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THE RELATION BETWEEN AMNIOTIC FLUID CONSTITUENTS AND HUMAN FETAL GROWTH

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ABSTRACT

To investigate the relation between amniotic fluid (amf) constituents and human fetal growth and birth weight (b.wt), amf was collected from 395 pregnant women undergoing routine amniocentesis at 14-16 weeks' gestation at the Royal Victoria (RVH), Jewish General (JGH), and St. Mary's (SMH) Hospitals. The fluid was analyzed for total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ß-hydroxybutyrate (ßHBA), and lactate. Maternal and neonatal data were collected from a questionnaire at the time of recruitment and from medical charts post-delivery. The mean b.wt in our population was 3409±552g. Birth weight differed significantly by infant gender, maternal height (ht), and prepregnancy weight (wt), as determined by one-way analysis of variance (ANOVA). Of the amf constituents measured, glucose showed strong evidence of being a potential predictor of b.wt, such that for each mmol/L increase in amf glucose a 119.4g increase in b.wt was observed. Lactate showed a similar but weaker tendency toward predictive value. Ongoing research is currently being done to further examine the role of human amf constituents in predicting b.wt, the goal being to develop a predictive model that would aid in preparing for and preventing aberrations in fetal growth.

SOMMAIRE

Pour examiner la relation entre les constituants du liquide amniotique et la croissance du foetus et le poids à la naissance, le liquide amniotique a été recueilli de 395 femmes enceintes subissant une amniocentèse de routine entre la 14^{ième} et la 16^{ième} semaine de grossesse aux hôpitaux Royal Victoria, Général Juif, et au Centre Hospitalier de St. Mary's. Le liquide a été analysé pour le total de protéine, albumine, urée, créatinine, acide urique, glucose, ß-hydroxybutyrate, et lactate. Les données maternelles et néonatales on été réunies à partir d'un questionnaire soumis lors du recrutement et des dossiers médicaux après l'accouchement. La moyenne du poids à la naissance de notre population était de 3409±552g. Le poids à la naissance a été significativement différent selon le genre l'enfant, la taille de la mère, et son poids avant la grossesse. Le glucose a démontré être un facteur potentiel dans la prédiction du poids à la naissance, tel que pour chaque augmentation du mmol/L en glucose, une augmentation de 119.4g dans le poids à la naissance a été observée. Le lactate a démontré une tendance similaire mais plus faible. Les recherches se poursuivent dans le but d'examiner le rôle des constituants du liquide amniotique humain dans la prédiction du poids à la naissance afin de développer un modèle du poids à la naissance qui aiderait à la prévention d'anomolies de la croissance foetale.

"The fear of the Lord is the beginning of knowledge; fools despise wisdom and instruction."

Proverbs 1:7, *The Bible*

"You work that you may keep pace with the earth and the soul of the earth. For to be idle is to become a stranger unto the seasons, and to step out of life's procession, that marches in majesty and proud submission towards the infinite. When you work you are a flute through whose heart the whispering of the hours turns to music. ... I say to you that when you work you fulfill a part of earth's furthest dream, assigned to you when that dream was born, and in keeping yourself with labour you are in truth loving life, and to love life through labour is to be intimate with life's inmost secret."

The Prophet, Gibran Kahlil Gibran

To my family...

my grandmother, who served not only as an inspiration but as a constant reminder that "a thesis is hard work"

my mother, without whose support, encouragement, advice, and love I would not have survived the journey - I know that if she could have done it for me, she would have

and...

my friends, who served as a sounding board when things were rough, and a source of motivation when I needed it most

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STATEMENT OF ORIGINALITY

In conjunction with the supervisor, the author developed the present research project. From that point forward the author was responsible for obtaining ethics approval (from McGill Univesity and the 5 hospitals involved in the study), recruiting the participants, collecting and transporting the amniotic fluid that was drawn at the hospitals by various physicians, performing the laboratory and statistical analyses, reading the medical charts of the participants, and data management.

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LIST OF ABBREVIATIONS

amf	amniotic fluid
ANOVA	Analysis of variance
BCAA	Branched-chain amino acids
BCP	Bromcresol purple
ßHBA	ß-hydroxybutyrate
BMI	Body mass index
b.wt	birth weight
GI	Gastrointestinal
ht	height
IUGR	Intrauterine growth retardation
JGH	Jewish General Hospital
LGH	Lakeshore General Hospital
MCH	Montreal Children's Hospital
rh	rhesus
RVH	Royal Victoria Hospital
SMH	St. Mary's Hospital
wt	weight

I. OVERVIEW

Fetal b.wt is a determinant of short- and long-term health status (Chervenak and Gabbe, 1996; Gluckman and Harding, 1997; Ounsted et al., 1988). Both low and high b.wts are associated with increased risk of morbidity and mortality (Bernstein and Gabbe, 1996). A variety of factors have been shown to affect intrauterine growth and fetal b.wt, such as infant gender, racial/ethnic origin, maternal ht and wt, maternal nutritional status, cigarette and alcohol consumption, maternal age, socioeconomic status, parity, caffeine consumption, and placental wt (Kramer, 1987). It is currently difficult to diagnose and predict aberrant b.wt early in gestation, if at all (Norman, 1982). Most methods available today are unreliable or contingent on the knowledge of exact gestational age, which is itself assessed, in part, using fetal size.

Amniotic fluid is the interface between baby and mother that is closest to the growing fetus, and as such, serves an important role in fetal development. At least three sources of amf have been elucidated: maternal blood, fetal blood and fetal urine (Levy and Montag, 1969). Amniotic fluid composition changes as pregnancy progresses, demonstrating the influences of the different sources at the various stages of gestation. Maternal diet has been shown to modulate amf composition (Kim and Felig, 1972; Koski and Fergusson, 1992). Amniotic fluid has many functions, including supplying energy (Bell et al., 1989) and nutrients (Schmidt, 1992) to the fetus. Fetal swallowing of amf has been established early in gestation and shown to increase until term (Trahair and Harding, 1992). Furthermore, amf ingestion has been demonstrated to affect fetal growth in the animal model (Buchmiller et al., 1994; Mulvihill et al., 1985a).

Given its close proximity to the growing fetus in utero, previous studies have both quantified some of the constituents found in amf and linked them with certain genetic and pathological processes. A number of amf constituents have been studied in relation to fetal growth and development. Positive associations have been found between b.wt and amf creatinine (Yong and Gui-Lan, 1982), uric acid (Weiss et al., 1974), and glucose (Mulvihill et al., 1985a). Other amf constituents including total protein (Cheng et al., 1996), albumin, urea nitrogen (Bissenden et al., 1979), ßHBA, and lactate (Koski and Fergussion, 1992) have not yet been thoroughly studied with respect to fetal growth and

b.wt.

Human amf is routinely extracted in high risk pregnancies via a procedure called amniocentesis. It is performed at 14-16 weeks' gestation in order to test for genetic abnormalities in women 35 years and older, as well as in other cases where there is an increased risk of genetic abnormality (Gosden et al., 1981). It is also done, although less commonly, at 35-37 weeks' gestation to assess rhesus (rh)-sensitized pregnancies or fetal lung maturation in women with gestational diabetes (Bennett, 1981).

The present study examined the relationship between human amf constituents and fetal growth early in gestation. Amniotic fluid was collected from women 35 years and older undergoing routine amniocentesis at 14 to 16 weeks' gestation. Nutrients and other metabolic indicators, including total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate were measured and their possible relation to b.wt was assessed, both in a descriptive manner (correlations), and in a more causal manner (multiple regression).

The following section reviews the present body of literature on fetal growth, amf, amniocentesis, and the role of specific amf constituents in fetal growth. Other known determinants of growth are also discussed.

II. LITERATURE REVIEW

1. Aberrations of Fetal Growth

The lowest infant mortality is associated with b.wts of 3500 to 4000g (Chase, 1967; Chase, 1969; Saugstad, 1981). Two extreme deviations from normal fetal growth exist: intrauterine growth retardation (IUGR) and fetal macrosomia or excess fetal growth. Both conditions have been linked with increased morbidity and mortality (Bernstein and Gabbe, 1996; Chervenak and Gabbe, 1996; Manning et al., 1989; Ritchie, 1995).

Intrauterine Growth Retardation:

Intrauterine growth retardation complicates 3-10% of all live births in the United States and Canada (Galbraith et al., 1979; McCormick, 1985). Intrauterine growth retardation is an abnormality of fetal growth and development resulting in a b.wt which is below the 3rd, 5th, or 10th percentile for gestational age, or a b.wt which is greater than 2 standard deviations below the mean for gestational age (depending on diagnostic criteria used).

The growth-retarded fetus is at a greater risk for mortality and morbidity (Bernstein and Gabbe, 1996; Ritchie, 1995), even during the newborn period (IOM, 1990). They are at increased risk of polycythemia, hypoglycemia, hypocalcemia, and birth asphyxia (Arora et al., 1987; Kramer et al., 1989; Ounsted et al., 1988; Usher, 1970), as well as having a higher probability of developing cerebral palsy (IOM, 1990). There is evidence that IUGR leads to small but persistent effects in stature, brain growth, and neurocognitive performance (Teberg et al., 1988). Intrauterine growth retardation is likewise attributed with a slightly higher risk of fetal and infant mortality (Arora et al., 1987; Haas et al, 1987; Koops et al., 1982; Kramer et al., 1989; Usher, 1970).

Many known causes of IUGR exist (DeSesso, 1987; Ritchie, 1995; Rosenberg, 1996). They can be divided into three main categories: maternal, fetal, and uteroplacenta. Maternal risk factors include: previous history of IUGR, alcohol use, smoking, drug use, anemia, malnutrition, prepregnancy wt <50kg, cyanotic heart disease, chronic

hypertension, diabetes mellitus (with vasculopathy), and connective tissue disease (Norman, 1982). Fetal risk factors include: genetic disorders, chromosomal abnormalities, congenital anomalies, and fetal infection (Norman, 1982). Uterine and placental risk factors include: Müllerian anomalies and placental insufficiency due to infarctions, infection, chorioangioma, multi-fetal pregnancy, circumvallate placenta previa, focal abruption, and marginal insertion of the cord. In almost half of the cases, however, the etiology is unknown (Norman, 1982).

There are three categories of IUGR, each reflecting the time of onset of the pathological process. Type 1, or symmetrical IUGR, begins early in gestation. It reflects a decreased growth potential and results in a fetus that is proportionally small for gestational age (Norman, 1982). Type 2, or asymmetrical IUGR, is a result of a later growth insult, generally occurring after 28 weeks' gestation. The neonate has restricted growth, most commonly caused by uteroplacental insufficiency (Norman, 1982). Intermediate IUGR, the third category, is a combination of types 1 and 2 IUGR, occurring during the middle period of gestation, at 20-28 weeks.

Intrauterine growth retardation can be diagnosed prenatally using a variety of biometric parameters. Ultrasound every 3-4 weeks, and up to every 2 weeks if diagnosis is suspected, is indicated beginning at 26 weeks of gestation (Norman, 1982). Comparisons of fetal growth can be made using biparietal diameter, transverse cerebellar diameter, abdominal circumference, head circumference, and femur length. Body proportionality can be assessed using ratios of the former measures. It is crucial, however, to ascertain gestational age as accurately as possible. Because gestational age is often unknown, and is inaccurate in up to 40% of cases, interpretation based on ultrasound measurements is difficult (Norman, 1982). Reduced amf volume is another measure that enhances prenatal diagnosis of IUGR. This method is most accurate from 27 to 42 weeks' gestation. Approximately 25% of all growth retarded fetuses remain undiagnosed until delivery (Norman, 1982).

Fetal growth retardation is linked to postnatal growth failure, long-term deficits in neurocognitive development, as well as a higher propensity for the development of cardiovascular and metabolic diseases, hypertension, and non-insulin dependent diabetes during adult life (Ariouat and Barker, 1993; Barker, 1991; Barker et al., 1993; Gluckman

and Harding, 1997; Godfrey and Barker, 1995; Godfrey et al., 1996; Jendryczko and Poreba, 1996; Seckl, 1997). Antenatal diagnosis of IUGR may allow for appropriate management and earlier obstetrical intervention and prevent the high mortality and morbidity associated with it.

Macrosomia:

Fetal macrosomia has been defined as a b.wt of over 4000 to 4500g (Chervenak and Gabbe, 1996). It has also been categorized as a b.wt above the 90th percentile for gestational age and gender (Chervenak and Gabbe, 1996; Manning et al., 1989).

Excessive fetal growth is associated with increased perinatal risk (Chervenak and Gabbe, 1996; Manning et al., 1989). During vaginal delivery of a macrosomic fetus, the newborn is at a greater risk for perinatal mortality and morbidity (Brudenell, 1989) including developing shoulder dystocia, traumatic injury, brachial plexis injury, asphyxia, and metabolic disorders such as hypoglycemia (Chervenak and Gabbe, 1996; Manning et al., 1989; Rosenberg, 1996). Antenatal detection, therefore, is important to allow for optimal selection of the route of delivery in order to reduce birthing risks. It is also important in order to allow for induction of labor at 38 weeks' gestation to avoid the increase in body size and wt that takes place in the last 2 weeks of pregnancy, as well as to minimize the increased risk of intrauterine death (Brudenell, 1989).

The most common causes of macrosomia are maternal diabetes and maternal obesity, however, other conditions, including: normal but increased genetic growth potential (Manning et al., 1989), erythroblastosis fetalis, other causes of fetal hydrops, and Beckwith-Wiedemann syndrome, may also precipitate such a deviation (Rosenberg, 1996).

Macrosomia as a result of maternal diabetes is characterized by an infant with selective organomegaly, including increases in both fat and muscle mass. This results in an asymmetric growth, with a disproportionate increase in abdomen and shoulder size, without an impact on growth of the brain (Chervenak and Gabbe, 1996). When macrosomia is the result of maternal obesity without glucose intolerance, the infant experiences a symmetric excess of growth with increases of both head and abdomen (Chervenak and Gabbe, 1996).

The clinical ability to identify large-for-gestational age (LGA) fetuses at term is poor, with only a 35% identification associated with symphysis-fundal ht measurements (Chervenak and Gabbe, 1996). Ultrasound is another way of identifying the macrosomic fetus, however its predictive value is also limited. Abdominal circumference is the most reliable parameter used with sonographic techniques. Head to abdominal circumference ratio can be used to identify asymmetric macrosomia, although its predictive value is very low and knowledge of the exact gestational age is required. The most predictive parameter that can be used is fetal length to abdominal circumference ratio, and knowledge of gestational age is not required (Chervenak and Gabbe, 1996).

Large-for-gestational age infants have an increased tendency of becoming obese later in life (IOM, 1990). This can be associated with abnormal glucose tolerance, as well as other chronic diseases. Most macrosomic babies exhibit normal physical and mental development, although neurologic impairment is possible where prolonged hypoglycemia occurs in the neonate (Brudenell, 1989).

2. Determinants of Fetal Growth and Birth Weight

Many studies examining factors that affect fetal growth and b.wt have been conducted. Factors that have been looked at extensively as having a direct, causal impact on growth in utero include infant gender, racial/ethnic origin, maternal ht, prepregnancy wt, paternal wt and ht, maternal b.wt, history of prior low b.wt infants, gestational wt gain and energy intake, general morbidity and episodic illness, malaria, cigarette smoking, alcohol consumption, and tobacco chewing (Kramer, 1987). Other factors, such as maternal age, socioeconomic status, parity, and caffeine consumption have been studied though the conclusions drawn have not been as consistent (Kramer, 1987).

Infant Gender:

Most studies concur that males have a higher b.wt and lower risk of IUGR and low b.wt than females (Armstrong, 1972; Kramer, 1987; Meyer et al., 1976; Taffel, 1980). Male babies are on average 200g heavier than their female counterparts (Norman, 1982). In a study of 183 singleton infants, male babies had statistically significantly

greater b.wts than females (3643±468g vs 3467±433g, p=0.009; Catalano et al., 1995).

Racial/Ethnic Origin:

It was shown that Blacks, Indians, and Pakistanis have lower b.wts than European and North American Whites, and that other ethnic groups such as North African Jews and North American Indians have higher b.wts (Kramer, 1987). Studies of Black women have reported deficits of mean b.wt of between 108g and 164 g (Horon et al., 1983; Showstack et al., 1984). Reports on North African women found mean b.wts in this population to be higher than those of Isreali, or Asian origins (Palti and Adler, 1977; Yudkin et al., 1983), but lower than those of European or North American origins (Palti and Adler, 1977). Higher mean b.wts have been found among North American Indians when compared to the general Canadian population (Munroe et al., 1984). Table 1 (p. 8) summarizes global estimates of mean b.wt and prevalence of low b.wt by country.

Table 1. Mean b.wts and prevalence of low birth weight (LBW) by country of origin

Country	Mean b.wt (g)	LBW Rate (%)
North America:		
Canada	3327	6.0
United States	3299	6.9
Europe:		
France	3240-3335	5.6
Federal Republic of Germany	3356	5.5
Italy	3445	4.2
Sweden	3490	4.0
United Kingdom	3310	7.0
Latin America:		
Brazil	3170-3298	9.0
Colombia	2912-3115	10.0
Mexico	3019-3025	11.7
Africa:		
Egypt	3200-3240	7.0
Nigeria	2880-3117	18.0
Zaire	3163	15.9
Asia:		
China	3215-3285	6.0
India	2493-2970	30.0
Iran	3012-3250	14.0
Iraq	3540	6.1
Japan	3200-3208	5.2
Pakistan	2770	27.0

Adapted from World Health Organization. The incidence of low birth weight: a critical review of available information, 1980; and The incidence of low birth weight: an update, 1984.

The lowest b.wts and highest prevalence of low b.wts are found in the Asian countries, followed by Africa, and Latin America. The highest b.wts and lowest rate of low b.wts are found in Europe and North America.

Since studies investigating the impact of racial/ethnic differences on b.wt have been done on a population basis, it is difficult to assess whether the effects are simply an indirect consequence of other confounding factors, such as cultural, environmental, and anthropometric differences. Most studies done have, however, controlled for such factors as socioeconomic status, maternal ht, prepregnancy nutritional status, and either energy intake or wt gain during pregnancy.

Maternal Height:

It is generally recognized that there is a significant positive correlation between

maternal ht and mean b.wt (Kramer, 1987). The effect appears to be approximately 7.8g per centimeter of maternal ht. Similarly, a significant inverse relationship has been reported between maternal ht and IUGR (Fedrick and Adelstein, 1978; Meyer et al., 1976; Scott et al., 1981).

Maternal Weight:

In a study of 183 singleton pregnancies, maternal prepregnancy wt and wt gain during pregnancy were strong predictors of b.wt (Catalano et al., 1995). The average magnitude of the effect on mean b.wt in women with adequate prepregnancy wt for ht was approximately 20 g/kg of total wt gain (Kramer, 1987). A clear trend of increased rates of IUGR and decreased mean b.wts with decreases in prepregnancy wt for ht in women with low gestational wt gain was demonstrated in various studies (Abrams and Laros, 1986; Frentzen et al., 1988; Mitchell and Lerner, 1989; Naeye, 1981a,b; Seidman et al., 1989; Winikoff and Debrovner, 1981). The relative risk for IUGR in women with low gestational wt gain (<7 kg) was found to be approximately 2 (Kramer, 1987). Underweight women appear to derive a greater benefit from a given gestational wt gain than do women with adequate or excessive wts (Abrams and Laros, 1986). Furthermore, women who are thinner before pregnancy tend to have smaller babies than do heavier women with the same wt gain (Kramer, 1987).

It was demonstrated that b.wt rises with increases of maternal wt between pregnancies and with greater wt gains during pregnancy (Billewicz and Thomson, 1973). Women with large gestational wt gain are at increased risk of having higher b.wt infants (Ounsted and Scott, 1981; Scholl et al., 1988; Udall et al., 1978). In obese women, the effect of gestational wt gain on fetal growth is weak or absent (Abrams and Laros, 1986; Brown et al., 1986; Frentzen et al., 1988; Harrison et al., 1980; Luke et al., 1981; Mitchell and Lerner, 1987; Naeye, 1981a; Rosso, 1985; Winikoff and Debrovner, 1981). Moreover, for the same wt gain, obese women have larger babies than those of non-obese women (Kramer, 1987).

Maternal Nutritional Status:

The effect of maternal nutritional status on the developing fetus has been studied

by a variety of investigators. It was suggested that maternal nutrition influences b.wt (Brasel and Winick, 1972; Falkner, 1981), and more specifically, that undernutrition is linked with fetal growth restriction in humans (Ariouat and Barker, 1993; Barker et al., 1993; Crawford et al., 1993; DeSesso, 1987; Luke, 1994; Mavalankar et al., 1994; Primhak and MacGregor, 1991; Thame et al., 1997). Maternal dietary protein restriction, alone and in conjunction with energy restriction, resulted in decreased fetal growth in many species, including sheep and rats (Fattet et al., 1984; Hill, 1984; Lederman and Rosso, 1980; Pond et al., 1988; Rosso, 1977a, b, 1980; Rosso and Streeter, 1979). Maternal food and nutrient supplementation during pregnancy were found to result in higher b.wts (Cox et al., 1981; Klein et al., 1976; Zibell-Frisk et al., 1990).

Cigarette Consumption:

Smoking has been shown to have a detrimental effect on fetal growth (Kramer, 1987). It is recognized as the single most important modifiable factor responsible for fetal growth retardation in developed countries (Kramer, 1987). Most studies concur that the b.wt is reduced on average by 200g among infants of smokers (Abel, 1980; Berkowitz, 1988), and that the effect is proportional to the frequency of smoking (Abel, 1980). It has been reported that women who stop smoking during pregnancy give birth to infants of similar wt to those who either did not smoke or stopped smoking before becoming pregnant (Naeye, 1981c; Papoz et al., 1982; Rush and Cassano, 1983).

Alcohol Consumption:

Alcohol is recognized as a potent teratogen during pregnancy. Fetal alcohol syndrome is characterized by prenatal or postnatal growth retardation, distinct facial anomalies, and mental deficiency (Rosett, 1980). Significantly lower b.wts have been associated with maternal alcohol consumption (Hingson et al., 1982; Zuckerman et al., 1983). An estimated decrease of 155g was associated with consumption of ≥2 drinks/day (Little, 1977; Mills et al., 1984; Olsen et al., 1983).

Maternal Age:

Maternal age has not been reported to influence the size of the baby at birth in

most studies done to date (DaVanzo et al., 1984; Mills et al., 1984; Pachauri and Marwah, 1970; Quick et al., 1981), however, increasing age may augment the effect of other risk factors (Meyer et al., 1976; Miller and Merritt, 1979). Once controlled for differences in gestational wt gain, prepregnancy wt, and other confounders, no significant difference was found in fetal growth in adolescents (Duenhoelter et al., 1975; Horon et al., 1983; Scholl et al., 1984), however, studies done in older women found higher rates of low b.wt among women 35 years and older (Eisner et al., 1979; Legg et al., 1970) and women 40 years and older (Kaminski et al., 1973). One study found the effect present only in first and second pregnancies (Meyer et al., 1976). Another study found no increase in IUGR in older women who had no other risk factors (Miller and Merritt, 1979). Only one study found a significant independent effect of maternal age on mean b.wt (Yudkin et al., 1983).

Socioeconomic Status:

Socioeconomic status alone has not been reported to have a significant effect on mean b.wt and intrauterine growth (DaVanzo et al., 1984; Donaldson and Billy, 1984; Kramer, 1987; Lechtig et al., 1975; Linn et al., 1982; Scott et al., 1981). It is likely, however, that it exerts indirect effects via nutritional status, access to health care, and toxic, anthropometric, or infectious factors.

Parity:

Parity has been widely recognized as having an effect on the size of the baby at birth (Kramer, 1987). An estimated 43.3g increase in b.wt per birth has been calculated (Kramer, 1987). Parity was found to be a strong predictor of b.wt in a study of 183 singleton pregnancies by Catalano et al. (1995). In a study of 6702 married women, the second baby was heavier than the first 60% of the time, although this trend did not persist in later pregnancies (Billewicz and Thomson, 1973). While spontaneous abortions did not appear to affect b.wts in subsequent pregnancies, when the first pregnancy ended in stillbirth, b.wts in subsequent pregnancies tended to be reduced considerably (Billewicz and Thomson, 1973).

Caffeine Consumption:

Caffeine consumption has been associated with a reduction in b.wt and an increased risk of low-birth-weight infants, especially in full-term pregnancies (Hogue, 1981; Martin and Bracken, 1987; Mau and Netter, 1974; Munoz et al., 1988; van den Berg, 1977; Watkinson and Fried, 1985). It remains uncertain, though, whether the effects seen in some of the studies were due to caffeine, another constituent of coffee, or associated characteristics of coffee drinkers. The level at which effects have been reported ranges from >150mg (Martin and Bracken, 1987) to >700mg per day (Hogue, 1981). Other studies have not found a significant association between caffeine consumption during pregnancy and b.wt (Brooke et al., 1989; Hingson et al., 1982; Linn et al., 1982; Tennes and Blackard, 1980).

Placental Weight:

Given that the placenta is at the root of the maternal/fetal interface, placental growth and the fetal/placental wt ratio has been suggested to influence patterns of fetal growth (Molteni, 1984). In this review, it was determined that mean fetal/placental wt ratios do not vary greatly between small-, average-, and large-for-gestational-age groups. It was therefore proposed that the placenta appears to select an ideal fetal growth rate that is independent of predetermined growth category. Nevertheless, a consistent increase in placental size was demonstrated in average- and large-for-gestational-age groups until 42 weeks of gestation.

Summary

Aberrations of normal fetal growth are associated with negative fetal outcomes both immediate and long-term. Infant gender and racial/ethnic origin have been shown to have an effect on fetal b.wt. Maternal ht, wt, and nutritional status, parity, and placental wt have been shown to have a positive association with b.wt, and maternal cigarette and alcohol consumption have been shown to have a negative association with b.wt. Weak or controversial associations have been demonstrated between b.wt and maternal age, socioeconomic status, and caffeine consumption. Despite the many factors known or

thought to influence b.wt and fetal growth that have been identified, unfortunately at the present time, diagnosis of either extreme in b.wt, IUGR or macrosomia, is not reliable. Given the various complications associated with both abnormalities, early detection is desirable. Some research focusing on the amf as a possible indicator of fetal growth has been conducted since it is a fluid compartment that is closest to the fetus.

3. Amniotic Fluid

Amniotic fluid is the liquid that surrounds the growing fetus in the amniotic sac. It serves many different functions, including temperature regulation and protection against external trauma, and has bacteriostatic properties that protect the fetus from infection (Ritchie, 1995). Amniotic fluid allows the fetus to move freely thereby facilitating fetal activity and breathing movements necessary for normal development (Ritchie, 1995). By transmitting sound freely, amf provides an excellent view of the fetus during ultrasound (Ritchie, 1995). The even distribution of the fluid allows for the force of uterine contraction during labor to be applied evenly to the cervix (Ritchie, 1995). Amniotic fluid may also play a critical role in supplying energy (Bell et al., 1989) and nutrients (Schmidt, 1992) to the growing fetus (Pitkin and Reynolds, 1975).

The Importance of Amniotic Fluid Ingestion by the Fetus:

Fetal swallowing is established early in human development (Trahair and Harding, 1992). The volume of amf swallowed by the fetus increases linearly until 28 to 30 weeks' gestation, and then rises exponentially during late gestation when the volume swallowed reaches approximately 750 mL/day (Pritchard, 1966) and was shown to be as high as 1006 mL/day at term, using a mathematic model of human amf dynamics (Mann et al., 1996). Lack of ingestion has not only led to increased amf volume (Karnak et al., 1996), but to decreases in somatic growth (Jacobs et al., 1989; Mulvihill et al., 1985a), while increased swallowing has lead to increases in growth in the rabbit model (Buchmiller et al., 1994; Mulvihill et al., 1986). Restoration of fetal swallowing has produced a reversal of these adverse effects on fetal growth, again in the rabbit model (Mulvihill et al., 1985a).

Amniotic fluid thus may serve as a considerable source of nutrients for the growing and developing fetus (Trahair and Harding, 1992). In order to clarify the nutritional value of swallowed amf to the fetus, Jacobs et al. (1989) studied 63 fetal rabbits that underwent esophageal ligation during the final trimester of pregnancy and found significant reductions in wt, as well as significant but less consistent reductions in crown-rump length and biparietal diameter. Results demonstrated that there is indeed nutritive value in swallowed amf, and that this value may be of particular importance when there is limited placental function. In the fetal rabbit, transamniotic feeding (n=30; Buchmiller et al., 1994) and intragastric infusion post-esophageal ligation (n=43; Mulvihill et al., 1986) with bovine amf resulted in fetal growth augmentation. This result suggested that increased ingestion of amf is responsible for the enhancement of growth since bovine and rabbit amfs are very similar in composition. In another study, fetal wt was significantly decreased in esophageal ligated (on day 24 of a normal 31 day gestation) fetal rabbits (n=13) compared with control rabbits (Buchmiller et al., 1993). Prevention of fetal swallowing at 23 days in rabbit fetuses (n=15) resulted in a 14% and 10% reduction in b.wt and crown-rump length, respectively, when compared with controls (Mulvihill et al., 1985a). These reductions were reversed by intragastric infusion of amf (n=7), suggesting that the fetus is dependent upon ingested amf for between 10-14% of its normal energy intake.

Inhibition of fetal amf ingestion has also negatively affected the growth and function of the fetal gastrointestinal (GI) tract in sheep (Avila and Harding, 1991) and rabbits (Buchmiller et al., 1993a; Karnak et al., 1996; Mulvihill et al., 1986; Yee et al., 1995). Restoration of fetal swallowing has produced a reversal of these adverse effects on gut function, again in the sheep (Trahair and Harding, 1995) and the rabbit models (Mulvihill et al., 1985a). Avila and Harding (1991) examined the influence of ingested amf in the development of the fetal intestine in a case-control study involving 12 sheep. In fetal sheep in which the ingestion of amf was inhibited, abdominal girth and wts of the GI tract, liver, and pancreas were reduced. This observation points to a possible role of amf swallowing in the growth and development of these fetal organs. In the absence of amf swallowing in fetal sheep, defects in enterocyte morphology, including abnormal or absence of microvilli, inappropriate cell extrusion, glycogen accumulation, and altered

lysosomal morphology were found (Trahair and Harding, 1992). A study, involving 4 fetal sheep in which amf ingestion was inhibited and 14 age- and breed-matched controls, was conducted by Trahair et al. (1986) to investigate the effects of ingested amf on small intestine development during late gestation. A ~25 % reduction in mucosal layer width at both sites in the small intestine was observed, greatly contributed to by a ~30% reduction in villus ht. Villus and crypt densities at the proximal and distal sites increased significantly. Their findings indicate that amf ingestion during late gestation has an effect on growth of the small intestine's mucosal elements. When ingestion was inhibited for longer periods of time (~80 days of a normal 145-148 day gestation) in the fetal sheep (n=11), not only was the growth of the GI tract significantly retarded, the small intestine being the most severely affected, with smaller villi in the proximal and distal regions, villus density increased and crypt density decreased, but progressive growth-retarding effects were also observed, becoming more pronounced as the period of absence of swallowing increased (Trahair and Harding, 1995). These effects were reversed upon restoration of fetal swallowing, even after relatively short periods of time (15 days).

Small intestinal length and midjejunal protein content were significantly decreased in fetal rabbits who underwent esophageal ligation on day 24 (n=13) when compared with control rabbits (Buchmiller et al., 1993). Esophageal ligation to prevent fetal swallowing on day 23 in the rabbit model (n=43) resulted in a 32% reduction in gastric wt, a 40% reduction in serum gastrin level, and a striking decrease in gastric acid concentration when compared with control fetuses (Mulvihill et al., 1986). In a study involving 56 fetal rabbits, esophageal ligation also on day 23 resulted in a 45% decrease in gastric wt and a 34% decrease in DNA content, not reversed by intragastric carrier infusion (Yee et al., 1995). Likewise, Karnak et al. (1996) investigated 24 fetal rabbits that underwent esophageal ligation at the same gestational period and found a significant decrease in gastric, small intestinal, and total GI tract wts. Lactase activity in the proximal small intestinal tissue was significantly decreased and fetal stomach tissues revealed marked histological alterations.

To evaluate the relation between IUGR and intestinal length in the human fetus, Shanklin and Cooke (1993) looked at several parameters in 100 infants from 12-42 weeks' gestation. They found that intestinal length increased ($p<0.0001$) with b.wt,

gestational age, and crown-heel length. The body wt to intestinal length ratio also increased with gestational age. Both intestinal length and body wt to intestinal length ratio decreased in IUGR infants (n=21), suggesting that this reduction in functional mass may contribute to the low wt attainment of IUGR infants.

The aforementioned body of literature makes clear the importance of investigating the composition of amf at various stages in gestation and what may lead to changes therein. The exact factors present in amf that have lead to changes in the growth and development of the fetus have yet to be ascertained, however possibilities include various nutrient and metabolic compounds.

Amniotic Fluid Composition:

The composition of the amf is not constant but changes as pregnancy progresses (Parvin, 1887). Studies have shown that there are at least three sources of amf, maternal blood, fetal blood, and fetal urine (Bevis, 1956; Levy and Montag, 1969; Seppälä et al., 1966; Usategui-Gomez and Morgan, 1966). In the first half of pregnancy, due to the permeability of fetal skin (Lind et al., 1972), the concentration of the diffusible solutes, eg., sodium, chloride, and urea, are closer to those of fetal serum than those of maternal serum. Also influencing amf composition at this stage are small amounts of fetal urine which is suggested by slightly lower concentrations of sodium and higher urea in the fluid as compared to fetal serum, that likely reflects the kidney's function of sodium removal and urea concentration even at this early stage. In the second half of pregnancy, as fetal skin becomes more and more impermeable, the fluid composition is increasingly influenced by fetal urine and maturation of renal function. Fetal kidneys, in their continuous contribution of fluid and electrolytes, play an essential role in the maintenance of amf volume, without which fetal development would be impossible (Lumbers, 1995). Amniotic fluid volume is increased by fetal urine from 12 weeks' gestation onwards (Käser et al., 1967). As pregnancy progresses, therefore, it follows that there is a downward trend in fluid osmolality and sodium concentration, and an increase in urea (Wu et al., 1995) and creatinine concentrations. The latter two are also an indication of enhanced fetal protein metabolism (Wu et al., 1995).

Role of Maternal Diet in Modulating Amniotic Fluid Composition:

Maternal diet during pregnancy has also been implicated in altering the composition of amf. Kim and Felig (1972) were among the first to study the effects of maternal energy deprivation on the levels of amf metabolic fuel. After 4 days of maternal starvation, increased amf ßHBA and acetoacetate concentrations were observed. Increased free fatty acid concentration was found in maternal plasma, but not in amf, reflecting their limited transport across the placental barrier.

The levels of amf amino acids are likewise affected by maternal diet. Maternal starvation in rats produced decreases in amf glucogenic amino acids: glycine and alanine, as well as the branched-chain amino acids (BCAA): valine, leucine, and isoleucine, and increases in the glycine/valine ratio (Bernstein et al., 1992). An 84-90hr maternal fast during the second trimester of pregnancy produced marked increases in maternal plasma and amf BCAA: valine, leucine, and isoleucine (in contrast to the previous study's findings), and a decrease in alanine (Felig et al., 1972). Maternal starvation did not change the rate of transfer of amino acids across the placental barrier. Tyson et al. (1976) similarly observed that the decrease in concentration of glucose and alanine in the amf following a 72hr maternal fast was parallel to that of the decrease in the maternal plasma.

Koski and Fergusson (1992) demonstrated that changes in maternal carbohydrate intake influence the composition of the amf in pregnant rat dams. As the level of carbohydrate decreased in the maternal diet, amf glucose was significantly decreased, while amf uric acid was significantly increased (Koski and Fergusson, 1992). The effect of prolonged maternal fasting in rats was investigated by Nowacka and Gorski (1988). A decrease in amf glucose after one day of fasting was observed, followed by a subsequent stabilization. Decreases of amf and maternal plasma glucose were observed after 4 days of maternal starvation (Kim and Felig, 1972). Lower levels of amf glucose have also been associated with fetal growth retardation in humans during late gestation (Marin and Hood, 1979).

Summary

Amniotic fluid, while playing a vital role in fetal survival is a dynamic fluid with

an ever changing composition that seems to be influenced not only by the period of gestation, but by maternal factors such as diet as well (Bernstein et al., 1992; Felig et al., 1972; Kim and Felig, 1972; Koski and Fergusson, 1992; Nowacka and Gorski, 1988; Tyson et al., 1976). Certain nutrients and metabolic indicators present in amf have been recognized to have an effect on fetal growth and development. They include: total protein (Jauniaux et al., 1994), albumin, urea nitrogen (Almeida and Kitay, 1988; Gluck et al., 1971; Koski and Fergusson. 1992). creatinine (Weiss et al., 1974; Wyatt et al., 1969; Yong and Gui-Lan, 1982), uric acid (Bissenden et al., 1979; Koski and Fergusson, 1992; Weiss et al., 1974), glucose (Drazancic and Kuvacic, 1974; Koski and Fergusson, 1992; Marin and Hood, 1979), β-hydroxybutrate, and lactate (Koski and Fergusson, 1992).

4. Role of Amniotic Fluid Constituents in Fetal Growth

Many studies have examined levels of different constituents in amf throughout gestation. Studies indicating that specific amf nutrients and metabolites may be important for fetal growth and development have also been done. In this section, the levels found in amf, the source(s), and the significance of the nutrient and its relation to fetal growth will be discussed with special emphasis on: total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate.

Protein:

The proteins in amf are essentially of maternal origin (Emara et al., 1978). Amniotic fluid levels of total protein are much lower than maternal serum concentrations throughout gestation (Benzie et al., 1974). Direct transfer through the amniotic membrane separating the exocoelomic from the amniotic cavity is limited during early gestation (Jauniaux et al., 1994). Transudation through fetal skin is thus thought to be the main contributor in the first trimester until the other fetal organs are mature and the skin becomes keratinized (Jauniaux et al., 1998).

Mean concentrations of total protein in amf of between 0.1 and 8.63 g/L have been reported in the literature. Table 2 (p. 19) summarizes previous studies on human amf protein levels throughout gestation.

Table 2. Amf total protein concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x} \pm$ SD (g/L)
Jauniaux et al., 1991	17	5-13	0.2±0.2
Campbell et al., 1992	29	7-12	0.97±1.18
Jauniaux et al., 1994	24	7-14	0.1±0.04
Jauniaux et al., 1998	20	11–14	3±3
	12	12–16	7±3 – series
Benzie et al., 1974	29	15	5±2
	61	19	5±1
	5	22	5±1
	19	34	4±2
	51	40	3±2
Emara et al., 1978	42	Term	8.63±3.24

[1] SD, standard deviation

As can be seen from the table, total protein concentration in amf is quite low and remains fairly stable throughout gestation, increasing slightly toward the middle of gestation and tapering off near the end of gestation. This is contrary to the finding by Jauniaux et al. (1994) of a positive correlation coefficient of 0.73 between gestational age and total protein in the amf in a sample of 47 pregnant women implying a steady increase in concentration throughout gestation.

The total protein levels reported in the studies by Jauniaux et al. (1991; 1994) are substantially lower than other reported values. The values obtained in their studies may not accurately reflect population values given the small sample sizes used. In 1998 the same researchers reported the level in amf in a group of 20 women at 11-14 weeks' gestation and found similar levels to other researchers. At that time, the group also measured serial total protein levels in a group of 12 women who were to terminate their pregnancies for psychosocial reasons. Values obtained in this manner were slightly higher than reports based on single measurements, during the same gestational period. Benzie et al. (1974) measured total protein at different gestational ages in different cohorts of women. They excluded samples that were contaminated with blood or meconium and the levels reported are consistent. The lower levels reported by this group during the later gestational period were explained by dilution. Emara et al. (1978) did not find the same drop in amf levels at term, this may be explained by contamination of the samples. The concentration of total protein expected in amf at 14-16 weeks' gestation is

5±2 g/L (Benzie et al., 1974).

Little is known about the effects of amf total protein on fetal growth, however, in a retrospective human study involving 56 neonates, the effect of amf protein absorption on fetal body wt could not be demonstrated clinically (Cheng et al., 1996). In a study of baboon fetuses, Brans et al. (1986) found no difference between amf protein concentrations of normally developed (n=7) and growth-retarded fetuses (n=5) between 173 and 176 days of gestation (6.00±1.75 g/L and 4.50±0.44 g/L, respectively), however, their sample size may have been too small to detect a significant difference.

Albumin:

Albumin constitutes 55-65% of amf protein (Burdett et al., 1982). The source of amf albumin is the same as amf total protein. Maternal serum albumin concentrations are also much greater than amniotic concentrations throughout gestation (Benzie et al., 1974).

Mean amf albumin concentrations of 0.50 to 5.05 g/L have been reported in the literature. Table 3 (p. 20) summarizes previous studies on human amf albumin levels throughout gestation.

Table 3. Amf albumin concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (g/L)
Campbell et al., 1992	24	7-12	0.50±0.98
Benzie et al., 1974	26	15	3±2
	58	19	3±1
	5	22	3±1
	12	34	2±1
	34	40	1±1
Emara et al., 1978	42	Term	5.05±2.72

[1] SD, standard deviation

As can be seen from the table, albumin concentration in amf is quite low and remains fairly stable throughout gestation, increasing slightly toward the middle of gestation and tapering off near the end of gestation. Campbell et al. (1992) found amf albumin levels to be much lower during early gestation than those reported by Benzie et al. (1974). Given the large standard deviation reported in that study, it is possible that the

method used for analysis was not optimal in the level of precision it was able to detect. In late gestation, Emara et al. (1978) found higher levels than Benzie et al. (1974), likely due to the fact that the latter group excluded samples that were contaminated with blood or meconium. As with amf protein, amf albumin concentration falls at the end of gestation again possibly due to dilution (Benzie et al., 1974). The concentration of albumin expected in amf at 14-16 weeks' gestation is 3±2 g/L (Benzie et al., 1974).

Although amf albumin has not been studied with respect to fetal growth and b.wt, as the principle protein in amf such a relationship is worth exploring.

Urea Nitrogen:

Urea nitrogen is a measure of protein degradation. Fetal urine is the main source of amf urea nitrogen. As the fetus grows and more fetal urine is added to the amf, the concentration of urea increases (Moore and Ward, 1970). Amniotic fluid levels throughout gestation were greater than maternal serum values (Benzie et al., 1974).

Mean amf urea nitrogen concentrations of 2 to 11.42 mmol/L have been reported in the literature. Table 4 (p. 22) summarizes previous studies on human amf urea nitrogen levels throughout gestation.

Table 4. Amf urea nitrogen concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (mmol/L)
Jauniaux et al., 1991	17	5-13	7.2±2.1
Campbell et al., 1992	38	7-12	3.32±0.86
Gulbis et al., 1996	12	8-11	2 to 6[2]
Jauniaux et al., 1998	20	11-14	6.07±1.78
	12	12-16	7.50±1.78 – series
Benzie et al., 1974	27	15	3.86±0.96
	58	19	3.89±0.75
	5	22	3.43±1.46
	20	34	6.18±2.14
	53	40	6.32±1.64
Lind et al., 1971	12	≤30	8.21[3]
	14	31-34	7.50[3]
	9	35-37	8.92[3]
	29	≥38	11.42[3]
Bissenden et al., 1979	32	Term	11.4±3.2
	13	Term	10.2±3.0
Cherry et al., 1969	18	Unreported	4.60±1.78
Raghav et al.,1985	75	Unreported	8.82±2.47

[1] SD, standard deviation
[2] range
[3] no SD reported

The mean concentrations found by Jauniaux et al. (1991, 1998) during early gestation were larger than other reported values, however, the levels obtained in their studies may not accurately reflect population values given the small sample sizes used. In the articles by Cherry et al. (1969) and Raghav et al. (1985) the gestational age at which the amf samples were taken was not specified, however the levels found indicate that the samples were taken during the early-middle and late-middle periods of gestation, respectively. The concentration of urea nitrogen expected in amf at 14-16 weeks' gestation is 3.86±0.96 mmol/L (Benzie et al., 1974).

Amniotic fluid urea nitrogen has mainly been studied as a predictor of respiratory distress syndrome (Almeida and Kitay, 1988; Gluck et al., 1971). Urea nitrogen was negatively correlated with fetal wt in rats (Koski and Fergusson, 1992). In a study of European (n=32) and Asian (n=13) women, term amf urea nitrogen values were similar in pregnancies with poor and normal fetal growth (Bissenden et al., 1979).

Creatinine:

Creatinine is a measure of muscle protein degradation. Fetal urine is also the source of amf creatinine. As the fetus grows and more fetal urine is added to the amf, the concentration of creatinine increases (Moore and Ward, 1970). The concentration of creatinine becomes proportionately greater than urea as gestation advances. Amniotic fluid levels throughout gestation were greater than maternal serum values (Benzie et al., 1974).

Mean amf creatinine concentrations of 20 to 590 μmol/L have been reported in the literature. Table 5 (p. 23) summarizes previous studies on human amf creatinine levels throughout gestation.

Table 5. Amf creatinine concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (μmol/L)
Jauniaux et al., 1991	17	5-13	27.7±5.9
Campbell et al., 1992	40	7-12	37.1±12.0
Gulbis et al., 1996	12	8-11	20 to 80[2]
Jauniaux et al., 1998	20	11-14	35.36±8.84
	12	12-16	61.88±8.84 – series
Doran et al., 1970	31	13-29.5	85.75[3]
	53	30-34.5	123.76[3]
	47	>35	166.19[3]
Yong and Gui-Lan, 1982	31	<20	42.43±35.36
Benzie et al., 1974	29	15	70.72±8.84
	61	19	70.72±8.84
	5	22	70.72±8.84
	20	34	150.28±35.36
	55	40	194.48±44.20
Lind et al., 1971	12	≤30	79.56[3]
	14	31-34	114.92[3]
	9	35-37	141.44[3]
	29	≥38	185.64[3]
Emara et al., 1978	42	Term	189.86±39.53
Bissenden et al., 1979	32	Term	590.0±180.0
	13	Term	520.0±90.0
Cherry et al., 1969	18	Unreported	167.96±132.60
Raghav et al.,1985	75	Unreported	175.92±38.28

[1] SD, standard deviation
[2] range
[3] no SD reported

The unusually high levels of amf creatinine reported by Bissenden et al. (1979) as compared to other reported values leads one to doubt the results of that study. The fact that they focused on the European and Asian populations specifically could be one explanation for such results. While nothing uniquely different about those populations is known, it is suggested that it may not be appropriate to compare the levels with the general population. Once again in the studies by Cherry et al. (1969) and Raghav et al. (1985), the levels found indicate that the amf was taken during the early-middle and late-middle periods of gestation, respectively. The concentration of creatinine expected in amf at 14-16 weeks' gestation is 70.72±8.84 μmol/L (Benzie et al., 1974).

Many studies looking at the relationship between amf creatinine and fetal maturity and/or b.wt have been done. Of the 10 human studies that have been conducted, 7 found a significant positive correlation between amf creatinine concentration and b.wt (Begneaud et al., 1969; Doran et al., 1970; Miodovnik et al., 1982; Roopnarinesingh, 1970; Weiss et al., 1974; Wyatt et al., 1969; Yong and Gui-Lan, 1982); only the study by Miodovnik et al. (1982) controlled for gestational age of the newborn. The remaining 3 studies found no correlation between the two (Bissenden et al., 1979; Cassady et al., 1975; Williams et al., 1981); all controlled for gestational age. Amniotic fluid creatinine progressively increased with increasing b.wt in a study of 130 pregnant women, however, it was not found to be predictive of b.wt (Yong and Gui-Lan, 1982). In a study by Weiss et al. (1974), changes in amf creatinine from 10 weeks' gestation to term (n=135) were correlated with b.wt up to 2500g ($r=0.783$, $p<0.001$). In b.wts greater than 2500g there was no relationship. Amniotic fluid creatinine concentrations of <132.6 μmol/L were indicative of b.wts of less than 2500g, and amf levels of ≥132.6 μmol/L were indicative of b.wts of 2500g or more, in mature fetuses, with a sensitivity of 91% in a study of 82 newborns delivered within 1 or 2 days after samples were obtained (Wyatt et al., 1969).

Doran et al. (1970) found a correlation coefficient of 0.66 (no p-value reported) between amf creatinine, taken within one week of delivery, and b.wt (n=47). A statistically significant correlation ($p<0.001$) was found between amf creatinine taken within 3 days of delivery (n=113) and b.wt (Begneaud et al., 1969). Roopnarinesingh (1970) found a significant correlation ($p<0.01$) between b.wt and amf creatinine concentration at the time of delivery in 87 pregnancies. Amniotic fluid 3-methyl histidine

to creatinine molar ratio, within 6 days of delivery, was significantly different ($p \leq 0.001$) between pregnancies resulting in newborns with b.wts $<10^{th}$ percentile (n=15) and pregnancies resulting in either b.wts $>10^{th}$ but $\leq 25^{th}$ percentile (n=7), or b.wts $>25^{th}$ but $\leq 75^{th}$ percentile (n=20; Miodovnik et al., 1982).

Alternatively, no correlation was found between amf creatinine taken within 3 days of delivery and b.wt (n=19; Williams et al., 1981). In a study of European (n=32) and Asian (n=13) women, amf levels of creatinine at term were found to be similar in groups carrying light-for-dates babies and those carrying normally grown babies (Bissenden et al., 1979). Cassady et al. (1975) found no difference between amf creatinine levels taken at >28 weeks' gestation from diabetic women whose pregnancies resulted in macrosomic (n=65) and growth retarded newborns (n=36).

Uric Acid:

Amniotic fluid uric acid is of fetal origin. As the fetus grows and more fetal urine is added to the amf, the concentration of uric acid increases (Weiss et al., 1974). Amniotic fluid levels throughout gestation were greater than maternal serum values (Benzie et al., 1974).

Mean amf uric acid concentrations of 237.92 to 1500 μmol/L have been reported in the literature. Table 6 (p. 26) summarizes previous studies on human amf uric acid levels throughout gestation.

Table 6. Amf uric acid concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x} \pm$ SD (μmol/L)
Doran et al., 1970	22	13-29.5	302.75[2]
	33	30-34.5	359.85[2]
	26	>35	381.86[2]
Benzie et al., 1974	28	15	237.92±59.48
	60	19	249.82±59.48
	5	22	321.19±71.38
	19	34	547.22±220.08
	53	40	618.59±178.44
Marks et al., 1968	23	Third trimester	342.01±13.68
Bissenden et al., 1979	32	Term	1 470.0±390.0
	13	Term	1 500.0±280.0
Cherry et al., 1969	18	Unknown	344.98±71.38

[1] SD, standard deviation
[2] no SD reported

The unusually high levels of amf uric acid reported by Bissenden et al. (1979) as compared to other reported values once again leads one to doubt their results. The fact that they focused on the European and Asian populations specifically could be an explanation for this difference. As previously mentioned, although nothing uniquely different is known about those populations, it may not be appropriate to compare the levels with the general population. The indication that the amf was taken during the early to mid period of gestation in the study by Cherry et al. (1969) is again affirmed by the levels of uric acid found. The concentration of uric acid expected in amf at 14-16 weeks' gestation is 237.92±59.48 μmol/L (Benzie et al., 1974).

Three studies have examined the relationship between amf uric acid and b.wt. Two studies found a negative correlation, one in animals (Koski and Fergusson, 1992), the other in humans (Bissenden et al., 1979), and the third study found a positive correlation (Weiss et al., 1974). Amniotic fluid uric acid was negatively correlated with fetal wt in rats at term (Koski and Fergusson, 1992). In a study of European (n=32) and Asian (n=13) women, term amf uric acid was significantly higher in women carrying light-for-dates babies (Bissenden et al., 1979). In a study by Weiss et al., (1974), amf uric acid concentration, taken between the 10th week of gestation and term, correlated with b.wt ($r=0.728$, $p<0.001$) up to 2500g (n=111). In newborns greater than 2500g no correlation was found.

Glucose:

The source of glucose in the amf is not known, though fetal urine is likely one source (Weiss et al., 1985). As the fetal kidneys mature, perhaps more glucose is resorbed leaving less in the amf. Amniotic fluid glucose concentration was positively correlated with maternal blood glucose level (Bai et al., 1969; Spellacy et al., 1973; Weiss et al., 1985; Wood et al., 1963). Glucose freely passes the placental barrier by facilitated diffusion and is used by the placenta and the fetus (Spellacy et al., 1973). The concentration was significantly higher in women with diabetes than in women without diabetes (Fallucca et al., 1995; Pederson, 1954; Spellacy et al., 1973; Weiss et al., 1985). With higher blood glucose concentrations crossing the placenta in maternal diabetes, the fetal kidney's threshold for reabsorption is probably exceeded, therefore, more is excreted through the urine and more appears in the amf.

Mean amf glucose concentrations of 0.69 to 3.20 mmol/L have been reported in the literature. Table 7 (p. 27) summarizes previous studies on human amf glucose levels throughout gestation.

Table 7. Amf glucose concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (mmol/L)
Campbell et al., 1992	26	7-12	3.20±0.51
Jauniaux et al., 1994	24	7-14	2.8±0.5
Weiss et al., 1985	26	14/15	2.49±0.66
	428	16/17	2.55±0.54
	537	18/19	2.45±0.51
	117	20/21	2.29±0.58
Benzie et al., 1974	27	15	2.60±0.52
	58	19	2.51±0.57
	5	22	2.02±0.49
	19	34	2.76±1.86
	51	40	1.79±1.19
Saleh et al., 1989	4	Third trimester	1.04±0.51
Seeds et al., 1979	40	Late third trimester	0.69±0.36

[1] SD, standard deviation

The concentration of glucose decreases as pregnancy progresses (Anteby et al., 1973; Bai et al., 1969; Marin and Hood, 1979; Schmid et al., 1969; Schreiner and Schmid, 1969; Spellacy et al., 1973), rising slightly between the 14-17th week of

gestation, and decreasing at the end of pregnancy (Weiss et al., 1985), with a minor increase at 34 weeks' (Benzie et al., 1974). The concentration of glucose expected in amf at 14-16 weeks' gestation is 2.60±0.52 mmol/L (Benzie et al., 1974).

All 4 studies done linking amf glucose to b.wt found a positive relationship; 2 studies were done in animals (Koski and Fergusson, 1992; Mulvihill et al., 1985a), the other 2 in humans (Drazancic and Kuvacic, 1974; Marin and Hood, 1979). Lower levels of human amf glucose in late pregnancy were found in association with fetal growth retardation (Drazancic and Kuvacic, 1974; Marin and Hood, 1979). In rats, amf glucose was shown to be positively associated with and predictive at term of fetal wt (Koski and Fergusson, 1992).

Continuous infusion of nutrient solutions of dextrose and amino acids intraamnioticially during the third trimester in fetal rabbits resulted in significantly higher fetal body, liver, and brain wts, crown-rump length, and brain protein (Mulvihill et al., 1985a). Amniotic fluid, serum, and gastric content glucose levels were significantly higher and a linear relationship between non-protein calories administered and fetal growth was seen in infused fetuses.

β-hydroxybutyrate:

Beta-hydroxybutyrate, appears to pass unutilized through the fetus to the amf compartment (Saleh et al., 1989). Ketone bodies are formed in the liver as a by-product of lipolysis due to insufficient insulin, or as a consepuence of low glucose availability resulting from starvation (Mayes, 1993). As a consequence, ketone body synthesis occurs more frequently in people with diabetes. The ketone body, ßHBA, is formed, in a reversible reaction, from acetoacetate in the presence of the enzyme, ßHBA dehydrogenase (Mayes, 1993). Alternately, acetoacetate is converted to acetone in a non-reversible, non-enzymatic reaction (Mayes, 1993).

Mean amf ßHBA concentrations of 50 to 190 µmol/L have been reported in the literature. Table 8 (p. 29) summarizes previous studies on human amf ßHBA levels during late gestation.

Table 8. Amf β-hydroxybutyrate concentration in humans during late gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (μmol/L)
Saleh et al., 1989	4	Third trimester	50±30
Seeds et al., 1979	25	Late third trimester	190±230

[1] SD, standard deviation

Amniotic fluid ßHBA has been studied in connection with glucose in diabetic pregnancies. Beta-hydroxybutyrate has not been studied with respect to fetal growth, but given its link with glucose utilization, it seems plausible that a link would exist between amf levels and b.wt, if only as a result of poor glucose metabolism.

Lactate:

Although the presence of lactate has been attributed mainly to anaerobic metabolism by the fetus, either during gestation or during delivery (Hendricks, 1957), the possibility of maternal origin has also been suggested (Derom, 1964; Rooth and Nilsson, 1964; Vedra, 1959) since high levels have been found in the first and second trimesters of pregnancy, before fetal urine could make a significant contribution (Scheiner and Gubler, 1963).

Mean amf lactate concentrations of 0.9 to 8.84 mmol/L have been reported in the literature. Table 9 (p. 29) summarizes previous studies on human amf lactate levels throughout gestation.

Table 9. Amf lactate concentration in humans at different weeks of gestation[1]

Reference	N	Weeks' gestation	$\bar{x}$ ± SD (mmol/L)
Jauniaux et al., 1994	24	7-14	0.9±0.2
Otey et al., 1967	12	16-44	8.84±5.65
Seeds et al., 1979	33	Late third trimester	8.6±2.3
Richey et al.,1995	28	Labor	3.37±8.34
Daniel et al., 1966	11	Labor	4.14[2]

[1] SD, standard deviation
[2] no SD reported

Lactate, as a marker of anaerobic metabolism, has been looked at in the amf mainly with respect to fetal asphyxia, namely that higher amf concentrations are associated with oxygen deprivation (Pietz et al., 1988; Ruth et al., 1988a, b). Only one study has looked at amf lactate in relation to b.wt (Koski and Fergusson, 1992). In their

rat model, amf lactate was not found to predict b.wt.

Summary

Positive associations have been shown between b.wt and amf creatinine, uric acid, and glucose. Other associations are not as clear or well defined as yet, therefore, further investigation of amf constituents during gestation is needed in order to determine whether such factors serve a role in providing an indication of fetal growth and b.wt or not. As greater findings in this field occur it can be foretold that the role of amniocentesis will increase.

5. Amniocentesis

Amniocentesis involves the removal of a small quantity of amf. It was first advocated as a diagnostic technique by Meness et al. in 1930 (Fairweather, 1978). Today, amniocentesis is performed most commonly during the 14-16 week period of gestation in order to determine fetal karyotype. Indications for such testing are: advanced maternal age (over 35 years), previous child with chromosome abnormality (including Down's syndrome), structural chromosomal abnormality in the parents, family history of chromosome disorder, previous child with neural tube defect, previous child with metabolic disorder, high maternal serum alpha-fetoprotein value, and genetic diseases such as Meckel syndrome, hemophilia, etc. (Gosden et al., 1981). In a later stage of pregnancy, 35-37 weeks' gestation, amniocentesis is done to assess rh-sensitized pregnancies or fetal lung maturation in women with gestational diabetes, for example (Bennett, 1981).

At present, analysis of specific constituents of amf has proven useful in a variety of situations, including detection of neural tube defects, chromosomal abnormalities, anemia, infection, inborn errors of metabolism, and lung function. Fibroblasts (fetal cells), cultured and arrested in metaphase, provide accurate chromosomal diagnoses (Chard and Macintosh, 1995; Chen et al., 1996; Dick, 1996; Groli et al., 1996; Ritchie, 1995).

The concentration of alpha-fetoprotein, acetylcholinesterase and the presence of rapidly adhering cells has been linked to open neural tube defects (Allen et al., 1996; Drugan et al., 1996; Ritchie, 1995). Where amf alpha-fetoprotein concentration is elevated early in the gestational period, a number of fetal abnormalities have been observed in addition to open spina bifida, such as anencephaly, intrauterine death, and Turner's syndrome (Brock, 1977). A number of other amf constituents have been measured with respect to detection of spina bifida and anencephaly (Pettit et al.. 1979). Mean amf calcium concentration was 35% higher ($p<0.05$) in spina bifida as compared with anencephaly. Higher concentrations of inorganic phosphate were observed in anencephaly ($p<0.001$) and a decreasing trend was found in spina bifida. Levels of protein were higher in anencephaly patients than in either controls ($p<0.005$) or spina bifida patients ($p<0.05$). Higher amf osmolality was observed in patients with spina bifida as compared to controls ($p<0.025$) or to patients with anencephaly ($p<0.05$). The residual contributions to osmolality by sodium, potassium, and glucose were higher in amf of pregnancies resulting in fetal spina bifida than in either controls or those resulting in fetal anencephaly ($p<0.001$). Combining these measures with the use of amf alpha-fetoprotein would provide for a better diagnostic tool for neural tube defects (Pettit et al., 1979).

Levels of amf bilirubin can provide an indirect measure of fetal red cell breakdown and thus anemia (Ritchie, 1995). The presence of white cells and bacteria has been useful in the diagnosis of chorioamnionitis (Ritchie, 1995). For the detection of inborn errors of metabolism, a number of prenatal diagnostic tests have been successful requiring a relatively small culture of amf cells (Bennett, 1981; Crawford, 1989). Along with others, they include diseases involving lipid metabolism, mucopolysaccharidoses and related disorders, amino acid metabolism, carbohydrate metabolism, and blood (Bennett, 1981; Crawford, 1989). Lecithin/sphingomyelin (L/S) ratio, as well as other amf measures, are good indicators of lung maturation and subsequent likelihood of the newborn to develop respiratory distress (Dubin, 1992; Gluck et al., 1971; Ritchie, 1995).

Amniocentesis has not been specifically used as an indicator of fetal growth as yet. There is, however, as mentioned earlier, a growing body of literature linking different concentrations of specific nutrients and metabolic indicators found in the amf to

the growth of the fetus, suggesting a potential important future role.

Summary

Much evidence exists about the potential role of amf nutrients and metabolic indicators in fetal growth and development. Current research indicates that a positive association exists between b.wt and amf glucose, creatinine, and uric acid up to a b.wt of approximately 2500g. The body of literature presented supports further investigation of amf levels of total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate at 14-16 weeks' gestation in relation to fetal growth abnormalities in the hopes of using such measurements as a means of early diagnosis of abnormal b.wt in the future. It is clear that other factors, such as infant gender, racial/ethnic origin, maternal ht, prepregnancy wt and wt gain during pregnancy, parity, cigarette, alcohol, and caffeine consumption must be taken into account when examining the effects of the amf constituents on fetal b.wt.

III. STATEMENT OF PURPOSE

1. Rationale

Deviations from the normal range of b.wts are associated with various risks, both to the mother and to the baby (Bernstein and Gabbe, 1996). Detection of abnormal b.wt is presently unreliable, especially early in gestation (Norman, 1982). Given the close proximity of the fetus to amf, it is a logical venue to pursue in search of an early diagnostic tool. Amniocentesis is routinely performed at 14-16 weeks' gestation in pregnant women 35 years of age and older, as well as in women with a history of genetic abnormality (Gosden et al., 1981). The procedure extracts more fluid than is needed for current genetic testing leaving open the possibility of further analysis on the fluid. The current body of literature about how early amf can serve as an indicator of fetal growth and b.wt has not yet been established. Past research suggests a strong positive link between fetal b.wt and amf creatinine (Yong and Gui-Lan, 1982) and glucose (Mulvihill et al., 1985a) in late gestation. Uric acid has been directly correlated with fetal b.wt up to 2500g in one human study (Weiss et al., 1974). Both amf uric acid and urea nitrogen have been negatively correlated with b.wt in rats during late gestation in another study (Koski and Fergusson, 1992). Amniotic fluid total protein and lactate have not been studied specifically in humans during early gestation with respect to fetal b.wt, and amf albumin (the principle protein in amf) and ßHBA have not yet been studied in relation to b.wt. The aim of this study is to examine the relation between varying levels of nutrients/metabolites in amf early in gestation and fetal b.wt. With the ability to foresee an undesirable b.wt, measures to prepare for the fetus' delivery and subsequent care could be taken, and/or antenatal care to prevent certain complications could occur.

2. Research Questions

1. Will measurements of amf constituents at 14-16 weeks' gestation be associated with or predictive of human fetal growth in utero?

2. Can any or all of these constituents be used to detect IUGR and/or macrosomia early in gestation?

3. Specific Objective & Hypotheses

The specific objective of this study is to determine whether the concentrations of amf total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate during early gestation will be associated with fetal b.wt in humans on a population basis. The a priori hypotheses being that:

- amf total protein, albumin, urea nitrogen, creatinine, uric acid, and glucose, at 14-16 weeks' gestation, will be positively associated with fetal b.wt and;
- amf ßHBA and lactate, at 14-16 weeks' gestation, will be negatively associated with fetal b.wt.

IV. MATERIALS AND METHODS

1. Experimental Design and Overview

This prospective study of 395 pregnant women was designed to elucidate the relationship between levels of amf total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate at 14-16 weeks' gestation and fetal b.wt.

Participants were recruited at the time of routine amniocentesis. A sample of the amf routinely collected at 14-16 weeks' gestation was obtained from women undergoing amniocentesis at the Royal Victoria, Lakeshore General (LGH), Jewish General, and St. Mary's Hospitals. Note that amniocentesis for patients at the LGH is done at the RVH, therefore recruitment at the RVH includes both hospitals. Levels of total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate were measured. After the delivery date, the participants' medical charts, located at the obstetric hospital where they gave birth, were reviewed to obtain pregnancy outcome-related information. Data about potential confounders was obtained both from a subject questionnaire given at the time of recruitment and through the medical charts.

2. Usual Protocol for Amniocentesis in the Montreal Area

The Montreal Children's Hospital (MCH) coordinates all prenatal testing for the Royal Victoria, Lakeshore General, Jewish General, and St. Mary's Hospitals. Approximately 2000 amniocentesis tests are performed each year in the Montreal area. The procedure is performed at the respective obstetric hospitals and the pregnant women bring their amf samples (~30cc in 2 tubes) to the MCH for analysis. The women must sign a consent form (Appendix 1, p. 102) allowing the hospital to do the analysis and confirming that the hospital will not distribute the fluid to a third party. The samples are centrifuged at 1000 rpm for 8 minutes to separate the cells, which are used for the genetic testing, from the fluid, which is discarded unless ultrasound results indicate the need for further testing. It is this portion of the amf that was used for this study, therefore additional consent was required in order to gain permission to access the sample.

3. Ethics Approval

Because the study involved more than one McGill teaching hospital it was necessary to obtain ethical approval first from McGill before the hospitals were approached. Having been accepted by McGill, approval at each hospital could be obtained either automatically (JGH), or from the chair of the board (RVH, LGH) if the hospital is represented on the McGill Ethics committee. therefore bypassing the committees at each hospital. In the event that the hospital is not represented on the McGill Ethics committee (MCH, SMH), the study would have to go before the hospital ethics committee for approval. Note that after McGill accepts the study protocol, the individual hospitals cannot make any major alterations to it, but can simply accept or refuse to allow the research to take place within their hospital.

University:

- *McGill University:*

Ethical approval was first obtained from the McGill Medical Ethics Committee which is part of the Institutional Review Board of the Faculty of Medicine. The committee meets on a monthly basis to review study protocols for approval. In order to reserve a time slot for the meeting, a registration form was sent to the ethics committee coordinator 2 months prior to the meeting. A ten-page proposal, including abstract, short literature review, expanded protocol with subject consent form (Appendix 2, p. 105) and questionnaire (Appendix 3, p. 110) was prepared. In addition, it was required that the study have a contact at the MCH who was aware of the study and the protocol. Dr. Louis Beaumier, M.D. was approached and accepted the role. Twenty copies of the proposal were submitted to Dr. Neil MacDonald, committee chair, and the committee members one month before the meeting that took place on January 26th, 1998. On February 12th, the committee's comments and revisions were received. Modifications and clarifications were made and the final approval letter was received on March 25th (Appendix 4, p. 113). With this letter of approval, the five hospitals implicated in the study protocol were contacted for approval.

Hospitals:

- *The Montreal Children's Hospital:*

When the secretary to the chair of the Ethics Committee at the MCH was contacted, it was made clear that the research protocol would require separate institutional review. A copy of the ten-page proposal with a letter from the coordinator of the genetics department at the MCH (the department directly involved in the research), and the approval letter from McGill, was submitted one month prior to the committee meeting that took place on April 27th, 1998. Approval was granted via a stamp that was placed on the bottom of the consent form (Appendix 2, p. 105).

- *The Royal Victoria Hospital:*

A copy of the ten-page research proposal and the letter of approval from McGill were submitted to the chair of Professional Services at the RVH. The committee did not have to meet and within 5 days, on April 14th, 1998, a letter of approval was received (Appendix 5, p. 116).

- *The Jewish General Hospital:*

Because approval from McGill was already obtained, the study received automatic approval from the JGH as confirmed on the telephone by the research/ethics coordinator.

- *St. Mary's Hospital:*

Since SMH is not represented on the McGill Medical Ethics Committee, the study protocol was submitted to the research coordinator to be approved by the committee 2 weeks prior to their meeting. Again, an on-site contact was required. Dr. Gary Luskey was approached and accepted the task. The ethics committee met on May 6th, 1998. A letter of approval (Appendix 6, p. 118) and stamped consent form (Appendix 7, p. 121) were received on May 27th.

- *The Lakeshore General Hospital:*

Approval at the LGH was not necessary for recruitment since recruitment for that hospital occurred at the RVH, but was needed for chart review after delivery. A copy of the ten-page research proposal along with the letter of approval from McGill were submitted to the Director of Professional Services. A letter of approval was received within one week, on July 29th, 1998 (Appendix 8, p. 126).

Confidentiality:

At no time during the study were the participants' names divulged to a third party. All information obtained about the participants, either from the subject questionnaires or the medical charts was kept confidential.

Once obtained, the study samples were aliquoted into several small tubes and labeled, so as not to be linked with the participant's name. Subsequent analyses were performed on the labeled samples. The name associated with the code was only used when reviewing the medical chart, after which time data obtained in this manner was likewise coded. The results of the analyses, as well as any information obtained from the chart was not discussed in association with the names of the parties involved. The researcher who had the list of codes with the names of the women was the same researcher who visited the hospitals at the time of amniocentesis and who reviewed the medical charts. This person is also a dietitian who is bound by a Professional Code of Ethics in terms of patient confidentiality.

4. Subject Recruitment

Four hundred and fifty subjects were recruited between April 30th and November 13th 1998. Since ethical approval at SMH had not yet been obtained, recruitment began at the RVH (LGH) and the JGH first. Recruitment at SMH began on May 28th, 1998.

All pregnant women undergoing amniocentesis and meeting the inclusion criteria of the study were asked to participate. The author initially visited the MCH twice weekly, in order to facilitate recruitment, answer questions, and meet face to face with the women. Mondays and Thursdays were chosen since they are the days that the most amniocentesis tests are done. Modifications were made after the first week and a half for

2 reasons: first, it was discovered that it was most often not the women themselves who were bringing the fluid to the MCH, but the spouse, friends, etc.; and second, the people bringing the fluid were in a rush to get back to their jobs, etc. Subsequently, the obstetric hospitals where the amniocentesis tests were being done were visited. This method was found to be much more efficient since it guaranteed that the women themselves would be seen. The time factor was also much more conducive to meet with the women, given that they were waiting for the amniocentesis and thus the author had the opportunity to explain the study, answer questions, etc.

At the JGH and the RVH, amniocentesis tests are performed any day from Monday to Friday. This made it impossible to meet with all the women undergoing amniocentesis at those 2 hospitals. As a consequence, each hospital was visited by the author according to the number of amniocentesis tests scheduled on a particular day.

The schedule of patients having amniocentesis at the JGH was obtained on a weekly basis either in person or on the telephone. The schedule of patients having amniocentesis at the RVH was obtained from the MCH on a weekly basis, however there were often great discrepancies between the schedule obtained and the actual appointments, therefore, that hospital was visited with less success.

Amniocentesis tests at SMH are only done on Thursday mornings, and thus the author was able to visit and meet with the women at that hospital with greater success.

In the author's absence, the secretaries at the different hospitals were responsible for giving the women undergoing amniocentesis the study cover letter (Appendix 9, p. 128), consent form, and questionnaire, along with the usual consent form for amniocentesis, which allows the MCH to do genetic testing. As per the cover letter, the women were instructed to bring the study's consent form to the MCH along with their fluid if they agreed to participate in the study. In cases where more information was required by the woman prior to agreeing to participate, the phone number of the author was provided and an address where the consent form could be mailed was given. Upon giving consent, the women were asked to fill out a short questionnaire in order to obtain some preliminary data for the study, such as maternal age, ht, prepregnancy wt, ethnic background, etc., as well as the name of the obstetric hospital and physician with which they were affiliated.

Participation Data:

A list of all women who underwent amniocentesis testing during the study period was obtained from the MCH. A list of eligible, but non-consenting women was made in order to generate information on the level of participation in the study. Table 10 (p. 41) summarizes that information.

Table 10. Participation rates by hospital

Hospital	Period of recruitment	Total Eligible N	Consenting N (%)	Nonconsenting N (%)
Royal Victoria	Apr. 30- Nov. 13, 1998	373	117 (31.4)	256 (68.4)
Lakeshore General	Apr. 30- Nov. 13, 1998	109	16 (14.7)	93 (85.3)
RVH incl. LGH[1]	Apr. 30- Nov. 13, 1998	482	133 (27.6)	349 (72.4)
Jewish General	Apr. 30- Nov. 13, 1998	254	150 (59.1)	104 (40.9)
St. Mary's	May 28- Nov. 13, 1998	172	167 (97.1)	5 (2.9)
Total	------	**908**	**450 (49.6)**	**458 (50.4)**

[1] sum total of subjects recruited at the RVH which includes subjects from the Lakeshore General Hospital, since LGH patients have amniocentesis at the RVH

The high level of participation at SMH is reflective of the fact that the author was able to meet with most of the women at that hospital.

5. Study Sample

Inclusion Criteria:

Subjects invited to participate in the study were pregnant females undergoing amniocentesis at 14-16 weeks' gestation at the Royal Victoria, Lakeshore General, Jewish General, or St. Mary's Hospitals, and who were 35 years of age or older at the time of delivery.

In view of seeking early detection of abnormal fetal growth, the research was conducted during the first period of routine amniocentesis, ie, 14-16 weeks' gestation and did not include women undergoing amniocentesis at >35 weeks'. Ninety to ninety-five percent of the profile of the women undergoing amniocentesis at 14-16 weeks' gestation is represented by advanced maternal age (≥35 years at the time of delivery). History of chromosome abnormality, in a previous child (eg, Down's syndrome), in the parents (eg, structural chromosomal abnormality), a family history of chromosome disorder, a previous child with neural tube defect, with metabolic disorder, a high maternal serum alpha-fetoprotein value (indicative of neural tube defect), and genetic diseases such as Meckel syndrome, hemophilia, etc. represent 5-10% of the profile of the women. This small percentage of women was excluded from the study on the basis that these factors may interfere with the results of the study since the constituents of interest may act differently in those specific cases and/or such conditions may alter b.wt.

All women carrying more than one fetus were excluded from the study given the inherent difficulty in measuring the effects of the various levels of the constituents on a single fetus and that b.wts are lower in this population of babies (Corney et al., 1981; Keith et al., 1980; Yarkoni et al., 1987).

Women with gestational diabetes were expected to have higher amf glucose values, and thus were not excluded from the study in the interest of not limiting the range of amf glucose concentration.

Post hoc exclusions were made based on the following: i) genetic abnormality

revealed through amniocentesis (n=47); ii) known presence of maternal diseases, such as human immunodeficiency virus (HIV) or hepatitis (n=3); iii) discovery of a multi-fetus pregnancy (n=5); and iv) samples that were mishandled, lost, broken, or not in sufficient quantity for analysis (n=0-60). Between 335 and 395 amf samples were therefore available for analyses. Due to missing or unavailable chart information (sometimes due to the fact that the woman delivered at another hospital or center; n=33) and pregnancies that resulted in miscarriages or abortions (n=9), outcome measurements were available for 353 of the 395 participants.

6. Experimental Measures

Collection and Storage of Amniotic Fluid:

Amniotic fluid samples for which consent was obtained were collected on a monthly basis from the MCH. They were transported in a cooler full of dry ice to the laboratory (~25min.) and immediately placed in a freezer where they were stored at -80°C until analysis. It is important to note that due to the fact that the fluid was transported from the hospital where amniocentesis took place to the MCH by the women themselves, it was difficult to control the length of time and temperature of this arm of the transportation procedure. While metabolic changes could have occurred during this time period, the magnitude of such changes is unknown and difficult to ascertain at this time. The volume of sample obtained depended on the volume taken at amniocentesis, but ranged from 5 to 20cc. They were labeled with the participant's name and arranged by date of amniocentesis.

All samples were thawed once on ice, vortexed, and aliquoted into 1.3 mL samples placed in 1.5 mL Eppendorf tubes. This was done 36 samples at a time and the fluid was always in an ice water bath. The tubes were coded at this time in numerical order by sample thawed. The fluid was immediately re-frozen at -80°C until analyzed.

Biochemical Analyses:

Sensitivity and specificity of the methods were verified for all the biochemical analyses done. Measurements were taken within the linear portion of the standard curves

of the kits used, and all procedures were performed under optimal conditions as outlined in the package inserts. All analyses were done in duplicate or triplicate as a marker of precision, and control solutions were used to assess accuracy.

- *Total Protein:*

Amniotic fluid total protein was analyzed using a Dionex MR5000/7000 Microplate Reader with the use of a microprotein-PR Sigma diagnostic kit (Procedure No. 611) which is a modification of the method of Fujita et al. (1983). The reagent was supplied ready for use, was stored at 2-8°C, and discarded after the expiration date shown on the label.

The chemical procedure is based upon measuring the shift in the absorption that occurs when pyrogallol red-molybdate complex binds basic amino acid groups of protein molecules. The increase in absorbance at 600nm is directly proportional to protein concentration in the sample. The manual procedure described in the package insert was adapted in order to decrease the reagent and sample volumes to fit into the microtiter plate wells for analysis on the microplate reader. The analysis was carried out at room temperature according to insert recommendations. The procedure is linear between protein levels of 0.01-1 g/L. The level of amf total protein was expected to be around 5±2 g/L, as reported in the literature (Benzie et al., 1974), therefore, the samples were diluted with 0.85% physiological saline in order to bring the measured levels into the linear portion of the standard curve. The concentrations of the standard solutions used were 0.15 g/L, 0.30 g/L, 0.50 g/L, 0.75 g/L, and 1.0 g/L.

- *Albumin:*

Amniotic fluid albumin was analyzed using a Dionex MR5000/7000 Microplate Reader with the use of an albumin (BCP) Sigma diagnostic kit (Procedure No. 625) which is a modification of the method of Pinnell and Northam (1978). The reagent was supplied ready for use, was stored at room temperature, and discarded after the expiration date shown on the label.

The chemical procedure is based upon the formation of a blue-purple complex when albumin reacts specifically with bromcresol purple (BCP). The intensity of the color at an

absorbance maximum of 600nm is proportional to the albumin concentration in the sample. The manual procedure described in the package insert was adapted in order to decrease the reagent and sample volumes to fit into the microtiter plate wells for analysis on the microplate reader. The analysis was carried out at room temperature according to insert recommendations. The procedure is linear up to albumin levels of 60 g/L. The level of amf albumin was expected to be around 3±2 g/L, as reported in the literature (Benzie et al., 1974). The concentrations of the standard solutions used were 1.0 g/L, 1.5 g/L, 2.5 g/L, 5.0 g/L, 7.5 g/L, 10.0 g/L, and 12.5 g/L.

- *Urea Nitrogen:*

Amniotic fluid urea nitrogen was analyzed on the Abbott Discrete VP Analyzer with the use of a diagnostic A-gent Reagent BUN (blood urea nitrogen) kit made by Abbott Laboratories (List No. 6007-03) which is a modification of the original method of Talke and Schubert (1965). The reagent was kept at room temperature until it was reconstituted with distilled water for use, after which it was kept at 2-8°C for no more than 3 days.

The chemical principle of the assay is based on the splitting of urea into ammonia and carbon dioxide by urease. The ammonia formed then combines with alpha-ketoglutarate in the presence of glutamic dehydrogenase and reduced NAD (nicotinamide adenine dinucleotide) to yield glutamate and NAD. The conversion of NADH to NAD at 37°C is measured and quantitatively related to the amount of ammonia formed, which is, in turn, quantitatively related to the amount of urea initially present. Absorbance is read at 340nm. The procedure is linear up to urea nitrogen levels of 24.99 mmol/L. The level of amf urea nitrogen was expected to be around 3.86±0.96 mmol/L, as reported in the literature (Benzie et al., 1974). The concentrations of the standard solutions used were 3.57 mmol/L and 17.85 mmol/L.

- *Creatinine*

Amniotic fluid creatinine was analyzed using a Dionex MR5000/7000 Microplate Reader with the use of a Stanbio Creatinine diagnostic kit (Procedure No. 0400) which is based on the Jaffé method (Jaffé, 1986). The reagents were supplied ready for use, stored at room temperature, and were discarded after the expiration date shown on the label.

The chemical procedure is based upon the formation of a reddish-brown complex when creatinine, in a picric acid protein-free solution, reacts with added alkali. The intensity of the color at an absorbance of 520nm is proportional to the creatinine concentration in the sample. The samples were deproteinized by mixing them with excess picric acid and centrifuging at high speed (12000 rpm for 5min.) until a clear fluid was obtained. The manual procedure described in the package insert was adapted in order to decrease the reagent and sample volumes to fit into the microtiter plate wells for analysis on the microplate reader. The analysis was carried out at room temperature according to insert recommendations. The procedure is linear up to creatinine levels of 884 µmol/L. The level of amf creatinine was expected to be around 70.72±8.84 µmol/L, as reported in the literature (Benzie et al., 1974). The concentrations of the standard solutions used were 44.2 µmol/L, 88.4 µmol/L, 110.5 µmol/L, 221.0 µmol/L, and 442.0 µmol/L.

- *Uric Acid*

Amniotic fluid uric acid was analyzed on the Abbott Discrete VP Analyzer with the use of a diagnostic A-gent Reagent Uric Acid kit made by Abbott Laboratories (List No. 6184-01) which is based on the forward uricase reaction. The reagent was kept at 2-8°C and was reconstituted with a supplied buffer solution before use, after which it was kept for no more than 7 days.

The chemical principles of the assay are based on the oxidation of uric acid by bacteria uricase to yield allantoin and peroxide. The peroxide is then reduced by ethanol in the presence of catalase to form acetaldehyde and water. The reagent works by measuring the degree of oxidation of NADH to NAD at 30°C in the presence of acetaldehyde and alcohol dehydrogenase. Absorbance is read at 340nm. The procedure is linear up to uric acid levels of 713.76 µmol/L. The level of amf uric acid was expected to be around 237.92±59.48 µmol/L, as reported in the literature (Benzie et al., 1974). The concentrations of the standard solutions used were 237.92 µmol/L and 535.32 µmol/L.

- *Glucose:*

Amniotic fluid glucose was analyzed on the Abbott Discrete VP Analyzer with the use of a diagnostic A-gent Reagent Glucose-UV kit made by Abbott Laboratories (List

No. 6082-03) which is a modification of the method described by Richterich and Dauwalder (1971). The reagent was kept at room temperature until reconstituted with distilled water for use, after which it was kept at 2-8°C for no more than 7 days.

The chemical principles of the assay are based on the phosphorylation of glucose by hexokinase and excess adenosine triphosphate (ATP) in the presence of magnesium ions. The reagent works by measuring the degree of reduction of NAD to NADH at 37°C during the subsequent oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase. Absorbance is read at 340nm. The procedure is linear up to glucose levels of 38.86 mmol/L. The level of amf glucose was expected to be around 2.49±0.66 mmol/L, as reported in the literature (Weiss et al., 1985). The concentrations of the standard solutions used were 5.551 mmol/L and 27.755 mmol/L.

- *β-hydroxybutyrate:*

Amniotic fluid ßHBA was analyzed on the Abbott Discrete VP Analyzer with the use of a ßHBA Sigma diagnostic kit (Procedure No. 310-UV) which is based on the method initially described by Williamson et al. (1962). The reagent was kept at 2-8°C. After being reconstituted with distilled water, the reagent was stored at 2-8°C for no more than 7 days.

The chemical principle of the assay is based on the oxidation of ßHBA to acetoacetate in the presence of ßHBA dehydrogenase. The reagent measures the reduction of NAD to NADH at 37°C. Absorbance is read at 340nm. The procedure is first run without the enzyme and then run in the presence of the enzyme. The concentration of ßHBA is thus calculated as the difference between the value in the presence of the enzyme and the value in the absence of the enzyme. The procedure is linear up to ßHBA levels of 7684 µmol/L. The level of amf ßHBA was expected to be between 50±30 µmol/L and 190±230 µmol/L, as reported in the literature (Saleh et al., 1989; Seeds et al., 1979). The assay uses a calibrator solution that has a concentration of 4800 µmol/L.

- *Lactate*

Amniotic fluid lactate was analyzed on the Abbott Discrete VP Analyzer with the use of a Lactate Sigma diagnostic kit (Procedure No. 735-10) which is based on the

conversion of lactic acid to pyruvate and hydrogen peroxide by lactate oxidase. The reagent was kept at 2-8°C. After being reconstituted with distilled water, the reagent was stored at 2-8°C for no more than 7 days.

The chemical principle of the assay is based on the oxidative condensation of chromogen precursors in the presence of hydrogen peroxide and peroxidase. The reagent measures the intensity of the color produced at an absorption maximum of 540nm. The reaction is carried out at 30°C. The procedure is linear up to lactate levels of 13.32 mmol/L. The level of amf lactate was expected to be around 8.84±5.65 mmol/L, as reported in the literature (Otey et al., 1967). The concentrations of the standard solutions used were 2.22 mmol/L and 8.88 mmol/L.

Questionnaire Information and Chart Review:

A one-page subject questionnaire (Appendix 3, p. 110) was given with the consent form of the study and was filled out at the time of recruitment. Each obstetrical hospital was visited monthly to review the medical charts of the participants who had delivered. A form (Appendix 10, p. 131) was developed and used to expedite this process and to increase consistency of information obtained from each chart. Information gathered via these two methods include parameters that may have an effect on fetal growth and b.wt. Birth wt has been shown to be higher among taller women and women with higher prepregnancy wt, with parity, male gender, certain racial/ethnic origins, etc. (Kramer, 1987; Molteni, 1984). As such, these factors must be considered in terms of potential confounders of the study. Information was divided into 3 categories: maternal data, antenatal data, and neonatal data.

- *Maternal Data:*

The subject questionnaire and the participant's medical chart were used to obtain maternal information that may affect fetal growth and b.wt. Prepregnancy wt, last wt predelivery, maternal ht, ethnic origin, parity, cigarette, alcohol, caffeine, and drug use during pregnancy, history of genetic abnormality, and information about maternal chronic diseases were the parameters gathered. Maternal date of birth was collected from the questionnaire in order to access eligibility, and from the medical chart as a cross

reference. The participant's postal code was obtained from the medical chart for use in deducing socioeconomic status information. Prepregnancy body mass index (BMI) was calculated as prepregnancy wt divided by maternal ht squared. When measured ht and wts were not available, self reported information was used, however, it was not thought to have an impact on the quality of the data; on average, little discrepancy was found between self-reported and measured wt and ht in large samples of the U.S. (Rowland, 1990) and Finnish (Jalkanen et al., 1987) populations.

- *Antenatal Data:*

The participant's medical chart was used to obtain antenatal information related to fetal growth and b.wt. Number of weeks' gestation at amniocentesis (by ultrasound), information about hospitalizations during the pregnancy, Rh compatibility, and method of parturition (spontaneous or induced / vaginal or cesarean section) were the parameters gathered.

- *Neonatal Data:*

The participant's medical chart was used to obtain neonatal data related to fetal growth and b.wt. Gestational age at birth, neonatal gender, b.wt, birth length, placental wt, APGAR score (at 1min., 5min., and 10min.), and condition at birth were the parameters gathered. Only the charts at the RVH included information about birth length and placental wt.

7. Sample Size

Different methods for calculating a required sample size are appropriate depending on the statistics of interest and the method of calculation. They include the following:

Correlation analysis:

On the basis that correlations of less than 0.4 are not clinically useful or meaningful, 75 samples are required for such an analysis using a two-tailed α=0.05 and β=0.10 and

accounting for a 20% dropout rate (Browner et al., 1988).

Multiple regression:

1. Tables 11 and 12 (p. 50) were derived using the formula:

$$N = \frac{\sigma^2 (Q_e^{-1} + Q_c^{-1}) (Z_\alpha + Z_\beta)^2}{\mu_1^2}$$, previously described by Lachin (1981)

where Q_e and Q_c: are sample fractions, ie, proportions of subjects in each group

Z_α = 1.96 (α=0.05, two-sided)

Z_β = 1.282 (90% chance of detection)

μ_1: minimal relevant difference to be detected

and a 3% incidence of IUGR[1].

Table 11. Calculated sample sizes required by constituent based on literature values

Amf constituent	σ	μ_1	**N**
Total protein (g/L)	2	2	**362**
Albumin (g/L)	2	1	**1445**
Urea nitrogen (mmol/L)	0.96	1	**333**
Creatinine (µmol/L)	8.84	25	**46**
Uric acid (µmol/L)	59.48	40	**799**
Glucose (mmol/L)	0.66	1	**158**
ßHBA (µmol/L)	30	20	**813**
Lactate (mmol/L)	5.65	2	**2883**

Table 12. Calculated sample sizes required by constituent based on analysis of a preliminary set of 180 samples

Amf constituent	σ	μ_1	**N**
Total protein (g/L)	1.4	2	**177**
Albumin (g/L)	1.2	1	**521**
Urea nitrogen (mmol/L)	0.89	1	**287**
Glucose (mmol/L)	0.59	1	**126**
ßHBA (µmol/L)	65.3	20	**3851**

2. One-hundred-and-eighty samples are required using the rule of thumb for regression analysis of having 10 subjects per variable in the model with an estimated 15 variables (8 amf constituents and study confounders) and accounting for a 20% dropout rate.

[1] At an incidence of 3%, and with a sample size of 400, approximately 12 infants with IUGR are expected. Since we are looking at b.wt as a continuous variable, even if by chance no babies with IUGR/MACROSOMIA are obtained, the data will nonetheless be worthwhile in terms of viewing levels of constituents along a growth curve.

8. Statistical Analyses

Statistical analyses was carried out using the SAS computer program, version 6.12. A p-value < 0.05 was used as a marker of statistical significance. Normality of the data was assessed using the Kolmogorov-Smirnov Normality Test, which is based on the skewness and kurtosis of the data as compared to the normal distribution (Snedecor and Cochran, 1976). Bartlett's Test was used to assess homogeneity of variances (Steel and Torrie, 1980). The analyses included the following:

- *Descriptive Analyses:*

A discussion of the source of the data used, as well as a description of maternal and neonatal characteristics was outlined. The mean concentrations of measured amf constituents was calculated. A discussion of how the concentrations and the b.wts differed by maternal and infant parameters using Student's t-test and one-way ANOVA was included.

- *Correlation Analyses*:

Each amf constituent was correlated with b.wt individually to determine which are related to b.wt. In order to determine which constituents are significantly related to each other to allow for the use of an appropriate model for regression analysis, the amf constituents were correlated with each other. The amf constituents were also correlated with the continuous maternal characteristics (ht, prepregnancy wt and BMI). Pearson correlations were used for normal and normalized data. Spearman correlations were used in cases where the data could not be normalized.

- *Multiple Regression:*

Multiple regression was used to assess the association between the non-correlated constituents and both b.wt and percentile of b.wt by gestational age. The models used were: *b.wt* = Δ *amf constituents* + *study confounders* and *percentile b.wt* = Δ *amf constituents* + *study confounders*.

V. RESULTS

1. Descriptive Analyses

Questionnaire vs Chart obtained Information:

Since some maternal data gathered was obtained through the questionnaires given at the time of recruitment and via the medical charts after delivery, the following section describes measures taken in cases where there were discrepancies between the two sources and in cases of missing data from one source. Table 13 (p. 53) summarizes this information. Decisions on what data source to use were made based on whether or not the author was present at the time that the questionnaires were filled, and on the plausibility and completeness of the information.

- The information on maternal ht was different in 30 participants, in all cases the chart data were used.

- For prepregnancy wt the information obtained from the questionnaire was different from the information obtained from the chart in 117 instances. In 113 instances the data from the questionnaires were used and in 4 instances the data from the charts were used. In 3 cases in which questionnaire information was missing and chart information was not, the chart information was used.

- There were 9 differences in the information obtained from the questionnaires versus the charts with respect to parity. In all cases the information from the questionnaires was used. In 2 cases in which questionnaire information was missing and chart information was available, the chart information was used.

- For ethnic origin, the information obtained from the questionnaire was different from the information in the chart in 16 instances. In 10 cases the information from the questionnaire was used, and in the remaining 6 cases the information from the chart was used.

- There were 4 instances in which the information on smoking was different between the questionnaires and the charts. In 1 case the questionnaire data was used, and in 3 cases the data from the charts were used.

Table 13. Summary of the source of maternal information in cases of differences between the data in pregnant women[1]

	No. of Differences	Questionnaire used	Medical Chart used	Missing quest. info. – Med. chart used
Maternal ht	30	0	30	---
Prepreg. wt	117	113	4	3
Parity	9	9	0	2
Ethnic origin	16	10	6	---
Smoking	4	1	3	---

[1] prepreg., prepregnancy; quest., questionnaire; info., information; med., medical

Maternal Characteristics:

Table 14 (p. 54) summarizes age, ht, and wt data of maternal participants.

Table 14. Age, height, and weight of maternal participants[1]

	N	$\bar{x} \pm SD$	min.	max.	median	25th %	75th %
Age (years)	386	38.1±2.4	35.0	46.0	37.0	36.0	40.0
Height (m)	393	1.62±0.07	1.37	1.83	1.62	1.57	1.68
Prepreg. Wt (kg)	390	62.2±12.0	38.0	113.5	60.0	54.5	68.0
Prepreg. BMI (kg/m^2)	389	23.6±4.23	15.4	45.6	22.7	20.7	25.7

[1] prepreg., prepregnancy; SD, standard deviation

Table 15 (p. 55) describes the data on parity, ethnic origin, and smoking status. Parity refers to all previous deliveries, including stillbirths, but excludes the present pregnancy. A parity of 0 indicates that the woman is primiparous. Women were grouped into 3 categories based on their country of origin. The number 1 refers to areas in which b.wts are known to be smaller than average (WHO, 1980; 1984), and includes women of South American, African, or Asian background. The number 2 refers to areas in which b.wts are known to be average (WHO, 1980; 1984), and includes women of North American, European, Central American, Middle Eastern, Russian, or Australian background. The number 3 includes Native North American women who traditionally have larger babies (Kramer, 1987). Because of the small sample size, the participant in group 3 was added to group 2 for the analyses. Women were likewise grouped into 3

categories based on their smoking habits as, smokers or those who smoked during the present pregnancy, ex-smokers or those who quit before this pregnancy, and non-smokers.

Table 15. Parity, ethnic origin, and cigarette consumption of maternal participants

	N	% of Total
Parity[1]	392	100
0	106	27.0
1	170	43.4
2	78	19.9
3	24	6.1
≥4	14	3.6
Ethnic origin	394	100
1[2]	97	24.6
2[3]	296	75.1
3[4]	1	0.0025
Smoking status	392	100
Smokers	33	8.4
Ex-smokers[5]	22	5.6
Non-smokers	337	86.0

[1] refers to all deliveries, including stillbirths, but excluding the baby presently under study
[2] South American, African, Asian
[3] North American, European, Central American, Middle Eastern, Russian, Australian
[4] Native North American
[5] refers to women who quit for this pregnancy

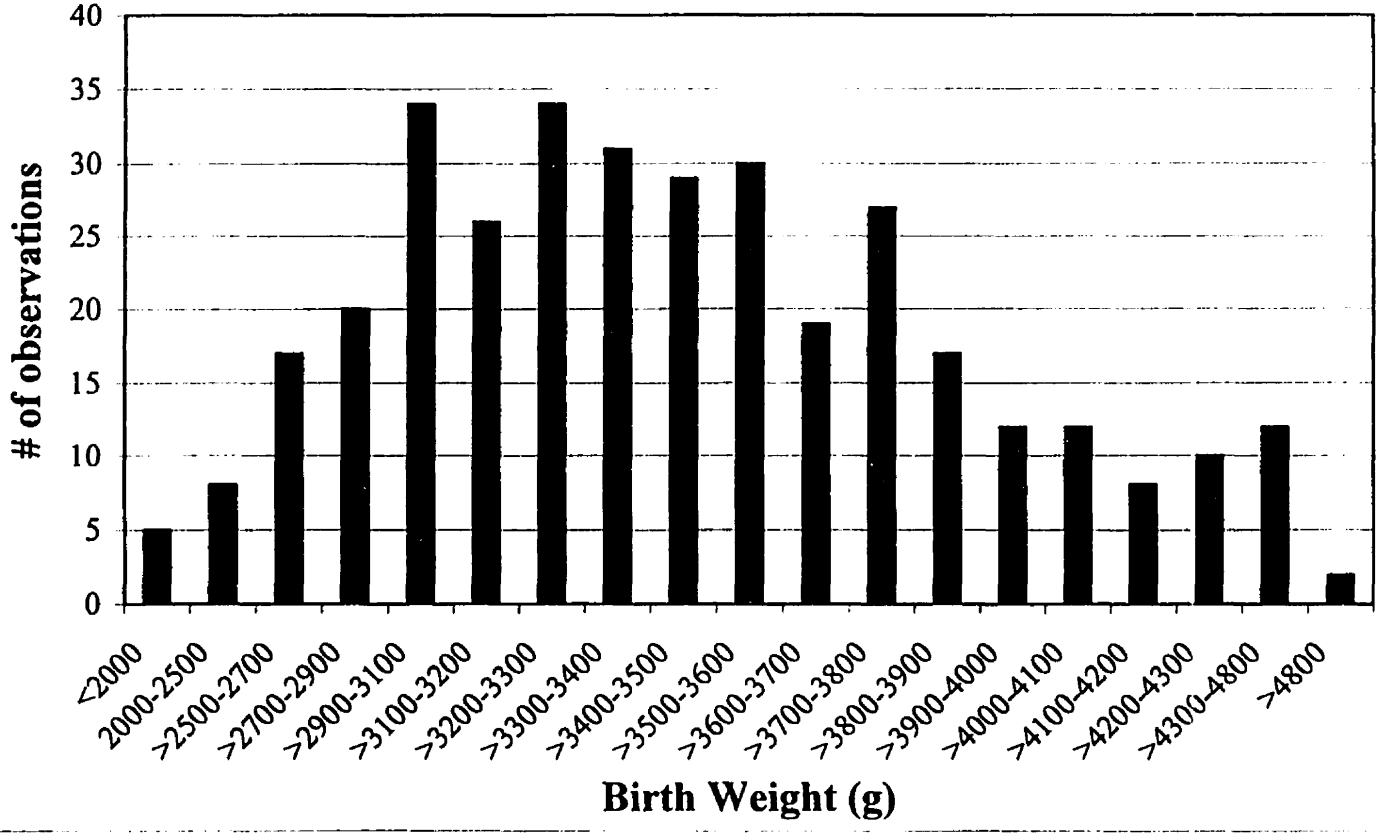
Neonatal Characteristics:

Fetal b.wts were obtained from 353 maternal charts. In 25 instances either the delivery information or the medical charts were unattainable, and in 8 instances the b.wt was missing from the medical chart. A total of 7 participants had an abortion, and 2 participants had a miscarriage. Twin pregnancies were excluded from the study (refer to Materials and Methods section, p. 42).

Of the 353 infants for which delivery information was available, 167 (47.3%) newborns were female and 186 (52.7%) were male. Gestational age ranged from 28.6 weeks to 42.1 weeks, with the average being 39.3±1.7 weeks. The 25th, 50th, and 75th percentiles were 38.6, 39.6, and 40.3 weeks, respectively. Eleven (3.2%) infants were under 36 weeks of gestation at birth. Three (0.8%) infants were 42 weeks of gestation or greater.

The distribution of b.wts in this population is shown in Figure 1 (p. 55).

Figure 1. Population Distribution of Birth Weights



A wide distribution was noted with b.wts ranging from 1130 g to 5230 g. The mean b.wt was 3409±552g. The 25th, 50th, and 75th percentiles were 3114g, 3420g, and 3732g, respectively. Six newborns (1.7%) weighed less than 2000g at birth, 13 (3.7%) weighed less than 2500g, 45 newborns (12.7%) had b.wts greater than 4000g, and 8 (2.3%) had b.wts greater than 4500g. The distribution was not normally distributed when both genders were treated as one population, as assessed by the Kolmogorov-Smirnov Normality Test. When sub-divided according to gender, the distribution for females was only normal after it was transformed by squaring the data. The distribution for males was normally distributed.

Table 16 (p. 58) describes the mean b.wt of this population by infant gender, maternal ht, prepregnancy wt and BMI, parity, ethnic origin, and cigarette consumption, established determinants of b.wt (Kramer, 1987). Data were divided into quartiles of ht, wt, and BMI. Because many women were of the same ht, the numbers in the ht quartiles are not even. Women were grouped into 3 categories based on their country of origin as described above.

Male babies weighed more than female babies ($t=48$, $df=351$, $p<0.0001$). Using one-way ANOVA, b.wt was statistically different among ht quartiles ($p<0.0008$). Using Student-Newman-Keuls all pairwise multiple comparison test, significant differences were between quartiles 1 and 2, 3 and 4, and 1 and 4 ($p<0.05$), suggesting that as maternal ht increased, b.wts also increased. Birth wt was different when divided by quartiles of maternal prepregnancy wt ($p=0.0006$). Post hoc comparison revealed significant differences between the 1st and the 2nd quartiles, the 1st and the 3rd quartiles, and the 1st and the 4th quartiles ($p<0.05$), indicating that women with prepregnancy wt less than 54kg had significantly smaller babies than heavier women. Birth wt was not significantly affected by prepregnancy BMI ($p=0.08$), parity ($p=0.52$), ethnic origin ($p=0.97$), or smoking status ($p=0.58$), but there were perhaps too few smoking mothers in the study ($n=33$) to reveal a difference.

Table 16. Mean b.wts by infant gender, maternal height, prepregnancy weight and BMI, parity, ethnic origin, and cigarette consumption[1]

	N	$\bar{x} \pm SD$ (g)
Infant gender	353	---
♀	167	3368.6 ± 525.4^{a}
♂	186	3445.7 ± 574.0^{b}
Maternal ht (m)	352	---
<1.57	64	3238.1 ± 565.6^{a}
1.57-<1.62	89	3458.9 ± 552.1^{bc}
1.62-<1.68	103	3324.6 ± 529.7^{ac}
≥1.68	96	3560.6 ± 518.7^{b}
Prepregnancy wt (kg)	351	---
≤54	83	3199.5 ± 598.4^{a}
>54-<61	97	3424.4 ± 451.9^{b}
61-<68	82	3472.0 ± 498.8^{b}
≥68	89	3524.6 ± 610.8^{b}
Prepregnancy BMI (kg/m^2)	350	---
<20.6	88	3362.4 ± 539.0
20.6-<22.7	88	3305.8 ± 530.8
22.7-<25.7	87	3452.2 ± 481.3
≥25.7	87	3499.9 ± 631.2
Parity[2]	351	---
0	96	3348.5 ± 617.0
1	156	3424.6 ± 539.9
2	67	3428.6 ± 502.9
3	20	3569.4 ± 500.0
≥4	12	3330.9 ± 562.4
Ethnic origin	353	---
1[3]	83	3407.2 ± 596.7
2[4]	270	3409.8 ± 538.8
Smoking status	351	---
Smoker	30	3316.7 ± 523.4
Ex-smoker[5]	20	3408.2 ± 578.8
Non-smoker	301	3425.0 ± 543.8

[1] SD, standard deviation
[2] refers to all deliveries, including stillbirths, but excluding the baby presently under study
[3] South American, African, Asian
[4] North American, European, Central American, Middle Eastern, Russian, Australian, Native North American
[5] refers to women who quit for this pregnancy

- ***Percentile birth weights by gestational age and gender:***

The b.wt percentiles by gestational age were determined for each newborn using b.wt percentile charts (Arbuckle et al., 1993). The distribution of percentiles of b.wts by gestational age is shown in Figure 2 (p. 59).

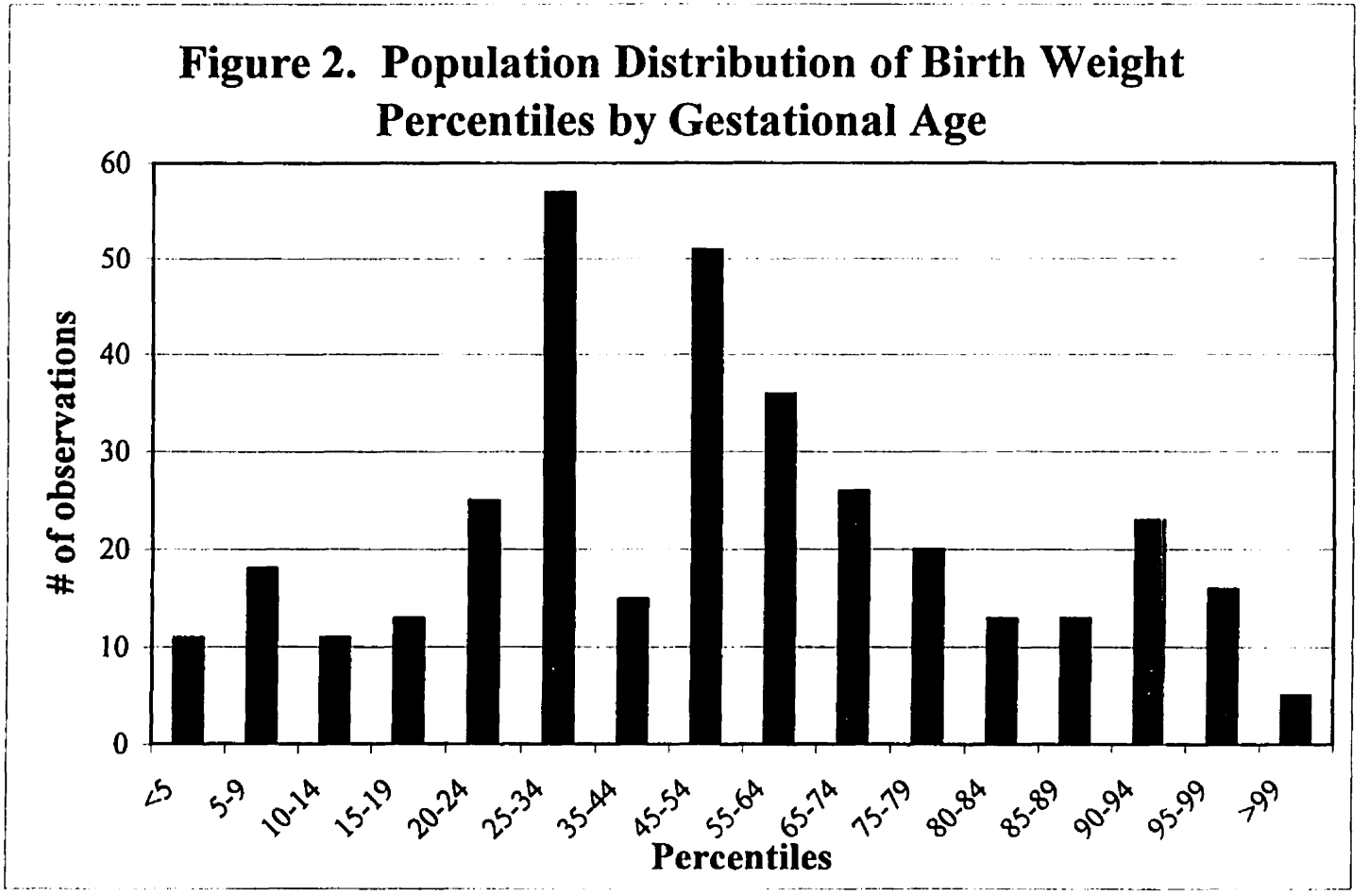


Figure 2. Population Distribution of Birth Weight Percentiles by Gestational Age
# of observations
60
50
40
30
20
10
0
<5
5-9
10-14
15-19
20-24
25-34
35-44
45-54
55-64
65-74
75-79
80-84
85-89
90-94
95-99
>99
Percentiles

Percentiles ranged from less than 1 to greater than 99. The mean percentile b.wt was 49.9 ± 28.2. The 25th, 50th, and 75th percentiles were 27.0, 49.5, and 75.0, respectively. Nine newborns (2.5%) were under the 5th b.wt percentile for gestational age, 27 (7.6%) were under the 10th percentile, 31 newborns (8.8%) were above the 90th percentile, and 9 (2.5%) were above the 95th percentile. The distribution was not normally distributed as assessed by the Kolmogorov-Smirnov Normality Test.

The newborns with b.wts less than the 10th percentile (N=27) were compared to the newborns with b.wts greater than the 90th percentile (N=31) for all the maternal, neonatal, and amf parameters using Student's t-test. The two groups differed significantly with respect to maternal ht (p=0.001) and prepregnancy wt (p=0.01), indicating that taller and heavier women had bigger babies.

Amniotic Fluid Constituents:

The mean concentrations and standard deviations of the measured amf constituents are described in Table 17 (p. 60). Some assays performed were done on a sub-sample of the total population due to lack of sufficient sample volume.

Table 17. Mean concentrations and standard deviations of measured amf constituents[1]

Amf constituent	N	$\bar{x} \pm SD$	min.	max.	median
Total protein (g/L)	373	6.02 ± 2.12	0.68	12.00	5.70
Albumin (g/L)	337	3.68 ± 1.04	1.27	6.78	3.58
Urea Nitrogen (mmol/L)	395	4.00 ± 1.01	0.99	8.02	3.91
Creatinine (µmol/L)	335	194.0 ± 13.4	134.3	254.0	194.6
Uric Acid (µmol/L)	335	209.1 ± 45.2	87.1	353.7	209.6
Glucose (mmol/L)	395	2.54 ± 0.59	0.80	5.21	2.49
ßHBA (µmol/L)	395	118.7 ± 74.9	0.0	317.3	120.6
Lactate (mmol/L)	335	9.87 ± 2.52	3.77	20.20	9.55

[1] SD, standard deviation

The distributions of the measured amf constituents are shown in Figures 3–10 (pp. 61-64).

Figure 3. Population Distribution of Amniotic Fluid Total Protein

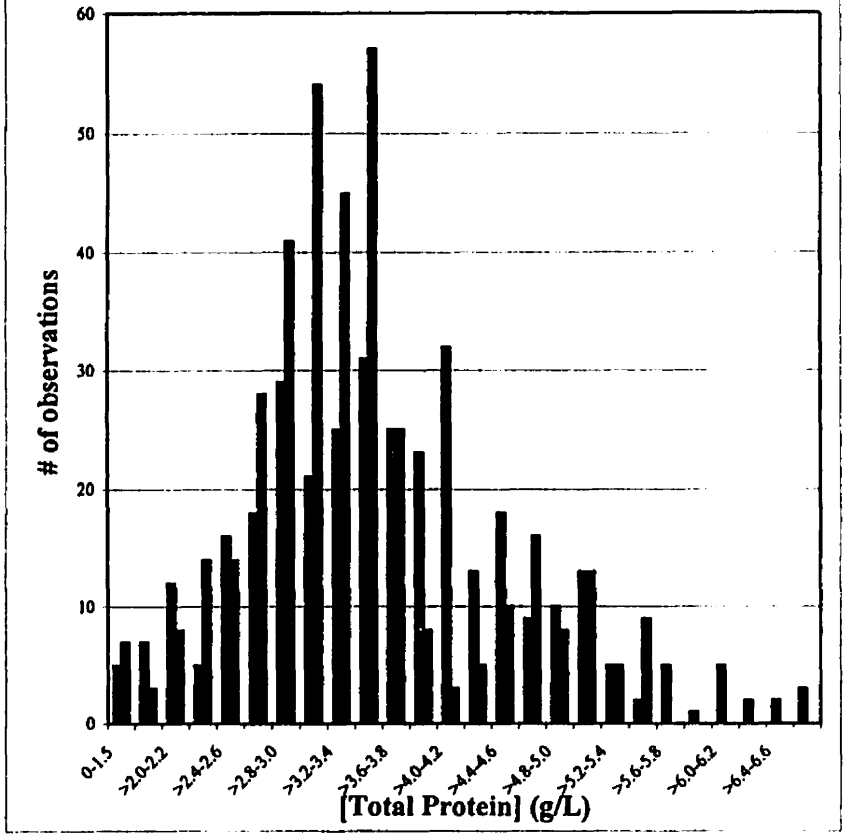
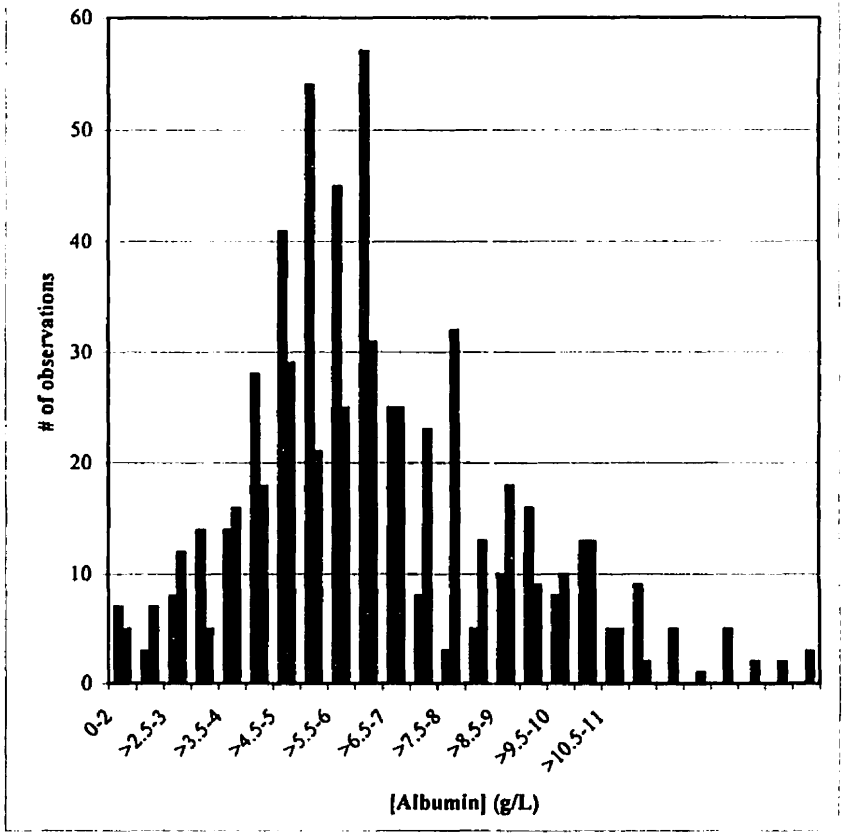


Figure 4. Population Distribution of Amniotic Fluid Albumin Concentration



Note: When the above charts were condensed to fit onto one page, some of the spaces between the bars and their respective labels were omitted, so that the second bar in Figure 3 corresponds to total protein concentrations of >1.5-2.0 g/L, the forth bar corresponds to total protein concentrations of >2.2-2.4 g/L, etc.

Figure 5. Population Distribution of Amniotic Fluid Urea Nitrogen

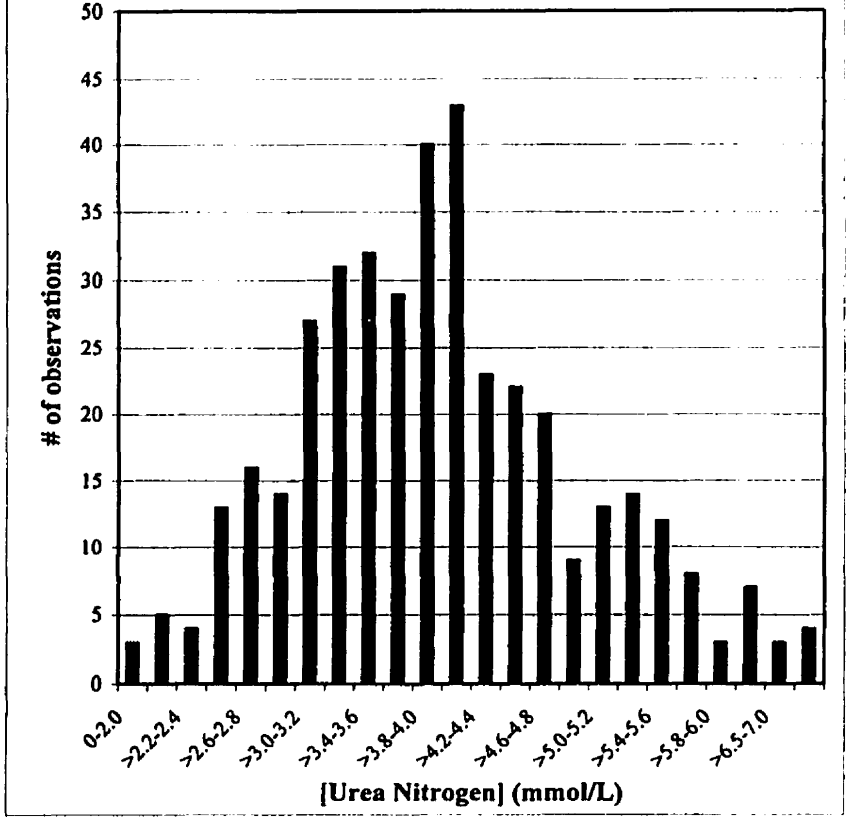


Figure 6. Population Distribution of Amniotic Fluid Creatinine

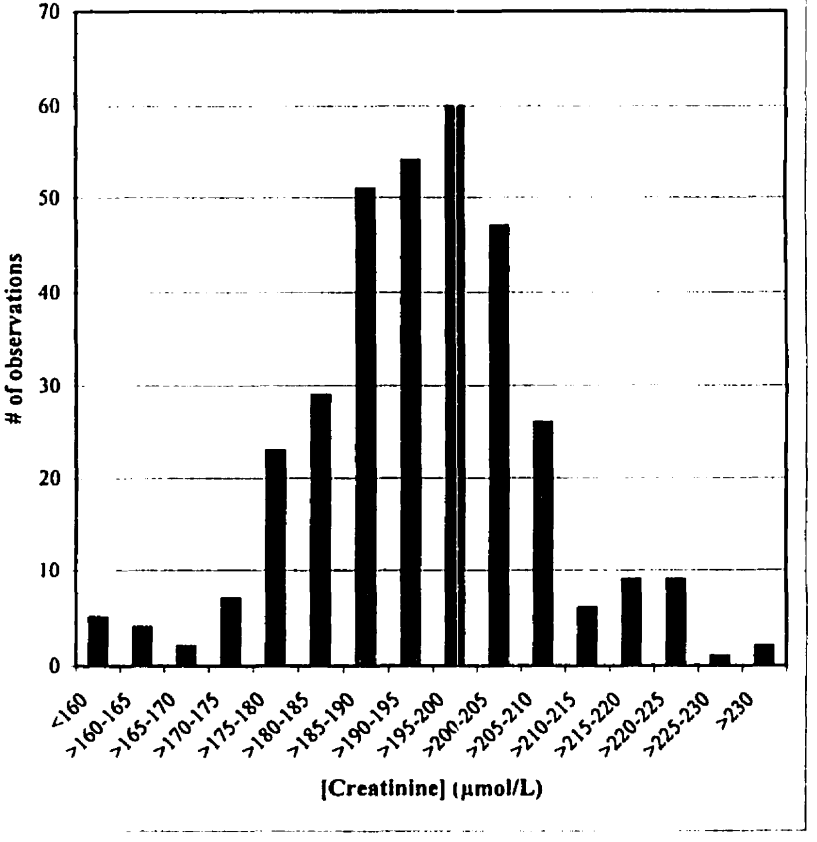


Figure 7. Population Distribution of Amniotic Fluid Uric Acid

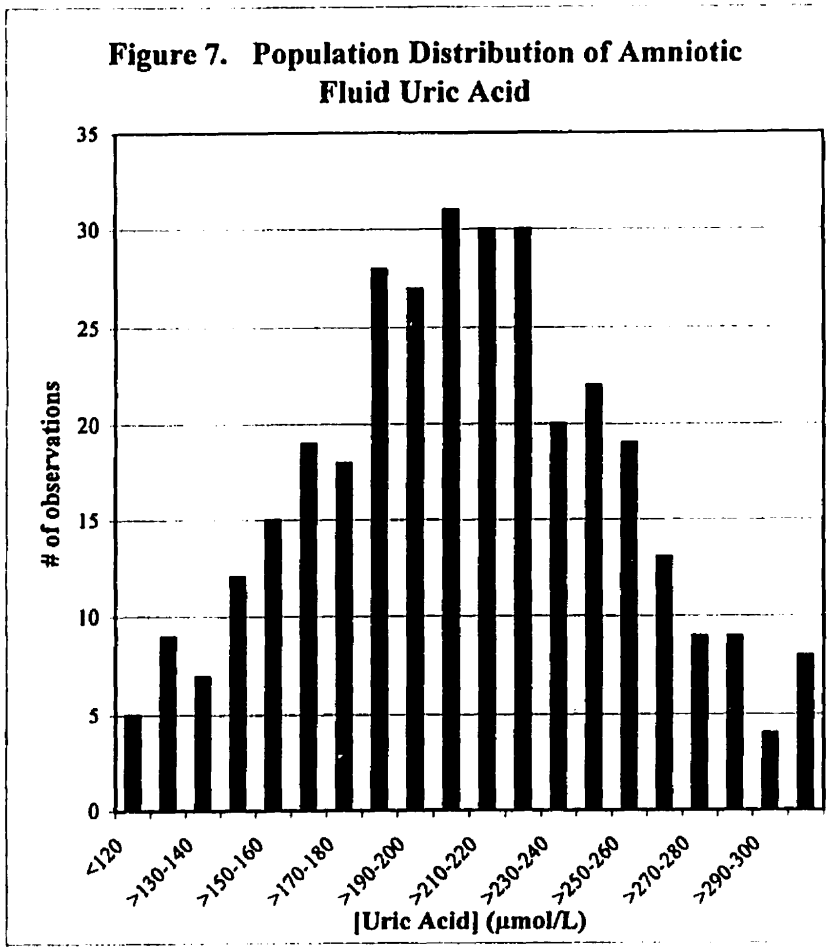


Figure 8. Population Distribution of Amniotic Fluid Glucose

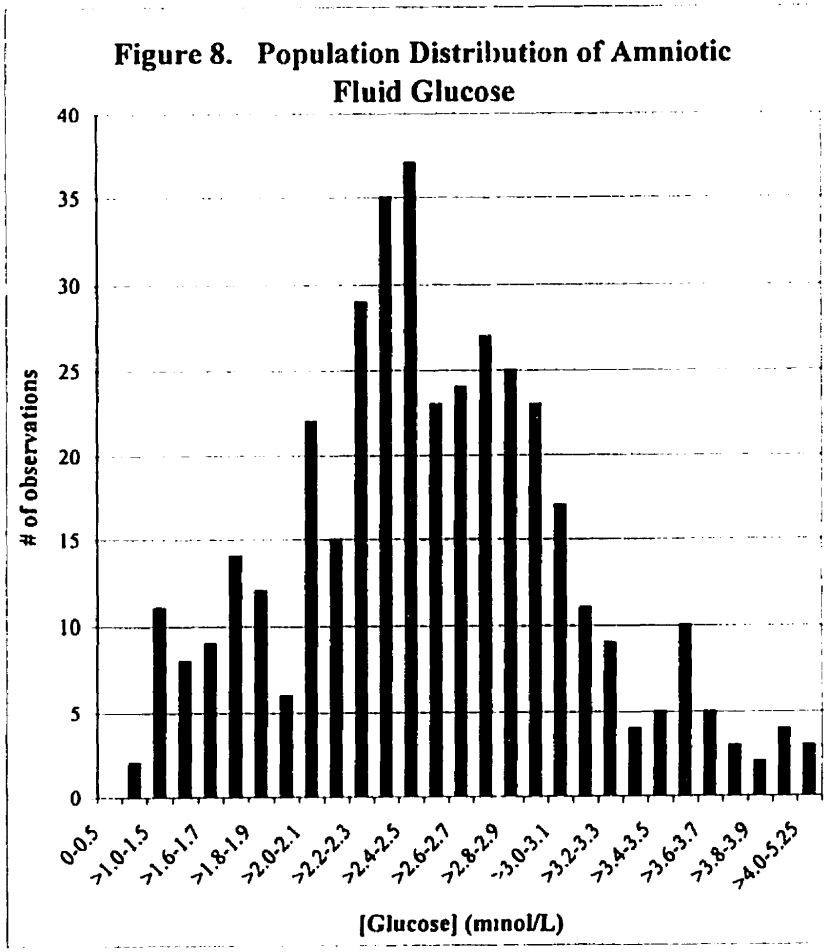


Figure 9. Population Distribution of Amniotic Fluid ß-hydroxybutyrate

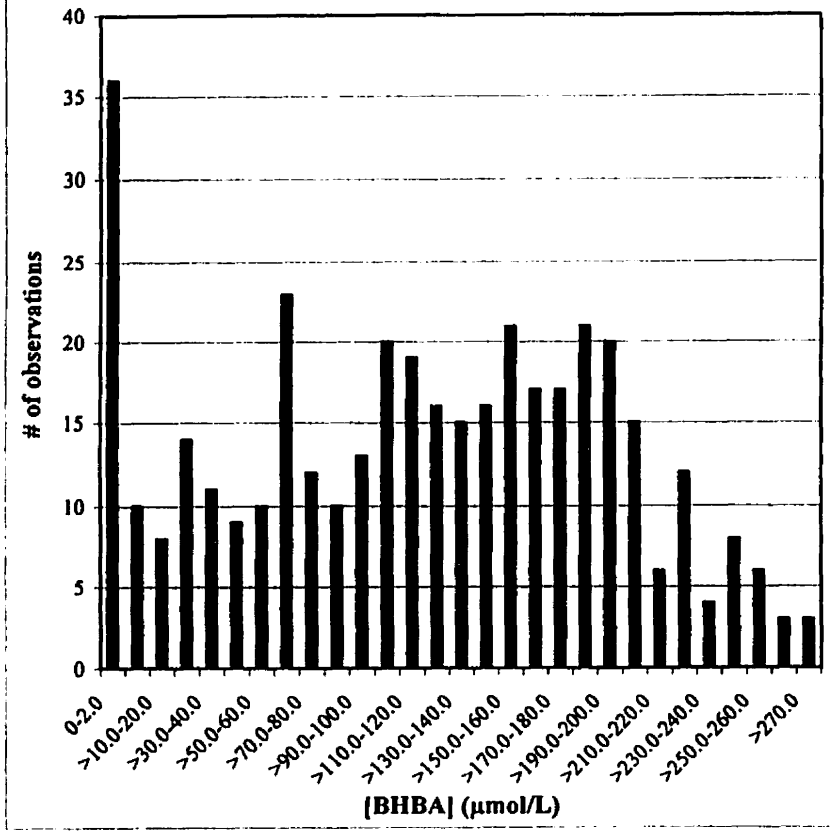
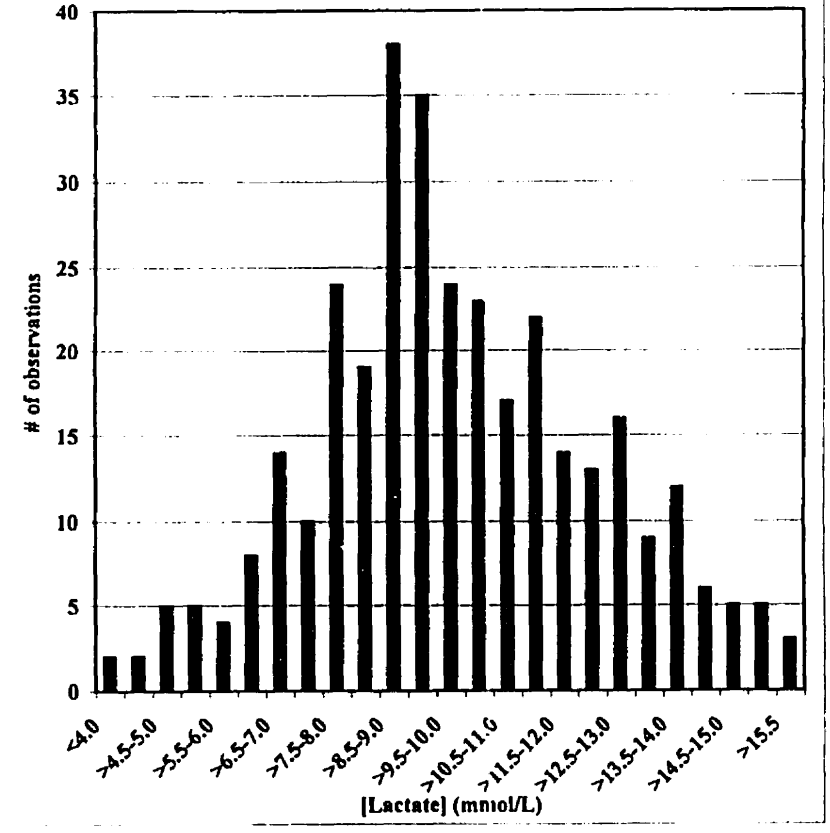


Figure 10. Population Distribution of Amniotic Fluid Lactate



A wide distribution was noted with all measured constituents indicating high variability within the population. Amniotic fluid uric acid and glucose were normally distributed as assessed by the Kolmogorov-Smirnov Normality Test, however, total protein, albumin, urea nitrogen, creatinine, ßHBA, and lactate were not normally distributed. The distributions of albumin and urea nitrogen were normalized using natural logarithmic transformations, and lactate distribution was normalized using a square root transformation. Total protein, creatinine, and ßHBA could not be normalized using square, natural log, log 10, reciprocal, exponential, square root, or arcsin square root transformations.

Table 18 (p. 67) describes the amf constituents on the basis of infant gender, maternal ht, prepregnancy wt and BMI, parity, ethnic origin, and cigarette consumption using one-way ANOVA. Student-Newman-Keuls all pairwise multiple comparison test was used to reveal the source of significant differences. Amniotic fluid total protein concentration differed by infant gender ($p<0.02$). Female babies had higher total protein concentrations than male babies. Amniotic fluid albumin concentration differed by ethnic origin ($p<0.04$). Participants in group 2 of ethnic origin (North American, European, Central American, Middle Eastern, Russian, and Australian), known to have higher b.wts, had a higher concentration of albumin than those in group 1 (South American, African, and Asian), known to have lower b.wts. Urea nitrogen concentration differed by maternal ht ($p=0.008$, quartile 1 vs 4, 2 vs 4), prepregnancy BMI ($p=0.02$, quartile 1 vs 4), and ethnic origin ($p<0.0001$). Women with a ht <1.62m (the two lowest quartiles) had significantly lower urea nitrogen concentrations as compared with taller women. The concentration of urea nitrogen was significantly higher in women with a prepregnancy BMI <20.6 (the lowest quartile) compared with women with a prepregnancy BMI ≥25.7 (the highest quartile). The concentration of urea nitrogen was higher in participants of ethnic origins known to have higher b.wts than in participants of ethnic origins known to have lower b.wts. Uric acid concentration differed by maternal prepregnancy wt ($p=0.02$, quartile 1 vs 4), prepregnancy BMI ($p=0.007$, quartile 1 vs 4, 2 vs 4, 3 vs 4), and parity ($p<0.04$, source unknown). The concentration of uric acid was significantly lower in women with a prepregnancy wt ≤54kg (the lowest quartile) as compared with women with a prepregnancy wt ≥68kg (the highest quartile). Women with a prepregnancy BMI

<25.7 (the three lowest quartiles) had significantly lower uric acid concentrations than women with a prepregnancy BMI ≥25.7 (the highest quartile). There was no established pattern for the relationship between uric acid and parity. Amniotic fluid glucose concentration differed significantly by maternal ht (p=0.001, quartile 1 vs 4, 2 vs 4), prepregnancy BMI (p=0.0008, quartile 1 vs 4, 2 vs 4), parity (p=0.004, parity 2 vs parity ≥4), and ethnic origin (p=0.0002). The concentration increased with decreasing maternal ht, increasing prepregnancy BMI, and was higher in participants of ethnic origins known to have lower b.wts than in participants of ethnic origins known to have lower b.wts. No pattern was identified for the relationship between glucose and parity, although the lowest glucose values, which occurred in parity 2, differed significantly from the highest values, which occurred in parity ≥4. A significant difference of ßHBA concentration was found by parity (p=0.03, source unknown). There was no established pattern for the relationship between ßHBA and parity. Neither creatinine nor lactate concentrations were significantly affected by the maternal and neonatal parameters measured. Maternal smoking status did not influence amf metabolite concentrations.

Table 18. One-way ANOVA of amf constituents by maternal and neonatal data[1]

	Tot.ptn (g/L)	Alb (g/L)	Urea N (mmol/L)	Creat (μmol/L)	Uric Acid (μmol/L)	Gluc (mmol/L)	ßHBA (μmol/L)	Lact (mmol/L)
Infant gender								
♀	6.24±2.09[a]	3.75±1.01	3.98±0.96	194.3±13.1	204.4±45.8	2.54±0.55	120.2±80.3	9.72±2.34
♂	5.72±1.96[b]	3.63±1.07	4.01±1.06	194.2±14.3	213.5±45.7	2.56±0.60	117.5±70.1	9.88±2.51
Mat. ht (m)								
<1.57	6.40±2.29	3.67±0.93	3.74±0.94[a]	192.6±11.0	201.6±50.1	2.69±0.56[a]	132.6±71.9	9.52±2.44
1.57-<1.62	6.00±1.91	3.70±1.02	3.90±0.95[a]	192.4±12.6	212.2±47.6	2.62±0.60[a]	119.3±70.5	9.86±2.48
1.62-<1.68	5.86±2.17	3.60±1.08	4.02±0.96[ab]	194.3±11.5	212.9±46.5	2.52±0.56[ab]	115.4±76.9	9.85±2.51
≥1.68	5.89±2.11	3.77±1.11	4.24±1.12[b]	196.4±17.5	207.1±36.6	2.37±0.61[b]	111.6±79.1	10.18±2.65
Prepreg. Wt (kg)								
≤54	6.10±2.27	3.90±1.12	3.97±1.00	191.4±10.0	198.8±43.0[a]	2.49±0.56	119.6±70.5	9.73±2.60
>54-<61	5.92±2.09	3.56±0.92	4.08±0.97	194.0±11.9	206.0±39.5[ab]	2.51±0.57	108.5±73.7	9.51±2.17
61-<68	6.19±2.10	3.70±1.08	3.91±0.97	193.9±17.9	210.6±46.1[ab]	2.52±0.58	131.9±76.3	9.74±2.72
≥68	5.97±2.06	3.64±1.07	4.02±1.14	196.3±13.6	220.3±50.1[b]	2.58±0.62	116.2±78.2	10.42±2.52
Prepreg. BMI (kg/m^2)								
<20.6	6.07±2.29	3.89±1.15	4.18±0.94[a]	192.8±12.4	199.4±39.5[a]	2.37±0.58[a]	112.5±69.4	9.86±2.44
20.6-<22.7	5.72±1.84	3.63±1.00	4.03±1.02[ab]	193.2±14.7	206.4±38.7[a]	2.48±0.55[a]	113.4±78.5	9.61±2.56
22.7-<25.7	6.29±2.21	3.70±1.00	4.05±1.08[ab]	195.1±13.0	206.9±48.9[a]	2.55±0.56[ab]	121.3±73.8	9.73±2.59
≥25.7	6.01±2.11	3.54±1.02	3.74±0.99[b]	194.6±13.8	223.0±49.9[b]	2.70±0.60[b]	126.1±78.4	10.18±2.44
Parity[2]								
0	5.82±1.99	3.81±1.00	4.02±0.98	192.6±13.5	216.2±44.2	2.53±0.57[ab]	9.59±2.48	99.5±72.5
1	6.10±2.01	3.61±1.06	4.04±1.00	195.4±13.6	203.8±41.5	2.58±0.55[ab]	10.06±2.43	125.0±77.0
2	6.10±2.38	3.72±1.14	3.91±1.04	192.4±14.4	205.0±45.6	2.36±0.62[a]	9.81±2.70	125.3±75.1
3	6.64±2.41	3.69±0.94	3.94±0.91	195.0±9.6	233.1±53.2	2.59±0.75[ab]	10.36±3.30	137.4±67.8
≥4	5.16±2.43	3.41±0.79	3.95±1.44	194.4±11.5	206.3±69.5	2.96±0.61[b]	9.33±1.42	121.0±57.7
Ethnic origin								
1[3]	6.03±2.25	3.48±0.87[a]	3.58±0.81[a]	192.1±12.3	210.5±46.9	2.73±0.59[a]	125.3±74.4	10.06±2.60
2[4]	6.01±2.08	3.75±1.09[b]	4.13±1.04[b]	194.7±13.8	208.7±44.7	2.47±0.58[b]	116.4±75.2	9.81±2.50
Smoking status								
Smoker	5.57±1.98	4.07±1.17	3.74±1.12	193.0±16.8	191.7±44.5	2.50±0.66	96.2±74.3	9.32±2.43
Ex-smoker[5]	5.11±2.42	3.44±1.24	3.74±0.91	187.7±15.1	212.7±37.4	2.54±0.55	121.5±66.4	8.96±2.57
Non-smoker	6.12±2.11	3.66±1.02	4.03±1.01	194.5±12.9	210.7±45.4	2.54±0.59	121.1±75.3	9.98±2.52

[1] values in cells are means ± SD; prepreg., prepregnancy
[2] refers to all deliveries, including stillbirths, but excluding the baby presently under study
[3] South American, African, Asian
[4] North American, European, Central American, Middle Eastern, Russian, Australian, Native North American
[5] refers to women who quit for this pregnancy

2. Correlation Analyses

Amniotic Fluid Constituents:

The amf constituents were correlated with each other. Spearman correlation coefficients were used for correlations involving non-normally distributed data, otherwise Pearson correlation coefficients were used. Table 19 (p. 68) describes such analyses. Statistically significant positive correlation coefficients were found between amf total protein and albumin (n=322, Spearman r=0.61, p<0.01), urea nitrogen and creatinine (n=334, Spearman r=0.38, p<0.01), creatinine and lactate (n=334, Spearman r=0.30, p<0.01), urea nitrogen and lactate (n=335, Pearson r=0.27, p<0.01), creatinine and uric acid (n=334, Spearman r=0.22, p<0.01), total protein and ßHBA (n=372, Spearman r=0.20, p<0.01), uric acid and ßHBA (n=335, Spearman r=0.20, p<0.01), urea nitrogen and uric acid (n=335, Pearson r=0.19, p<0.01), albumin and urea nitrogen (n=336, Pearson r=0.17, p<0.01), and glucose and ßHBA (n=394, Spearman r=0.13, p=0.01). A statistically significant negative correlation coefficient was found between glucose and lactate (n=335, Pearson r=-0.16, p<0.01).

Table 19. Pearson and Spearman correlation coefficients of the amf constituents[1]

	Tot.ptn[2]	Alb.[3]	Urea N[3]	Creat.[2]	Uric acid[4]	Gluc.[4]	ßHBA[2]	Lact.[5]
Tot.ptn (g/L)	1	**0.61***	**0.07**	**-0.02**	**0.06**	**0.08**	**0.20***	**-0.06**
Alb. (g/L)		1	**0.17***	**-0.002**	**-0.01**	**0.05**	**0.07**	**0.03**
Urea N (mmol/L)			1	**0.38***	**0.19***	**0.05**	**0.005**	**0.27***
Creat. (µmol/L)				1	**0.22***	**-0.0004**	**0.11**	**0.30***
Uric acid (µmol/L)					1	**0.06**	**0.20***	**0.08**
Gluc. (mmol/L)						1	**0.13***	**-0.16***
ßHBA (µmol/L)							1	**-0.07**
Lact. (mmol/L)								1

[1] Spearman correlation coefficients are bold, otherwise Pearson correlation coefficients were used; tot.ptn, total protein; alb., albumin; urea N, urea nitrogen; creat., creatinine; gluc., glucose; lact., lactate
[2] data that could not be normalized
[3] data that was normalized by natural logarithm
[4] data that was normal
[5] data that was normalized by squaring
* statistically significant correlation coefficients (p<0.05)

Each amf constituent was individually correlated with the continuous variables: maternal ht, prepregnancy wt and BMI. Spearman correlation coefficients were used when the data were not normally distributed, otherwise Pearson correlation coefficients

were used. Statistically significant ($p<0.05$) positive correlations were found between maternal ht and urea nitrogen (n=393, Pearson r=0.15), maternal ht and creatinine (n=333, Spearman r=0.14). A statistically significant ($p<0.05$) negative correlation was found between maternal ht and glucose (n=393, Pearson r=-0.18). Prepregnancy wt correlated significantly ($p<0.01$) and positively with uric acid (n=332, Pearson r=0.22), and creatinine (n=331, Spearman r=0.15), and negatively with urea nitrogen (n=390, Pearson r=-0.11). Significant ($p<0.05$) positive correlations were found between maternal prepregnancy BMI and uric acid (n=331, Pearson r=0.24) and prepregnancy BMI and glucose (n=389, Pearson r=0.16), and a significant negative correlation was found between prepregnancy BMI and urea nitrogen (n=389, Pearson r=-0.19).

Birth Weights:

Spearman correlation coefficients were used to determine how well the amf constituents correlated with b.wt since b.wt was not normally distributed. Table 20 (p. 69) describes such analyses. None of the correlation coefficients were statistically significant.

Table 20. Spearman correlation coefficients of the amf constituents with b.wt[1]

	Tot.ptn[2] (g/L)	Alb.[3] (g/L)	Urea N[3] (mmol/L)	Creat.[2] (µmol/L)	Uric acid[4] (µmol/L)	Gluc.[4] (mmol/L)	ßHBA[2] (µmol/L)	Lact.[5] (mmol/L)
B.wt (g)	0.007	-0.05	0.09	0.02	0.06	0.02	0.05	0.12

[1] tot.ptn, total protein; alb., albumin; urea N, urea nitrogen; creat., creatinine; gluc., glucose; lact., lactate
[2] data that could not be normalized
[3] data that was normalized by natural logarithm
[4] data that was normal
[5] data that was normalized by squaring

3. Regression Analyses

Multiple regression analyses was done using proc GLM. Several models were fit using either b.wts or percentiles of b.wt by gestational age as the dependent variables. The ability to predict percentile b.wt may be more clinically useful since it removes gestational age from the list of independent variables. A stepwise regression was also performed to examine the strongest predictors more closely.

- ***Maternal and Neonatal Characteristics as Predictors of Birth Weight:***

In order to examine the effects of the maternal and neonatal characteristics on b.wt, a basic model was run that included all the parameters except the amf constituents. Table 21 (p. 70) describes such analyses. The ß-estimates explain the variation in the dependent variable for every unit change in the particular independent variable. Gestational age ($p=0.0001$) and maternal ht ($p<0.04$) were the only significant parameters when b.wt was the dependent variable, and parity ($p=0.02$) was the only significant effect when percentile b.wt was the dependent variable. The model with b.wt as the dependent variable captured more of the variability than the model with percentile b.wt as the dependent variable ($r^2=0.42$ vs 0.13), because of the strong relationship between gestational age and b.wt.

Table 21. Maternal and neonatal parameters as predictors of b.wt[1]

Dependent variable:	**Birth weight (g)**		**Percentile birth weight**	
	R^2= 0.42	**N=345**	**R^2=0.13**	**N=338**
INDEPENDENT VARIABLES	ß-ESTIMATE	p-value	ß-ESTIMATE	p-value
Gestational age (wks)	201.9*	0.0001	---	---
Infant gender (M/F)	80.7	0.07	-3.98	0.18
Maternal height (cm)	34.5*	0.04	1.78	0.08
Prepreg. wt (kg)	-24.1	0.24	-0.80	0.53
Prepreg. BMI (kg/m^2)	79.8	0.14	2.81	0.41
Parity[2]	16.6	0.16	1.33*	0.02
Ethnic origin[3]	-87.2	0.16	-4.95	0.22
Smoking status[4]	72.3	0.22	4.20	0.27

[1] prepreg., prepregnancy
[2] parity 0 vs 1 vs 2 vs 3 vs ≥4
[3] group 1 vs group 2
[4] smokers vs ex-smokers vs non-smokers
* statistically significant

Since prepregnancy wt and prepregnancy BMI are two ways of expressing the same information, the models were run with only one of the two at a time. When prepregnancy wt was taken out of the models, gestational age ($p=0.0001$), maternal ht ($p=0.0001$), and prepregnancy BMI ($p=0.005$) were statistically significant in the model with b.wt as the dependent variable ($r^2=0.42$, N=345). Statistical significance didn't change in the model with percentile b.wt as the dependent variable. When prepregnancy BMI was taken out of the models, gestational age ($p=0.0001$), maternal ht ($p=0.002$), and prepregnancy wt ($p=0.008$) were statistically significant in the model with b.wt as the dependent variable ($r^2=0.41$, N=345). In the model with percentile b.wt as the dependent

variable (r^2=0.13, N=338), parity (p=0.02) and maternal ht (p=0.0001) were statistically significant. The model that included prepregnancy BMI captured slightly more of the variability than the model that included prepregnancy wt.

- ***Each Amniotic Fluid Constituent, with Maternal and Neonatal Parameters as Predictors of Birth Weight in Separate Regression Models:***

To evaluate the additional contribution of the amf constituents to the basic model of known predictors, each amf constituent was run individually in a model with b.wt as the dependent variable, and the maternal and neonatal parameters as independent variables. Table 22 (p. 72) describes the ß-estimates and p-values of the amf constituents. Although none of the constituents achieved statistical significance, glucose had a strong tendency toward reaching significance (p=0.06).

Table 22. ß-Estimates of amf constituents as predictors of b.wt[1]

Model[2]	p-value of amf constit-uent	ß-ESTIMATES								R^2	N
		Amf constituent	Gest. Age (wks)	Infant gender (M/F)	Mat. ht (cm)	Prepreg. BMI (kg/m^2)	Parity[3]	Ethnic origin[4]	Smok. Status[5]		
Tot.ptn (g/L)	0.77	3.50	194.5	86.9	15.0	17.2	21.0	-80.6	71.2	0.40	326
Alb. (g/L)	0.55	15.0	190.2	69.0	16.6	18.0	11.3	-80.6	89.9	0.40	296
Urea N (mmol/L)	0.21	30.2	197.8	80.1	14.3	18.5	18.5	-97.9	68.6	0.42	345
Creat. (µmol/L)	0.93	0.17	189.5	68.4	16.5	17.6	11.1	-79.0	87.3	0.39	294
Uric Acid (µmol/L)	0.67	0.25	189.5	67.0	16.5	17.0	12.5	-75.8	85.0	0.40	295
Gluc. (mmol/L)	0.06	78.7	201.3	77.8	15.3	15.2	21.7	-64.5	71.1	0.42	345
βHBA (µmol/L)	0.46	0.23	198.2	81.8	14.9	16.9	16.3	-80.9	74.8	0.42	345
Lact. (mmol/L)	0.32	10.6	189.8	68.2	16.1	17.0	11.4	-72.1	83.7	0.40	295

[1] tot.ptn, total protein; alb., albumin; urea N, urea nitrogen; creat., creatinine; gluc., glucose; lact., lactate; gest., gestational; mat., maternal; prepreg., prepregnancy
[2] each amf constituent was run in separately in a model with the maternal and neonatal parameters (gestational age, infant gender, maternal height, prepregnancy BMI, parity, ethnic origin, and smoking status)
[3] parity 0 vs 1 vs 2 vs 3 vs ≥4
[4] group 1 vs group 2
[5] smokers vs ex-smokers vs non-smokers

- ***Amniotic Fluid Constituents as Predictors of Birth Weight:***

A model including the amf constituents as independent variables, without the maternal and neonatal parameters was also run, in order to examine their independent effects on b.wt. Table 23 (p. 73) describes such analyses. Because total protein and albumin are highly correlated (r=0.55), the models were run without albumin. Albumin was excluded since it was measured on a smaller sample size than total protein. None of the constituents achieved statistical significance with b.wt as the dependent variable. Lactate was the only constituent to reach significance (p=0.05) when percentile b.wt was the dependent variable. Both models fit poorly as demonstrated by low r^2 values of 0.03, indicating that alone, the amf constituents are not explaining much of the variability in b.wts.

Table 23. Amf constituents as predictors of b.wt[1]

Dependent variable:	**Birth weight (g)**		**Percentile birth weight**	
	R^2=0.03	**N=286**	**R^2=0.03**	**N=279**
INDEPENDENT VARIABLES	ß-ESTIMATE	p-value	ß-ESTIMATE	p-value
Total protein (g/L)	0.80	0.96	0.46	0.60
Urea Nitrogen (mmol/L)	19.76	0.58	2.34	0.21
Creatinine (µmol/L)	-3.55	0.19	-0.08	0.56
Uric Acid (µmol/L)	0.61	0.40	0.01	0.75
Glucose (mmol/L)	73.30	0.20	3.05	0.33
ßHBA (µmol/L)	0.57	0.23	0.02	0.38
Lactate (mmol/L)	27.00	0.06	1.50*	0.05

[1] all the amf constituents, except albumin, were put in the same model; none of the maternal and neonatal parameters were included

* statistically significant

- ***Maternal, Neonatal, and Amniotic Fluid Parameters as Predictors of Birth Weight:***

A model that included all the parameters in the study was run. Table 24 (p. 74) describes such analyses. Again, amf albumin was excluded due to its colinear relationship with total protein. When b.wt was the dependent variable, gestational age (p=0.0001), maternal ht (p=0.03), and glucose concentration (p=0.02) achieved statistical significance. When percentile b.wt was the dependent variable, maternal ht (p=0.05) and parity (p=0.05) were the only variables to reach significance. The model with b.wt as the dependent variable was again better fitting than the model with percentile b.wt as the dependent variable (r^2=0.40 vs 0.17), reflecting the strong predictive effect of gestational

age.

Table 24. Maternal, neonatal, and amf parameters as predictors of b.wt[1]

Dependent variable:	Birth weight (g)		Percentile birth weight	
	R^2=0.40	N=281	R^2=0.17	N=275
INDEPENDENT VARIABLES	ß-ESTIMATE	p-value	ß-ESTIMATE	p-value
Gestational age (wks)	190.4*	0.0001	---	---
Infant gender (M/F)	62.16	0.21	-4.84	0.17
Maternal height (cm)	18.66*	0.0001	1.31*	0.0001
Prepreg. BMI (kg/m^2)	12.09	0.09	0.58	0.18
Parity[2]	12.11	0.25	1.08*	0.04
Ethnic origin[3]	-52.37	0.53	-4.91	0.32
Smoking status[4]	58.60	0.37	2.46	0.60
Total protein (g/L)	-0.56	0.97	0.26	0.76
Urea Nitrogen (mmol/L)	1.79	0.96	1.72	0.38
Creatinine (µmol/L)	-1.99	0.38	-0.10	0.45
Uric Acid (µmol/L)	0.21	0.74	-0.007	0.87
Glucose (mmol/L)	119.4*	0.02	3.62	0.26
βHBA (µmol/L)	0.33	0.40	0.02	0.43
Lactate (mmol/L)	20.68	0.09	1.18	0.12

[1] prepreg., prepregnancy
[2] parity 0 vs 1 vs 2 vs 3 vs ≥4
[3] group 1 vs group 2
[4] smokers vs ex-smokers vs non-smokers
*statistically significant

- ***Forward and Backward Stepwise Regressions:***

In order to further examine the strongest predictors of b.wt and percentile b.wt, both backward and forward stepwise regression analyses were performed. Surprisingly, both forward and backward methods gave the same results and rank order of significant parameters. With b.wt as the dependent variable, the most predictive parameters were gestational age (ß-estimate: 190.3, p<0.0001), maternal ht (ß-estimate: 15.2, p<0.0001), amf glucose (ß-estimate: 114.4, p=0.02), and prepregnancy BMI (ß-estimate: 14.1, p=0.02), suggesting that older babies from taller, heavier women with higher amf glucose levels are larger. When all four parameters were included in a model the r^2 was 0.35. With percentile b.wt as the dependent variable, the most predictive parameter was maternal ht (ß-estimate: 1.0, p<0.0001), however, the model did not fit well as demonstrated by an r^2=0.07.

- ***Amniotic Fluid Glucose as a Predictor of Birth Weight:***

Of all amf constituents measured, glucose showed strong evidence of being a potential predictor of b.wt over and above the effects of the maternal and neonatal parameters. This positive relation with b.wt was explored further by examining the effect of glucose in the lowest quartile of b.wt compared with that in the highest quartile to ascertain whether the effect would be sustained at low and high b.wts. Table 25 (p. 75) describes such analyses. When stratified by b.wt quartile, the strongest predictors of lower b.wt were gestational age (p=0.0001) and ethnic origin (p=0.05). The higher r^2=0.61 is reflective of the fact that the model included many variables to explain b.wts over a smaller range (b.wts <3114g) in a small sample (N=87). Using the same model except with the highest quartile of b.wt as the dependent variable, the strongest predictor was prepregnancy BMI (p=0.007), but glucose demonstrated a strong trend toward significance (p=0.08). The model did not capture very much of the variability as demonstrated by the low r^2=0.25.

Table 25. Maternal and neonatal parameters and amf glucose as predictors of b.wt[1]

Dependent variable:	**1st Quartile of b. wt (<3114g)**		**4th Quartile of b. wt (>3732g)**	
	R^2=0.61	**N=87**	**R^2=0.25**	**N=86**
INDEPENDENT VARIABLES	ß-ESTIMATE	p-value	ß-ESTIMATE	p-value
Gestational age (wks)	151.1*	0.0001	19.0	0.55
Infant gender (M/F)	-7.36	0.86	96.4	0.18
Maternal height (cm)	6.63	0.13	3.60	0.40
Prepreg. BMI (kg/m^2)	-10.5	0.20	19.9*	0.007
Parity[2]	33.5	0.43	-36.1	0.31
Ethnic origin[3]	144.6*	0.05	51.3	0.55
Smoking status[4]	7.04	0.92	46.7	0.70
Glucose (mmol/L)	65.9	0.26	102.3	0.08

[1] prepreg., prepregnancy
[2] parity 0 vs 1 vs 2 vs 3 vs ≥4
[3] group 1 vs group 2
[4] smokers vs ex-smokers vs non-smokers
* statistically significant

Gestational age had a less important effect in the higher b.wt range, but was very predictive in the lower range of b.wt. Maternal ht was no longer a predictor of fetal growth, suggesting that its effect was strongest in the middle range of b.wts. This analysis also demonstrates that women with a higher prepregnancy wt, and higher glucose

concentrations have bigger babies. Though the effect of glucose was not sustained in the lower b.wt range, it approached significance in the higher range (p=0.08). Nevertheless, glucose did not demonstrate as big of an effect in the lower or higher ranges as it did over the entire range of b.wts. The low r^2 of 0.25 in the highest quartile of b.wt model implies that the model is not capturing much of the variability in the higher b.wt range, and the lower sample size of both of the models may also have affected the findings.

It is important to recognize that due to the lack of knowledge about potential predictors of b.wt, the regression analyses were performed in an exploratory manner. In the model that included known predictors, only 42% of the variability in b.wt was captured, leaving 58% of the variability unexplained. Several important findings were revealed in this study: first, gestational age, maternal ht, prepregnancy BMI, and amf glucose were the strongest predictors of b.wt in this population; second, future studies should focus on amf glucose, and perhaps lactate, to further investigate their ability to predict b.wt; and third, percentile b.wt models never captured a high amount of the variability due to the strength of gestational age as a predictor of b.wt. Since a priori knowledge of gestational age at birth would not be necessary, predicting b.wt relative to gestational age, ie, percentile b.wt, is of higher clinical value as described earlier, and therefore it would have been the preferred model.

VI. DISCUSSION

Predicting low and high b.wts is clinically important, and is currently very difficult at an early stage of pregnancy. This project was the first large human study to examine the relation between amf constituents, in particular, total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, ßHBA, and lactate, at 14-16 weeks of gestation, and fetal b.wt. This study confirmed that gestational age, infant gender, maternal ht, prepregnancy wt and BMI, and parity are important determinants of b.wt as previously reported (Kramer, 1987). Stepwise multiple regression revealed that the strongest predictors of b.wt in our population were gestational age ($p<0.0001$), maternal ht ($p<0.0001$), prepregnancy BMI ($p=0.02$), and amf glucose ($p=0.02$). Due to the large scope and magnitude of this research project, it was not possible to address the second part of the first research question, regarding predictability, and the second research question, regarding detection of growth abnormalities, at this time. Once this field of research becomes narrower in terms of which amf constituents are most strongly associated with growth, assessing predictability and the use of such measurements as a diagnostic tool will be more feasible.

This was the first human study to show that amf glucose may potentially predict b.wt, even early in gestation. In rank order, glucose came third, before prepregnancy BMI, as a predictor. For every mmol/L increase in amf glucose, a 119.4g increase in b.wt was noted (Table 24, p. 74). It is recognized that although this study did examine the effect of glucose at the higher amf concentrations it did not do so separately for the women with and without gestational diabetes. The link between gestational diabetes and higher b.wt could be mediated by exposure of the fetus to higher amf glucose levels since higher maternal blood glucose levels have been shown to lead to higher amf glucose (Koski and Fergusson, 1992), and since glucose is the principle metabolic fuel. Previously, amf glucose at term was shown to be positively associated with and predictive of fetal wt in the rat model (Koski and Fergusson, 1992). Past human research has also found positive relations between amf glucose and b.wt, and more specifically, lower levels of amf glucose in association with growth retardation (Drazancic and Kuvacic, 1974; Marin and Hood, 1979), however, all focused on late gestation and

neither looked at predictability. Using multiple regression in the lower range of b.wts, this study did not find that glucose was as strong a predictor of low b.wts as it was over the entire range of b.wts, possibly because the effect is not manifest at such an early stage of gestation, or due to the small sub-sample size used for the analysis that focused on the lowest quartile of b.wts. Using a similar analysis in the highest quartile of b.wts, there was a stronger indication that amf glucose was a potential predictor in the higher range of b.wts ($p=0.08$, Table 25, p. 75). Interestingly, amf glucose was related to maternal ht and prepregnancy BMI, known maternal predictors of b.wt (Kramer, 1987). One-way ANOVA revealed that as maternal ht decreased, and BMI increased, amf glucose increased. It is suggested that perhaps taller women need to consume more calories to attain and maintain a higher amf glucose level. Heavier women may consume more calories allowing them to maintain higher glucose levels. Whether maternal ht and prepregnancy BMI mediate the effect of glucose, or vice versa, or whether the effects are independent is unknown at this time.

The exploratory nature of this first large human study necessitated the use of several different regression models in an attempt to capture the most variability in b.wt. The best fit was achieved using the maternal (height, prepregnancy weight and BMI, parity, ethnic origin, and smoking status) and neonatal (gestational age and gender) parameters as the independent variables ($r^2=0.42$, Table 21, p. 70). Although adding the amf constituents did not improve the variability captured by the model (Tables 22 and 24, pp. 72 and 74), amf glucose was nevertheless a significant factor using stepwise regression. Great emphasis was placed on the stepwise regression model despite its smaller reported r^2 ($r^2=0.35$, p. 74) since it is a more powerful statistical tool. As previously mentioned, the regression models never captured more than 17% of the variability when percentile b.wt was used as the dependent variable (Table 24, p. 74). Because of the greater clinical relevance of having percentile b.wt, as opposed to b.wt, as the outcome variable, future research should concentrate on models that include other amf constituents as independent variables.

The sample sizes used in the study were limited by amf sample volume and the availability of b.wt data. In order to verify that the sample sizes used were sufficient to examine statistically significant differences, sample sizes required for each amf

constituent based on the standard deviations found in this study and desired detectable differences were calculated. Table 26 (p. 79) summarizes this information. As before an incidence of IUGR of 3%, and a two-sided p-value of 0.05, with a 90% chance of detection, were used. The minimum relevant differences used for the calculations were based on a clinical judgment after examining the population means and variability observed within the population for each constituent. With the exception of creatinine and glucose, the sample sizes were not sufficient in our population. The sample sizes used for total protein, albumin, and urea nitrogen were close, and since the results obtained were similar to previous studies, it was not thought to impact on the findings. Insufficient sample sizes may have had an impact on the findings for uric acid and lactate. As demonstrated by the magnitude of the sample size required for ßHBA, there is no point in examining it further as a predictor of b.wt.

Table 26. Sample sizes required for statistical significance for each amf constituent based on results of the present study[1]

Amf constituent	σ	μ_1[2]	N required	N studied[3]	Difference[4]
Tot.ptn (g/L)	2.12	2	406	334	-72
Alb. (g/L)	1.04	1	391	301	-90
Urea N (mmol/L)	1.01	1	369	353	-16
Creat. (µmol/L)	13.4	25	104	299	195
Uric Acid (µmol/L)	45.2	40	462	300	-162
Gluc. (mmol/L)	0.59	1	126	353	227
βHBA (µmol/L)	74.9	20	5066	353	-4713
Lact. (mmol/L)	2.52	2	574	300	-274

[1] tot.ptn, total protein; alb, albumin; urea N, urea nitrogen; creat., creatinine; gluc., glucose; lact., lactate
[2] minimal relevant difference to be detected
[3] sample size for which both constituent and b.wt information was available
[4] refers to the difference between the N studied and the N required;
negative numbers indicate insufficient N studied

Storage of the amf at -80°C was not thought to affect amf composition early in gestation. One study assessing the effects of freezing at -20°C and -70°C found little change in composition of homeostatically regulated constituents such as glucose, urea, uric acid, and creatinine, especially at -70°C (Zaidman et al., 1992). Another study looking at the effects of repeated freezing and thawing on early amf total protein composition found that rapid freezing and thawing up to 10 times in the space of a few days did not alter the concentration (Keniston et al., 1975). As previously mentioned in

the Materials and Methods section (p. 43), storage conditions previous to the time that the fluid was in the author's possession were unknown. This may have led to metabolic changes in the fluid, although this was not assessed, and the magnitude of such changes could potentially be different for different samples depending on the way the fluid was handled by the different participants.

The concentrations of the amf constituents measured in this study were similar to past literature values (Benzie et al., 1974; Otey et al., 1967; Saleh et al.. 1989; Weiss et al., 1985), with the exception of creatinine (Benzie et al., 1974). The value for creatinine found in this study was nearly three times greater than previously reported (194.0±13.4 μmol/L vs 70.72±8.84 μmol/L; Benzie et al., 1974) and, in fact, was comparable to that found in the same study when measured at 40 weeks' gestation (194.48±44.20 μmol/L). Past studies that measured amf creatinine in early gestation (Benzie et al., 1974; Campbell et al., 1992; Cherry et al., 1969, Doran et al., 1970; Gulbis et al., 1996; Jauniaux et al., 1991; 1998; Lind et al., 1971; Yong and Gui-Lan, 1982), however, have involved very small sample sizes compared to the present study (N=12-40 vs N=335), and as such may not accurately represent a larger population.

Amniotic fluid albumin constituted 61% of total protein in the present study, comparable to that found in other studies (Burdett et al., 1982), however, outliers in this study were identified such that the concentration of albumin was greater than total protein in 19 cases. The differences between the albumin and total protein concentrations ranged from –2.7 g/L to –0.012 g/L, and in 7 cases the difference was less than 1 g/L. One explanation for this occurrence is the possibility that substances that interfere with the chemical reactions upon which the analyses are based were present in the amf. Despite previous findings that deep freezing and thawing and re-freezing doesn't affect protein concentration, it should be mentioned that total protein and albumin were measured at different times, on different aliquots of fluid that had been thawed and re-frozen different amounts of times. Albumin was, nevertheless excluded from the regression analyses because of its colinear relationship with total protein.

Amniotic fluid lactate was not significantly associated with b.wt in this study, although it approached statistical significance as a potential predictor in the regression model with all the amf and maternal and neonatal parameters (p=0.09, Table 24, p. 74).

Lactate achieved significance in the model with the amf constituents alone (p=0.05, Table 23, p. 73), suggesting that its effect was not strong enough to be seen over and above the other predictors. The lack of significant findings may be attributed to an insufficient sample size (N studied=300 vs N required=574), as described above.

The present study did not find significant associations between b.wt and amf ßHBA or uric acid. Two previous studies done during late gestation, one in rats (Koski and Fergusson, 1992) and one in humans (Bissenden et al., 1979), had found a significant negative correlation between amf uric acid and growth. One human study done between the 10^{th} week of gestation and term, found a positive relation between amf uric acid and fetal growth up to 2500g, afterwhich, no association was observed (Weiss et al., 1974), however the study involved aborted fetuses for the period prior to 20 weeks' gestation and was based on correlation analyses. The lack of a significant finding in the present study suggests that either uric acid is not a good predictor in humans early in gestation or that the sample size of this study was too small to detect it (N studied=300 vs N required=462), as described above. Beta-hydroxybutyrate has never been studied in relation to fetal growth, and as mentioned, investigating its potential as a predictor would require an extremely large sample size.

Measures of protein content and metabolism were examined with respect to b.wt early in gestation. The results of our study concur with a small animal study that found no relation between amf total protein concentration and b.wt (Brans et al., 1986). Albumin concentration was similarly not found to have a significant association with fetal growth in this study, and no previous literature comparing the two parameters exists. It is important, however, to keep in mind that the albumin values obtained in this study, though similar to other reported values, were questionable due to previously mentioned technical problems in the laboratory analysis. As substrates for protein synthesis, amf amino acid levels may be a more appropriate indicator to measure in future pursuits. Previously, amf urea nitrogen was negatively correlated with fetal wt in rats (Koski and Fergusson, 1992), however the results of our study were similar to another human study that found no relation between the two (Bissenden et al., 1979). Despite a sufficient sample size (N studied=299 vs N required=104), our study did not corroborate previous studies that found a significant positive association between b.wt and amf creatinine

(Begneaud et al., 1969; Doran et al., 1970; Miodovnik et al., 1982; Roopnarinesingh, 1970; Weiss et al., 1974; Wyatt et al., 1969; Yong and Gui-Lan, 1982), however, all 7 studies were done in late gestation or at term, strongly suggesting creatinine as a potential predictor in late gestation. Nevertheless, other studies also done during late gestation or at term found no correlation between the two (Bissenden et al., 1979; Cassady et al., 1975; Williams et al., 1981). Interestingly, one-way ANOVA revealed that the protein markers in this study changed with other known determinants of b.wt. Female babies had higher total protein levels than male babies, albumin concentration was higher in participants of ethnic origins known to have higher b.wts, urea nitrogen was higher in women with a ht $\geq$1.62m (the highest 2 quartiles), in women with a prepregnancy BMI <20.6kg/m^2 (the lowest quartile) as compared to women with a prepregnancy BMI $\geq$25.7kg/m^2 (the highest quartile), and in participants of ethnic origins known to have larger babies. Despite these significant changes, this study demonstrated that amf markers of protein metabolism were not good predictors of b.wt early in gestation but may be modulated by known established predictors.

The participant characteristics in this study were similar to those in another recent study of pregnant women in the Montreal area (Synder et al., 1994) with respect to the number of female babies (47.3% in our study vs 45.7%), number of babies weighing $\geq$4000g (12.7% in our study vs 13.8%), and number of babies weighing $\geq$4500g (2.3% in our study vs 3.0%). Birth wt by gender in this study was similar to that reported for the Canadian population between 1986 and 1988 (♀: 3369±525g in our study vs 3335±522g, ♂: 3446±574g in our study vs 3464±556g; Arbuckle et al., 1993). A slightly lower number of babies weighed less than 2500g than that reported by Arbuckle et al. (3.7% vs 5.8%).

The participant characteristics were different to those found in the study by Synder et al. (1994) with respect to mean b.wt, prepregnancy BMI, parity, and cigarette consumption. Since the Synder et al. study (1994) involved women with gestational diabetes, differences in b.wt and BMI were expected. The mean b.wt (3409±552g vs 3542±481g) and the average prepregnancy BMI (23.6kg/m^2 vs 25.6kg/m^2) in our study were lower than those reported by Synder et al. The number of multiparous women was slightly higher in this study (73%) compared to 60.8% in the study by Snyder et al., the

reason being that the women studied in this project were by definition over 35 years of age at the time of delivery and as such were likely to have more children. The percent of women who smoked while pregnant was lower in this study than that reported by Snyder et al. (8.4% vs 22.7%).

The effects of the maternal and neonatal parameters on b.wt seen in this study were similar to previously established trends. Comparing the magnitude of effects of maternal ht, prepregnancy wt, and parity on b.wt found in this study with those reported in the meta-analysis by Kramer (1987), a greater effect was seen for ht and wt in this study (34.5 g/cm vs 7.8 g/cm, 24.1 g/kg vs 9.5 g/kg), and a smaller effect was seen for parity (16.6 g/birth vs 43.3 g/birth). In the report by Norman (1982), male babies were on average 200g heavier than their female counterparts. In the present study, male babies were on average 80g heavier. Abel (1980) and Berkowitz (1988) concurred that b.wt is reduced by an average of 200g among smoking mothers compared with non-smoking mothers. Babies of smoking mothers in this study weighed an average of 72g less than those of non-smoking mothers. The lack of variability in the data on ethnic origin and cigarette consumption (n=33 for smokers) is one plausible reason why their effects on b.wt were attenuated in this study.

VII. CONCLUSION & IMPLICATIONS

Diagnosis of growth retardation and macrosomia is currently unreliable during early gestation when corrective measures could potentially be taken to prevent their occurrences. The present study is an important first step in investigating the use of amf constituents to predict human fetal growth. It put forth substantial evidence that amf glucose, and perhaps lactate, may be potentially important predictors of human fetal b.wt even during early gestation. Simultaneous, ongoing research is looking at other potential indicators such as insulin (Krew et al., 1994; Weiss et al., 1984) and insulin-like growth factors (Wang and Chard, 1992) as potential predictors of b.wt. We suspect that combining glucose, and perhaps lactate, in a model with insulin and insulin-like growth factors will lead to a regression model that captures more variability in b.wt and would

lead to a higher degree of predictability. As mentioned earlier, developing a model to predict percentile b.wt by gestational age would be even more clinically useful since it is does not rely on gestational age at birth. This should therefore be the emphasis of future studies.

The implications of finding a predictive model would be twofold: one, that measurement of certain amf constituents during routine amniocentesis at 14-16 weeks' gestation can be used for the early detection of IUGR / macrosomia; and second, that manipulation (via maternal diet, intraamniotic infusion, etc.) of levels of these constituents may prevent or protect against the occurrence of IUGR / macrosomia. Future human research should, therefore, focus on determining what factors are driving the changes in the amf constituents in order that intervention is made possible. Animal studies have pointed to maternal diet as influencing amf composition (Bernstein et al., 1992; Koski and Fergusson, 1992; Nowacka and Gorski, 1988), however, further studies in humans are required. The present study is a pioneer in this field as it focused on humans early in gestation, and provides a basis for the continuation of such pursuits.

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APPENDIX 1

Montreal Children's Hospital Amniocentesis Consent Form

CONSENTEMENT A L'AMNIOCENTESE, CHORIOCENTESE ET AU DIAGNOSTIC PRENATAL / CONSENT FOR AMNIOCENTESIS, CHORIONIC VILLI SAMPLING & PRENATAL DIAGNOSIS

Par la présente, j'accorde mon consentement au Programme McGill de Diagnostic Prénatal et à un médecin habilité par ce Programme, pour faire une amniocentèse / un prélevement du placenta (CVS).

Avant de signer le présent consentement, on m'a expliqué la nature, le but et les effets possibles de l'intervention et j'ai lu le verso. Je comprends parfaitement cette description et je suis au courant des risques que cette intervention comporte, y compris le risque de fausse couche. On m'a également déclaré que l'information recueillie permettra à mon médecin de poser un diagnostic, bien qu'il n'y ait pas de garantie que cette information puisse fournir un diagnostic définitif. Je comprends que cette analyse peut révéler des informations touchant à la paternité. Sauf erreur, je comprends qu'un résultat normal n'élimine pas la présence d'anomalies autres que celles qui sont recherchées par les analyses effectuées. Je sais qu'après le diagnostic, les échantillons qui restent seront détruits, ou bien sauvegardés pour raison de contrôle de qualité seulement, en aucun cas, ils ne me seront remis.

I hereby give consent to the McGill Prenatal Diagnosis Programme and to a physician appointed by the Programme to perform amniocentesis/cvs.

Prior to signing this consent, the nature, purpose, and possible effects of the procedure have been explained to me and I have read the overleaf. I fully understand this description and have been made aware of the inherent risks, including the risk of miscarriage. I have also been informed that the information obtained will help my doctor to arrive at a diagnosis although there is no guarantee that this information can provide a definitive diagnosis. I understand that there is the possibility of the test revealing a condition that relates to paternity. I also understand that a normal finding cannot rule out the presence of disorders other that those for which the procedure is to be performed. I accept that following this medical diagnosis, any remaining cells will be destroyed or kept for quality assurance purposes only and in no event will be returned to me.

Signature_____

Témoin/Witness_____

Date_____

RESUME DE L'INFORMATION SUR L'AMNIOCENTESE ET LA BIOPSIE CHORIONIQUE (CVS)
SUMMARY OF AMNIOCENTESIS & CHORIONIC VILLI SAMPLING INFORMATION

	AMNIOCENTESE / AMNIOCENTESIS	BIOPSIE CHORIONIQUE CHORIONIC VILLI SAMPLING
PROCEDURE	Liquide amniotique prélevé avec une aiguille et une seringue/Amniotic fluid removed by needle & syringe	Villosité chorioniques prélevées avec un cathéter et une seringue/Chorionic vill removed by catheter & syringe
CALENDRIER/TIMING	14-18 semaines/wks	9-12 semaines/wks
RISQUE D'AVORTEMENT SPONTANE/RISK OF MISCARRIAGE	0.5%	1%
CHANCE DE REUSSITE DU DIAGNOSTIC/CEANCE OF SUCCESSFUL DIAGNOSIS	Environ 99% Approximately 99%	Environ 95%. Si infructueux, CVS peut être suivie d'une Aniocentèse/ Approximately 95%. If unsuccessful, can follo with amniocentesis
TEMPS NECESSAIRE POUR LE DIAGNOSTIC/ TIME REQUIRED FOR DIAGNOSIS	Environ 3 semaines/ About 3 weeks	3 semaines/wks
PRECISION/ACCURACY	99.8%	97.7%
RESULTATS/RESULTS	OBTENUS AU BUREAU DU MEDECIN OBTAINED THROUGH YOUR DOCTOR'S OFFICE	

APPENDIX 2

Study Consent Form (English and French Versions)

APPENDIX 3

Study Subject Questionnaire (English and French Versions)

McGill

School of Dietetics and Human Nutrition
Faculty of Agricultural and Environmental Sciences
McGill University
Macdonald Campus

École de diététique et nutrition humaine
Faculté des sciences de l'agriculture et de l'environnement
Université McGill
Campus Macdonald

Tel.: (514) 398-7842
Fax: (514) 398-7739

21,111 Lakeshore
Ste-Anne-de-Bellevue
Québec, Canada H9X 3V9

111

Questionnaire

Please answer this brief questionnaire. You are reminded that all the information provided will be kept strictly confidential.

Name:_____ Telephone number:_____

Date of birth:_____ Height:_____feet_____inches *or* _____meters

Ethnic background:
North American ____ South American ____
European ____ African ____
Middle Eastern ____ Asian ____
Other _____

Number of children I have already given birth to: _____

The following information pertains to this pregnancy only:

Weight prior to pregnancy:_____ pounds *or* _____ kg

I am in my _____th week of pregnancy Due date:_____

Hospital where I will deliver:
Royal Victoria ____
Lakeshore General ____
Jewish General ____
St. Mary's ____
Other _____

Name of Obstetrician/Gynecologist:_____

I am a smoker:
Yes ____
if Yes, while pregnant I smoke _____ cigarettes / day
Yes, but stopped while pregnant ____
No ____

While pregnant, I consume an average of:
0-1 alcoholic drinks / week _____
2-5 alcoholic drinks / week _____
6-10 alcoholic drinks / week _____
11-15 alcoholic drinks / week _____

0-1 cups of coffee/tea / week _____
2-5 cups of coffee/tea / week _____
6-10 cups of coffee/tea / week _____
11-15 cups of coffee/tea / week _____

I am currently taking medication (prescribed by my doctor or over-the-counter): Yes___ No___
If you checked yes. please specify _____
_____

THANK-YOU

APPENDIX 4

Ethics Approval: McGill Medical Ethics Institutional Review Board

APPENDIX 5

Ethics Approval: Royal Victoria Hospital

APPENDIX 6

Ethics Approval: St. Mary's Hospital

APPENDIX 7

Study Consent Form: St. Mary's Hospital (English and French Versions)

APPENDIX 8

Ethics Approval: Lakeshore General Hospital

APPENDIX 9

Study Cover Letter (English and French Versions)

APPENDIX 10

Study Chart Form

Name:_____ DOB:_____
Postal code:_____
Ethnic Origin:_____

Gravity___ Parity___ Aborta___
Ht: _____ Pre-pregnancy wt: _____ Wt at term: _____
Wt at _____wks':_____
Meds:_____
Smoking Habits:_____ quit:_____
Coffee Habits:_____ quit:_____
Alcohol Habits:_____ quit:_____

Amniocentesis: _____wks' gestation

Hospitalized during pregnancy:_____ reason:_____
_____ reason:_____
_____ reason:_____
Infection/fever gestational age:_____reason:_____
Toxemia/preeclampsia/htn _____
Rh compatibility:_____ received shot____

Delivery: Induction ____ reason:_____
C-section ____ reason:_____
_____wks' gestation
Date: _____
Male___ Female___
Wt:_____g Placenta wt:_____g Birth length:_____cm
Apgar 1____ 5____ 10_____
Condition at birth:_____

Abortion____ Miscarriage___ date: _____
reason:_____

History: premature/IUGR/low b. wt.: child___ twin/triplet wt_____ gestational age___
child___ twin/triplet wt_____ gestational age___
child___ twin/triplet wt_____ gestational age___

Maternal chronic disease:_____ trt:_____
_____ trt:_____
_____ trt:_____

Comments:_____
_____
_____
_____