5.3)	40 (31.0)	
≥37 weeks	116 (55.8)	33 (55.0)	7 (36.8)	76 (58.9)	
Maternal age					<0.01
<20 years	20 (9.6)	2 (3.3)	1 (5.3)	17 (13.2)	
20-34 years	148 (71.2)	37 (61.7)	12 (63.2)	99 (76.7)	
35-39 years	28 (13.5)	11 (18.3)	6 (31.6)	11 (8.5)	
40+ years	12 (5.8)	10 (16.7)	0 (0.0)	2 (1.6)	
Education					<0.01
≤ 11 years	78 (37.9)	3 (5.0)	3 (16.7)	72 (56.3)	
12 years (or GED)	46 (22.3)	5 (8.3)	3 (16.7)	38 (29.7)	
13+ years	82 (39.8)	52 (86.7)	12 (66.7)	18 (14.1)	
Maternal race/ethnicity					<0.01
White	102 (49.0)	31 (51.7)	14 (73.7)	57 (44.2)	
Black	85 (40.9)	13 (21.7)	3 (15.8)	69 (53.5)	
Hispanic	17 (8.2)	14 (23.3)	1 (5.3)	2 (1.6)	
Other	4 (1.9)	2 (3.3)	1 (5.3)	1 (0.8)	
Maternal smoking[c]					<0.01
0 cigarettes	137 (66.5)	53 (88.3)	14 (73.7)	70 (55.1)	
1-9 cigarettes	31 (15.1)	4 (6.7)	2 (10.5)	25 (19.7)	
10+ cigarettes	38 (18.5)	3 (5.0)	3 (15.8)	32 (25.2)	
Pre-pregnancy BMI					0.20
<18.5	9 (4.6)	3 (5.0)	0 (0.0)	6 (5.1)	
18.5-24.9	97 (49.2)	23 (38.3)	10 (52.6)	64 (54.2)	
25.0-29.9	56 (28.4)	16 (26.7)	6 (31.6)	34 (28.8)	
30.0-34.9	21 (10.7)	10 (16.7)	2 (10.5)	9 (7.6)	
35.0+	14 (7.1)	8 (13.3)	1 (5.3)	5 (4.2)	
Parity					<0.01
Primiparous	62 (30.0)	39 (65.0)	4 (21.0)	19 (14.8)	

Multiparous	145 (70.0)	21 (35.0)	15 (79.0)	109 (85.2)	
Individual Neonatal Characteristics (N=416)					
Birthweight, grams	2,353 (46)	2,465 (84)	1,838 (221)	2,376 (52)	0.02
Placental weight, grams	337.8 (6.7)	347.9 (13.8)	314.5 (35.0)	336.8 (7.0)	0.60
Thickness, cm	2.0 (0.0)	2.2 (0.1)	2.1 (0.2)	1.9 (0.0)	<0.01
Surface area, cm^2	214.3 (3.4)	218.3 (7.4)	200.8 (16.7)	214.4 (3.6)	0.61
Difference in Diameters, cm	3.8 (0.1)	3.6 (0.2)	5.8 (0.7)	3.6 (0.2)	<0.01

Abbreviations: BMI – Body Mass Index; CPP – Collaborative Perinatal Project; NICHD– *Eunice Kennedy Shriver* National Institute of Child Health and Human Development study; SCRN – Stillbirth Collaborative Research Network Study; SE – Standard error

[a] *P*-value from Chi-square test, Fisher's exact test, or F-test, two sided
[b] Zygosity was not evaluated in the SCRN, *P*-value compares NICHD and CPP, excluding those with unknown zygosity
[c] Average number of cigarettes per day prior to pregnancy

Table 4.2. Descriptive Characteristics for Placental Variables and Birthweight for the Total Sample and Stratified by Study (N=208).

Characteristic Mean (SE) or N (%)	Overall (N=208)	NICHD (N=60)	SCRN (N=19)	CPP (N=129)	*P*-value[a]
Analytic Differences (Twin A – Twin B)					
Difference in birthweight, grams	0.4 (27.2)	-18.2 (37.9)	27.8 (62.9)	5.1 (39.1)	0.88
Difference in placental weight, grams	7.5 (5.5)	19.1 (9.3)	-2.6 (14.3)	3.7 (7.4)	0.39
Difference in thickness, cm	0.1 (0.0)	-0.1 (0.1)	0.1 (0.2)	0.1 (0.0)	0.02
Difference in surface area, cm^2	0.0 (3.6)	10.6 (6.7)	3.4 (11.7)	-5.1 (4.6)	0.15
Difference in difference in diameters, cm	0.3 (0.2)	0.0 (0.4)	1.2 (1.0)	0.3 (0.3)	0.36
Absolute Differences (Larger twin – Smaller twin)					
Difference in birthweight, grams	282.5 (18.8)	221.9 (24.7)	185.2 (45.8)	325.0 (26.6)	0.01
Difference in placental weight, grams	56.7 (3.8)	53.9 (6.5)	42.3 (10.2)	60.1 (5.1)	0.36
Difference in thickness, cm	0.3 (0.0)	0.3 (0.1)	0.4 (0.1)	0.3 (0.0)	0.34
Difference in surface area, cm^2	40.5 (2.2)	39.9 (4.2)	33.0 (8.5)	41.9 (2.6)	0.51
Difference in difference in diameters, cm	2.4 (0.1)	2.5 (0.2)	3.2 (0.7)	2.2 (0.2)	0.10

Abbreviations: CPP – Collaborative Perinatal Project; NICHD– *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Study; SCRN – Stillbirth Collaborative Research Network Study; SE – standard error

[a] *P*-value from F-test or Fisher's exact test, two sided

SUPPLEMENTAL MATERIAL

Table S4.1. Difference in Birthweight in Grams as a Function of Difference in Placental Characteristics Within a Twin Pair for the Total Sample (N=208) and Stratified by Gestational Age.

	Thickness[a]		Surface Area[b]		Difference in Diameters[c]	
	β	95% CI	β	95% CI	β	95% CI
Total sample						
Model 1[d]	35.7	-71.1, 142.5	158.5	53.1, 263.9	9.5	-8.4, 27.4
Model 2[e]	91.7	-19.7, 203.1	175.0	67.1, 282.9	9.3	-8.4, 26.9
Model 3[f]	91.5	-17.6, 200.5	188.7	83.5, 293.9	8.5	-8.8, 25.8
By gestational age group						
Model 1[d]						
<32 weeks	50.6	-78.3, 179.5	57.7	-94.3, 209.8	-7.8	-27.2, 11.6
32-36 weeks	-63.0	-202.7, 76.7	66.5	-76.1, 209.0	1.7	-25.2, 28.5
>36 weeks	82.5	-85.0, 249.9	212.1	50.9, 373.3	15.3	-11.9, 42.5
Model 2[e]						
<32 weeks	63.0	-70.1, 196.1	60.7	-93.1, 214.5	-10.5	-30.6, 9.6
32-36 weeks	-42.8	-199.2, 113.6	48.6	-111.1, 208.4	1.9	-25.2, 29.0
>36 weeks	167.7	-10.6, 346.1	226.4	64.2, 388.7	16.6	-10.3, 43.6
Model 3[f]						
<32 weeks	42.6	-93.8, 178.9	101.2	-59.8, 262.2	-8.4	-28.1, 11.2
32-36 weeks	-7.7	-169.1, 153.7	45.5	-115.5, 206.6	0.0	-27.0, 27.1
>36 weeks	124.7	-52.1, 301.5	237.0	77.5, 396.4	13.4	-13.0, 40.1

Abbreviations: CI – confidence interval; TA – Twin A; TB – Twin B

[a] Estimate reflects a 1 cm difference in thickness within a twin pair

[b] Estimate reflects a 100 cm^2 difference in surface area within a twin pair

[c] Estimate reflects a 1 cm difference in the difference in diameters within a twin pair

[d] Model 1 contains each placental variable modeled individually (three separate models)

[e] Model 2 contains all placental variables modeled together

[f] Model 3 contains all placental variables and sex discordance

Table S4.2. Difference in Birthweight in Grams as a Function of Difference in Placental Characteristics Within a Twin Pair Restricted to Same-Sex Twin Pairs (N=111) and Stratified by Sex.

	Thickness[a]		**Surface Area[b]**		**Difference in Diameters[c]**	
	β	95% CI	β	95% CI	β	95% CI
Same-sex twin pairs (N=111)						
Model 1[d]	149.3	-13.6, 312.2	247.4	99.6, 395.2	2.1	-21.7, 28.9
Model 2[e]	178.6	21.6, 335.6	264.3	117.7, 410.8	8.2	-14.3, 30.8
Same-sex male pairs (N=54)						
Model 1[d]	284.3	38.0, 530.6	356.6	76.1, 637.1	-6.9	-44.7, 30.9
Model 2[e]	283.7	44.0, 523.3	353.0	80.7, 625.2	5.1	-30.1, 40.2
Same-sex female pairs (N=57)						
Model 1[d]	-57.0	-264.5, 150.5	173.8	22.0, 325.7	14.9	-14.1, 43.8
Model 2[e]	-9.2	-213.0, 194.7	176.6	20.8, 332.4	16.3	-11.9, 44.5

Abbreviations: CI – confidence interval

[a] Estimate reflects a 1 cm difference in thickness within a twin pair

[b] Estimate reflects a 100 cm^2 difference in surface area within a twin pair

[c] Estimate reflects a 1 cm difference in the difference in diameters within a twin pair

[d] Model 1 contains each placental variable modeled individually (three separate models)

[e] Model 2 contains all placental variables modeled together

Table S4.3. Change in Birthweight in Grams as a Function of Change in Placental Characteristics of Interest using Weighted[a] Marginal Models to Account for Correlation Within a Twin Pair (N=416).

	Thickness[b]		**Surface Area[c]**		**Difference in Diameters[d]**	
	β	95% CI	β	95% CI	β	95% CI
Model 1[e]	108.1	11.4, 204.9	323.6	231.7, 415.5	12.6	-2.32, 27.5
Model 2[f]	194.9	107.6, 282.2	372.9	275.0, 470.9	11.9	-2.8, 26.7
Model 3[g]	101.9	29.1, 174.8	226.2	138.5, 313.8	10.5	-2.5, 23.5
Model 4[h] - Males	100.4	29.1, 171.7	233.4	148.7, 318.1	10.6	-2.8, 24.0
Model 4[h] - Females	67.2	-25.4, 159.7	173.3	88.7, 257.8	4.6	-12.9, 22.0

Abbreviations: CI – confidence interval

[a] In the SCRN Study, births <32 weeks' gestation were oversampled. To account for this key difference in study design, we applied weights to make the gestational age distribution of the SCRN sample reflect that of the NICHD sample (10% <32 weeks' gestation). The weights were scaled such that the sum of the weights reflected the 19 births in the SCRN Study. All births in the CPP and NICHD studies were given a weight of one.

[b] Estimate reflects a 1 cm increase in thickness

[c] Estimate reflects a 100 cm^2 increase in surface area

[d] Estimate reflects a 1 cm increase in the difference in diameters

[e] Model 1 contains each placental variable modeled individually (three separate models)

[f] Model 2 contains all placental variables modeled together

[g] Model 3 contains all placental variables and covariates (maternal age, race/ethnicity, smoking, height, weight, parity, gestational age, sex, sex discordance, and study)

[h] Model 4 contains all placental variables and covariates (see Model 3) plus interaction between sex and placental characteristics

Table S4.4. Descriptive Characteristics Stratified by Monozygotic and Dizygotic for the Twin Pairs With Known Zygosity (N=169) from the CPP and NICHD Studies.

Characteristic N (%)	Monozygotic (N=18)	Dizygotic (N=151)	*P*-value[a]
Sex			<0.01
Male/Male	7 (38.9)	30 (19.9)	
Female/Female	11 (61.1)	31 (20.5)	
Male/Female	0 (0.0)	90 (59.6)	
Gestational age			0.05
<28 weeks	3 (16.7)	4 (2.7)	
28-31 weeks	0 (0.0)	8 (5.3)	
32-36 weeks	7 (38.9)	49 (32.4)	
≥37 weeks	8 (44.4)	90 (59.6)	
Maternal age			
<20 years	3 (16.7)	15 (9.9)	0.72
20-34 years	13 (72.2)	106 (70.2)	
35-39 years	1 (5.6)	19 (12.6)	
40+ years	1 (5.6)	11 (7.3)	
Education			0.18
≤ 11 years	9 (52.9)	57 (37.8)	
12 years (or GED)	1 (5.9)	38 (25.2)	
13+ years	7 (41.2)	56 (37.1)	
Maternal race/ethnicity			0.49
White	6 (33.3)	70 (46.4)	
Black	9 (50.0)	65 (43.0)	
Hispanic	3 (16.7)	13 (8.6)	
Other	0 (0.0)	3 (2.0)	
Smoking			0.87
0 cigarettes	12 (66.7)	98 (65.8)	
1-9 cigarettes	2 (11.1)	24 (16.1)	
10+ cigarettes	4 (22.2)	27 (18.1)	
Pre-pregnancy BMI			0.17
<18.5	0 (0.0)	9 (6.3)	
18.5-24.9	11 (61.1)	66 (30.1)	
25.0-29.9	2 (11.1)	43 (30.1)	
30.0-34.9	2 (11.1)	16 (11.2)	
35.0+	3 (16.7)	9 (6.3)	
Parity			0.23
Primiparous	8 (44.4)	46 (30.5)	
Multiparous	10 (55.6)	105 (69.5)	

Abbreviations: BMI – Body Mass Index

[a]*P*-value comparing monozygotic and dizygotic twins, from chi-square or Fisher's exact test, two sided

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CHAPTER 5: ASSOCIATIONS BETWEEN PLACENTAL MORPHOLOGY AND COGNITIVE ASSESSMENTS IN SINGLETONS AND DICHORIONIC TWINS

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ABSTRACT

Background: Poor placental function may result in growth restriction, which is associated with adverse cognitive outcomes. The purpose of our study was to estimate associations between placental morphology and intelligence quotient (IQ) in children.

Methods: We estimated associations separately in singletons and dichorionic twins using linear regression. Placental and IQ data on 514 singletons were obtained from the Alabama Fetal Growth Study, conducted in 1986-1988. Data on 82 sets of dichorionic twins were obtained from the Collaborative Perinatal Project, conducted in 1959-1966. Placental variables included thickness, surface area, difference in diameters, and abnormal umbilical cord insertion. The Wechsler Preschool and Primary Scale of Intelligence-Revised was administered to singletons at age five and the Wechsler Intelligence Scale for Children was administered to twins at age seven. Both assessments provide measures of full, verbal, and performance IQ.

Results: There were no statistically significant associations between measures of placental morphology and IQ. However, magnitudes of the associations diverged between males and females. Among males, a 1 cm increase in thickness was associated with a 4.8-point increase in IQ (95% confidence interval [CI]: -0.4, 10.0), whereas the same association among females was -1.4 (95% CI: -6.1, 3.3). Similar relationships were observed among same-sex twins.

Conclusions: Features of placental morphology were not statistically significantly associated with IQ. Sex-specific differences in the magnitudes of the associations in both singletons and twins are consistent with reported differences in placental development. Our results support the hypothesis that placentas of males may have less reserve capacity than placentas of females.

INTRODUCTION

The fetal period is a critical time for brain development.[53] Studies have reported that low birthweight is associated with reduced intelligence quotient (IQ) in childhood and early adulthood.[129, 130] One study utilizing monozygotic twins, which allows for efficient control of shared confounders, found that the heavier twin has a verbal IQ that averages half a standard deviation higher than the lighter twin.[131] Similarly, fetal growth restriction is associated with reduced cognitive ability and worse behavioral outcomes.[132-134] Further, effects may differ between males and females. Although not well understood, males are thought to be more vulnerable to insults, supported by higher rates of morbidity and mortality among males as compared to females.[135]

Altered brain development in utero may be affected by placental development and function, given the critical role of the placenta in regulating the growth and development of the fetus.[6] Features of placental morphology have been associated with both IQ and mental health outcomes in childhood.[50, 51] One proposed mechanism for adverse cognitive outcomes is disruption in placental production of neuropeptides, which are important for fetal brain development.[52] Additionally, the brain has a high rate of growth during late gestation, which makes it particularly sensitive to inadequate oxygen and nutrient supply.[53] Another mechanism suggests that vascular endothelial growth factor regulates angiogenesis in both the placenta and the developing brain, and may additionally have neurotrophic effects.[54] Thus measures of placental morphology that are dependent on angiogenesis, such as thickness and surface area, may serve as indicators for angiogenesis in the brain.[50, 136] Further, there may be sex-specific differences in placental development and function, including differences in levels of vascular endothelial growth factor.[56, 59]

Two studies have evaluated features of placental morphology in relation to cognitive outcomes in childhood, both of which are based on data from the Collaborative Perinatal Project, a cohort study conducted from 1959-1966.[50, 67] Niswander and Gordon (1972) reported that placental lesions are associated with neurologic abnormalities at one year of age.[67] However, this study did not adjust for gestational age and did not consider sex-specific differences in placental or fetal development. Additionally, the authors used a broad definition of neurologic abnormality. Misra et al. (2012) reported a positive association between placental thickness and higher IQ at age seven in models adjusted for socioeconomic status, race, parity, gestational age, and age at IQ evaluation.[50] The authors also found positive associations between both longest and shortest diameters and higher IQ in females but not in males. The authors did not control for maternal smoking and body size, which may confound the association between placental morphology and cognitive outcomes.[137, 138] Further study of this relationship is necessary using analytic techniques to adjust for important confounders.

The purpose of our study was to evaluate the associations between placental morphology and measures of cognitive development in children while accounting for key confounders. We evaluated associations in both singletons and dichorionic twins. Dichorionic twins have been suggested as a unique opportunity investigate the developmental programming of health outcomes due to their shared confounders, including maternal and pregnancy characteristics.[21, 120] Further, we investigated reported sex-specific differences in placental function by evaluating interaction between the features of placental morphology and sex.[59] We hypothesized that features of placental morphology would be associated with cognitive development, and that these associations would differ by sex.

METHODS

Study Sample - Singletons

The Alabama Fetal Growth (AFG) Study was a population-based cohort study that enrolled women in Alabama between January 1986 and March 1988.[139] The purpose of the study was to evaluate risk factors associated with being small for gestational age (SGA), as well as assessing adverse childhood outcomes associated with being SGA. A sample of para 1 and para 2 pregnant women who were <26 weeks' gestation were recruited when presenting for prenatal care at the Jefferson County Health Department (n=1,593). Additionally, 323 women were invited to participate at the hospital following delivery for a total sample size of 1,916. Of these women, 1,254 were selected to participate in one and five-year follow-up studies (Figure 5.1). All women delivering live born infants identified as SGA (n=199) or <34 week's gestation (n=44) or twins (n=54) or part of the prenatal random sample (n=296) or enrolled at the hospital (n=323) were selected for follow-up. Additionally, a random sample of the control sample was selected for follow-up (n=338). Although the 323 women enrolled at the hospital were selected to participate in the follow-up study, they were not included in this analysis because they did not have placental examinations. We also excluded twins, which resulted in 877 participants eligible for inclusion. Our analysis was restricted to the 514 women with a complete placental examination who also completed the five-year follow-up study. All women provided written informed consent and the study was approved by the appropriate Institutional Review Board. Additional details of the study design and recruitment strategy have been published.[139-141]

Study Sample – Twins

We utilized dichorionic twin pregnancies with unfused placentas from the Collaborative Perinatal Project (CPP). The CPP was a longitudinal cohort study that enrolled women from

1959-1966 at twelve study sites in the U.S. Children were followed through age seven. The purpose of the study was to evaluate pregnancy exposures and their relationship to adverse perinatal outcomes, including cerebral palsy and other developmental disabilities.[67] Details of the study design have been published.[67] Of the 615 sets of twins enrolled in the CPP, 521 had a complete placental examination. Of these, 318 were identified as dichorionic and 141 were dichorionic with unfused placentas. Twins with fused placentas (157 fused, 20 missing information) were excluded because they may have lower birthweights as a result of placental crowding leading to restricted placental growth, rather than as a result of intrinsic individual placental development.[23] Our analysis included the 82 sets of twins with complete seven-year follow-up data (Figure 5.2).

Placental Measures

Information on maternal, pregnancy, and neonatal characteristics were obtained from maternal interview and medical chart abstraction. The features of placental morphology of interest were obtained from the placental examination, conducted as part of the AFG Study and the CPP. Placental variables considered in this analysis included thickness, surface area, difference in diameters, and abnormal umbilical cord insertion. Protocols for the studies, particularly for the variables of interest, were similar.

Thickness - Measured to the nearest 0.5 cm.

Surface area – Determined based on the formula for the area of an ellipse using the recorded maximum (a) and minimum (b) diameters (area = $ab\pi/4$).

Difference in Diameters – Measured by subtracting the minimum diameter from the maximum diameter. Differences of zero indicate circular placentas while larger differences indicate increasingly oval placentas.

Umbilical Cord Insertion - Pathologists measured the distance from the insertion site to the nearest placental edge and recorded if the insertion was membranous. Umbilical cords with a measured distance of 0 cm and those considered membranous were classified as abnormal. All other umbilical cords were classified as normal insertions. This variable was not included in the analysis of dichorionic twins.

Cognitive Assessments

Both studies used variations of the same cognitive assessment designed to evaluate intelligence. The AFG Study used the Wechsler Preschool and Primary Scale of Intelligence – Revised (WPPSI-R), administered to children at five years of age. The CPP used the Wechsler Intelligence Scale for Children (WISC), administered at seven years of age. The assessments consist of subscales categorized as verbal or performance-based and yield a global measure of full IQ as well as measures of verbal IQ and performance IQ. These assessments are reliable and internally valid, and are normed to have a mean of 100 and a standard deviation of 15.[142, 143]

Statistical Analysis - Singletons

We used multiple imputation to account for missing covariates and stabilized inverse probability weights to account for missing placental examinations and loss to follow-up.[144] First, we imputed missing covariates using ten imputations to ensure that weights could be calculated for all observations. Imputed covariates included smoking status (4.9% missing), marital status (4.7% missing), maternal education (4.1% missing), maternal body mass index (BMI; 1.8% missing), and maternal alcohol use (0.8% missing). Next, we evaluated factors potentially related to availability of the placental examination and participation in the five-year follow-up, including time of day of delivery, weekend delivery, gestational age, parity, maternal age, race, maternal education, annual household income, marital status, BMI, maternal smoking, and maternal

alcohol use. These factors were evaluated separately within each group of the follow-up study (SGA sample, prenatal random sample, control random sample, <34 weeks' gestation sample). Among the SGA group, parity and marital status were related to inclusion; among the prenatal random sample, race, marital status, and maternal smoking were related to inclusion; and among the control random sample, race, marital status, maternal education, and maternal alcohol use were related to inclusion. There were no factors related to follow-up among the group of births <34 weeks' gestation. The stabilized inverse probability weights ensured that the distribution of covariates of the 514 women with placental data and five-year outcome data reflected the distribution of covariates of the 877 women who were eligible for inclusion in the analysis.

Prior to model building, we assessed placental variables and the measures of IQ for linear relationships. Following this, we conducted weighted linear regression using the imputed data to evaluate the associations of IQ with placental characteristics and covariates. Estimates from the ten imputations were combined using Rubin's Rule.[145] We controlled for gestational age (24-27 weeks, 28-31 weeks, 32-36 weeks, ≥37 weeks), parity (1, 2), maternal age (<20 years, 20-34 years, ≥35 years), race (African-American, Caucasian), maternal education (0-11 years, 12 years, ≥13 years), marital status (not married or cohabiting, cohabiting, married), maternal BMI (<18.5, 18.5-24.9, 25-29.9, 30-34.9, ≥35 kg/m^2), maternal smoking (0, <10, ≥10 cigarettes per day during the first trimester), maternal alcohol use (0, ≥1 drink during the first trimester), and sex. We also evaluated interaction between the features of placental morphology and sex. In general, we evaluated four models for each of the three IQ measures:

Model 1 – Each placental variable modeled individually.

Model 2 – All placental variables modeled together.

Model 3 – All placental variables and covariates, as previously described.

Model 4 – All placental variables and covariates, plus interaction between placental variables and sex.

For these models, estimates of association reflect a 1 cm increase in thickness, a 100 cm^2 increase in surface area, and a 1 cm increase in difference in diameters.

Statistical Analysis – Twins

To evaluate associations within dichorionic twin pairs, we used linear regression to model the difference in the cognitive assessment within the twin pair as a function of their differences in placental characteristics. We determined differences by subtracting the second born twin (twin B) from the first born twin (twin A). This resulted in normally distributed variables with expected mean differences of zero. By constraining the intercept to pass through the origin, the results were robust to the method of subtraction.[128] This method inherently controls for all shared confounders, such as gestational age and parental IQ. We considered sex discordance as a potential confounder. We also conducted a secondary analysis restricted to same-sex pairs to evaluate differences in the associations by sex. In general, we evaluated three models for each outcome measure:

Model 1 – Each difference in placental characteristic modeled individually with difference in the cognitive measure.

Model 2 – All differences in placental characteristics modeled together with difference in the cognitive measure.

Model 3 – All differences in placental characteristics modeled together with difference in the cognitive measure, controlling for sex discordance (this model was not evaluated in the secondary analysis restricted to same-sex pairs).

For these models, estimates of association reflect a 1 cm difference in thickness, 100 cm^2 difference in surface area, and a 1 cm difference in difference in diameters within a twin pair. In all analyses, a p-value of <0.05 was used to determine statistical significance. Analyses were performed using SAS version 9.4 (SAS Institute INC., Cary, North Carolina).

RESULTS

Descriptive Statistics - Singletons

In the AFG Study, 81.1% of children were born at term (≥37 weeks' gestation) and 51.5% were male (Table 5.1). Most mothers were between 20-34 years old (86.2%), identified as African-American (73.0%), had a BMI within the normal range (52.9% 18.5-24.9 kg/m^2), and did not smoke or drink alcohol in the first trimester (54.2% and 54.3%, respectively). 26.0% of mothers did not complete secondary school or obtain a GED and 71.2% of women had an annual household income <$10,000.

The average placenta weighed 524.8 grams, had a thickness of 2.1 cm, had a surface area of 244.7 cm^2, and had a difference in maximum and minimum diameters of 2.6 cm (Table 5.1). In 16.6% of deliveries, the umbilical cord was inserted abnormally (membranous and marginal insertions). Cognitive assessments were administered at an average age of 5.5 years. The mean measure of full IQ was 82.0 (standard deviation (SD): 12.3). Measures of verbal and performance IQ were similar (verbal: mean: 83.7, SD: 11.8; performance: mean: 84.1, SD: 13.6).

Descriptive Statistics – Twins

In the CPP, most mothers of twins had a term delivery (68.3%), were between 20-34 years old (76.8%), identified as Caucasian (51.2%), received <12 years of education (53.1%), had a BMI within the normal range (55.8%), and did not smoke in the first trimester (58.8%)

(Table 5.1). The average placenta weighed 351.4 grams, had a thickness of 1.9 cm, had a surface area of 217.7 cm^2, and a difference in diameters of 3.5 cm. The average absolute difference within a twin pair was 64.4 grams for placental weight, 0.3 cm for placental thickness, 43.5 cm^2 for surface area, and 2.2 cm for difference in diameters (Table S5.1). Cognitive assessments were administered at an average age of 7.1 years (Table 5.1). The average full IQ was 92.1, the average verbal IQ was 91.4, and the average performance IQ was 95.2.

Analytic Results – Singletons

There were no statistically significant associations between the features of placental morphology and the measures of IQ in models adjusted for covariates (Figure 5.3, Table S5.2). However, estimates of associations for thickness and umbilical cord insertion appeared to diverge for males and females, although this was not statistically significant. A 1 cm increase in thickness was associated with a 4.8-point increase in full IQ among males (95% CI: -0.4, 10.0) and a 1.4-point decrease among females (95% CI: -6.1, 3.3; p-value for interaction range in 10 imputations: 0.06, 0.14). This difference was more pronounced for performance IQ (males: 5.5, 95% CI: -0.8, 11.7; females: -1.2, 95% CI: -6.9, 4.5) as compared to verbal IQ (males: 3.4, 95% CI: -1.0, 7.8; females: -0.9, 95% CI: -4.9, 3.0; Figure S5.1, Figure S5.2, Table S5.2). Similarly, among males, abnormal umbilical cord insertion was associated with a 3.2-point decrease in full IQ as compared to normal umbilical cord insertion (95% CI: -6.7, 0.3); whereas the same association among females was 0.1 (95% CI: -3.5, 3.7; p-value for interaction range in 10 imputations: <0.01, 0.23; Figure 5.3, Table S5.2).

Analytic Results – Twins

Similar to the singleton results, there were no statistically significant associations between differences in features of placental morphology and difference in IQ within a twin pair (Table 5.2, Figure S5.3). In the sample restricted to same-sex twins, the estimates of associations appeared to diverge within male and female pairs (Table 5.2, Figure S5.4). Among male pairs, a difference in placental thickness of 1 cm was associated with a 10.9-point difference in full IQ (95% CI: -11.3, 33.0); the same association among female pairs was 0.5 (95% CI: -10.1, 11.0; p-value for interaction: 0.61). Similarly, a 100 cm^2 increase in surface area was associated with a -9.6-point difference in full IQ among males (95% CI: -24.0, 4.8) and a 2.4-point difference among females (95% CI: -7.1, 12.0; p-value for interaction: 0.36). The magnitudes of the associations for thickness and surface area among males were stronger for performance IQ as compared to verbal IQ (Table 5.2).

DISCUSSION

There were no statistically significant associations between the features of placental morphology and measures of IQ among either singletons or dichorionic twins. However, among singletons, the magnitudes of the associations for thickness and umbilical cord insertion were stronger among males as compared to females. Similarly, among same-sex twins, a difference in placental thickness demonstrated a stronger association within male pairs as compared to female pairs. In both singletons and twins, the magnitudes of the associations were also generally stronger for performance IQ as compared to verbal IQ. These results suggest that there may be sex-specific differences in associations between placental variables and cognitive outcomes, and that males may be more sensitive to placental development. This is consistent with studies suggesting that placentas of males have less reserve capacity than placentas of females.[59]

Misra et al. (2012) reported a positive association between placental thickness and higher IQ at age seven in both males and females, which is inconsistent with our results.[50] Our results of no association between surface area and IQ in both males and females are consistent with the results of Misra et al. (2012). However, the authors reported positive associations between both longest and shortest diameters and higher IQ in females but not in males. These differences may be due to differences in modeling strategy. Misra et al. (2012) stratified by sex and included socioeconomic status, race, parity, gestational age, and age at IQ assessment in adjusted models. Our null results may be due to adjustment for additional important covariates, such as maternal smoking and body size.

Our results may have limitations in their generalizability based on the selection criteria of the studies. The AFG Study oversampled women delivering growth-restricted infants and the mothers were predominantly low income and African-American. Additionally, the average IQ in the AFG Study was over one standard deviation below the normed mean of 100. Similarly, differences in growth patterns between singletons and dichorionic twins may limit the generalizability of the results from the twin sample.[124] Studies have also demonstrated that twins have lower IQs than singletons.[146, 147] However, the individual fetal-placental relationship may be similar between singletons and dichorionic twins.[21] Further, neonatal brain structure is similar in singletons and twins.[148] This is supported by the consistency of our results within singletons and twins.

Another limitation of our analysis was the relatively small sample size. We analyzed data on 514 singleton children from the AFG Study and 82 sets of dichorionic twins from the CPP. These sample sizes may not have been adequate to detect small differences, particularly differences in the relationships between males and females. This was especially true for the

analysis restricted to same-sex twins, which was based on 38 sets of twins. Due to the small sample size of twins, we were also unable to evaluate associations in monozygotic twins, which would allow for improved control of confounding due to shared genotype. Additionally, the small sample size may have contributed to the low variability in the features of placental morphology within a twin pair. This was particularly true for placental thickness; 56.3% of twins had no difference in placental thickness, which may explain our overall null findings.

Strengths of our analysis included the use of both singletons and dichorionic twins. Evaluating similar relationships in these samples allowed us to investigate the robustness of observed relationships. Additionally, we were able to control for important covariates that previous studies did not consider, including maternal smoking and body size. Dichorionic twins also allowed for improved control of shared covariates, including unknown or unmeasured confounders such as parental IQ. We also considered verbal and performance subsets of IQ using well-validated measures, which have not previously been evaluated in the context of placental morphology.[142, 143]

While our results are not suggestive of statistically significant associations between placental morphology and IQ, our results indicate that these relationships may meaningfully differ between males and females, and that performance IQ may be more sensitive to placental function than verbal IQ. This is consistent with studies reporting differences in both placental development and long-term health outcomes between males and females. Future research should utilize larger, modern, population-based cohorts to estimate these associations. In addition to evaluating full IQ, studies should also consider evaluating specific components of cognition to better identify potential mechanisms. A better understanding of the role of the placenta in

cognitive development, and sex-specific differences in this relationship, is important given the long-term implications and impact on quality of life.[62]

FIGURES AND TABLES

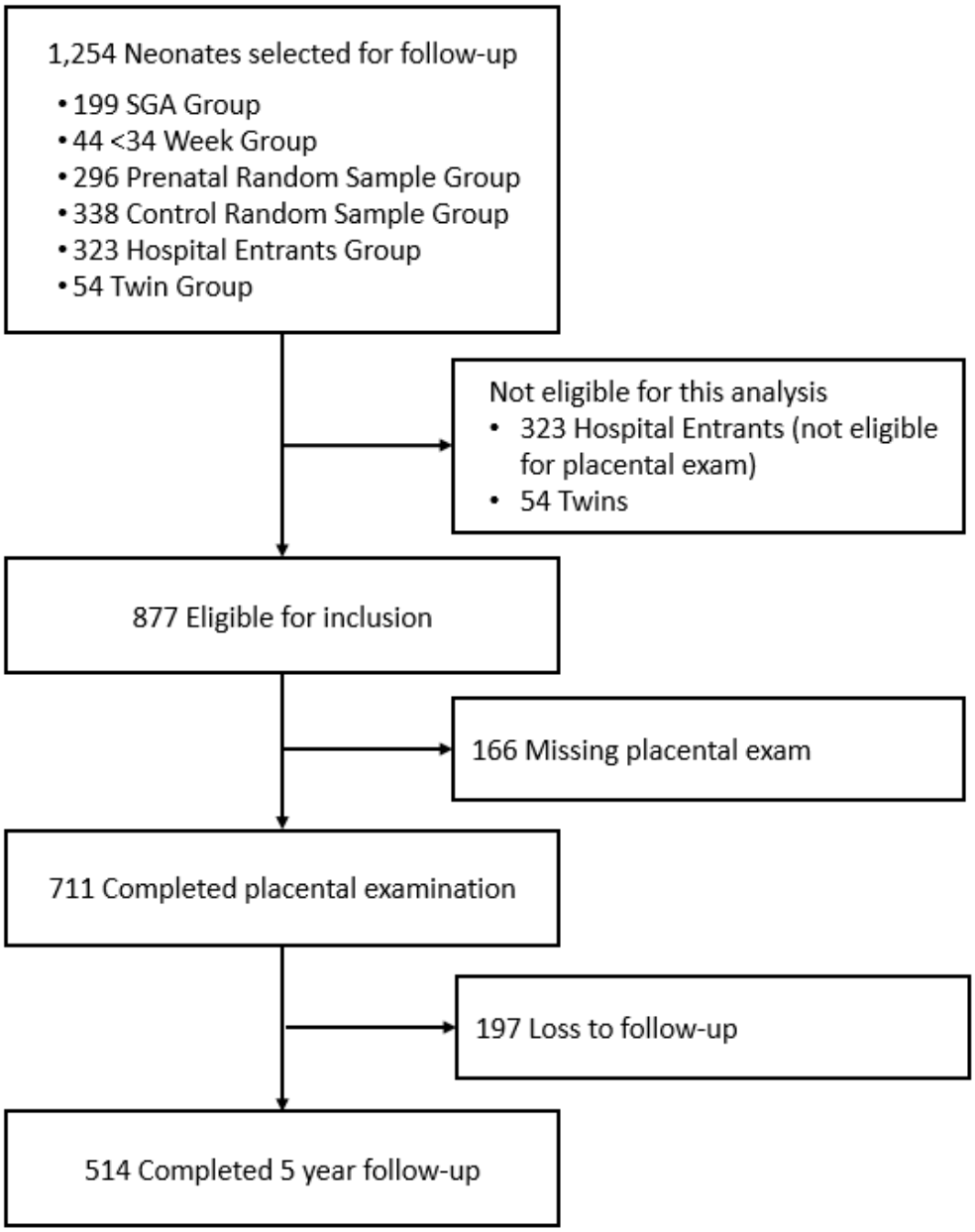

Figure 5.1. Enrollment and inclusion in the Alabama Fetal Growth Study.

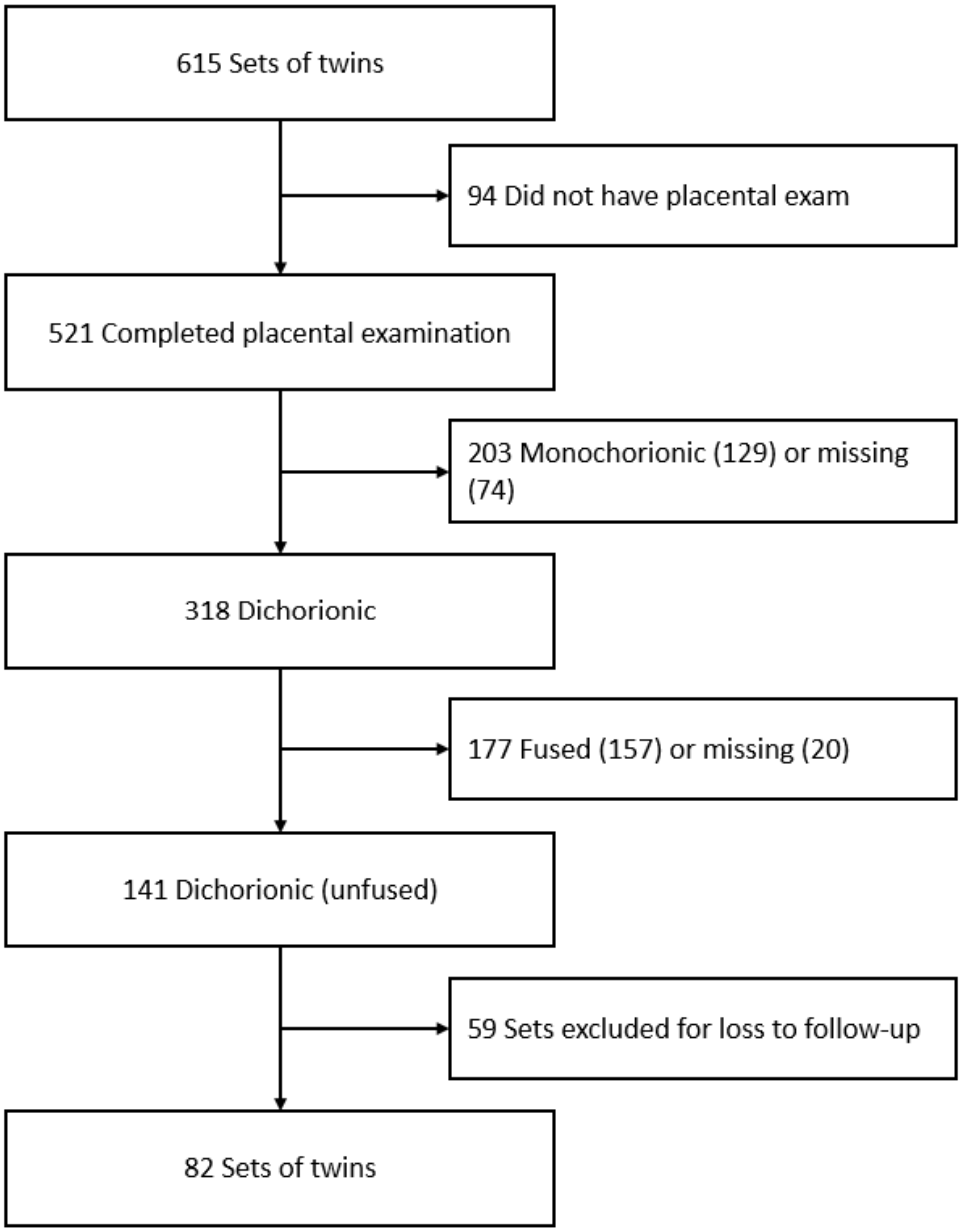

Figure 5.2. Enrollment and inclusion among twins in the Collaborative Perinatal Project.

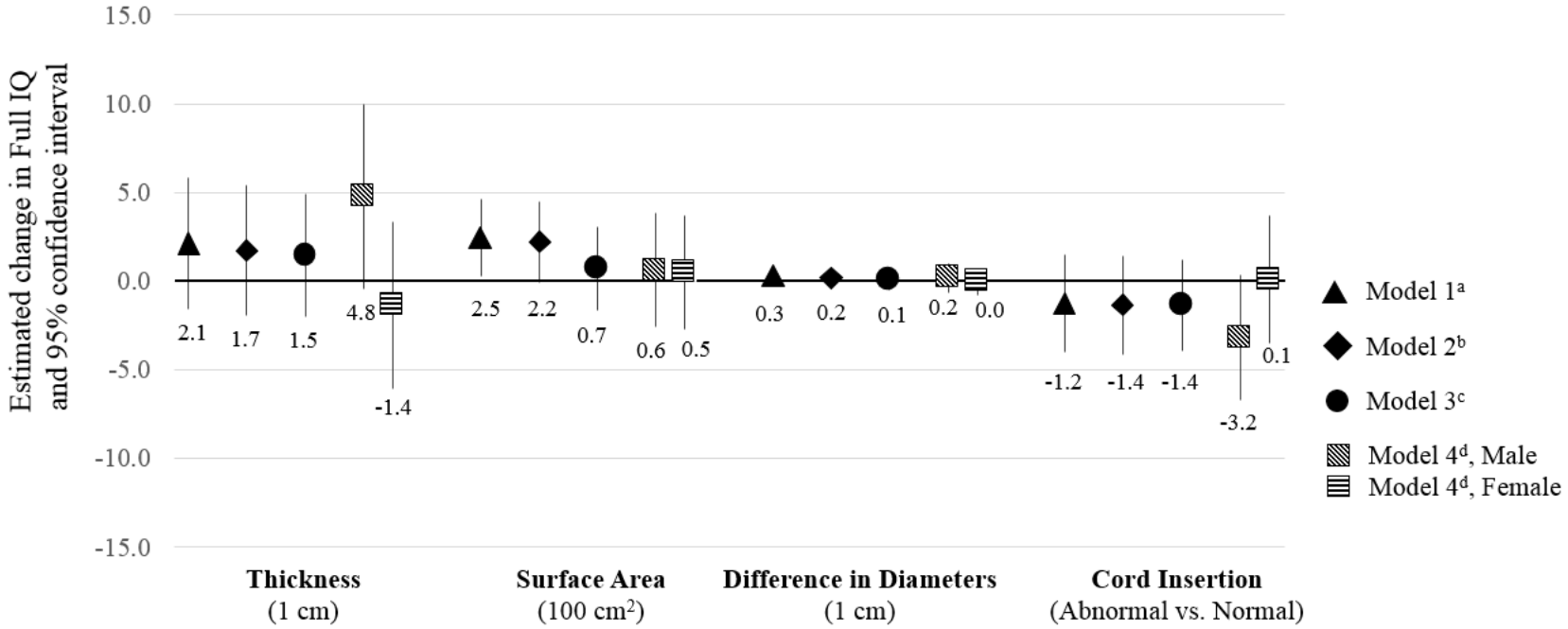

Figure 5.3. Associations between features of placental morphology and full IQ in the Alabama Fetal Growth Study (N=514).

[a] Model includes each placental variable modeled individually (four separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and covariates (sex, gestational age, parity, maternal age, race/ethnicity, maternal education, marital status, body mass index, smoking, alcohol use)
[d] Model includes all placental variables and covariates with interaction of placental variables and sex

Table 5.1. Descriptive characteristics of singletons from the Alabama Fetal Growth (AFG) Study (N=514) and dichorionic twins from the Collaborative Perinatal Project (CPP, N=82 sets).

Characteristic	AFG Study (Singletons) N (%) or Mean (SE)	CPP (Twins) N (%) or Mean (SE)
Maternal and Pregnancy Characteristics	**N=514**	**N=82 sets**
Gestational Age		
24-27 completed weeks	2 (0.4)	0 (0.0)
28-31 completed weeks	16 (3.1)	1 (1.2)
32-36 completed weeks	79 (15.4)	25 (30.5)
≥37 completed weeks	417 (81.1)	56 (68.3)
Parity[a]		
0	0 (0.0)	12 (14.8)
≥ 1	514 (100.0)	69 (85.2)
Maternal Age at Delivery, years		
<20	62 (12.1)	10 (12.2)
20 – 34	443 (86.2)	63 (76.8)
35 – 39	9 (1.7)	7 (8.5)
≥40	0 (0.0)	2 (2.5)
Maternal Race/Ethnicity		
Caucasian	139 (27.0)	42 (51.2)
African-American	375 (73.0)	39 (47.6)
Hispanic	0 (0.0)	1 (1.2)
Maternal Education		
0 – 11 (none/primary/some secondary)	128 (26.0)	43 (53.1)
12 (completed secondary or GED)	220 (44.6)	24 (29.6)
13+ (college)	145 (29.4)	14 (17.3)
Annual Household Income		
< $5,000	164 (37.0)	
$5,000 - $9,999	152 (34.2)	
$10,000 - $14,999	91 (20.5)	
≥ $15,000	37 (8.3)	
Marital Status		
Not married or cohabitating	244 (49.8)	
Cohabitating	32 (6.5)	
Married	214 (43.7)	
Maternal BMI[b], kg/m^2		
<18.5	81 (16.1)	3 (3.9)
18.5 – 24.9	267 (52.9)	43 (55.8)
25 – 29.9	94 (18.6)	21 (27.3)

30 – 34.9	31 (6.1)	6 (7.8)
≥35	32 (6.3)	4 (5.2)
Maternal height, cm	163.0 (0.3)	161.3 (0.8)
Maternal pre-pregnancy weight, kg	62.7 (0.7)	64.2 (1.5)
Maternal Smoking Status[c]		
Did not smoke	265 (54.2)	47 (58.8)
< 10	89 (18.2)	16 (20.0)
≥ 10	135 (27.6)	17 (21.2)
Alcohol Use[d]		
Did not drink	277 (54.3)	
≥ 1 drink	233 (45.7)	
Individual Child Characteristics	**N=514**	**N=164[e]**
Placental weight, grams	524.81 (6.94)	351.38 (6.96)
Maximum diameter, cm	18.89 (0.11)	18.43 (0.21)
Minimum diameter, cm	16.34 (0.09)	14.95 (0.18)
Thickness, cm	2.10 (0.01)	1.94 (0.05)
Surface area, cm^2	244.66 (2.43)	217.66 (4.20)
Difference in diameters, cm	2.57 (0.09)	3.45 (0.19)
Umbilical cord insertion, abnormal	84 (16.6)	6 (3.7)
Sex, male	264 (51.5)	76 (46.3)
Age at cognitive assessment, years	5.47 (0.42)	7.13 (0.08)
Full Scale IQ	82.04 (12.25)[f]	92.11 (1.63)
Verbal IQ	83.68 (11.78)[f]	91.36 (1.54)
Performance IQ	84.12 (13.58)[f]	95.15 (1.58)

Abbreviations: AFG – Alabama Fetal Growth; BMI – body mass index; CPP – Collaborative Perinatal Project; IQ – intelligence quotient; SE – standard error

[a] All women in AFG Study were para 1 (66.0%) or para 2 (34.0%)
[b] Pre-pregnancy BMI
[c] First trimester smoking status
[d] First trimester alcohol use
[e] Standard errors account for correlation within a twin pair using the Taylor series method
[f] Number in parentheses reflects the standard deviation rather than the standard error

Table 5.2. Associations between differences in features of placental morphology and difference in full, verbal, and performance IQ among dichorionic twins in the Collaborative Perinatal Project (N=82 sets) and restricted to same-sex dichorionic twins (N=38 sets).

	Thickness (1 cm difference)		**Surface area** (100 cm^2 difference)		**Difference in Diameters** (1 cm difference)	
	β	95% CI	β	95% CI	β	95% CI
Full IQ						
Model 1[a]	-2.9	-9.1, 3.8	0.3	-6.2, 6.8	0.9	-0.3, 2.0
Model 2[b]	-3.3	-10.8, 4.2	-0.6	-7.1, 6.0	0.8	-0.4, 2.0
Model 3[c]	-4.1	-12.1, 3.8	-0.5	-7.1, 6.2	0.7	-0.5, 1.9
Verbal IQ						
Model 1[a]	0.7	-5.1, 6.4	0.8	-4.9, 6.4	0.7	-0.3, 1.7
Model 2[b]	0.7	-5.8, 7.3	0.2	-5.6, 5.9	0.7	-0.3, 1.78
Model 3[c]	1.0	-5.6, 7.5	0.4	-5.4, 6.1	0.7	-0.3, 1.7
Performance IQ						
Model 1[a]	-4.5	-12.4, 3.3	3.0	-4.7, 10.7	0.7	-0.7, 2.1
Model 2[b]	-4.8	-13.7, 4.2	2.2	-5.7, 10.0	0.5	-0.9, 2.0
Model 3[c]	-6.3	-15.7, 3.0	2.1	-5.8, 10.1	0.4	-1.1, 1.8
Restricted to same-sex twins						
Full IQ						
Model 1[a]	-0.8	-9.3, 7.6	0.7	-6.1, 7.6	-0.9	-1.6, 1.4
Model 2[b]	-1.0	-9.9, 7.9	0.7	-6.3, 7.8	-0.1	-1.7, 1.5
Model 4[d] - Males	10.9	-11.3, 33.0	-9.6	-24.0, 4.8	1.9	-0.6, 4.5
Model 4[d] - Females	0.5	-10.1, 11.0	2.4	-7.1, 12.0	-1.2	-3.6, 1.1
Verbal IQ						
Model 1[a]	2.6	-5.6, 10.8	1.5	-5.1, 8.1	-0.3	-1.8, 1.1
Model 2[b]	2.3	-6.2, 10.9	1.4	-5.4, 8.2	-0.2	-1.7, 1.3
Model 4[d] - Males	2.4	-20.2, 25.1	-1.5	-16.2, 13.3	0.4	-2.3, 3.0
Model 4[d] - Females	4.4	-6.4, 15.2	2.6	-7.2, 12.4	-0.8	-3.2, 1.6
Performance IQ						
Model 1[a]	-4.6	-15.0, 5.9	0.0	-8.5, 8.5	0.1	-1.7, 2.0
Model 2[b]	-4.6	-15.5, 6.4	0.1	-8.6, 8.8	0.0	-1.9, 1.9
Model 4[d] - Males	17.8	-8.2, 43.8	-17.0	-33.9, -0.1	3.3	0.3, 6.3
Model 4[d] - Females	-3.8	-16.2, 8.7	2.3	-9.0, 13.5	-1.7	-4.4, 1.1

Abbreviations: IQ – intelligence quotient

[a] Model includes each placental variable modeled individually (three separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and sex discordance
[d] Model includes all placental variables and interaction with sex

SUPPLEMENTAL MATERIAL

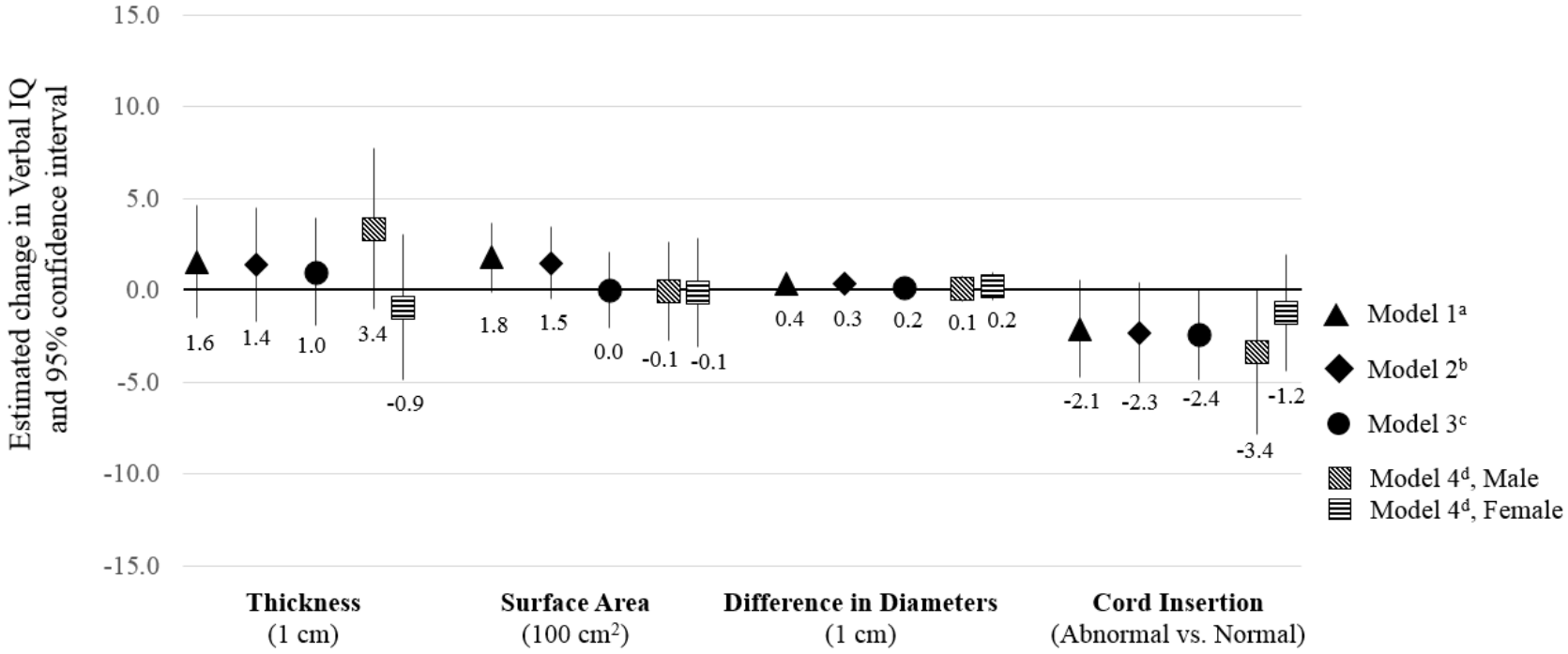

Figure S5.1. Associations between features of placental morphology and verbal IQ in the Alabama Fetal Growth Study (N=514).

[a] Model includes each placental variable modeled individually (four separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and covariates (sex, gestational age, parity, maternal age, race/ethnicity, maternal education, marital status, body mass index, smoking, alcohol use)
[d] Model includes all placental variables and covariates with interaction of placental variables and sex

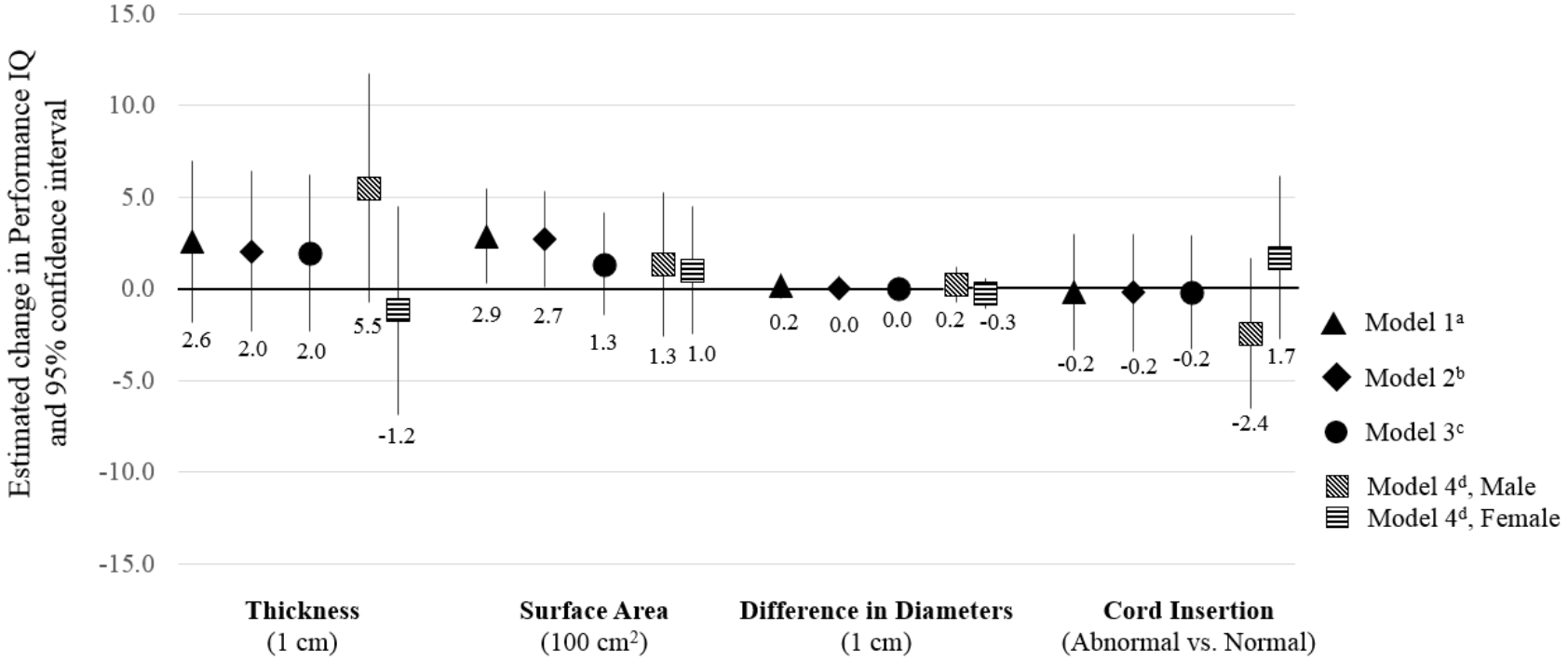

Figure S5.2. Associations between features of placental morphology and performance IQ in the Alabama Fetal Growth Study (N=514).

[a] Model includes each placental variable modeled individually (four separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and covariates (sex, gestational age, parity, maternal age, race/ethnicity, maternal education, marital status, body mass index, smoking, alcohol use)
[d] Model includes all placental variables and covariates with interaction of placental variables and sex

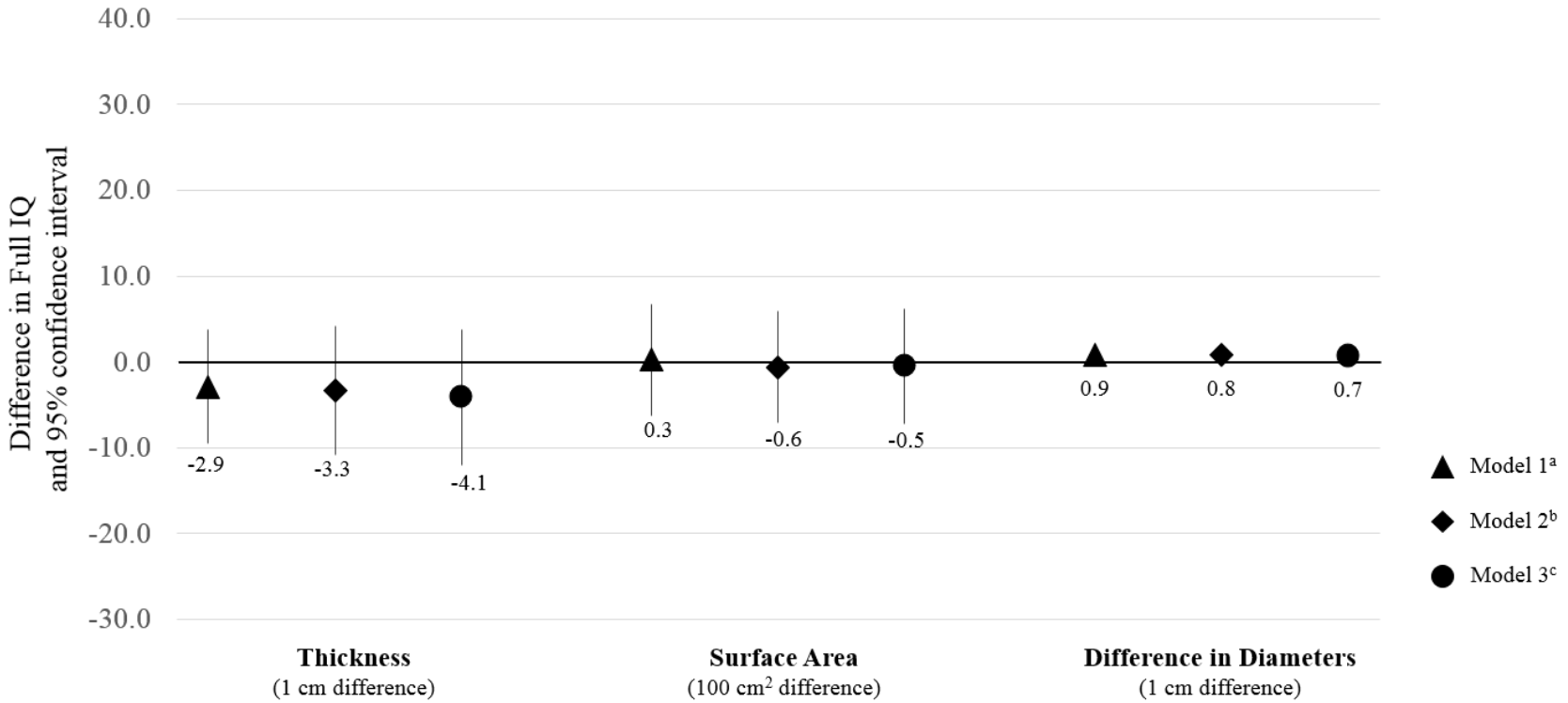

Figure S5.3. Associations between differences in features of placental morphology and difference in full IQ among dichorionic twins in the Collaborative Perinatal Project (N=82 sets).

[a] Model includes each placental variable modeled individually (three separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and sex discordance

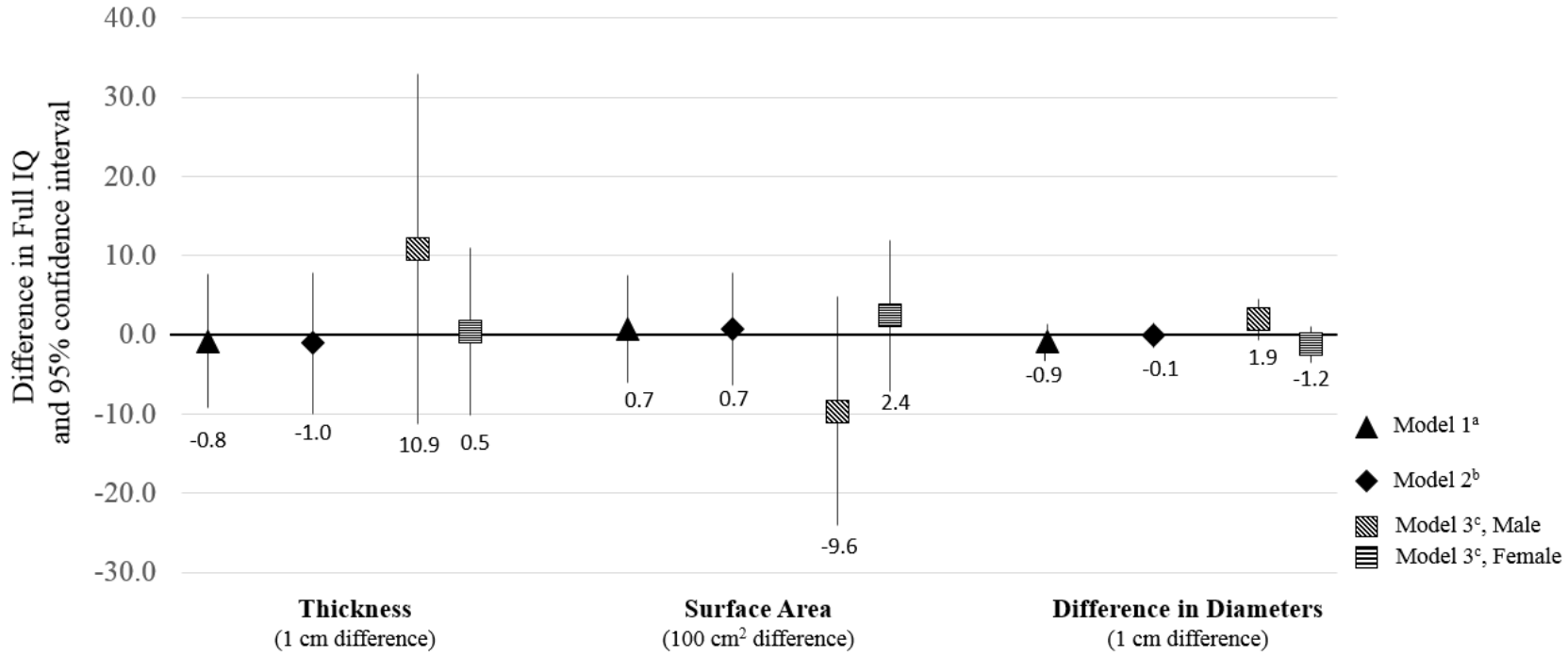

Figure S5.4. Associations between differences in features of placental morphology and difference in full IQ among same-sex dichorionic twins in the Collaborative Perinatal Project (N=38 sets).

[a] Model includes each placental variable modeled individually (three separate models)
[b] Model includes all placental variables modeled together
[c] Model includes all placental variables and interaction with sex

Table S5.1. Differences in placental variables within dichorionic twins in the Collaborative Perinatal Project (N=82 sets).

Characteristic	N (%) or Mean (SE) N=82 sets
Analytic Differences (Twin A – Twin B)	
Difference in birthweight, grams	47.07 (50.84)
Difference in placental weight, grams	3.73 (10.15)
Difference in thickness, cm	0.13 (0.05)
Difference in surface area, cm^2	-1.09 (5.94)
Difference in difference in diameters, cm	0.44 (0.33)
Difference in full IQ	1.27 (1.65)
Difference in verbal IQ	-0.79 (1.44)
Difference in performance IQ	2.26 (1.96)
Absolute Differences (Larger measure – Smaller measure)	
Difference in birthweight, grams	359.51 (31.88)
Difference in placental weight, grams	64.42 (7.08)
Difference in thickness, cm	0.29 (0.05)
Difference in surface area, cm^2	43.49 (3.33)
Difference in difference in diameters, cm	2.22 (0.22)
Difference in full IQ	11.17 (1.10)
Difference in verbal IQ	9.66 (0.95)
Difference in performance IQ	13.06 (1.32)

Table S5.2. Associations between features of placental morphology and measures of cognitive function using weighted linear regression in ten multiply imputed datasets from the Alabama Fetal Growth Study (N=514).

	Thickness (1 cm)		**Surface Area** (100 cm^2)		**Difference in Diameters** (1 cm)		**Umbilical Cord Insertion** (Abnormal vs. Normal)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Full IQ								
Model 1[a]	2.1	-1.6, 5.8	2.5	0.3, 4.7	0.3	-0.3, 0.8	-1.2	-4.0, 1.5
Model 2[b]	1.7	-2.0, 5.4	2.2	-0.1, 4.5	0.2	-0.4, 0.8	-1.4	-4.2, 1.4
Model 3[c]	1.5	-2.0, 5.0	0.7	-1.7, 3.1	0.1	-0.5, 0.6	-1.4	-3.9, 1.2
Model 4[d] - Males	4.8	-0.4, 10.0	0.6	-2.6, 3.8	0.2	-0.7, 1.0	-3.2	-6.7, 0.3
Model 4[d] - Females	-1.4	-6.1, 3.3	0.5	-2.7, 3.7	0.0	-0.8, 0.7	0.1	-3.5, 3.7
Verbal IQ								
Model 1[a]	1.6	-1.5, 4.7	1.8	-0.1, 3.7	0.4	-0.1, 0.8	-2.1	-4.8, 0.5
Model 2[b]	1.4	-1.7, 4.5	1.5	-0.5, 3.5	0.3	-0.2, 0.8	-2.3	-5.0, 0.4
Model 3[c]	1.0	-1.9, 3.9	0.0	-2.1, 2.1	0.2	-0.3, 0.7	-2.4	-4.9, 0.1
Model 4[d] - Males	3.4	-1.0, 7.8	-0.1	-2.8, 2.6	0.1	-0.6, 0.8	-3.4	-7.8, 0.1
Model 4[d] - Females	-0.9	-4.9, 3.0	-0.1	-3.1, 2.8	0.2	-0.5, 1.0	-1.2	-4.4, 2.0
Performance IQ								
Model 1[a]	2.6	-1.9, 7.0	2.9	0.3, 5.4	0.2	-0.5, 0.8	-0.2	-3.4, 3.0
Model 2[b]	2.0	-2.4, 6.4	2.7	0.1, 5.3	0.0	-0.6, 0.7	-0.2	-3.5, 3.0
Model 3[c]	2.0	-2.3, 6.2	1.3	-1.4, 4.1	0.0	-0.6, 0.6	-0.2	-3.3, 2.9
Model 4[d] - Males	5.5	-0.8, 11.7	1.3	-2.6, 5.2	0.2	-0.7, 1.2	-2.4	-6.6, 1.7
Model 4[d] - Females	-1.2	-6.9, 4.5	1.0	-2.5, 4.5	-0.3	-1.1, 0.6	1.7	-2.8, 6.1

Abbreviations: IQ – intelligence quotient

[a] Model includes each placental variable modeled individually (four separate models)

[b] Model includes all placental variables modeled together

[c] Model includes all placental variables and covariates (sex, gestational age, parity, maternal age, race/ethnicity, maternal education, marital status, body mass index, smoking, alcohol use)

[d] Model includes all placental variables and covariates with interaction of placental variables and sex

ACKNOWLEDGEMENTS

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CHAPTER 6: DISSERTATION CONCLUSIONS AND IMPLICATIONS

The placenta plays a crucial role in regulating healthy fetal development.[106] Poor placental development may result in failure of the placenta to meet the oxygen and nutrient needs of the fetus, which is believed to result in poor growth in utero.[6] Neonates who experienced suboptimal fetal growth are at greater risk of perinatal morbidity and mortality, as well as cognitive delays in childhood and cardiovascular disease in adulthood.[12, 35, 41, 45] Features of placental morphology, including thickness, surface area, shape, and umbilical cord insertion, are associated with placental function and efficiency.[3, 4] Evaluating these features in the context of fetal growth and cognitive outcomes may help to elucidate potential mechanisms related to these processes. Further, these features of gross placental morphology develop at different periods of gestation and therefore may provide insight into critical periods of development. This dissertation builds on existing literature by evaluating the associations of features of placental morphology with both fetal growth (relationship 1, Figure 6.1) and cognitive development (relationship 2, Figure 6.1).

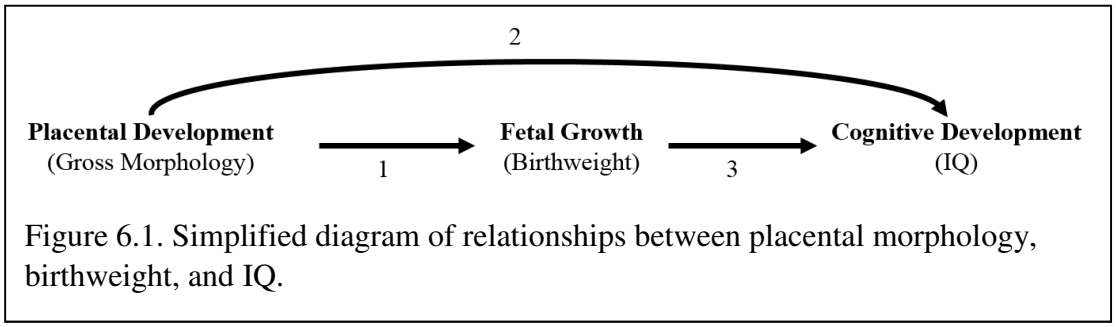

Figure 6.1. Simplified diagram of relationships between placental morphology, birthweight, and IQ.

First, we investigated relationships between features of placental morphology and birthweight separately in singletons and dichorionic twins (relationship 1, Figure 6.1; Chapters 3 & 4). Placental morphology evaluated among singletons included thickness, surface area, difference in diameters, shape, and umbilical cord insertion. Results indicated that placental

thickness and surface area were independently associated with birthweight. Further, associations were attenuated after adjustment for maternal and pregnancy characteristics. We also considered sex-specific differences in the associations and found no evidence of interaction. Results were similar among dichorionic twins. Models in dichorionic twins considered difference in birthweight as a function of differences in placental characteristics (placental characteristics included differences in thickness, surface area, and difference in diameters). However, although not statistically significant, when sex-specific differences were considered, the estimates for same-sex male twin pairs and same-sex female twin pairs appeared to diverge for thickness and surface area, with the associations among male pairs having larger magnitudes as compared to the associations among female pairs.

Next, we evaluated the relationships between features of placental morphology and IQ separately in singleton and dichorionic twin children (relationship 2, Figure 6.1; Chapter 5). Among the singleton sample, there were no statistically significant associations between features of placental morphology (thickness, surface area, difference in diameters, and umbilical cord insertion) and IQ at age five. When we considered interaction by sex, the estimates appeared to diverge in magnitude for placental thickness and umbilical cord insertion, with stronger associations observed among males and null associations observed among females. Similarly, differences in placental characteristics within a twin pair were not associated with difference in IQ at age seven. However, among same-sex pairs, estimates appeared to diverge between male and female pairs for thickness and surface area, with stronger estimates observed among male pairs as compared to female pairs. However, the confidence intervals were wide due to the small number of same-sex twin pairs (n=38).

When investigating both birthweight and cognitive outcomes, there was stronger evidence for sex-specific differences in the associations among the dichorionic twin samples as compared to the singleton samples based on the differences in the estimates between males and females or same-sex male twin pairs and same-sex female twin pairs. Further, estimates were consistently stronger among males as compared to females. Studies have hypothesized that placentas of males are more efficient but have less reserve capacity, which makes them more sensitive to stressors and puts them at increased risk of becoming undernourished.[59] Twin pregnancy may act as a stressor to fetal and placental development, as twins must compete for space and resources within the uterus. The added stress of a twin pregnancy may explain why evidence for sex-specific differences was stronger among twins as compared to singletons. Additionally, the direction of the differences (stronger associations among males) is consistent with the hypothesized differences in susceptibility to stressors between males and females.

Taken together, these results indicate associations between placental morphology and birthweight (relationship 1, Figure 6.1) but are not suggestive of overall associations between placental morphology and cognitive outcomes (relationship 2, Figure 6.1). Existing literature supports the association of birthweight and/or fetal growth restriction and IQ (relationship 3, Figure 6.1).[134, 149] Further, we found weak evidence of a relationship between birthweight and IQ in both the singleton and twin samples considered in this analysis (Table 6.1). There are several potential explanations for why we may have observed evidence of relationships between placental morphology and birthweight, and birthweight and IQ, but not an overall relationship between placental morphology and IQ.

One explanation may be that the association between fetal growth and cognitive development that has been reported in the literature is not dependent on gross placental

morphology. For this to be the true, the association between placental morphology and birthweight would need to be spurious. This could be due to an unknown or unmeasured confounder that affects both placental development and birthweight (Figure 6.2). To explain the association between placental morphology and birthweight within a twin pair, the confounder would also need to differ within a twin pair. Given these characteristics, genetic differences may be a plausible confounder, as the majority of the twins in our analysis were dizygotic, meaning that our analysis in twins only partially controls for genetic factors. However, if true, we would have expected associations to be attenuated among twins as compared to singletons due to partial control for genetics, an observation not present in our results.

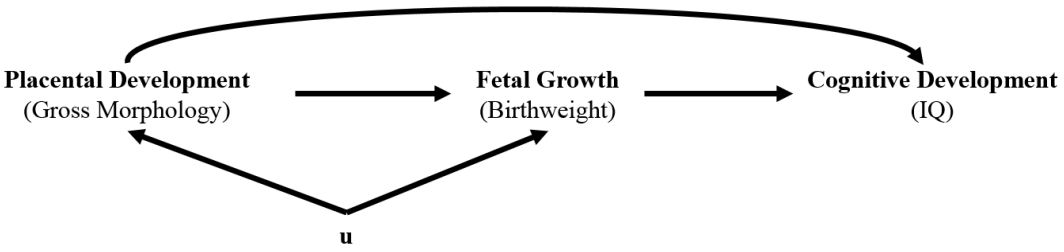

Figure 6.2. Simplified diagram of relationships between placental morphology, birthweight, and IQ, with unknown and/or unmeasured confounder.

If the association between placental morphology and birthweight is true, lack of an association between placental morphology and IQ may be due to the measures used and the characteristics of the study sample. There was low variability in some of the placental measures evaluated. In particular, in all four of our study samples, there was evidence of digit preference, with most pathologists rounding placental thickness to the nearest 0.5 cm rather than 0.1 cm, as was indicated in study protocols. To account for this, all placental thickness measures were rounded to the nearest 0.5 cm. This resulted in reduced variation in placental thickness,

especially among twins. Between 50-60% of twins had no difference in placental thickness. Additionally, the singleton analysis of IQ was based on a study conducted at a county health department in Birmingham, Alabama that oversampled women at risk of delivering a small-for-gestational age infant. At age five, the children had an average IQ that was more than one standard deviation below the normed mean of 100 (normed standard deviation: 15; mean of study sample: 82.0, standard deviation of study sample: 12.3). The reduced variability among the participants, including reduced variability in IQ, may have affected our ability to detect an association. However, the Scarr-Rowe hypothesis suggests that the heritability of IQ is dependent on socioeconomic status, with reduced heritability demonstrated in individuals of lower socioeconomic status.[150, 151] Our sample was predominantly low-income which should have improved our ability to detect an association due to the reduced influence of genetics.

Another explanation may be the bidirectional nature of the relationship between placental function and fetal growth. One of the key limitations of our work, and of similar population-based studies of the postnatal placenta, is the use of cross-sectional data due to the use of birthweight and postnatal placental measures. The placenta has the ability to adapt and respond to stressors and considering postnatal placental measures does not allow for determining the directionality of the association. For example, abnormal placental shape may result due to disruptions in early placental development or later in pregnancy as an adaptation to an adverse uterine environment and poor fetal growth.[21, 95] While abnormal placental shape may be indicative of abnormal placental development, the specific cause of the abnormal shape cannot be determined from postnatal measures.

Finally, lack of an overall association between placental morphology and IQ may be due to the underlying etiology. Associations between fetal growth and cognitive development may be

driven by factors related to fetal growth restriction, which may not be fully captured by birthweight. This is supported by the weak associations we observed between birthweight and IQ (Table 6.1). The features of placental morphology that we evaluated, while associated with birthweight, may not be strongly associated with true fetal growth restriction and subsequent cognitive development. Similarly, there may be underlying differences in the etiology between males and females. Sex-specific differences in placental and fetal development likely contributed to our ability to detect an overall association. Our results support the hypothesis that male fetuses are more sensitive to stressors and that the placentas of males have less reserve capacity and adaptive ability. Null associations in females may reflect their improved ability to adapt and respond to stressors. Heterogeneity in the underlying mechanisms may explain why we did not observe an overall association between placental morphology and IQ.

This dissertation has several analytic strengths. We controlled for maternal and pregnancy covariates that may act as confounders, which has been a limitation of similar work. In the singleton analyses, these variables were incorporated in the models. In the dichorionic twin analyses, covariates shared within a twin pair were controlled for by design. Further, evaluating these associations separately in singletons and dichorionic twins allowed for comparison of the results in these populations. Additionally, investigating relationships in dichorionic twins is advantageous because they have a higher prevalence of suboptimal fetal growth than singletons.[19] We also used data weights to account for loss to follow-up and multiple imputation to account for missing information on covariates. Another strength includes the consideration of sex-specific differences. Recent literature has demonstrated sex-specific differences in placental development and function.[58] However, many studies do not consider this when estimating associations.

Overall, we identified strong associations between placental thickness and surface area with birthweight. Further, there may be sex-specific differences in the relationships of placental thickness and surface area with birthweight and IQ among dichorionic twins, potentially due to the inherent stress of a twin pregnancy. Interestingly, placental thickness and surface area may reflect different aspects of placental function and are established at different times during pregnancy. Thickness is the main dimension of placental growth during the third trimester and may reflect the vascularization and branching of the chorionic villi.[63, 72] Surface area is generally established by the third trimester and may reflect the number of spiral arteries supplying the placenta.[5, 63, 72, 107] Strong associations with factors that reflect different aspects of placental development, potentially during different periods, may reflect critical windows of development and the adaptive ability of the placenta. While these results may be helpful in identifying potential mechanisms, it is important to note the limitations of using postnatal placental measures and the adaptive ability of the placenta.

Future studies should consider longitudinal measures of fetal and placental development across pregnancy. Evaluating placental development and function, and corresponding fetal development, would facilitate determining the directionality of the observed relationships. Further, longitudinal measures would allow for investigation of the adaptive ability of the placenta. Longitudinal studies are becoming more feasible as advances are made in imaging technology, which allows for non-invasive assessment of real-time placental development and function, including the ability to assess specific characteristics such as perfusion and spiral artery remodeling.

Future studies should also consider more specific measures of placental function, fetal growth, and cognitive outcomes. The measures of gross placental morphology, fetal growth

(birthweight), and cognitive function (IQ), used in our work are broad and fairly crude. Further, many of our placental measures required assuming that all placentas are elliptical in shape (surface area, difference in diameters, and centrality of umbilical cord insertion). However, we did find that this assumption did not introduce a meaningful degree of error for surface area calculations (Chapter 2). Evaluating specific components of placental function, fetal growth, and cognitive development, including markers of placental development, deviations from fetal growth potential, and measures of brain development or specific components of cognition, would allow for better understanding of the potential mechanisms that may be driving reported associations.

Finally, given the critical role of the placenta in regulating fetal growth, studies should continue to consider the role of placental function and efficiency in the developmental programming of long-term health outcomes. Many studies of developmental programming use fetal growth, often birthweight, as a measure of the intrauterine environment and adverse fetal development. Future research should work to evaluate more specific markers of the intrauterine environment, including incorporating placental development and function, and considering sex-specific differences in these relationships. A better understanding of what a healthy intrauterine environment looks like is necessary to identify potential ways to promote healthy pregnancy. Given the variety of adverse health outcomes now attributed to developmental programming, identifying and promoting healthy pregnancy may be an important form of primary prevention for many long-term adverse health outcomes.

TABLES

Table 6.1. Associations between full scale IQ and birthweight using linear regression among singletons in the Alabama Fetal Growth Study (n=514) and same-sex twins in the Collaborative Perinatal Project (n=38).

	Singletons[a] (1000 gram increase in birthweight)		**Twins**[b] (1000 gram difference in birthweight)	
Model 1[c]	2.71	0.93, 4.48	1.46	-6.65, 9.58
Model 2[d]	2.23	-0.14, 4.60		
Model 3[e] – Males	2.75	0.04, 5.46	11.89	-3.59, 27.37
Model 3[e] – Females	1.78	-1.13, 4.68	3.78	-20.55, 28.11

[a] Singleton models use weighted linear regression in the multiply-imputed dataset evaluate IQ as a function of birthweight and covariates

[b] Twin models use linear regression to model difference in IQ as a function of difference in birthweight within a twin pair (no-intercept model)

[c] Model 1 is the unadjusted model

[d] Model 2 adjusts for gestational age, parity, maternal age, maternal race/ethnicity, maternal education, marital status, maternal pre-pregnancy BMI, maternal smoking, maternal alcohol use, and infant sex (not evaluated in twins, since these characteristics are inherently controlled for)

[e] Model 3 is the adjusted model with interaction by sex

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