The effect of iron status during pregnancy on hearing functions in the newborn.

by

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Abstract

Background: Iron deficiency, anemia, and iron excess have been associated with altered hearing functions in children and adults. Animal studies suggest that iron deficiency during pregnancy negatively affect fetal auditory development. The relationship between maternal iron status and auditory functions in healthy term newborns has not been clearly elucidated among humans. The goal of this pilot study was to determine the relationship between markers of iron status during pregnancy and brainstem auditory function in healthy neonates.

Methods: Pregnant women who gave birth at the Montfort Hospital were recruited to take part in this study (n=6). Within two weeks after birth, their newborn's hearing function was assessed by wave amplitude, latency and inter-latency from the Auditory Brainstem Response (ABR) test. Markers of iron status, namely hemoglobin (Hb) and mean corpuscular volume (MCV), were collected retrospectively for the first and second trimester from the women's medical chart.

Results: Overall, no significant relationship was observed between maternal Hb and MCV concentrations and newborns auditory function. Although two significant Spearman correlations were observed (MCV and inter-latency I-V; r=0.87; p=0.005 and Hb and amplitude V; r=0.89; p=0.04), these findings may be due to chance because of multiple testing and the small sample size.

Conclusion: Although iron is a key nutrient involved in the brain and auditory system development, we were not able to demonstrate a relationship between iron status during pregnancy and newborn hearing function. Prospective or intervention studies with a larger sample size and with more specific iron markers (ex. ferritin) are required to confirm these findings.

Résumé

Contexte : La déficience en fer, l'anémie et l'excès en fer ont été associés à des anomalies auditives chez les enfants et les adultes. Des études chez les animaux suggèrent que la déficience en fer pendant la grossesse peut avoir un impact négatif sur le développement du système auditif du fœtus. La relation entre le statut maternel en fer et les fonctions auditives du nouveau-né en santé né à terme n'a pas été clairement élucidée chez les humains. L'objectif de cette étude pilote était de déterminer la relation entre les marqueurs du statut en fer pendant la grossesse et la fonction auditive du tronc cérébral chez les nouveau-nés.

Méthodes : Des femmes enceintes ayant accouché à l'Hôpital Montfort ont été recrutées pour participer à cette étude (n=6). Dans un délai de deux semaines suivant la naissance, la fonction auditive de leur nouveau-né a été mesurée par l'amplitude, la latence et l'interlatence des ondes du test du potentiel évoqué auditif du tronc cérébral (PEATC). Les marqueurs du statut en fer, soit l'hémoglobine (Hb) et le volume glomérulaire moyen (VGM), ont été collectés rétrospectivement pour le premier et le deuxième trimestre dans le dossier médical des femmes.

Résultats : Globalement, aucune relation significative n'a été observée entre les concentrations maternelles d'Hb et de VGM et la fonction auditive des nouveau-nés. Bien que deux corrélations de Spearman aient été observées (VGM et l'interlatence I-V; r=0.87; p=0.005 et l'Hb et l'amplitude V; r=0.89; p=0.04), ces résultats pourraient être dus à la chance à cause des tests multiples et de la petite taille de l'échantillon.

Conclusion : Bien que le fer soit un nutriment clé impliqué dans le développement du cerveau et du système auditif, nous n'avons pu établir de relation entre le statut en fer durant la grossesse et les fonctions auditives des nouveau-nés. Des études prospectives ou interventionnelles avec un plus grand échantillon et des marqueurs du statut en fer plus spécifiques (ex. ferritine) sont nécessaires pour confirmer ces résultats.

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Thesis Outline

Chapter 1: An overview of the project is described in the introduction. Fundamental concepts and relevant studies related to iron status and metabolism as well as to hearing functions are summarized in the literature review. This background information is then followed by the study rationale, the aim of the project, the hypothesis and specific objectives.

Chapter 2: The methodology is described in the second chapter. This section includes information on the study sample, methods used for the recruitment, data collection and measurements as well as the statistical analyses used.

Chapter 3: The results of this investigation are presented in this chapter.

Chapter 4: A summary of the findings and a discussion of the results are presented in this section, as well as comparisons with other relevant studies. A summary of the strengths and limitations of the study is also presented in chapter 4.

Chapter 5: The conclusions of the project, along with the relevance and implications of our findings, as well as suggestions for future research areas are discussed in the last chapter.

List of Abbreviations

OAE	Otoacoustic Emission
ABR	Auditory Brainstem Responses
HB	Hemoglobin
MCV	Mean Corpuscular Volume
HCT	Hematocrit
STFR	Serum Transferrin Receptor
IDA	Iron Deficiency Anemia
ID	Iron Deficiency
NHANES	National Health and Nutrition Examination Survey
TSAT	Transferrin Saturation
ESAs	Erythropoietin-Stimulating Agents
EAR	Estimated Average Requirements
RDA	Recommended Daily Allowance
DRI	Dietary Reference Intake
UL	Upper Intake Level
WHO	World Health Organization
SSNHL	Sudden Sensorineural Hearing Loss

CHAPTER 1

INTRODUCTION

It is widely accepted that the prevalence of iron deficiency anemia (IDA) is the most common form of nutritional deficiency in the world[1–3]. IDA is one of the most prevalent nutritional deficiencies during pregnancy. In fact, studies have shown that the occurrence of IDA among pregnant women in industrialized and developing countries is still around 18% and 50%, respectively[1–4].

Nutritional requirements during the gestational period increase significantly in order to ensure optimal fetal growth and development[5]. Indeed, it is difficult to support the increased iron requirements during pregnancy with diet alone[3]. Healthy diet before and during pregnancy can play a vital role in the development and delivery of a healthy baby[6]. Since iron supplementation during pregnancy has been shown to decrease the prevalence of IDA and its related adverse health consequences[7], Health Canada recommends pregnant women to take a daily multivitamin supplement containing 16-20 mg of iron[8]. Despite these public health recommendations, the prevalence of IDA among pregnant women remains high[2]. This could be explained by the fact that some pregnant women might take supplements containing insufficient amounts of iron, take supplements intermittently and/or only during certain trimesters[9]. The development of IDA during pregnancy can also depend on their iron stores at the beginning of their pregnancy, their rate of iron absorption and the form of iron found in supplements[9].

Insufficient or excessive nutrient intakes during pregnancy or early infancy may have short- and long-term negative impacts on offspring's health and well-being[10]. Among these nutrients, iron is critical in ensuring numerous important functions for normal fetal development, including the brain and auditory system[11–13]. Additionally, excess or insufficient iron intake during pregnancy can have

dramatic effects on maternal and fetal health such as increasing the risks of gestational hypertensive disorders, neonates being small for gestational age, prematurity, fetal death and maternal morbidity[14,15].

Studies examining the consequence of maternal IDA during pregnancy have also shown adverse impacts on subsequent children's health and development[12,16,17]. It is well known that IDA in early childhood can have a negative impact on infant's cognitive development[16]. Indeed lower cognitive performance and school achievement, as well higher behavioural problems[18], and intellectual disabilities can stem from IDA in childhood[18].The relationship between iron and cognitive/intellectual performances can partly be explained by the role of iron in neuronal and glial energy metabolism, neurotransmitters synthesis and myelination[19]. These iron-related functions also play an essential role in auditory neural maturation during the prenatal period[19]. In fact, iron is required for myelination of the auditory nerves which occurs during the fetal and neonatal periods[13]. For example, a prospective cohort study suggests that low iron status in preterm infants negatively affect their auditory functions[19]. Furthermore, a few studies on premature and term infants have shown that latent iron deficiency (ID) during pregnancy is associated with abnormal auditory neural myelination maturation at birth which leads to impaired hearing functions[13,20].

At the other end of the spectrum, some pregnant women have high iron intakes and blood levels (Hb concentrations above 130 g/L)[215,22], which can be due to iron supplementation and has been related to negative outcomes such as gestational hypertension, pre-eclampsia, eclampsia, low birth weight, and low Apgar score[15,23,24]. While some studies have shown a relationship between iron overload and altered hearing functions in children and adults[25–27], no studies have examined the effect of high maternal iron markers on hearing functions in newborns.

Despite the major role of iron in brain especially on the central nerves system development, the effect of ID and IDA as well as excess iron during pregnancy on fetal brain's neurophysiological

outcomes, including auditory function development, has not been well studied in healthy term human neonates. Thus, the goal of the proposed study is to examine the relationship between iron status during pregnancy and hearing functions in healthy newborns.

1. LITERATURE REVIEW

1.1 Iron

1.1.1 The physiology of Iron (intake, absorption, transport and storage)

Iron consumed in food is present in two forms: heme and non-heme iron[28]. Heme iron is found in hemoglobin (Fe^{2+} or ferrous iron) in animal food sources, such as meat, poultry, fish, and seafood[28,29]. Non-heme iron (Fe^{3+} or ferric iron) is present in plant-based foods, such as legumes, nuts, enriched grain products like cereals and bread, as well as dried fruit[28,29]. Overall, the absorption rate of heme iron from food is superior to non-heme iron and is estimated to be at 25% and 17%, respectively[30]. In omnivorous (meat eating) populations, heme iron is estimated to contribute to around 10-15% of their total iron intakes, but because of its increased absorption, heme iron represents around 15-35% of the total iron absorbed by the body[28,31]. There are also several other components of the diet that can impact iron absorption. For example, the phytates (myo-inositol hexakisphosphate) found in cereals and vegetables, act as the main inhibitors of iron absorption[32]. Polyphenols, which are present in plant-derived foods and beverages, such as vegetables, fruits, some cereals, legumes, tea, coffee, and wine[33,34], as well as calcium and proteins from dairy products[35–37], can also inhibit iron absorption. For instance, tea can decrease iron absorption by up to 90%[33]. Therefore, in order to maximize iron absorption, foods and beverages rich in these components should be consumed in-between iron-rich meals[33]. On the other hand, there are some components of the diet, like vitamin C (ascorbic acid) that can increase iron absorption. Indeed, vitamin C can increase iron absorption by around 35-40% by promoting the reduction of ferric iron to ferrous iron[38]. Therefore, including good sources of vitamin C in iron-rich meals can also be an effective strategy for increasing the absorption of non-heme iron as well as for overcoming the adverse impact of iron absorption inhibitors.

As an essential metal, iron plays an important role in Hb synthesis by erythrocytes, oxidation-reduction reactions, and cellular proliferation[39]. About two thirds of total body iron, which equals

around 3-4 g, is bound to the hemoglobin of red blood cell, which circulate in the body and supplies oxygen to the organs and tissues[40]. Iron also plays a significant role in cytochromes within the mitochondria, mediating the transfer of electrons in the electron transport chain[41]. It is also part of heme-containing enzymes such as catalase, xanthine oxidase, and glutathione peroxidase and acts as an enzymatic cofactor for aconitase, NADH dehydrogenase, succinate dehydrogenase alpha-glycerophosphate dehydrogenase[41]. Iron homoeostasis is largely achieved through the regulation of dietary iron absorption[41]. Once consumed, iron is absorbed by the enterocytes (nutrient-absorbing cells located on the surface of the small intestinal villi)[42] via the heme-carrier protein 1 (ferrous iron) and the divalent metal transporter 1 (ferric iron) which are located on enterocytes' apical membrane[41]. During gestation, iron is actively transported across the placenta in order to meet iron requirements for adequate fetal development[43–46].

The absorbed iron can be stored as ferritin or transferred through the mucosal cell and exported across the basolateral membrane by ferroportin[41,47]. According to body's requirements, absorbed iron can go to the tissues or be stored in the circulation[41,48]. The iron exported across the basolateral membrane is oxidized from ferrous to ferric iron by ferroxidase hephestin located at the surface of the membrane[41,48]. Transport of iron between tissues in the body is mediated by a protein called transferrin, [40]. It carries up to two ferric ions per molecule which can then be taken up by the cells via transferrin receptors[40,41]. As excessive iron concentrations can affect organs' function adversely, circulating levels of iron are well controlled by several factors including the hormone hepcidin which regulates the iron metabolism of storage and transport[49]. Iron is primarily stored in ferritin (hemosiderin), a large protein that can bind as many as 4,500 atoms of iron in the form of hydrated ferric oxide[41,47]. This regulation is controlled by hepcidin, a peptide synthesized in the liver in response to increased iron stores or inflammatory signals[41,50]. Hepcidin binds to ferroportin (the cellular iron export channel) in enterocytes and causes its degradation and internalization[50,51]. This thereby blocks dietary iron uptake and the

efflux of recycled iron from splenic and hepatic macrophages, and the release of iron from storage in hepatocytes[41,50,51]. Plasma iron and iron stores stimulate hepcidin synthesis by inhibiting erythropoietic activity[40,41]. This ensures that extracellular plasma iron concentration and iron stores are stable and covers erythropoietic iron requirements[40,41]. Hepcidin concentrations rise during inflammation and lead to iron sequestration in macrophages[40]. Therefore, anemia of inflammation can be the consequence of hypoferremia[40,51].

1.1.2 Markers of iron status during pregnancy

Different markers exist for assessing the iron status during various periods of life such as the critical gestational period. For example, the diagnosis of anemia is made with a decreased blood hemoglobin (Hb) concentration obtained by a full blood count[52]. Hb is an iron-containing protein in red blood cells which role is to carry oxygen[52]. Thus, low Hb concentrations result in a poorer ability for the blood to carry oxygen[52]. Although Hb is the most common indicator of poor iron status, it cannot be used to distinguish IDA from other causes of anemia[52,53].

Thresholds for anemia rely on sex, age, life cycle stage (ex. pregnancy), altitude, and smoking status[52]. IDA is diagnosed in adult men when their Hb concentration is less than 130 g/L, whereas, the value for adult women is less than 120 g/L. During pregnancy, this cut point is lowered to 110 g/L due to hemodilution[52]. To make a suitable assessment of ID, it is preferable to use several iron status indicators in combination because the diagnosis of ID is complex[54]. For example, hematocrit (Hct) less than 33% can also be used in combination with the conventional Hb cut-off for anemia (110 g/L) [55].

Iron status is routinely measured during the first and second trimester of pregnancy and sometimes just before birth by using hemoglobin, hematocrit and mean corpuscular volume (MCV) in the serum[56]. Hct and MCV are biochemical markers used to differentiate the type of anemia such as micro/macrocytic megaloblastic and sickle cell anemia[57]. Another more reliable but more expensive

iron status marker is serum ferritin, and is used as an indication of the iron stores[58]. In fact ferritin is considered as the 'gold standard' marker for the diagnosis of IDA during the gestational period[59,60]. Indeed, an observational study assessing laboratory markers of iron status in almost 500 pregnant women supports serum ferritin as the best indicator of biochemical ID overall[59]. Serum ferritin cannot only generate a precise response to the depletion of body's iron storage during the ID period, but it is also an acute phase protein[59]. In fact, many physiological states such as pregnancy, lactation, the period of infant's growth, inflammation, and tumour growth can affect serum ferritin concentrations[59,61]. Moreover, low concentrations of serum ferritin is specifically useful to distinguish IDA from other forms of anemia[58]. Thus, although Hb, MCV and Hct are routinely measured during pregnancy from readily available blood count, ferritin which is more expensive to measure, is often only used when suspicion of ID or IDA[59,60].

Serum transferrin receptor index (sTfR-index), which is the ratio of transferrin receptors to log ferritin, is another sensitive and specific indicator to distinguish between ID and physiological anemia and may offer stability in the assessment of iron stores from early pregnancy to full term birth[59]. When used alone, the sTfR is not sensitive to ID in early pregnancy[59]. Moreover, sTfR is a good biomarker for the erythropoietic capacity and can be used for determining iron storage deficiency in subclinical ID requiring only a small amount of serum or a small sample from finger pick[61]. Additionally, the sTfR compared with serum ferritin is less susceptible to be influenced by body inflammation and underlying acute and chronic infection[62]. Despite sTfR's practical clinical value and usefulness as a biomarker of IDA during pregnancy[59], the measurement of sTfR is not required for a diagnosis of IDA in routine practice[63].

Pregnancy affects the concentrations of some, but not all of the iron biomarkers[53]. Pregnancy is characterized by a poorer level of Hb and serum ferritin which is explained by lower iron availability to red blood cells and lower iron store[53]. However, some studies showed that the sTfR level is usually

higher than serum ferritin when the iron status is low in the body[64,65]. Additionally, the National Health and Nutrition Examination Survey (NHANES) revealed significantly lower sTfR levels in pregnant women in comparison with non-pregnant women[53]. In fact, sTfR may be downregulated during pregnancy in order to transfer iron from the mother's tissues to the fetus[53]. Therefore, even though other markers exist to measure iron status, those previously mentioned are the ones most frequently used in research and clinic.

1.1.3 Iron status during pregnancy; from deficiency to excess

If the dietary iron intake is insufficient to meet the body iron requirements, ID will most likely develop over time[54]. Blood losses and increased iron demands are other important factors that can increase the risk of ID [66]. IDA is the most severe form of ID and occurs when dietary iron intakes are insufficient and the iron stores depleted[1,5].

The causes and consequences of ID and IDA vary by trimester. It is primarily during the second and third trimesters of pregnancy that the iron requirements are increased in order to meet the needs of the growing placenta and fetus, to account for the expansion of maternal red blood cell mass, and Hb volume[15]. In fact, it is estimated that iron requirements are tripled towards the end of pregnancy[54,67]. Thus, this increased iron demand puts pregnant women at a higher risk of developing ID or IDA[5,68].

Although the majority of clinical and observational studies focused on the effects of the lower end of the spectrum of iron status, there are also health concerns to be raised at the higher end of that spectrum (iron excess). A low dose of iron supplement (30mg/day) during pregnancy in both anemic and non-anemic women has been shown to improve maternal iron status and also protect their newborns from IDA[69]. However, overtreatment should be avoided because of the adverse gestational outcomes such as eclampsia, pre-eclampsia and low birth weight[70]. A systematic review with a meta-

analysis of 21 randomized controlled trials and 15 quasi-experimental studies (n=5490), has shown that compared with daily iron supplementation, intermittent iron supplementation (two or three times a week), was associated with fewer side effects, while producing similar maternal and fetal outcomes[71]. In parallel with excessive iron intake from supplements, high Hb concentrations during pregnancy (≥130 g/L)[5], which is an indicator of iron status, have also been related to adverse pregnancy outcomes such as gestational hypertensive disorders (ex. preeclampsia), oxidative damage during pregnancy, neonate being small for gestational age, prematurity, fetal growth restriction and fetal death[22,56,72].The achievement of an optimal maternal iron status during pregnancy is of critical importance to help prevent pregnancy-related complications and adverse health outcomes in pregnant women and their neonates.

1.1.4 High iron status physiology and its impact on health

Very high Hb concentrations (i.e. 180 g/L) can cause high blood viscosity, which can lead to both compromised oxygen delivery to tissues and cerebrovascular complications[15,24]. Similar to what is observed with severe anemia, an inadequate amount of oxygen is transported to tissues, and can result in hypoxia[73]. Hemochromatoisis is the most prevalent heritable condition in populations of European descents and is characterized by abnormally high Hb concentration due to genetic mutations[74]. In this genetic disorder, the body absorbs excessive amounts of iron, which can result in tissue and organ damage[74]. Interestingly, a study of individuals with chronic mountain sickness or polycythemia, which arise from a combination of high-altitude residency and poor pulmonary function, illustrated that long-term survival with a Hb > 200 g/L is not possible[73]. Studies in adult rats have shown that iron overload was associated with rapid growth, increased body weight and insulin resistance [75,76]. Additionally, these rats had low serum and tissue copper concentrations, with induced

copper-deficiency anemia and cardiac hypertrophy[77]. In summary, not only ID but also iron overload can disturb normal iron-dependent physiology and body homeostasis.

1.1.5 Prevalence of ID, IDA and anemia

Undernutrition and micronutrient deficiency are important problems worldwide, particularly among the pregnant and infant populations[18]. Risks of ID and IDA are higher among certain groups, namely infants, young children, adolescents, women of childbearing age and pregnant women because of increased demands for growth and development[1,5,78]. ID is less prevalent among lactating women because of amenorrhea during exclusive breastfeeding[79]. According to the NHANES, pregnant women have significantly lower ferritin concentrations (ferritin= 40 ng/mL) in comparison with non-pregnant women (ferritin= 60ng/mL)[53], which may be partly due to hemodilution (increase in blood volume), but also to insufficient iron intake and/or storage[53].

ID is the most prevalent form of malnutrition and the leading cause of anemia in the world, including in developed countries[80]. In developed countries, pregnant women usually have a good nutritional status in comparison to those living in developing countries, where women might be severely undernourished and suffer from multiple micronutrient deficiencies[81,82]. Furthermore, it is widely accepted that IDA is the most common single nutrient disorder in the world and has been at the centre of global concern for many years[83–85]. Indeed, 30-34% of the United States' pregnant women suffer from ID or IDA during the third trimester despite routine prenatal supplementation[86,87]. In the offspring, the peak prevalence of ID occurs during early childhood, between 6 months and 3 years of age, because of their rapid growth and higher likelihood of insufficient dietary iron intake[85]. For example, a study performed among 434 nine-month-old infants in Vancouver (Canada) found that the prevalence of IDA (Hb $\leq$ 110 g/L) and low iron stores (serum ferritin < 10 μg/L) was 7% and 24%, respectively[88]. Research has also found a significant relationship between the prevalence of IDA and

breastfeeding duration in infants[88]. Results have shown that increasing breastfeeding duration significantly decreases the prevalence if IDA [88]. For instance, another study in the United States also showed that IDA was decreased by around 3% with increased breastfeeding duration or the use of iron-fortified infant formula and cereals[89]. Therefore, IDA is a concern not only in pregnant women, but in infants as well[88]. An analysis of the NHANES 2007-2010 data indicated that the prevalence of ID, IDA and anemia among 1-5 year-old children in the United States were 7.1%, 1.1% and 3.2%, respectively[90]. However, the World Health Organization (WHO)'s Global Burden of Disease (GBD) 2000 project reported that 7% of North American children suffer from anemia[80]. According to another WHO survey (1993-2005) representing 18.8% of the global population, 24.8% of the general population in the world suffered from anemia and 47.4% of the world burden of anemia has belonged to preschool-aged children[82].

1.1.6 The physiology of inadequate iron status and its health impacts on pregnant women

IDA and subclinical ID during pregnancy have been associated with adverse outcomes in women such as; poor gestational weight gain, complications during delivery, increased maternal morbidity, and preterm delivery[14,91,92]. Nowadays, IDA is becoming more and more widespread especially among pregnant women[93,94]. Inadequate iron status can vary in severity going from iron depletion to iron deficient erythropoiesis, and IDA[93]. IDA is the most serious stage of the spectrum, where Hb synthesis decreases and result in a microcytic hypochromic anemia because of the body's lack of iron storage[93]. When ID and iron deficient erythropoiesis occur, iron storage depletion is observed despite the normal range of Hb values which is defined as a subclinical ID[93].

ID first leads to the depletion of tissue iron stores accompanied with lower ferritin concentrations during which clinical symptoms are minimal[41,93]. ID is observed in two main forms: absolute or functional[54]. Absolute ID occurs when the iron stores of the body are low or exhausted

commonly due to poor dietary intake or occult bleeding[54,95]. The amount of stored iron is no longer adequate to meet the demands for erythropoiesis[54,95,96]. However, functional ID is characterized by total body iron storage at a normal level or even raised, as defined by the presence of stainable iron in the bone marrow together with a serum ferritin value within normal limits[54,96,97]. As a result, transferrin saturation is often low in these patients despite a normal or elevated ferritin concentration[98]. This condition is caused by the use of erythropoietin-stimulating agents which rapidly deplete circulating iron[95]. It can also be due to inflammation-related pathologies, including inflammatory bowel diseases or rheumatoid arthritis[95,98]. These inflammatory conditions decrease the availability of transferrin-bound iron in the blood[95,98]. Thus, iron mobilization and transport are low in both absolute and functional ID[95,98].

Interestingly, absolute and functional ID can happen at the same time[54]. Clinical signs and symptoms of both ID and IDA include paleness (45-50%)[99,100], fatigue (44%)[101], and dyspnea and headache (63%)[102]. The most common symptoms of IDA during pregnancy include pale skin, rapid heartbeat, shortness of breath, dizziness, trouble concentrating, and feeling tired or weak[103]. Indeed, all of the clinical features of ID and IDA will depend on the severity of the condition[54]. On the other hand, clinical signs and symptoms of high intake of iron during gestation include constipation and impaired zinc absorption[5]. Thus, both high and low iron marker concentrations have been associated with adverse health outcomes in the pregnant women and their child as well.

1.1.7 Iron status during pregnancy and its known impacts on the infants and children

Nowadays, anemia is becoming widespread not only in pregnant women, but also among infants [94,104]. WHO estimated that half of all types of anemia was attributed to ID and IDA[105]. In infancy and early childhood, IDA is associated with impaired temperature regulation[106], behaviour and cognitive development[107]. Additionally, ID and IDA have also been shown to be related to a declined

resistance to infection due to a reduction in oxygen transportation and a decrease in cellular oxidative capacity[108] and with impaired immune function [109].

Of importance, ID and IDA during pregnancy have been related to adverse outcomes on infants and children who were born from anemic mothers[14]. Indeed, there is a strong association between severe maternal anemia (Hb < 40 g/L) and increased prenatal maternal mortality and low birth weight[110–113]. Furthermore, an important consequence of maternal ID or IDA is to adversely impact fetal iron status[91,114]. For example, a study on pregnant women and their infants illustrated that around 61% of infants born from women with IDA during their gestation suffer from IDA at the age of six months even if they did not have IDA when they were born[93]. Gestational ID or IDA not only decreases infants iron stores, but can also increase the risk of developing subsequent later onset anemia[115,116], delayed mental development[117], poor neurocognitive development[56,91], and intellectual disability during their childhood[118,119]. Maternal iron stores provide 375-475 mg of iron to the fetus which then makes up for 40 to 70% of iron found in Hb for the first two years[62].

Not only insufficient iron during pregnancy has been associated with negative maternal and infant health outcomes, but high markers of iron status have also been shown to have deleterious effects. In fact, there is a U-shaped relationship between Hb concentration and prematurity, low birth weight, and fetal death; where the risk is increased if Hb concentration is lower than 90 g/L or higher than 130 g/L[5]. For example, a U-shaped curve was observed in a large British pregnancy cohort (n= 153,602), between Hb concentration and the proportion of low birth weight babies, where low and high Hb concentrations were associated with higher proportions of babies weighing less than 2500g[112]. Indeed, excessive iron intake during pregnancy achieved by iron supplementation has been related to gestational hypertension, pre-eclampsia, eclampsia, gestational diabetes and low birth weight[24,72,120]. In addition, Rioux et al. revealed in a literature review that high Hb concentrations along with iron supplementation have been associated with negative outcomes such as low birth weight, fetal death,

and prematurity[69,72,120]. This may be partly due to free-radical damage caused by iron excess during pregnancy[121]. Adverse impacts seem to increase sharply when Hb concentration is above 130 g/L[22]. Thus the higher end of the iron status spectrum is not more desirable than the lower end regarding maternal and fetal health outcomes.

According to two randomized controlled trials, iron supplementation meeting the pregnancy requirements during the gestational period can decrease the risk of low birth weight and preterm births[119,122]. However, a Chinese study has shown that although prenatal iron supplementation could decrease gestational ID and IDA among some of these women, most women still had ID (66.8% by serum ferritin <15μg, 54.7% by body iron <0 mg/kg) and more than 45% of newborns were still suffering from ID, despite supplementation[123]. Therefore, entering pregnancy with good iron stores and maintaining them through iron supplementation is essential to meet the iron needs of both the women and her fetus and prevent childhood anemia and its subsequent adverse health outcomes[56].

Research advances on the effects of anemia at different stages of gestation on infant health outcomes have led to two important findings. Firstly, women who are not anemic in their early pregnancy have infants with better physical growth than infants from women who were anemic in that period of time[124]. Secondly, women who are not anemic in their later gestational period have infants with higher attention and social interaction abilities (measured by the degree of alertness and responses to animate and inanimate visual and auditory stimuli) than infants of women who suffered from anemia at this stage of pregnancy[124]. Thus, avoiding anemia at all stages of pregnancy is crucial to ensure subsequent infant and child health.

1.1.8 Iron requirements and recommendations during pregnancy

Iron requirements depend on various factors aiming to maintain normal basal concentrations stores[5,67]. In fact, according to the Institute of Medicine, the Estimated Average Requirements (EAR) is

"*a nutrient intake value that is estimated to meet the requirement of half the healthy individuals in a group*"[125]. For pregnant women, the EAR for iron is 18 mg/day and 23 mg/day for their second and third trimester[79]. The Recommended Dietary Allowances (RDAs) is "*the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a group*"[125]. The RDA for iron among non-pregnant, pregnant and lactating women is 18, 27, and 9 mg/day, respectivly[5]. Since it is impossible to know the iron requirements on an individual basis, the Institute of Medicine recommends that all pregnant women take 27 mg/day of iron throughout their pregnancy[5]. The differences in requirements between non-pregnant and pregnant women, and between trimesters is mainly due to increased blood volume, red blood cell and Hb production and to fulfil the demand for fetal growth and development[67]. During pregnancy, women who develop IDA are recommended to take higher iron supplementation[69]. This is illustrated by the rise in the iron supplement intake in the second and third trimester of the Canadian pregnancy APrON cohort [126].

Although some research findings suggest that among pregnant women with sufficient iron stores can meet the increased iron requirement with healthy eating patterns[127], in general diet alone cannot support the increase in iron requirements during pregnancy[128]. Therefore, in order to help reduce the incidence of IDA during this period, Health Canada recommends all pregnant women to consume 16-20 mg of iron in supplements per day[8,128]. This range is based on statistical modelling of iron intake among Canadian pregnant women of the Canadian Community Health Survey (cycle 2.2) which would avoid both insufficient and excessive intake during pregnancy[128]. However, most common brands of prenatal supplements provide 27 mg of iron for pregnant women[129], which is in accordance with the RDA.

The Tolerable Upper Intake Level (UL) for pregnant women is 45 mg/day of iron[5], a level based on gastrointestinal distress and oxidative stress[130]. In normal pregnancy, oxidative stress is

increased[131], especially when the antioxidants level is low[132]. Therefore, iron supplementation during pregnancy can induce maternal oxidative stress and raise the production of free radicals[121]. In fact, high doses of iron supplements during pregnancy has been associated with fetal growth retardation[21]. Some experts do not even recommend iron supplementation during pregnancy[56] unless justified by suboptimal serum ferritin concentrations[130].

1.1.9 Dietary iron intake during pregnancy

The Scientific Review Committee of Canada[129,133] and the US Institute of Medicine recognize that many women have insufficient iron stores and are unable to meet their needs during pregnancy[134]. As mentioned earlier (section 1.8), daily iron supplementation is recommended in order to reduce the risk of IDA in pregnant women[135]. Among many Canadian pregnant women, iron intakes from food alone seem to be insufficient in comparison with the RDA[128,136,137]. Thus, Canada's Food and Drugs Act has made flour fortification with iron and folic acid mandatory since 1976 and 1997, respectively[138–140]. Iron enrichment of foods is an important public health intervention in order to improve the population's iron status[141]. Indeed, the purpose of the enrichment of food with iron coupled with iron supplementation, especially during pregnancy, is to supply enough iron to normalize Hb level and replenish iron storage to prevent the negative effects of IDA on the fetuses and infants. For example, a literature review done by Allen et al. (1997) reveals that iron supplementation (20-100 mg/day) during the gestational period improves maternal iron status during both the pregnancy and postpartum periods[110]. Iron supplementation during pregnancy can also protect children from anemia at birth and at 3, 6, and 12 months of age[142–144].

Several studies have examined the iron intake from food and/or supplements in North American pregnancy cohorts. The Canadian APrON pregnancy cohort study (n=599) showed that the mean intake of iron from supplements alone was 28 mg, 30 mg and 39 mg for the first, second and

third trimester, which corresponds to 104%, 112% and 143% of the RDA, respectively[126]. These values underestimate their real intake of iron since they do not take into account iron from food sources. However, the Canadian London pregnancy cohort study (n=2,019) indicated that the mean intake of iron from food alone was 13 mg and dietary supplements of iron was 19 mg among pregnant women and the mean total daily consumption of iron was 32 mg/day, thus surpassing the RDA [145]. The NHANES 1999-2006 survey showed that among pregnant women (n=1,296), the prevalence of pregnant women who took iron (mean supplement content of 48 mg/day) containing supplements during their first, second and third trimester was approximately 55-60%, 76-78% and 89% respectively[146].

There is some evidence showing that some pregnant women have a high iron intake from diet and supplements. This can partly be explained by the fact that pregnant women are taking supplements containing the RDA (27 mg/day) for iron in addition to having a diet rich in iron from fortified flour and iron-rich foods (e.g., red meat, poultry, and fish)[147]. The NHANES 1999-2006 survey reported that 72% of pregnant women were taking iron supplements, and among them, the median and mean total daily consumption of iron were 58 and 78 mg/day, respectively[87]. These values are well above the Tolerable Upper Intake Level (UL) of 45 mg/day, which is the safe limit set for iron intake during the gestational period[87]. Additionally, statistical modelling based on the Canadian diet showed that, when iron intakes from supplement is above 27 mg/day, it is estimated that 33% of pregnant women would be at risk of having iron intake above the UL[128]. High iron supplementation can increase Hb concentrations above 130 g/L, which is the cut point for high Hb values[21,22]. For example, a study on iron supplementation in non-anemic pregnant women showed that 61% of pregnant women who were taking 60 mg iron supplement daily had high Hb concentrations (≥135 g/L)[148]. Hb concentrations above this cut point has been associated with various adverse consequences, such as low birth weight, pre-eclampsia, eclampsia, low Apgar score, and gestational hypertension[15,23,24].

Nevertheless, both ends of the spectrum, whether it is insufficient or excessive iron intake, appear to be prevalent in North American pregnant women and can have adverse health outcomes on both maternal and fetal health.

1.2 Hearing

1.2.1 The development of the brain and the auditory system

The brain development starts early during pregnancy[149,150]. Indeed, neural growth, migration, and myelination begin simultaneously at different sites of the central nervous system, such as the cerebral cortex and neocortex, visual and auditory cortex, hippocampus and cerebellum, during this period[149–151].

Between 23 and 25 weeks of gestational age all of the ear's structures, including the cochlea, are in place[152–154]. In the human fetus, auditory information can be perceived at approximately week 26 of life[155]. Hair cells in the cochlea are fine-tuned for specific frequencies and can translate vibratory acoustic stimuli into an electrical signal to the brainstem between 26 and 30 weeks of gestational age[156]. Starting at 30 weeks of gestational age the auditory system is mature enough to recognize complex sounds and distinguish between different speech phonemes[153,157] which may correspond to the beginning of speech and language development[158]. Finally, auditory processing which facilitates learning and memory formation occurs by 35 weeks of gestational age[159,160].

Many sounds that are audible in the womb are generated internally by the mother's digestion, breathing, heart rate and physical movements[159]. Fetuses can also respond to sounds originated outside of the womb, like voices and music[161]. These sounds can stimulate the inner ear using a bone conduction mechanism[161]. A fetus can hear sound frequencies because the cochlea region is already developed[160,162]. Due to the fact that maternal tissue and fluid act as filters for high-frequency sounds,

the developing cochlear hair cells are protected from potentially damaging noise[162,163] making the womb an optimal and protective environment for auditory maturation. The first step for the necessary fine-tuning of the hair cells is a gradual exposure to low-frequency sounds to process high-frequency patterns of human speech, like intonation, pitch, and intensity[164].

After birth, the newborn is able to hear the high-frequency sounds related to the language processing[161]. Actually, several studies have illustrated that after birth, newborns prefer their mother's voice over an unknown female voice within only hours after birth[165,166]. This is due to the critical role of prenatal hearing experiences and selectively response to their mother's speech with detectable changes in their heart rate[165,166]. This suggests that human fetuses and neonates can recognize their mothers' voices[157,167]. Recognition memory in infancy has been shown to predict cognitive functioning and intelligence during childhood[168,169].

1.2.2 The role of iron status on brain development

As mentioned earlier, iron plays an important role in brain development[170]. Multiple developmental processes of the brain can be affected by ID during pregnancy and the postnatal period[44,45,171]. Indeed, the timing of maternal anemia during pregnancy may have different health implications. ID during pregnancy, especially in the last trimester has adverse impacts on fetal brain development[117,172,173]. Moreover, maternal ID within the third trimester can have long-term consequences including intellectual development delay during childhood, diminished neonatal general autonomous response, motor performance and self-regulation capabilities[12,117,173,174]. This may be due to the fact that the third trimester is a rapid developmental period for neuron myelination, synaptic function, monoamine metabolism, dendritic growth, neurotransmission, energy metabolism, and central nerve system development[44,124,174]. Such neurologic changes might also have impacts on attention, audiovisual responses and other cognitive and behavioural functions[175]. Other studies showed that ID

during the fetal or postnatal periods could change brain structure, neurochemistry, and cognitive functioning, and lead to long-term cognitive and motor impairment that cannot be corrected by iron supplementation after birth[16,176,177]. In support of these findings in humans, a study conducted on mice demonstrated that chronic marginal iron intake during pre- and early postnatal development can not only impact on the brain iron concentrations, but can also have functional effects on behavioural and cognitive development[178].

In humans, the hippocampus is one of the major brain structures which develops rapidly within the late fetal and early neonatal periods and its maturation and neural structure for recognition memory can be impaired by ID[179,188]. For example, a study done in two-month-old infants showed that ID occurring during the fetal-neonatal period was associated with lower mother's voice recognition compared with iron-sufficient infants, thus showing the negative impact on their memory recognition[179]. Another study in two-month-old infants with ID showed that they were less efficient at encoding stranger's voice and at updating their memory[180]. Other studies support the relationships between ID in infancy and altered recognition memory[107,181–183]. Moreover, arousal or engagement skills among iron deficient infants were shown to be lower than the iron sufficient infants[107,184]. This is supported by findings in rodents showing that ID occurring around the perinatal period affects the hippocampus neural structure and has deleterious effects on memory and learning[170]. Therefore, insufficient iron intake within the brain developmental period has a critical impact on mental, cognitive and behavioural functions[170].

A review of the literature on the impacts of pre- and postnatal ID on brain and cognitive development showed that there is a relationship between prenatal ID and delayed neurocognitive development and the risk of psychiatric illness[185] such as schizophrenia, mood disorder[186], major depressive and personality disorders[187]. Because the brain continues to develop rapidly right after birth, infants who had insufficient iron supply in utero and within the first 6 months of life may have deficits

in their neurocognitive development, including impairments in learning and memory[185]. Finally, cognitive abnormalities can persist up to 10-year-old even after iron repletion in infancy[183]. However, a meta-analysis revealed that iron supplementation in children and adolescent between 6-18 year-old taking iron supplements ranging from 17-260 mg/d (or 2-4 mg/kg/d) for a period of 8 to 21 weeks could increase attention and concentration, regardless of baseline iron status[188]. Maternal anemia can develop because of low intake of animal food sources which results in insufficient intakes of iron, as well as folic acid, and vitamin B_{12}[81]. In this context of multiple micronutrient deficiencies, the effect of maternal anemia at different trimesters of gestation on fetal and child brain and physical growth is less clear[124].

1.2.3 The role of iron in auditory functions

During the last trimester of pregnancy, progressive development of the fetal auditory nerve system occurs, and iron is an essential mineral for its development[45,159,189]. Iron is an important mineral for the development of the inner ear structures[152] and is involved in the maturation and development of the auditory nerves system such as the synthesis of neurotransmitters and the myelination of nerve fibres[190,191]. Brain ID occurring during the third trimester of pregnancy can impact the nerve myelination[13,192]. Importantly, within the prenatal period, the myelination of the auditory neural pathway can be used as a surrogate for overall brain myelination[189]. For example, infants who were born at ≤35 weeks of gestation and were ID in utero had altered auditory neural maturation (hypo myelination or delayed maturation of neurons leading to latencies in pathway transmission)[13] According to a study done on 285 marginally low birth weight infants who received iron supplements from 6 weeks to 6 months of age demonstrated that iron supplementation had no positive or negative impact on auditory functions[193].

There are several types of hearing loss such as conductive hearing loss, sensorineural hearing loss, retro cochlear and mixed hearing loss[194]. Sudden Sensorineural Hearing Loss (SSNHL) is one of the most common types of hearing affection causing deterioration of hearing function and is caused by sudden alterations of the vestibular cochlear nerve, inner ear, or central processing centres of the brain[195]. Even though most SSNHL are idiopathic, some are caused by autoimmune diseases, viral infections, labyrinthine membrane rupture, and vascular insults[195–201]. Among the different causes of SSNHL, vascular events are the most important ones[202]. In hematological disorders including IDA and sickle cell disease have been associated with SSNHL[202,203]. For example, a retrospective cohort study that was performed on 20,113 children and adolescents aged 4 to 21 years old has shown that the prevalence of SSNHL among patients who suffered from IDA was significantly higher than those without IDA[204]. Similarly, a population-based study conducted among a sample 16,016 adults, comparing a group suffering from SSNHL (n=4,004) with a control group (n=12,012), showed that the prevalence of IDA among SSNHL group was significantly higher than in the control group[202]. Subsequently, a case-control study was conducted to compare patients with sickle cell anemia (n=28) and patients without any hematological problems (n=28)[27]. They observed a higher prevalence of SSNHL among patients with sickle cell anemia in comparison with the control group[27]. Interestingly, a case study reported that a 26-year-old woman who suffered from sickle cell anemia and iron over loaded since her childhood and also had SSNHL[205]. Although these study results cannot prove causality, they suggest a link between IDA and SSNHL.

1.2.4 The impact of IDA during pregnancy on infant auditory functions

Animal studies, particularly using the guinea pig model, found that a mild maternal IDA during gestation and lactation had a negative impact on the auditory functions and nervous system development in the offspring[206,207]. In these studies, the auditory brainstem response (ABR) test, a

neurological and non-invasive assessment tool, has been used for evaluating auditory neural myelination and to assess postnatal brain maturation and differentiate between peripheral and central hearing disorders[208]. In one of these studies conducted by Jougleux et al., pregnant and lactating guinea pigs were fed an iron sufficient diet (n=10; 144 mg iron/kg) or an iron-deficient diet (n=10; 11.7 mg iron/kg) and the ABR test was conducted 24 days after birth on their offspring fed with a post-weaning sufficient iron diet[206]. Prenatal IDA was associated with an increased hearing threshold, corresponding to a hearing loss, in response to all tone pipes tested when compared to offspring born from non-ID dam [206]. The sensorineural hearing loss was also shown by differences in the ABR latency-intensity curves revealing a worse auditory acuity in IDA compared to the iron sufficient siblings[206]. Similarly, another study conducted a distortion product otoacoustic emission (DPOAE) task on young guinea pigs on postnatal day 9 and showed that mild maternal IDA during pregnancy and lactation resulted in hearing impairment, reduced hair cells and raised apoptotic cochlear hair cell numbers in the offspring[209]. Therefore, studies on guinea pigs demonstrate that iron status can affect cochlear and brainstem development during the prenatal and early postnatal periods and may play a role in the establishment of the adults' normal auditory function[209,210].

To our knowledge, only three studies examined the relationship between ID during pregnancy and hearing functions in human newborns. One prospective cohort study was done on 80 premature newborn infants who were born between 27 and 33 weeks of gestation[20], among which 35 had latent ID (11-75 ng/ml cord serum ferritin) and 45 had normal iron status with (> 75 ng/ml) at birth[20]. The ABR test was done between 24 and 48 hours after birth and revealed that infants with in utero latent ID had abnormal auditory neural maturation as indicates prolonged absolute wave latencies I, III and V and declined frequencies of mature ABR waveforms, in comparison with infants who had a normal in utero iron status[20]. The other study was performed on 45 infants who were born at 35 ≥ weeks demonstrated that latent ID at birth in infants (n=12; 11-75 ng/ml cord serum ferritin) was related to abnormal

auditory neural myelination when compared with infants with normal iron status (n=33; >75 ng/ml)[13]. Indeed, measures from the ABR test revealed prolonged I-III, III-V, and I-V interpeak latencies [13]. The other prospective cohort study was conducted on 90 premature and mature newborns with a gestational age ≥34 weeks, among which 60 suffered from latent ID (serum ferritin ≤75 ng/mL) and 30 had normal iron status (serum ferritin >75 ng/mL) at birth[19]. The ABR test which was performed within 48 hours after birth showed that newborns with latent ID had abnormal auditory maturation as demonstrated by significantly prolonged absolute wave V latencies, III–V and I-V interpeak latencies compared to newborns with normal iron status[19].

Two studies have examined the relationship between iron status and hearing functions in older infants and children[211,212]. One was performed in 6-month-old Chilean babies (n=55) and the other among 2.5-year-old Columbian children (n=25) and both studies revealed that IDA impaired auditory functions as shown by longer inter-peak latencies, central conduction time, altered morphological definition and response threshold from the otoacoustic emission (OAE) and the ABR tests[211,212]. OAE can measure the function of the outer hairs cells of the cochlear and it is usually part of a newborn hearing screening program[213–215]. Although the essential role of iron on auditory function development is clear, to our knowledge, the effect of iron status during pregnancy (both low and high levels) on hearing functions in healthy term human newborns has not been well studied.

1.2.5 The Impact of iron excess during childhood/adulthood on auditory functions

While clinical evidence suggests that sensorineural hearing loss can stem from IDA, other studies show that it can also originate from iron overload[26,216–219]. Iron overload is a clinical complication characterized by the excessive absorption and accumulation of iron within the body's tissues and organs, and is observed in certain pathologies such as siderosis, thalassaemia, and sickle cell anemia[26,216,218,219].

For example, a study on Angolan children (n=61) aged over 5 years old who suffered from sickle cell anemia[220] indicated that they had a 36% higher rate of bilateral hearing loss, compared to healthy children (n=61)[221]. Similarly, a prospective study was conducted among adolescents and adult patients who suffered from sickle cell anemia with ages ranging between 16 and 46 years (n=46) compared to control subjects between 16 and 39 years of age (n=29)[222]. Significantly greater hearing loss was found among sickle-cell anemia patients in comparison to control subjects, as well as with increasing disease severity and aging[222]. According to a review and meta-analysis covering the past 30 years[25], the prevalence of hearing impairment in sickle cell disease ranged from 0% (by Wiliams et al[223]) to 66% (by Onakoya et al[224]). Despite the clinical evidence in humans of various age groups suggesting a sensorineural hearing loss in pathologies associated with iron overload, to our knowledge, no study has examined the impact of high physiological iron concentrations during the gestational period on the development of the fetus's auditory system and function.

1.3 Study Rational and Relevance

In summary, it is known that healthy nutrition before and during pregnancy can play a vital role in the development and delivery of a healthy baby[6,225]. Iron is a crucial mineral required for early brain development and function including auditory neural maturation[19]. Indeed, insufficient iron such as ID and IDA or excessive iron concentrations have been shown to have adverse effects on pregnancy outcomes, including the brain and auditory system development[14,15,206].

Despite the documented evidence regarding the negative impact of anemia on auditory function in animals, children and adults, just a few studies examined the impact of ID or IDA during pregnancy on the development of auditory abnormalities in human neonates[13,19,20]. According to the APrON cohort study among North American pregnant women (n=599), most women were achieving

the RDA for iron from supplement at all trimesters but 19% were above the UL at their third trimester[126].

Despite the fact that iron status during pregnancy plays a critical role in the development of fetal auditory nerves system, the impact of iron status during pregnancy on hearing function has not been well studied, particularly in humans. Thus, the goal of this pilot study is to examine the relationship between iron status during pregnancy and hearing functions in human newborns.

A basic hearing test, the OAE, is currently performed routinely in Ontario in every newborn right after birth[226,227]. This test is not specific, and no information is provided on the newborn's hearing neurophysiological development[226,227].

The health care professionals must have better tools to manage iron status among pregnant women in order to prevent the consequences of maternal iron deficiency or excess in the neonates. If our hypothesis is confirmed by our study and replicated in further ones, pregnant women diagnosed with ID, IDA or iron excess should systematically be followed up with their newborn with more advanced hearing tests at birth. Thus, this study, as well as future studies that could arise from this one has the potential contribute to promote the healthy development of infants, especially hearing development, during the pre and perinatal periods.

1.4 Research Aim, Hypothesis and Specific Objectives of the Project:

Aim:

To determine the relationship between the iron status during pregnancy and auditory functions in the newborn.

Hypothesis:

Low and high concentrations of iron markers during pregnancy negatively impact the development of the fetus's auditory nerve system thereby altering hearing functions in the newborn.

Specific objectives:

To determine the relationship between:

1) iron status markers (Hb and MCV) concentrations during the first and second trimesters of pregnancy and cochlear functions as measured by the OAE test in newborns.

2) iron status markers (Hb and MCV) concentrations during the first and second trimesters of pregnancy and the auditory neural myelination as measured by the ABR in newborns.

CHAPTER 2

METHODOLOGY

2.1 Study Design

This pilot research project was a clinical investigation of the relationship between iron markers (Hb and MCV) and hearing functions (cochlear and brainstem) as evaluated by two tests in neonates. The maternal iron markers (Hb and MCV) were obtained retrospectively from pregnant women's medical chart in the first and second trimesters. The two hearing tests were assessed in newborns within two weeks after birth either at the Hospital Montfort (*Institut de recherche de l'Hôpital Montfort* research facility or hospital room) or at the participant's home.

2.2 Study Sample and Recruitment

The targeted population consisted of pregnant women in their second and third trimesters that had to give birth at the Montfort Hospital in order to have access to their medical chart. Although women could also be recruited just after birth in their hospital room, we preferred to recruit them prenatally in order to have more time to explain the project to them, answer their questions and obtain their informed consent.

Once the pregnant women received information on our study (see recruitment means below), they were asked to contact the research team by phone or email if they wanted more information or were interested in participating. These women were then contacted by the Master's student (Mona Doorsian) or a research assistant (for francophone participants) to explain the project and ensure that the inclusion/exclusion criteria were met. Exclusion criteria were chosen because they could affect newborn hearing functions or their ability to receive hearing tests after birth or could later maternal iron

status. Pregnant women were excluded if they had gestational diabetes, gestational hypertension, pre-eclampsia, eclampsia, infections during pregnancy or at birth (ex. toxoplasmosis, tuberculosis, HIV, septicemia, cytomegalovirus, herpes, simplex, rubella, chicken pox, shingles, and syphilis), and those who had consumed drugs, alcohol and other illicit substances during pregnancy.

To be part of the study all infants had to be born at term (≥37 weeks), without any abnormalities which can affect hearing, such as intrauterine growth restriction, infections (ex. rubella, toxoplasmosis, herpes simplex, cytomegalovirus, syphilis, tuberculosis, HIV, varicella, Zona or sepsis), craniofacial anomalies, chromosomal disorders (ex. Down, Usher, Pendred or Wolfram syndrome), or a condition affecting their nervous, cardiac, respiratory, renal or hepatic system. Infants suffering from a severe jaundice, defined as receiving phototherapy or having bilirubin concentrations >10 mg/dl, were also excluded since it could affect hearing functions[228]. In addition, a newborn was excluded if he/she was admitted to the neonatal intensive care unit. All eligibility criteria were self-reported by women except for severe jaundice which was assessed from the newborn hospital medical chart.

Pregnant women were recruited by different means, mainly at the Montfort Hospital during their second or third trimesters. The recruitment means included using flyers distributed by physicians following pregnant women at the Montfort Hospital or was added to the information package given before discharge by nurses to mothers who had just given birth. In addition, short messages were posted on TV screens at the Montfort Hospital, on the Montfort Family Birthing Centre website and in the Montfort newsletter. The flyers were also posted on the Montfort Hospital billboards and in the waiting room of the Montfort Academic Family Health Team Clinic. We also had a kiosk in the corridor near the main entrance of the Montfort Hospital exhibiting the study information with a student or a volunteer present to answer the pregnant women's questions. The recruitment flyers were also distributed at the end of prenatal classes at the Vanier Community Health Centre and the Ottawa

public Health (held in public libraries in the City of Ottawa). Finally, we also advertised using social media including targeted ads on Facebook, Kijiji and Twitter.

In order to give women more incentives to participate in the study, we added a $20 gift certificate at a pharmacy, reimbursed parking fees, had a healthy snack as well as nutrition education regarding infant feeding from birth to one-year old during their visit. To improve the recruitment rate, we subsequently added the possibility to do the hearing tests in their hospital room before discharge or at their home after discharge. Our original aim was to recruit 58 women-newborn dyads (see sample size calculation below). However, due to substantial challenges faced with the recruitment, only 8 woman-newborn dyads completed the study. Yet, 2 of them were excluded because: 1) of maternal infection (herpes) which was mentioned only in her questionnaire during the visit but omitted during the screening call; and 2) the ABR test yielded invalid results. Therefore, only 6 dyads were kept for the analyses.

Of the 38 pregnant women who initially contacted us to participate in the research project, 13 women withdrew from the study due to a lack of interest or time, and the other ones were not eligible because they had either pre-eclampsia, gestational hypertension, gestational diabetes, herpes simplex or an infection during pregnancy (n=9). Other dyads were excluded because their baby had severe jaundice, was admitted to the Intensive Care Unit, was born prematurely, or was large for gestational age (n=5). Finally, either the participant (n=1) or the student in audiology (n=2) did not show up or was not available for the appointment and two (n=2) other eligible women were excluded because of technical problems with the equipment.

The recruitment for this study proved to be very challenging. In order to overcome these challenges, we changed the recruitment strategies and added new means (ex. prenatal classes, home visits as discussed above) to offer more flexibility and convenience for the participant. Additionally, our subjects had to deliver only at the Montfort Hospital in order to gain access to their medical chart.

This has excluded many potential participants met during the prenatal classes around the city who were giving birth elsewhere. Moreover, when we hosted our kiosk in the main entrance of the Montfort Hospital, we could not approach women walking by as requested by the Research Ethics Board of the hospital. We needed to wait for them to come to us which decreased our potential to reach out to many women. Another challenge was to schedule the visit date and time with the mother and the research team members. In order to do so, we had to coordinate the schedule of the undergraduate and/or graduate students to do the hearing test and the nutrition education session in either French or English, according to the participant's preferred language. Because of schedule conflicts between the participants and the team members (courses, internship and part-time job), we were not able to book an appointment for three participants. Finally, to have a better understanding of the audiology tools, I have participated in one practical observation, which lasted 45 minutes, given by Dr. Amineh Koravand's lab assistant, two audiology practical trainings given by Dr. Amineh Koravand, and had a theoretical and practical training given by an audiology student (Debbie Gagné-Béland). Furthermore, I have observed several ABR testings done by the audiology students who were involved in this project, in order to replace the audiology students when they were not available. Unfortunately, due to the timeline constrains, I had not finally had a chance to learn and perform the hearing tests on babies. However, due to timeline constraints, I did not have the opportunity to perform the hearing tests on babies.

2.3 Data Collection

Once the women consented to participate in the study, a member of the research team started the weekly follow-up at their 36^{th} week of pregnancy. The contact was by phone or email in order to confirm their interest, eligibility criteria and to document whether birth had occurred. After delivery, we verified their baby's eligibility to participate in the study and sent the second consent form for both

parents (if applicable) to consent for the participation of their newborn. We then arranged an appointment for eligible mother and newborn within the first two weeks after birth.

During the visit, the consent form was first checked to ensure that both parents (if applicable) had signed the document and ensure that all questions had been answered. Afterward, the hearing tests (ABR and OAE) were performed on the baby during his/her sleep and it took around 40 minutes (more details are provided below). Meanwhile, the mother was asked to answer a four-page questionnaire about the mother's and her baby's general health, breastfeeding or formula feeding habits, sociodemographic characteristics, maternal iron-containing supplement intake and dietary patterns (ex. omnivorous, vegetarian or vegan) during pregnancy. Once the hearing tests were completed, the mother received nutritional information on children's healthy eating from zero to one-year-old according to Health Canada's recommendations, as well as a language development pamphlet. Finally, the parking ticket was offered if they came to the research facility and the healthy snack and $20 gift certificate was given to them in compensation for their time.

2.4 Iron Marker Measurements

The two maternal iron marker concentrations, Hb and MCV, were collected retrospectively for the first and second trimesters of the pregnant women's medical chart. These markers are routinely measured in the first trimester between the 11-14 weeks and during the second trimester at 28 weeks. These markers were assessed according to standard laboratory techniques in use where the blood samples were sent. These sites may have varied since some pregnant women were followed by their family physicians in their first two trimesters and transferred to a physician at the Montfort Hospital at their third trimester.

2.5 Evaluation of the Auditory Function

2.5.1 The Otoacoustic Emission (OAE) test

This test is meant to measure cochlear function[213]. The otoacoustic emissions are low-intensity sounds emitted by the cochlea in response to external acoustic stimulation[214]. A specialized ear tip is first fitted into the infant ear canal. Then, the instrument sends a low-intensity sound and the cochlear response is recorded by the microphone in the ear tip. This test is very sensitive and responsive to any physiological abnormalities that can impact the normal functioning of the outer hair cells of the cochlea[214]. The specificity and sensitivity of this test are higher than 90%[229]. The ear canal sound can be analyzed by an OAE test by using spectrum analyzers to assess continuous emissions as well as transient stimulation to measure delayed waveforms[229,230]. In clinical settings in Ontario, the OAE, which is the simplest and quickest hearing test, is routinely used to screen for auditory abnormalities in newborns[226,227]. However, when performed in hospitals, this test only provides a Pass/Fail result and is not necessarily always reported in the medical records. Therefore, it was necessary to do the OAE test on our newborn participants in order to have more sensitive and in-depth analyses of multi-frequency measurements. This test took approximately 5 minutes per ear to complete and is considered safe and painless. The OAE test was done during the neonate spontaneous nap. Abnormalities are diagnosed with an OAE threshold higher than 20 decibels (dB) of sound pressure level (SPL)[213]. This system can also be helpful for characterizing cochlear mechanical status before performing long term assessment[230].

We performed the OAE test on 4 newborns, but unfortunately, we had to stop doing the test because the results were not valid. These unreliable OAE test results can be explained by several reasons. First, newborns can have too much wax (cerumen) in the external ear canal or fluid accumulation in their middle ear space behind their ear drum which can alter the results[215]. Also, the audiology students encountered technical problems with the equipment preventing them from

performing the test. We thus decided to stop doing the test to avoid wasting the participants' time and the anxiety related to non-conclusive test results. The first objective of this thesis could thus not be verified (i.e. the relationship between iron markers and cochlear functions).

2.5.2 The Auditory Brainstem Responses (ABR)

The ABR test is a non-invasive and objective neurophysiological assessment tool, and has been used for evaluating auditory neural myelination in older infants and children as a function of iron status[212,231,232]. The ABR latencies measure conduction speed in the auditory system from the cochlea to the inferior colliculus in the upper brainstem[193,231,233]. These latencies decline sharply from late pregnancy to the first 3 to 6 months of life and continue to decrease slightly within the first 3 to 5 years of life[233].

Three waves (I, III, and V) of the ABR test can be observed in late preterm and term newborns soon after their birth[234]. Wave I is generated peripherally in the auditory nerve[235]. Wave III reflects the firing of axons exiting the cochlear nuclear complex in the brainstem[234,235]. Wave V primarily reflects an action potential generated by axons from the lateral leminiscus at a more rostral brainstem location[235]. Indeed, Wave III and V latencies can be reflective of both peripheral and central maturation in the auditory system[234]. Wave I, III, and V latencies and I-V interpeak latency (central conduction time) have been suggested as useful measures of the hearing functions, as they are more easily identifiable and reproducible[233]. Wave latency and the interpeak latency show the speed of neural transmission[236]. The nerve conduction velocity, an index of myelination, at different levels of the auditory pathways can be measured by the interpeak latencies between waves I-III, III-V, and I-V[237,236]. Although latency is more reliable than amplitude for evaluating the auditory brainstem function, some researchers also consider the wave amplitude as a measure of the number of neurons firing when interpreting the ABR resultsl[236,238].

2.6 Sample Size:

The sample size for our project was calculated using nQuery Advisor®. Fifty-eight participants were estimated to be sufficient to perform multiple linear regression analyses to examine the association between our iron status markers (Hb and MCV – continuous independent variables) and auditory function markers (amplitudes and latencies – continuous dependent variables). We estimated that 58 participants would enable us to test for a squared multiple correlation (r^2) of at least 0,20, with up to 5 covariates in the model (based on previous similar studies adjusting for covariates such as bilirubin concentrations, infant sex, maternal diabetes and gestational age)[13,20,212] with a level of significance (α) of 0.05 and a Power of 80%. Two studies with a sample size of 55 neonates and 45 six-month-old infants were able to find a difference in auditory function between normal and iron-deficient infants[13,212]. Unfortunately, major challenges in our recruitment and timeline did not enable us to reach the desired sample size. We thus did not have enough Power to test multiple regression models adjusted for covariates.

2.7 Statistical Analyses

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., NC, USA). Exploratory analyses were first done to examine variable distribution (i.e. range, mean, median, standard deviation, interquartile range), to find missing values or possible outliers or data entry errors. Frequencies (absolute and percentage) were also examined for categorical variables. Because of the small sample size (n=6) and the lack of normality of the distribution for continuous variables, we used non-parametric tests to assess the associations between our predictors (Hb and MCV) and outcomes

(amplitudes, latencies and inter-latencies) and to compare medians of markers of hearing functions between groups (ex. anemic vs non-anemic pregnant women). In order to test for our main objectives, we used Spearman correlations to determine the relationship between the mother's Hb and MCV concentrations during the first two trimesters as the independent variables and newborn left and right ear latencies, inter-latencies, and amplitudes at supra-threshold (65 dB) and threshold (25 dB) levels as the dependent variables. The Wilcoxon-Mann-Whitney test was used to test the difference between two or more groups (ex. anemic vs non-anemic pregnant women) and iron markers for each of the first and second trimester. The Wilcoxon-Mann-Whitney-rank-sum test (the equivalent of a paired t-test for continuous and normally distributed independent variables) was used to compare Hb and MCV concentrations within the same individuals in the first and second trimesters.

We also looked at whether the general characteristics of the mothers or newborns were associated with either our predictors and outcomes or both to find some potential confounders. The chi-square test was used to test for differences between categorical predictor and outcome variables (ex. anemia (yes/no) during pregnancy and intake of a prenatal multivitamin containing iron supplement during pregnancy (yes/no)). However, due to the small sample size, we did not have enough Power to detect such associations neither to adjust for covariates in our full models. All statistical tests were deemed significant when $p < 0.05$. All analyses were performed using the SAS software version 9.3.

2.8 My Contribution to this Research Project

I contributed to this research project since the beginning of the participants recruitment. I first did the online TCPS 2 tutorial – course on research ethics – provided by the government of Canada (http://www.pre.ethics.gc.ca/eng/education/tutorial-didacticiel/) and required by the Montfort Hospital Research Ethics Board for all research staff. Afterward, I started the recruitment process including; posting project flyer on Montfort Hospital billboards and in the waiting room of the Montfort

Academic Family Health Team Clinic and updated them every three months. I also held a kiosk advertising the study at Montfort Hospital and answered the women's questions. Additionally, I distributed recruitment flyers at prenatal classes in held by Ottawa Public Health (in public libraries in the City of Ottawa). Moreover, I advertised the study on social media including targeted ads on Facebook, Kijiji, and Twitter.

During the recruitment process, I checked all emails and voicemail messages, then contacted anglophone participants to explain the study and the procedures involved. I also entered all information collected during phone calls or emails (contact information, inclusion/exclusion criteria) and questionnaires in the research database RedCap (Research Electronic Data Capture). After arranging appointments with participants at Montfort Hospital or participants' home, I prepared a folder for each participant containing the consent form, questionnaire and pamphlets to be distributed snacks, gift-card and parking pass (as appropriate).

During visits, I greeted the participants, collect the signed consent form, answered their questions and explain what would happen during the visit. After the hearing test, I gave nutritional information on children's healthy eating from birth to 1 year to participants (mothers or both parents). I also had theoretical and practical training for the ABR and OAE hearing tests from the audiology group in order to be able to do the hearing tests on my own, but I did not get the chance to do them. I worked my thesis and learned how to use the SAS software using fictitious data during this period as well. Upon approval from my thesis advisory members, I started the statistical analysis of the data collected up to February 2017. I then interpreted the results and finalized the writing of my thesis.

CHAPTER 3

RESULTS

The general characteristics, including sociodemographic factors, of the women participating in our study are shown in Table 1. Overall, mothers were in their mid-thirties; two thirds had no other child, all of them and their partner had a post-secondary diploma and most were highly educated and had a high family income. About one third of mothers suffered from anemia (self-reported) during their pregnancy or their life or both 33.3% (n=2/6). Among the two women who self-reported having suffered from anemia during their pregnancy, only one used both prenatal multivitamin containing iron supplement and prescribed iron supplements. The other woman reported using only a prenatal multivitamin containing iron. Infants' characteristics are shown in Table 2. All infants were born at term, with a normal weight (between the 10^{th} and 90^{th} percentile), two thirds were girls and the majority were either exclusively or partially breastfed.

We then examined whether there were some associations between the general characteristics described above and the main predictors (Hb and MCV) and outcomes of interest (ABR latencies, inter-latencies and amplitudes). We found no association between any potential confounders and the predictors and outcomes of interest (data not shown).

We also wanted to investigate whether Hb and MCV differed between trimesters and between women who self-reported taking supplements or not, or those being anemic or not during pregnancy or their lifetime. There was no significant difference between Hb (median±interquartile range - QR) for the first and second trimester (123±12 vs. 115.5±16 g/L, respectively; p=0.62) or for MCV (90.08±4.9 vs. 94.15±5.9 fL, respectively; p=0.43). We then compared Hb and MCV concentrations between iron-containing prenatal multivitamin supplement users and non-users (Table 3), women who have developed anemia or not during pregnancy (Table 4) and during their lifetime (Table 5) for both

trimesters. No statistical difference was observed between any of the groups compared, although a trend was seen for Hb, where women who self-reported having developed anemia during pregnancy had lower Hb concentrations than those without anemia ,at the second trimester during their pregnancy (p=0.06; Table 4). Among the two women who self-reported being anemic (IDA) during their pregnancy, one of them was in fact not anemic in the first trimester and only mildly anemic (ID) in the second trimester based on her Hb concentrations (1^{st} trimester 123 g/L; second trimester 106 g/L) and the other one was mildly anemic (ID) in the first trimester and not anemic (IDA) in the second trimester (1^{st} trimester 103 g/L; second trimester 112 g/L). These are based on the following Hb reference cut points for pregnant women: normal ≥110 g/L, mild anemia 100-109 g/L, moderate anemia 70-99 g/L, severe-anemia < 70 g/L[52]. In addition, two women have also self-reported having been anemic during their lifetime. Based on their Hb values, one of them was indeed anemic (mild anemia) in the first trimester (Hb=103 g/L), but not in the second trimester (Hb=112 g/L). For the other subject, her Hb values for the first trimester was missing and she had normal Hb level concentrations the second trimester (Hb=118 g/L). However, except one missing MCV value for the first trimester, other pregnant women had normal MCV concentrations during the first and second trimesters of pregnancy (normal range for pregnancy: 85 to 97.8 , and 85.8 to 99.4 fL respectively)[238].

We then wanted to describe the newborns' auditory functions and determine whether the values were within the normal limits. Those values were assessed with the automatic feature in the computer program based on the UCLA (University of California) norm values. As shown in Table 6, latencies, inter-latencies and interaural differences of the different waves at supra-threshold (65 dB) and threshold (25 dB) levels for the right and left ears were within the normal limits as determined by the computer software. Similarly, the amplitudes at supra-threshold and threshold level of waves I, V and their ratio were also within the normal limits for both ears (Table 7).

Next, we wanted to investigate the association between the Hb and MCV concentrations and the newborn's right and left ear latencies and inter-latencies at supra-threshold and threshold levels for the first two trimesters (objective 2). Overall, no significant relationship (Spearman correlations) was observed between the Waves I, III, V latencies at supra-threshold, Wave V latency at threshold level, and inter-latency of waves I-III, I-V, III-V with Hb and MCV concentrations for both ears in both trimesters (Table 8 to 11). However, we found one significant correlation between maternal MCV concentrations and the right ear inter-latency I-V during the first trimester ($p=0.005$, Table 10). This finding, however, might be due to chance due to multiple testing.

We then examined the correlations between Hb and MCV and the newborns right and left ears' amplitudes of wave I, V, at supra-threshold (65 dB), amplitude of wave V at the threshold level (25 dB), and amplitude of ratio V/I (Tables 12 to 15). Similarly, we observed no significant relationship other than between maternal Hb concentrations and newborn left ear amplitude of wave V at the threshold level during the first trimester ($p =0.04$, Table 13).

Finally, we tested the differences in auditory function values as measured by the ABR test (latencies, inter-latencies and amplitudes) between iron-containing prenatal multivitamin supplement users and non-users, as well as between anemic versus non-anemic women (during pregnancy or their lifetime). Although we found no significant difference between groups in any hearing-related variables tested, we illustrate some examples of the findings in Figures 1 to 3. Figure 1 shows the comparison of inter-latency of wave I-V for the newborn's right ear between iron-containing prenatal multivitamin supplement users (n=5) and non-user (n=1) during pregnancy. Similarly, no statistical difference between groups was found for the left ear (data not shown). We also show the comparison of inter-latency of wave I-V for the newborn's right ear between anemic (n=2) and non-anemic (n=4) women during their pregnancy (Figure 2) and during their lifetime (Figure 3). Again, no statistical difference was observed between groups and similar results were found in the left ear.

Table 1. General characteristics of the mothers in the study sample (n = 6).

Mothers	**n**	**Mean±SD[a] or %[b]**
Age[a, c]		36±3.0
Number of children[b]		
1	4	66.6%
2	2	33.3%
Mode of delivery[b]		
C-section	2	33.3%
Vaginal	4	66.7%
Self-reported anemia during lifetime[b]		
Yes	2	33.3%
No	4	66.6%
Self-reported anemia during pregnancy[b]		
Yes	2	33.3%
No	4	66.6%
Prenatal multivitamin containing iron intake[b]		
Yes	6	100%
No	0	0%
Prescribed iron supplement intake[b]		
Yes	1	16.6%
No	5	83.3%
Mother's education[b]		
Primary school	0	0%
High school	0	0%
College	1	16.6%
Some university training	1	16.6%
Under graduate	1	16.6%
Graduate	3	50%

Father's education[b]		
Primary school	0	0%
High school	0	0%
College	2	33.3%
Some university training	1	16.6%
Under graduate	0	0%
Graduate	3	50%
Family-income[b]		
$10 000 – 39 999	0	0%
$40 000 – 59 999	0	0%
$60 000 – 99 999	1	16.6%
$100 000 -150 000	1	16.6%
>150 000	4	66.6%

Data presented as means ± SD [a] and frequencies [b] (n and %).
[c] One participant did not mention her age (n=5).

Table 2. General characteristics of the newborns in the study sample (n = 6).

Newborns	**n**	**Mean±SD[a] or %[b]**
Sex[b]		
Boys	2	33.3%
Girls	4	66.6%
Birth weight (kg)[a]		3.6±0.5
Gestational age (weeks)[a]		39.8±1.2
Type of feeding[b]		
Exclusive breastfeeding	2	33.3%
Partial breastfeeding	3	50.0%
Formula	1	16.6%

Data presented as means ± SD [a] and frequencies [b] (n and %).

Table 3. Comparison of maternal iron markers between iron-containing prenatal multivitamin supplement users and non-users for the first two trimesters (n = 6).

	1st Trimester			2nd Trimester		
	Median±QR	Median±QR	p values	Median±QR	Median±QR	p values
	Users	Non-Users		Users	Non-Users	
n	5	1		5	1	
Hb (g/L)	125±19*	116±0	0.45	113±6	128±0	0.55
MCV (fL)	89.3±3.5*	95.5±0	0.14	93.9±4.7	95.6±0	0.55

P values were obtained by using the Wilcoxon-Mann-Whitney Rank-Sum test. Abbreviations: QR=interquartile range; Hb=hemoglobin; MCV=mean corpuscular volume. g/L=gram/litre; fL=US fluid ounce.*One missing value.

Table 4. Comparison of maternal iron markers between women who self-reported having developed or not anemia during their pregnancy for the first two trimesters (n = 6).

	1st Trimester			2nd Trimester		
	Median±QR	Median±QR	p values	Median±QR	Median±QR	p values
	Anemic	Non-Anemic		Anemic	Non-Anemic	
n	2	4		2	4	
Hb (g/L)	113±20	128±20	0.18	109±6	123±15	0.06
MCV (fL)	90.9±4.9	90.1±7.7	0.73	90.7±6.4	95.0±3.8	0.11

P values were obtained by using the Wilcoxon-Mann-Whitney Rank-Sum test. Abbreviations: QR=interquartile range; Hb=hemoglobin; MCV=mean corpuscular volume; g/L=gram/litre; fL=US fluid ounce.

Table 5. Comparison of maternal iron markers between women who did or did not develop anemia during their lifetime among pregnant women for the first two trimesters (n = 6).

	1st Trimester			2nd Trimester		
	Median±QR	Median±QR	p value	Median±QR	Median±QR	p value
	Anemic	Non-Anemic		Anemic	Non-Anemic	
n	2	4		2	4	
Hb (g/L)	103±0.0*	125.5±12.5	0.15	115±6	120.5±21	0.48
MCV (fL)	88.5±0.0*	91.7±5.5	0.45	90.9±6.9	94.7±4.1	0.35

P values were obtained by using the Wilcoxon-Mann-Whitney Rank-Sum test. Abbreviations: QR=interquartile range; Hb=hemoglobin; MCV=mean corpuscular volume. g/L=gram/litre; fL=US fluid ounce.

Table 6. Right and left ear latencies and inter-latencies in the newborns (n = 6).

	Right ear median±QR	Left ear median±QR
Latencies at supra-threshold[a]		
Wave I	1.9±0.2	1.8±0.1
Wave III	4.4±0.3	4.4±0.5
Wave V	6.7±0.2	6.9±0.6
Latency at threshold*[b]		
Wave V	8.5 ± 0.3	8.5±0.3
Interaural differences of wave V*	0.1± 0.05	0.1±0.05
Inter-latencies[c]		
I-III	2.5±0.2	2.7±0.2
I-V	4.8±0.08	4.9±0.6
III-V	2.4±0.1	2.2±0.3

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; inter-latency is at 65dB[c]; Abbreviations: dB=decibels; QR=interquartile range.

Table 7. Right and left ear amplitudes in the newborns (n = 6).

	Right ear median±QR	Left ear median±QR
Amplitude at supra-threshold[a]		
Wave I	0.1±0.03	0.1±0.05
Wave V	0.1±0.04	0.2±0.06
Amplitude at the threshold level*[b]		
Wave V	0.1±0.04	0.1±0.01
Amplitude of ratio V/I[a]	1.1±1.2	1.2±0.6

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; Abbreviations: dB=decibels; QR=interquartile range

Table 8. Spearman correlations between maternal hemoglobin concentrations and newborn's right ear latencies and inter-latencies for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	P values	r	P values
Latency at supra-threshold[a]				
Wave I	0.10	0.87	0.14	0.78
Wave III	0.30	0.62	0.25	0.62
Wave V	0.10	0.87	0.25	0.62
Latency at threshold*[b]				
Wave V	0.80	0.20	0.50	0.39
Inter-latency[c]				
I-III	0.35	0.55	0.20	0.69
I-V	0.05	0.93	0.02	0.95
III-V	0.41	0.49	0.49	0.32

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; inter-latency is at 65dB[c]; dB=decibels.

Table 9. Spearman correlations between maternal hemoglobin concentrations and newborn left ear latencies and inter-latencies for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	P values	r	P values
Latency at supra-threshold[a]				
Wave I	0.50	0.39	0.25	0.62
Wave III	0.70	0.18	0.002	0.95
Wave V	0.20	0.74	0.08	0.78
Latency at threshold*[b]				
Wave V	0.20	0.74	0.08	0.87
Inter-latency[c]				
I-III	0.50	0.39	0.008	0.87
I-V	0.10	0.87	0.11	0.82
III-V	0.60	0.28	0.02	0.95

Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; inter-latency is at 65dB[c]; dB=decibels.

Table 10. Spearman correlations between maternal MCV concentrations and newborn right ear latencies and inter-latencies for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	P value	r	P value
Latency at supra-threshold[a]				
Wave I	0.80	0.10	0.40	0.30
Wave III	0.60	0.28	0.50	0.26
Wave V	0.80	0.10	0.60	0.20
Latency at threshold*[b]				
Wave V	0.80	0.20	0.10	0.87
Inter-latency[c]				
I-III	0.56	0.32	0.63	0.17
I-V	0.87	0.005	0.55	0.25
III-V	0.15	0.80	0.11	0.82

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; inter-latency is at 65dB[c]; dB=decibels.

Table 11. Spearman correlations between maternal MCV concentrations and newborn left ear latencies and inter-latencies for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	P value	r	P value
Latency at supra-threshold[a]				
Wave I	0.30	0.62	0.60	0.20
Wave III	0.00	1.00	0.14	0.78
Wave V	0.60	0.28	0.14	0.78
Latency at threshold[b]				
Wave V	0.10	0.87	0.31	0.54
Inter-latency[c]				
I-III	0.20	0.74	0.08	0.87
I-V	0.50	0.39	0.05	0.91
III-V	0.70	0.18	0.02	0.95

Supra-threshold is at 65dB[a]; threshold is at 25dB[b]; inter-latency is at 65dB[c]; dB=decibels.

Table 12. Spearman correlations between maternal hemoglobin and newborn's right ear amplitudes for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	p value	r	p value
Amplitude at supra-threshold[a]				
Wave I	0.80	0.10	0.085	0.87
Wave V	0.60	0.28	0.37	0.46
Amplitude at the threshold level*[b]				
Wave V	0.63	0.36	0.20	0.74
Amplitude of ratio V/I[a]	1.00	0.87	0.08	0.87

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b].

Table 13. Spearman correlation between maternal hemoglobin and newborn left ear amplitudes for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	p value	r	p value
Amplitude at supra-threshold[a]				
Wave I	0.50	0.39	0.48	0.32
Wave V	0.73	0.15	0.18	0.72
Amplitude at the threshold level*[b]				
Wave V	0.89	0.04	0.69	0.12
Amplitude of ratio V/I[a]	0.60	0.28	0.65	0.15

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b].

Table 14. Spearman correlations between maternal MCV and newborn's right ear amplitudes for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	p value	r	p value
Amplitude at supra-threshold[a]				
Wave I	0.50	0.39	0.25	0.62
Wave V	0.20	0.74	0.17	0.74
Amplitude at the threshold level*[b]				
Wave V	0.63	0.36	0.66	0.21
Amplitude of ratio V/I[a]	0.20	0.74	0.42	0.39

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b].

Table 15. Spearman correlations between maternal MCV and newborn left ear amplitudes for the first two trimesters (n = 6).

	1st Trimester		2nd Trimester	
	r	p value	r	p value
Amplitude at supra-threshold[a]				
Wave I	0.10	0.87	0.02	0.95
Wave V	0.05	0.93	0.00	1.0
Amplitude at the threshold level*[b]				
Wave V	0.22	0.71	0.09	0.86
Amplitude of ratio V/I[a]	0.00	1.00	0.14	0.78

*One missing value. Supra-threshold is at 65dB[a]; threshold is at 25dB[b].

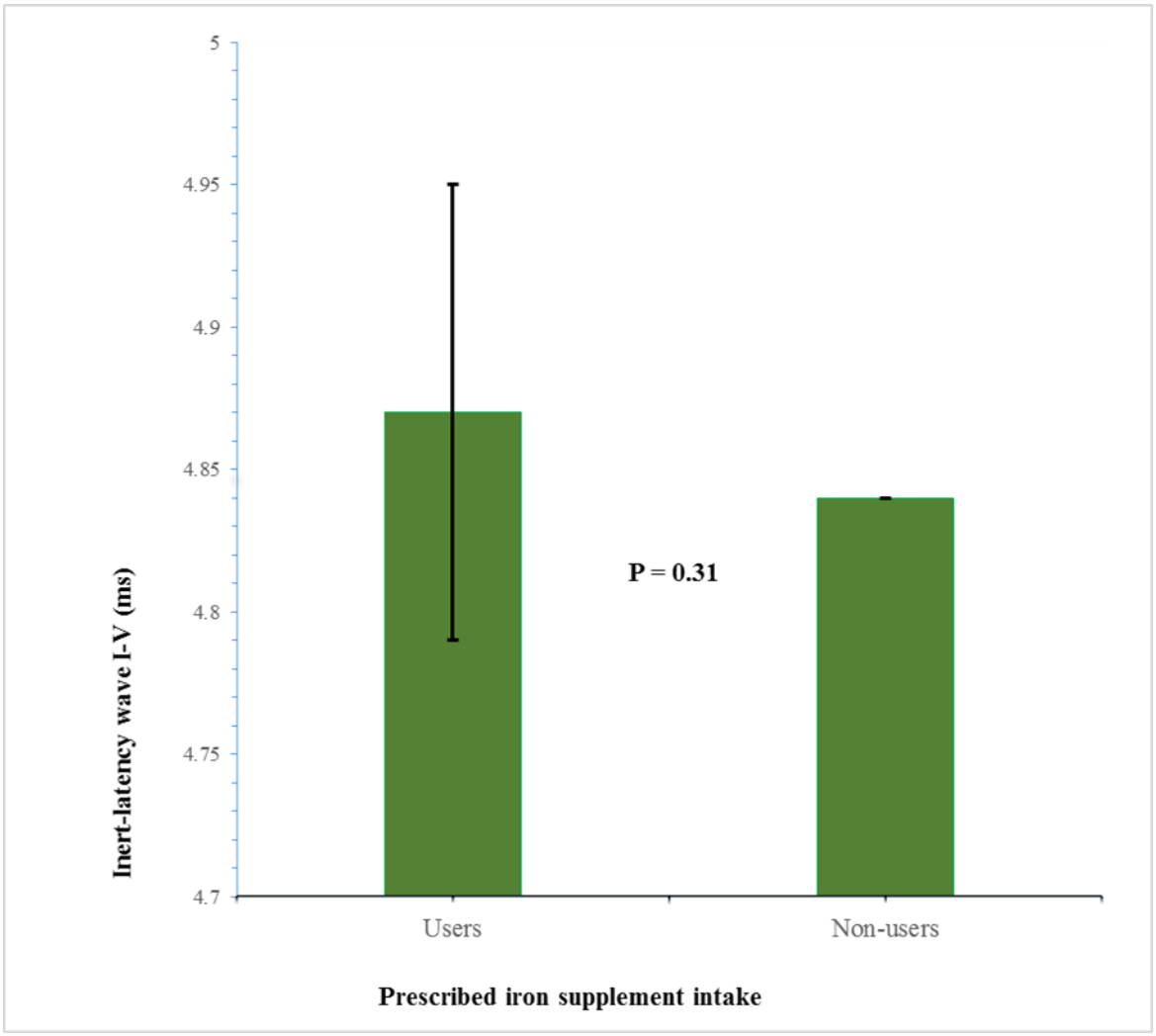

Figure 1. Comparison of inter-latency of wave I-V for the newborn's right ear between iron-containing prenatal multivitamin supplement users (n=5) and non-user (n=1) during pregnancy. Values shown are median ± interquartile range for users (4.87±0.08) and non-users (4.84 ±0.00).

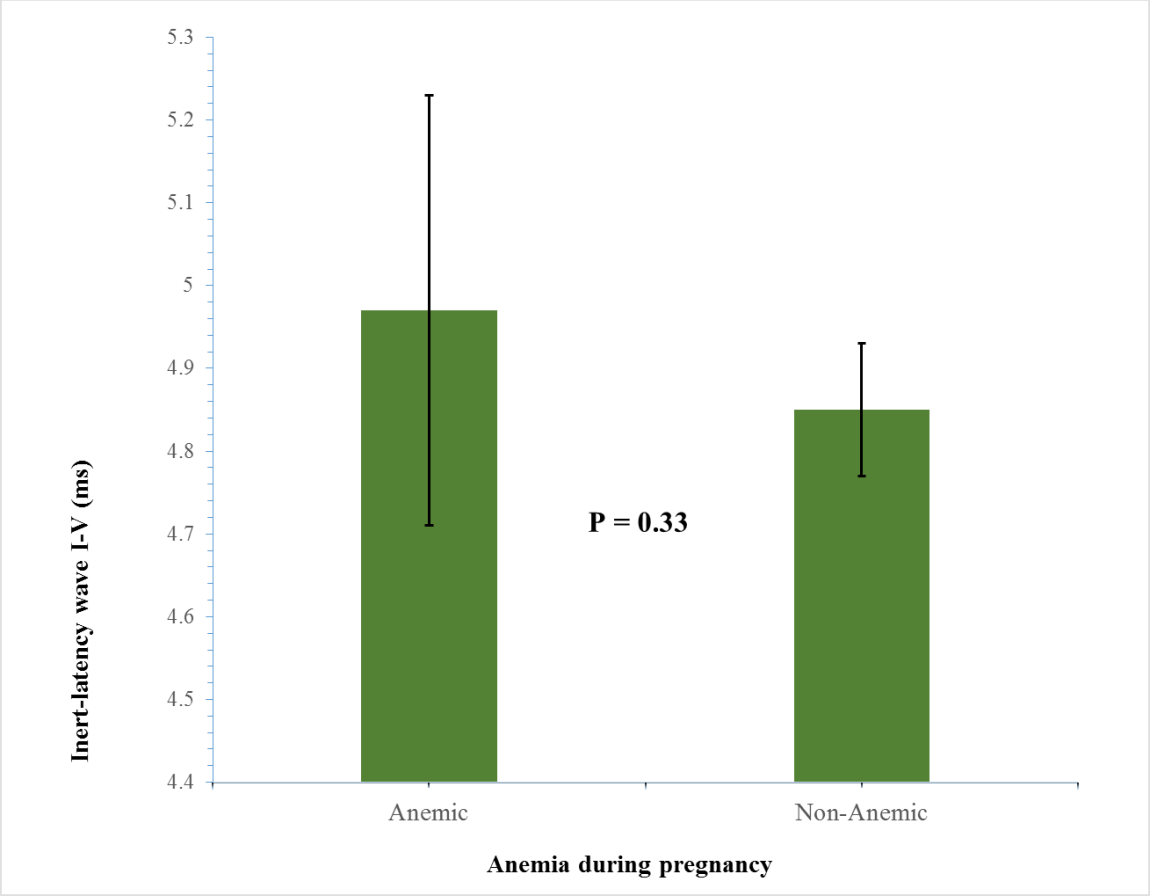

Figure 2. Comparison of inter-latency of waves I-V for the newborns' right ear between anemic (n=2) and non-anemic (n=4) women during pregnancy. Values shown are median ± interquartile range for anemic (4.97±0.26) and non-anemic (4.85 ±0.08) women.

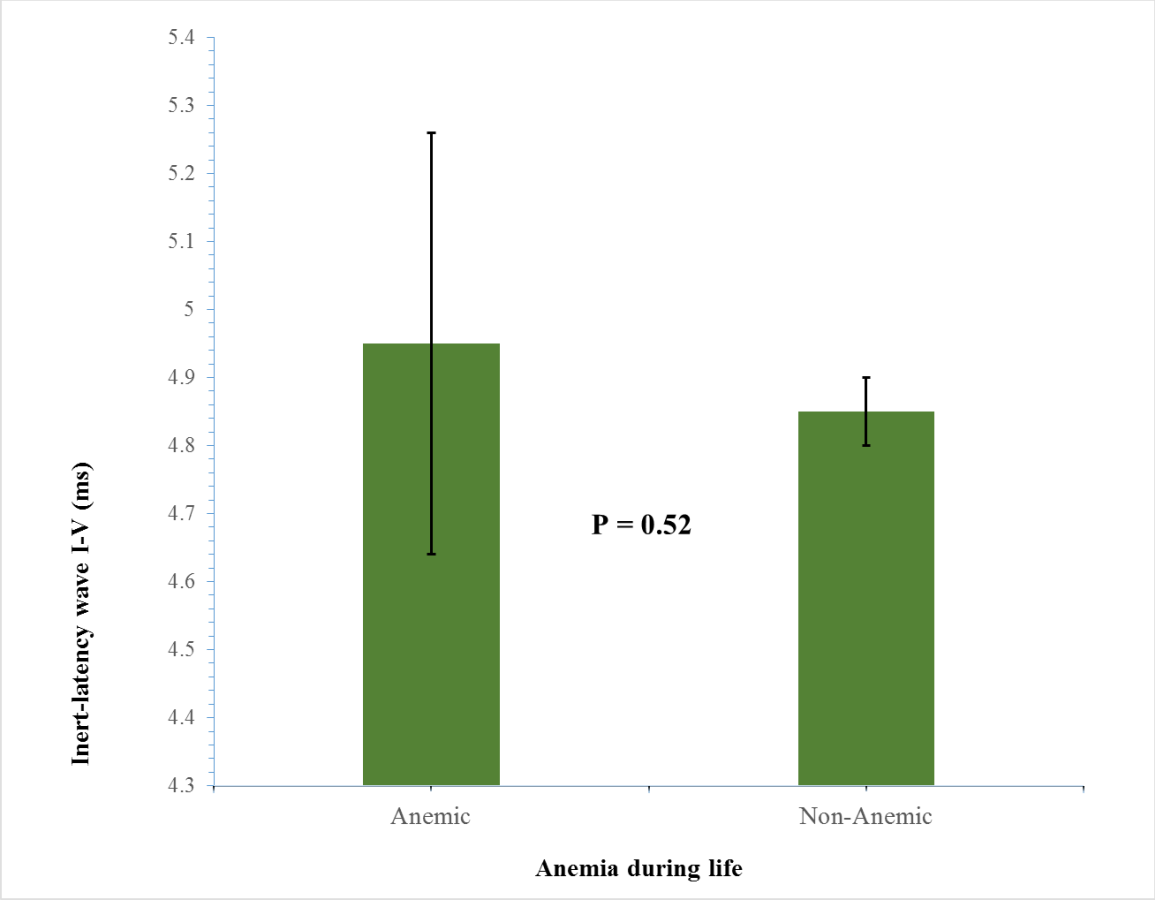

Figure 3. Comparison of inter-latency of wave I-V for the newborns' right ear between anemic (n=2) and non-anemic (n=4) women during their life. Values shown are median ± interquartile range for anemic (4.95±0.31) and non-anemic (4.85 ±0.05).

CHAPTER 4

4. DISCUSSION

4.1 Summary of our Findings

The goal of this pilot study was to examine the relationship between iron markers concentrations during pregnancy and auditory functions in newborns. This relationship was investigated among 6 dyads of mother and child by using Hb and MCV measured during the first two trimesters of pregnancy and collected from the mother's medical record and by performing a hearing test (auditory brainstem response) within the first two weeks after birth in their newborn. We were not able to assess cochlear function in all the newborns due to physiological and technical issues, thus we were not able to evaluate our first objective. For the second objective, overall, we did not find any convincing evidence of a relationship between markers of iron status in the first two trimesters and the newborns' hearing functions (using the ABR test) measured within two weeks after their birth. In addition, we did not find any difference in auditory functions between the self-reported anemic and non-anemic women (during their pregnancy and lifetime) or between iron-containing prenatal multivitamin supplement users and non-users. Even though we found two significant correlations, that is between MCV and inter-latency I-V of the right ear as well as Hb and amplitude V for the left ear – both during the first trimester - we believe that it is probably due to chance due to multiple testing and to the small sample size. Additionally, because of the small sample size, we could not evaluate the effect of extreme values of iron status (both high and low) on auditory functions in the newborns to infirm or confirm our hypothesis**.**

4.2 Education and Socioeconomic Status

Our participants had a high socioeconomic status which is not reflective the overall population of pregnant women in Québec and Ontario. Indeed, all mothers and fathers in our sample had a post-secondary degree with two thirds of mothers having at least an undergraduate degree and half of the fathers having a graduate degree. In addition, two thirds of the participants had a family income greater than $150,000. In comparison, according to Statistics Canada (2014),[239,240] the median income of families living in Québec and Ontario was $73,870 and $78,790, respectively, which is approximately half than in our sample. Similarly, 27% and 33% of Quebecers and Ontarians have completed at least an undergraduate degree (Statistics Canada, 2015)[241], which is much lower than the majority of our sample. According to a population-based study in Quebec, Canada, women with higher educational levels and those living in the wealthier neighborhoods are less vulnerable to adverse birth outcomes[242]. In addition, since we only had 6 participants in our sample, the generalizability of our results cannot be conferred to the rest of our target population.

4.3 Maternal Iron Status and Newborn Auditory Function

We wanted to examine whether low and high maternal Hb and MCV concentrations negatively impact the development of the fetus's auditory nervous system, thereby resulting in auditory abnormalities in neonates. As summarized previously, no association was found between pregnant women's Hb and MCV concentrations during pregnancy and newborns' auditory function as measured by the ABR test within two weeks after birth. A group of researchers have investigated the association between mild maternal IDA during pregnancy and lactation and auditory functions in guinea pigs[206,207]. The study was performed on twenty-two female guinea pigs which were fed either an iron sufficient diet (n=11) or an iron-deficient diet (n=11), corresponding to 144 and 11.7 mg of iron/kg of body weight, respectively[206]. Then, an iron sufficient diet was given to all offspring in both groups from

postnatal day 9 until the ABR test was performed on postnatal day 24[206]. A significant difference was found between the hearing thresholds of the offspring whereby iron sufficient offspring had lower hearing thresholds, thus better auditory acuity, than those who suffered from IDA[206]. Another animal study was performed on twenty female rats were randomly assigned to two different dietary groups in order to assess the role of ID (in the absence of anemia) on the hearing function and cochlear pathophysiology on different developmental stages[243]. Rats were fed a control diet (n=10; 103.95±31.71 mg iron/kg) or an iron-deficient diet (n=10; 25.27±9.08 mg iron/kg)[243]. The ABR test was performed twice on postnatal day 21 to measure the young rats' hearing function with different latency noise exposures[243]. Although, no relationship was found between ID and ABR threshold, amplitude of wave I at 70, 80, and 90 dB were decreased significantly in ID group in comparison with the control group[243]. Therefore, these animal studies suggest that ID or mild IDA during pregnancy have a negative impact on hearing acuity in the offspring[206,207].

Only a few investigators have studied the effect of maternal iron status using a proxy postnatal measure (cord blood) on newborns' auditory functions in humans[13,19,20]. For example, two studies examined the relationship between cord blood serum ferritin concentrations and auditory brainstem evoked response interpeak latencies, an index of auditory neural myelination, in infants[13,19]. Results showed that latent ID during pregnancy was related to abnormal auditory neural myelination maturation at birth[13,19]. Similarly, another observational cohort study compared premature and term infants (≥ 34 wk gestational age) with a normal iron status at birth (serum ferritin >75 ng/mL) (n=67) to infants with latent ID (serum ferritin ≤ 75 ng/mL) (n=23) and found abnormal auditory maturation, as shown by significant prolonged wave V latencies, III-V interpick latencies, and I-V interpeal latencies, among the ID infant[19]. Finally, a prospective cohort study was also made on 153 very premature infants (27-33 weeks of gestational age) using the same ferritin cut point to define ID at birth (75 ng/ml) and found that latent ID in the newborn was also associated with abnormal auditory neural

maturation, as demonstrated by significant prolonged absolute wave latencies I, III, and V and declined frequency of mature ABR waveforms[20]. The cut-off point of 75 ng/ml for cord blood ferritin concentration has also been shown to be associated with altered neurobehavioral and psychomotor development in preterm as well as term infants[244,245]. However, to our knowledge, the relationship between iron markers during different trimesters of pregnancy and hearing functions in term infants had not been examined to date.

Another vulnerable group for altered auditory functions are infants of diabetic mothers[181]. Indeed, these infants are at risk of prenatal brain ID that may target the developing hippocampus and alter auditory recognition memory[181]. Infants from diabetic pregnant women have been shown to have higher glycosylated fetal Hb values, increased Hb and erythropoietin concentrations and increased erythrocyte/storage iron ratios[246]. Due to the inverse correlation between erythropoietin concentrations and serum iron values, the erythrocyte and iron storage in infants born from diabetic mothers was significantly lower than infants born from nondiabetic mothers[246]. Indeed, one study compared the event-related potentials between 32 healthy newborn infants and 25 infants born from diabetic women[247]. The event-related potentials assess the integrity of neural pathways for auditory recognition memory in response to sounds[248,249]. For example, it can evaluate the neonatal auditory cortical responses to the mother's voice in comparison to a stranger's voice[181,247]. This study showed that there was a difference in event-related potential patterns between both groups of infants[247]. In fact, infants of both groups could recognize their mothers' voice, but a negative slow wave was elicited by the stranger's voice in the control group but not in the infants from the diabetic mothers[247]. There was also a significant association between this negative slow wave and the Mental Developmental Index performed at 1 year-old[247]. A longitudinal study also investigated the effect of ID on auditory recognition memory in infants born from women with gestational diabetes (n=32)[181]. They found that when compared to infants of diabetic mothers with ID (n=10; serum ferritin concentrations $\leq$34 μg/L)

those with sufficient iron stores (n=22; cord ferritin >34 μg/L) had lower cord ferritin concentrations, shorter maternal voice recognition paradigm and peak latencies, a significant negative slow wave associated with the strangers' voices in comparison with the mothers' voice and impairments in their motor skills development at one year of age[181]. Results from these two human studies, although they both had a small sample size, suggest that infants born from diabetic pregnant mothers had an altered iron status which in turn was associated with impaired hearing functions and subsequent altered mental development. This may partly be explained by the suboptimal iron status associated with gestational diabetes leading to an insufficient amount of oxygen brought to the fetus, creating a hypoxic state, and to impaired brain and hearing functions.

While iron insufficiency has been associated with hearing alterations, pathologies characterized by iron overload such as siderosis, thalassaemia and sickle cell anemia have also been linked to adverse hearing functions[26,216,218,219]. For example, the case study of a 38-year-old man with a history of siderosis, demonstrated progressive hearing loss over two years due to altered functions of the auditory-vestibular system[250]. Similarly, a study of the auditory functions of 75 children with thalassaemia indicated that, although there was a relationship between sensory neural hearing loss and high ferritin concentrations, no association was found between sensory neural damage and Hb concentrations[216]. However, to our knowledge, no study has examined the effect of high iron status during pregnancy on hearing functions in the newborn. Unfortunately, we did not have any pregnant women with excessive Hb concentrations in our small sample. Thus we could not confirm or infirm our hypothesis.

The association between latent ID during pregnancy and the neonatal period and acute and long-lasting detrimental effects on neurodevelopment seems to be a growing concern[16,181,244,245]. Although iron status and IDA can be corrected with iron therapy, the hearing alternations are irreversible[231]. Furthermore, because the iron depletion in the brain and other tissues occurs long before

red blood cells concentrations drop[251], early identification and treatment of ID before IDA develops is crucial. This is of particular importance since anemia is more prevalent during the third trimester of pregnancy[14] and it is during this same last trimester that the development of the fetal nervous system is accrued[45,189]. Latent ID during pregnancy and the neonatal period lay the foundation for abnormal long-term language, motor, cognitive, and behavioural functioning[172,174,176]. Unfortunately, latent ID is very common among pregnant women[67]and infants, especially in premature newborns[19,20,80,252]. Therefore, a large population of newborn infants are at risk of hearing problems despite the preventable nature of this nutritional deficiency[181,245,252,253]. The early diagnostic and treatment of ID and IDA during pregnancy would be crucial to prevent abnormal auditory development and function.

4.4 Strengths and Limitations of the Study

The major strength of our study is the objective assessment of the auditory neural myelination using the ABR test. This test was performed by one of the two trained MSc Audiology students without knowledge of the subjects' Hb and MCV concentrations. The observational nature of the study also better reflects a real life setting with iron markers collected by standardized procedures in recognized laboratories. The major limitation of our study is the small sample size. The lack of significant relationships between Hb or MCV and different measures of auditory functions are most likely due to the minimal spread of values of the variables tested between participants and the lack of power to detect our relationships of interest. Indeed, although two women self-reported having anemia during pregnancy, only one of them had confirmed anemia based on her Hb concentrations. However, it is possible that their ferritin concentrations were low despite apparently normal or borderline Hb concentrations. Also, we analyzed only Hb and MCV values from the pregnant women's medical chart during the first two trimesters which are nonspecific IDA markers. Ferritin is not routinely measured during pregnancy so it was not an available marker for the retrospective part of our pilot study.

Unfortunately, the Hb and MCV are not measured routinely during the last trimester when ID is most prevalent according to the World Health Organization (WHO)[82]. Nonetheless, it is measured at 28th weeks of gestation which is at the end of the second trimester and beginning of the 3rd trimester. In addition, no newborn had a hearing impairment which limited our ability to test our hypothesis. Furthermore, we were not able to use the OAE test in order to assess newborns' cochlear function which prevented us from testing our first objective.

The ABR hearing test was done within the first two weeks after birth to reduce the influence of postnatal iron intake (through breastfeeding or formula) or other environmental factors (ex. virus) on hearing functions. In fact we wanted to emphasize on the relationship between iron status during pregnancy rather than any other postnatal factors could have on hearing functions. Therefore, due to this short window of time, we lost some eligible women because of schedule conflicts or lack of mother's time. To circumvent this problem, we offered the hearing test in different settings (research facility, hospital room and at home) as well as many other incentives as previously described. Also, since the babies' hearing functions were already tested in the hospital by the OAE test, this might have limited women's interest in participating in the study and affected our recruitment when the test was deemed normal.

Although the ABR and OAE screening tests are the two professional and complementary tools widely adopted and accepted nowadays, they still have some significant limitations and drawback[254]. In auditory neuropathy, one type of hearing loss, the outer hair cells within the cochlea are present and functional, but sound information is not properly transmitted to the auditory nerve and brain[255]. Diagnosis of auditory neuropathy requires both OAE, which is the simplest and quickest test as well as the ABR screening test, which is a more in-depth brain hearing assessment, since they do not measure the same compartments (cochlea vs brainstem)[256]. Thus, both OAE and ABR tests are needed to have a complete assessment of hearing functions. Another drawback is that the hearing screening procedure

often results in up to 16.6% of false positive tests[257]. Furthermore, the definite diagnosis often takes months[254]. Thus false positive tests may be the cause of prolonged unnecessary diagnostic procedure[254,258]. Parents should thus be aware of the risk of false positive before giving their informed consent[259,260]. The best time and condition to obtain reliable results for both the ABR and OAE tests are during the child's nap in a quiet environment since both tests can be adversely influenced by the motion artifacts[254]. In our study, the environment was not always controlled since the tests have been performed in different environments; that is in the laboratory, the hospital room or the participants' home. All of these limitations greatly inflate the risk of type II error and our ability to make any definite conclusions.

CHAPTER 5

5. CONCLUSION

Despite the fact that iron status during pregnancy plays a critical role in the development of fetal auditory nerves system, the impact of iron status during pregnancy on hearing function has not been well studied, particularly in humans. We are not able to conclude with this pilot project whether there is a relationship between markers of iron status (Hb and MCV) in pregnant women and the development of auditory function in our small group of newborns. These analyses will need to be performed in the final sample size (n=58) to confirm the trends observed.

All things considered, even though we could not find any significant results among our six dyads of participants, results from previous studies, although mostly done in preterm infants for ID and in adults for iron excess, would support our hypothesis which stipulates that low and high concentrations of iron markers during pregnancy negatively impact the development of the fetus's auditory nerve system thereby altering hearing functions in the newborns. Based on the studies previously reviewed, we can speculate that both offends of the iron status spectrum could be associated with longer or shorter amplitudes and latencies. Indeed, studies on guinea pigs demonstrated that offspring with IDA had higher ABR thresholds, meaning worse auditory acuity and also higher amplitudes which indicated delayed peripheral and brainstem neural transmission times and neural synchrony than iron sufficient group[206,207].

Health care professionals must have better tools to manage iron status among pregnant women in order to prevent the consequences of maternal iron deficiency or excess in the neonates. If the results obtained by the full sample size (n=58) are promising, a subsequent study could be developed using a larger sample size in a multi-centre setting with participants from different socioeconomic backgrounds to better represent the targeted population of newborns in Canada. In addition, not only Hb and MCV,

but also more precise iron markers such as maternal serum ferritin, cord serum ferritin, serum transferrin receptor (STFR), and hematocrit (HCT) should be used for diagnosing ID or IDA during pregnancy. Additionally, these measurements should be taken in the three trimesters in a prospective manner. Further research should also investigate how to improve the medical follow-up with regards to nutrition in order to prevent iron deficiency/excess during pregnancy. This could be done by developing simple tools to identify women at risk of ID and IDA which could assess their dietary patterns (ex. vegetarian or vegan women), their prenatal supplement intake habits and whether they have been previously anemic in their life. Additionally, we need better strategies to prevent IDA during pregnancy because so far prescribing supplements containing iron does not appear to entirely prevent IDA. Findings from these kinds of studies have the potential to contribute to the development and the implementation to promote the healthy development, including brain and auditory functions, of infants during the pre- and perinatal periods.

5.1 Implications

Currently, the basic OAE hearing test is performed routinely in Ontario in every newborn right after birth[226,227]. This test is done in many other Canadian provinces, such as British Columbia, Nova Scotia, New Brunswick and Prince Edward Island, but other provinces either do not perform the test or do it only in high-risk babies[261]. However, this test does not provide information on the newborn's hearing neurophysiological development and the results are not always recorded in medical charts. As previously discussed, apart from one study examining the association between pregnancy latent iron deficiency in utero with ABR test results in newborn ≥ 35 weeks of gestation age, no other study has linked the maternal iron status and brainstem responses in healthy term newborns. Thus, research like the one we performed is necessary to evaluate the need to perform hearing tests in all Canadian

newborns and to identify under which circumstances they should be made (ex. among women with inadequate iron status during pregnancy).

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Appendix A: Research Ethics Board (REB) Approvals and Permissions

The Research Ethics Board (REB) Approvals can be found in the following appendices:

1. Research Ethics Board (REB) of the Montfort Hospital.

   File Number: BFB-01-09-15

2. The University of Ottawa: Health Sciences and Science Research Ethics Board.

   File Number: A04-16-04

3. Research Ethics Board of the Ottawa Public Health

   File Number: 225-16

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Hôpital Montfort

Un hôpital d'enseignement affilié à l'Université d'Ottawa

A teaching hospital affiliated with the University of Ottawa

Avis d'approbation éthique
Comité d'éthique de la recherche (CÉR) de l'Hôpital Montfort

Le 7 avril 2016

Chercheuse principale :

Bénédicte Fontaine-Bisson
École des Sciences de la nutrition
Université d'Ottawa
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Titre du projet : « Effect of Iron Deficiency an Excess during pregnancy on the development of auditory abnormalities in Human neonates »

Numéro du dossier : BFB-01-09-15

Date de début : 7 avril 2016

Date de fin : 6 avril 2017

En conformité avec l'Énoncé de politique des trois conseils — Éthique de la recherche avec des êtres humains (EPTC 2), décembre 2014, le Conseil canadien des normes, les bonnes pratiques cliniques : directives consolidées, Conférence internationale sur l'harmonisation des exigences techniques relatives à l'homologation des produits pharmaceutiques à usage humain (ICH-GCP E6), la Loi de 2004 sur la protection des renseignements personnels sur la santé, les lois et règlements applicables en Ontario, je confirme que le Comité d'éthique de la recherche (CÉR) de l'Hôpital Montfort a étudié et approuvé les documents suivants pour les dates de début et de fin mentionnées ci-dessus :

- Protocole de recherche, soumis le 1er septembre 2015
- Affiche de recrutement (FR et EN), soumis le 22 mars 2016
- Script de recrutement (version EN) soumise le 17 mars et (version FR) soumise le 30 mars 2016
- Questionnaire Fer et audition, soumis le 17 février 2016
- Brochure Fer et audition, soumise le 1er septembre 2015
- Formulaire de consentement pour femme enceinte (FR et EN) version soumise le 21 mars 2016
- Formulaire de consentement pour nouveau-né, FR et EN), version soumise le 21 mars 2016
- Formulaire de consentement pour nouveau-né de mère monoparentale (FR et EN) version soumise le 21 mars 2016

Le CÉR de l'Hôpital Montfort est constitué et exerce ses activités d'une manière conforme à la Loi sur les aliments et les drogues, la Partie C, Titre 5, du Règlement sur les aliments et drogues et aux règlements applicables, la partie 4 du Règlement sur les produits de santé naturels; partie 3 du Règlement sur les instruments médicaux ainsi qu'au « Codes of Federal Regulations » des États-Unis.

Le protocole de l'étude ne peut être modifié sans une approbation préalable du CÉR sauf s'il est question de la sécurité immédiate des participants ou de logistique administrative comme un changement de numéro de téléphone. Vous devez aviser le CÉR immédiatement de tout changement, événement indésirable ou nouvelle information pouvant augmenter le risque de l'étude, modifier le cours de l'étude ou atteindre la sécurité des participants. Les modifications au projet et aux outils de recrutement doivent être soumises au CÉR.

Veuillez nous acheminer **quatre semaines avant la date d'échéance de cet avis d'approbation**, un rapport final afin de fermer le dossier ou de faire une demande de renouvellement du certificat d'approbation éthique de l'étude.

Si vous avez des questions, vous pouvez communiquer avec le bureau du CÉR de l'Hôpital Montfort au 613-746-4621, poste 2221 ou par courriel à ethique@montfort.on.ca.

Christine Landry, B.Pharm, M. Sc., PharmD
Vice-présidente du Comité d'éthique de la recherche — Hôpital Montfort

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

April 25, 2016

Bénédicte Fontaine-Bisson
School of Nutrition Sciences
University of Ottawa
bfontaine@uottawa.ca

Co-investigators: France Rioux, University of Ottawa
Amineh Koravand, University of Ottawa

Re: U of O Ethics file no. A04-16-04 – "The effect of iron status during pregnancy on the newborn's auditory functions"

Dear Dr. Fontaine-Bisson and colleagues,

Thank you for the approval documents from the Montfort-REB (REB Protocol # BFB-01-09-15) for your project named above.

This is to confirm that, in accordance with the agreement between the University of Ottawa and The Montfort-REB, the University of Ottawa has authorized this board to act as Board of Record for the review and oversight of research involving human subjects conducted at or through the hospital.

We remind you of your obligation to:
- Follow all procedures of the Montfort-REB including reporting and renewal procedures;
- Submit to the authority of the Montfort-REB and that you are subject to the Montfort-REB requirements, including, without limitation, the requirement to modify or stop the research on demand of the Montfort -REB.

If you have any questions, please contact our ethics office at 562-5387.

Sincerely yours,

Catherine Paquet
Director, Office of Research Ethics and Integrity

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Ottawa | Public Health
Santé publique

November 30, 2016

Research Ethics Board
Ottawa Public Health
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Ottawa, ON K2G 6J8

Bénédicte Fontaine-Bisson, DtP | RD, PhD
Professeure agrégée | Associate Professor
Ecole des sciences de la nutrition | School of Nutrition Sciences
Université d'Ottawa | University of Ottawa
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bfontain@uottawa.ca

Dear Dr. Fontaine-Bisson:

Re: The effect of iron status during pregnancy on newborn's auditory functions.

I am pleased to inform you that the Ottawa Public Health Research Ethics Board (REB) has reviewed and accepted your research proposal entitled, 'The effect of iron status during pregnancy on newborn's auditory functions.' You are granted Category 1 Approval and may begin data collection per the schedule you have established.

You are reminded to inform the Board if you have any major changes in your proposal by completing Appendix F (attached). At the end of your study, you are to submit an end of project report using Appendix H (attached). Please submit the completed forms as indicated to the Ottawa Public Health Research Ethics Board Secretariat via email.

The term of approval ends on November 30, 2017. Should you require additional time, please contact the REB secretariat to obtain a renewal document which much be submitted in order to extend the time frame of the project.

On behalf of the Board, we wish you well in your research. Please do not hesitate to contact the Research Secretariat at oph.ethics@ottawa.ca or by phone at 613-580-6744, extension 23595 (prior to December 1) and at extension 16542 (after December 1), if you require further information.

Sincerely,

Marguerite Souliére
Chair, Research Ethics Board
Ottawa Public Health

Attachments: 2

c.c. Dawn Grakist, OPH

Appendix B: Research Information and Consent Form for the Pregnant Mother

INFORMATION AND CONSENT FORM FOR THE PREGNANT MOTHER

Project Name: The effect of iron status during pregnancy on auditory functions in the newborn.

Principal Researcher's Name:
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In case of emergency: Contact Dr. Fontaine-Bisson at (613) 562-5800 #3482.

Funding source : *Institut de recherche de l'Hôpital Montfort.*

Conflict of interest: The researchers declare having no conflict of interest associated with this study.

2. INTRODUCTION

Before agreeing to participate in this research project, please take the time to read and carefully consider the following information. This document explains the purpose of this research project, its procedures, benefits, risks and drawbacks. We encourage you to ask any and all questions you consider relevant to the person who gave you this document.

The goal of this pilot project is to explore the relationship between women's iron status during pregnancy and newborn's hearing functions.

3. INVITATION TO PARTICIPATE

You are invited to participate in the above-named research project conducted by Bénédicte Fontaine-Bisson, Associate Professor at the School of Nutrition Sciences at the University of Ottawa and Affiliated Researcher at l'*Institut de recherche de l'Hôpital Montfort.*

You are free to participate in the study or not. Your decision to participate or withdraw from the study will not affect the quality of service provided to you now or in the future in any way.

4. PURPOSE OF THE STUDY

The purpose of the study is to determine if an indicator of blood iron levels (hemoglobin) during pregnancy has an influence on hearing (sound perception) in newborns.

5. INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

- Be a pregnant woman giving birth to a single baby at term (≥37 weeks of pregnancy).
- Being followed by a physician from the *Hôpital Montfort* or the Montfort Academic Family Health Team.
- Plan to give birth at the *Hôpital Montfort*.

Exclusion Criteria

If you or your newborn suffer from one of the following conditions, you are not eligible to participate in the study:

Mother

- Gestational diabetes;
- Arterial gestational hypertension;
- Pre-eclampsia – eclampsia;
- Infections during pregnancy or at birth (ex. toxoplasmosis, tuberculosis, HIV, septicemia, cytomegalovirus, herpes, simplex, rubella, chicken pox, shingles, syphilis);
- Use of drugs, alcohol and other elicit substances during pregnancy;

occur while your newborn is sleeping and does not present any risk except for a slight discomfort should he/she wake up during the procedure.

9. DATA CONSERVATION

The data gathered from yours and your child's medical records as well as the results associated with your newborn's hearing test will be kept in a secure manner. The only information taken from the mother's medical record will be markers of iron status (hemoglobin and mean corpuscular volume). Only information concerning the presence of severe jaundice (bilirubin concentration or phototherapy use) will be taken from the newborn's record. The paper documents will be kept in a key-locked filing cabinet at the *Institut de recherche de l'Hôpital Montfort*. Access to the *Institut de recherche de l'Hôpital Montfort* is restricted and only authorized research personnel who have a magnetic access card can enter. Only research personnel authorized by the principal investigator will have access to this magnetic access card and key to the filing cabinet. The information obtained will be transferred to a secure database on the server of *Hôpital Montfort* and compiled in a password-protected computer. Ten years after the end of the study, the paper documents of the study will be destroyed in a secure manner and the electronic files will be erased.

10. CONFIDENTIALITY AND ANONYMITY

You have the researcher's assurance that any information shared with her team will be kept strictly confidential except when required by law. The information will be used only for analysis to determine if iron status during pregnancy influences hearing functions in the newborn, while respecting confidentiality. The details identifying you will be separated from the research data, which will be coded. However, it is possible that research records identifying you will be examined, in the presence of the researcher, by a representative of the organization sponsoring or funding the research (*Institut de recherche de l'Hôpital Montfort*) and the Research Ethics Board for research control purposes. However, you are assured that no record identifying you, by name or initials or with your contact information, will be permitted to leave the secured research database of the *Hôpital Montfort*. Anonymity is guaranteed in that any and all identifying details will be replaced by a code. The information identifying you will be entered in a folder separate from the other data in the secured database. Access to this information will be limited to members of the research team and used only to contact you.

11. VOLUNTARY PARTICIPATION

Your participation in the study is voluntary. You are free to withdraw at any time or to refuse to answer certain questions, without exposing yourself to any negative consequences. If you choose to withdraw from the study, the data gathered up to that time will be deleted. You will be informed at the appropriate time if any new information arises that might affect your interest in continuing to participate in the study.

12. REIMBURESEMENT/COMPENSATION

Parking costs associated with your travel, if applicable, will be reimbursed and a $20 gift-certificate will be offered to you.

Newborn

- Newborn is small or large for gestational age;
- Medical conditions in the newborn susceptible to affect hearing (severe jaundice, one of the infections mentioned above, craniofacial anomalies, a genetic, nervous system, cardiac, respiratory, hepatic or renal disorder);
- Was admitted to the neonatal intensive care unit.

6. PARTICIPATION

Should you accept to participate in this study, you will receive weekly 5-10 minute phone calls beginning in the 36th week of pregnancy and lasting until birth to verify the inclusion and exclusion criteria. You will also be called after giving birth in order to plan a meeting that will take place within two weeks after delivery. This meeting can be held in either the clinical research facilities at *Hôpital Montfort* (room 1E104), in your hospital room before discharge or at your place, according to your preference. During the meeting, a trained person will perform an exhaustive hearing test on your newborn (lasting 30 minutes). The test results will then be interpreted by a Master's student in Audiology. You will also be asked to complete a questionnaire that includes questions on your general health and iron supplement intake during pregnancy. This session will take less than 1 hour.

Alternatives to participation:

You are free to participate in this study or not. If you decide not to participate, you will continue to receive the scheduled care from your family doctor.

You are free to withdraw from the study at any time.

7. BENEFITS

Your participation in this research means that your child will be evaluated on his/her ability to hear different sounds. An interpretation of the hearing test results will be sent to you if you want. If the test results are abnormal, they will be transferred to your family doctor. Information concerning children's healthy eating, hearing and language development will be provided to you during the meeting. During the meeting (at the hospital or at home), you will also receive a healthy snack and a $20 gift-certificate to compensate for your time. You will receive a parking pass if you choose to come to the meeting in the hospital research facility after hospital discharge. In general, your participation in this study will generate preliminary results on whether or not the iron status during pregnancy influences hearing in the newborn. The confirmation of these results by other studies could lead decision-makers and medical associations to revise their guidelines on optimal iron status during pregnancy as well as diagnostic hearing procedures in the newborn.

8. RISKS

Your participation in this research entails that you provide personal information. The details provided will remain entirely confidential and only the principal researcher and members of the research team will have access to this information. The hearing test will

13. NOTIFICATION OF RESULTS

The results of this study may be published in scientific journals or presented at conferences. You can choose, in the indicated portion of the questionnaire that you will complete at the meeting, if you wish to receive the scientific publications that follow this project.

In the consent form for the participation of your newborn, you will be asked to indicate if you wish to receive the results from the hearing test.

In the case that a valid and significant scientific result that concerns your child's health (e.g. abnormal hearing test results) is found for which preventative measures or a treatment is available (e.g. a follow-up with a specialist), your family doctor: will be informed.

I understand that the communication of this information has certain risks for me and my family such as anxiety and stress (ex. Learning that my child has hearing problems).

14. CIVIL LIABILITY

Your consent to participate in this study does not affect your right to seek legal recourse in any matter whatsoever. If your participation causes you any prejudice, you reserve the right to take any legal recourse against the various research partners.

15. CONTACTS

For all additional information concerning this study, you can contact our research team by telephone or email.

613-746-4621, ext 6403
feraudition@montfort.on.ca

For all information on the ethical aspects of this research, you can contact the Hôpital Montfort Research Ethics Board, 745-A Montreal Road, suite 102, Ottawa, Ontario by telephone at 613-746-4621, extension 2221 or by email at ethique@montfort.on.ca.

CONSENT

Acceptance: You accept to participate in this research conducted by Professor Bénédicte Fontaine-Bisson.

> *I was explained the nature and development of the project. I have read the consent form and have been provided a copy. I have had the opportunity to ask questions which were answered to my satisfaction. I authorize the archives service to give to the research team only the information from my medical record mentioned in point 9 of this current form (data conservation). . I accept to participate in this research project.*

Appendix C: Research Information and Parental Consent Form for the Newborn

Hôpital Montfort
Institut de recherche

uOttawa

INFORMATION AND SINGLE-PARENT MOTHER CONSENT FORM FOR THE NEWBORN

Project Name: The effect of iron status during pregnancy on auditory functions in the newborn.

When the participant is a child, parental consent must be obtained.

Principal Researcher's Name:
Bénédicte Fontaine-Bisson, DtP, PhD
Affiliated researcher at l'*Institut de recherche de l'Hôpital Montfort*
Associate Professor, School of Nutrition Sciences,
Faculty of Health Sciences, University of Ottawa
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Amineh Koravand, PhD
Assistant Professor
School of Rehabilitation
Faculty of Health Sciences
University of Ottawa
(613) 562-5800 #8300
amineh.koravand@uOttawa.ca

In case of emergency: Contact Dr. Fontaine-Bisson at (613) 562-5800 #3482.

Funding source : *Institut de recherche de l'Hôpital Montfort.*

Conflict of interest: The researchers declare having no conflict of interest associated with this study.

Last update : January 25, 2017

2. INTRODUCTION

Before agreeing to involve your newborn in this research project, please take the time to read and carefully consider the following information. This document explains the purpose of this research project, its procedures, benefits, risks and drawbacks. We encourage you to ask any and all questions you consider relevant to the person who gave you this document.

The goal of this pilot research project is to explore the relationship between women's iron status during pregnancy and newborn's hearing functions.

3. INVITATION TO PARTICIPATE

You are invited to participate in the above-named research project conducted by Bénédicte Fontaine-Bisson, Associate Professor at the School of Nutrition Sciences at the University of Ottawa and Affiliated Researcher at l'*Institut de recherche de l'Hôpital Montfort*.

You are free to involve your newborn in the study or not. Your decision to involve or withdraw your newborn from the study will not affect the quality of service provided to him/her now or in the future in any way.

4. PURPOSE OF THE STUDY

The purpose of the study is to determine if an indicator of blood iron levels (hemoglobin) during pregnancy has an influence on hearing (sound perception) in newborns.

5. INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

- Your newborn must be born at term (≥37 weeks of pregnancy) at the *Hôpital Montfort*.

Exclusion Criteria

If your newborn suffers from one of the following conditions, he/she is not eligible to participate in the study:

- Newborn is small or large for gestational age;
- Medical conditions in the newborn susceptible to affect hearing (severe jaundice, one of the following infections: toxoplasmosis, tuberculosis, HIV, septicemia, cytomegalovirus, herpes, simplex, rubella, chicken pox, shingles, syphilis; craniofacial anomalies, a genetic, nervous system, cardiac, respiratory, hepatic or renal disorder);
- Was admitted to the neonatal intensive care unit.

2. INTRODUCTION

Before agreeing to involve your newborn in this research project, please take the time to read and carefully consider the following information. This document explains the purpose of this research project, its procedures, benefits, risks and drawbacks. We encourage you to ask any and all questions you consider relevant to the person who gave you this document.

The goal of this pilot research project is to explore the relationship between women's iron status during pregnancy and newborn's hearing functions.

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You are invited to participate in the above-named research project conducted by Bénédicte Fontaine-Bisson, Associate Professor at the School of Nutrition Sciences at the University of Ottawa and Affiliated Researcher at l'*Institut de recherche de l'Hôpital Montfort*.

You are free to involve your newborn in the study or not. Your decision to involve or withdraw your newborn from the study will not affect the quality of service provided to him/her now or in the future in any way.

4. PURPOSE OF THE STUDY

The purpose of the study is to determine if an indicator of blood iron levels (hemoglobin) during pregnancy has an influence on hearing (sound perception) in newborns.

5. INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

- Your newborn must be born at term (≥37 weeks of pregnancy) at the *Hôpital Montfort*.

Exclusion Criteria

If your newborn suffers from one of the following conditions, he/she is not eligible to participate in the study:

- Newborn is small or large for gestational age;
- Medical conditions in the newborn susceptible to affect hearing (severe jaundice, one of the following infections: toxoplasmosis, tuberculosis, HIV, septicemia, cytomegalovirus, herpes, simplex, rubella, chicken pox, shingles, syphilis; craniofacial anomalies, a genetic, nervous system, cardiac, respiratory, hepatic or renal disorder);
- Was admitted to the neonatal intensive care unit.

6. PARTICIPATION

Should you accept to involve your newborn in this study, an exhaustive hearing test will be performed by a trained person on him/her within two weeks after birth either in the clinical research facilities of the *Hôpital Montfort* (1E104) or in your hospital room before discharge or at your place, according to your preference. The test results will then be interpreted by a Master's student in audiology. The hearing test will take a maximum of 30 minutes, is safe and painless for the newborn. No known prejudice is linked to the participation of your child in this study.

Alternatives to participation:

You are free to involve your newborn in this study or not. If you decide not to have him/her participate, your newborn will continue to receive the scheduled care from your family doctor.

You are free to withdraw your newborn from the study at any time.

7. BENEFITS

Your newborn's participation in this research means that he/she will be evaluated on his/her ability to hear different sounds. An interpretation of the hearing test results will be sent to you if you want. If the test results are abnormal, they will be transferred to your family doctor. Information concerning healthy eating, hearing and language development of the child will be provided to you during the meeting. During the meeting (at the hospital or at home), you will also receive a healthy snack and a $20 gift-certificate to compensate for your time. You will receive a parking pass if you choose to come to the meeting in the hospital research facility after hospital discharge. In general, your newborn's participation in this study will generate preliminary results on whether or not the iron status during pregnancy influences hearing in the newborn. The confirmation of these results by other studies could lead decision-makers and medical associations to revise their guidelines on optimal iron status during pregnancy as well as diagnostic hearing procedures in the newborn.

8. RISKS

Your newborn's participation in this research entails that you provide personal information. The details provided will remain entirely confidential and only the principal researcher and members of the research team will have access to this information. The hearing test will occur while your newborn is sleeping and does not present any risk except for a slight discomfort should he/she wake up during the procedure.

9. DATA CONSERVATION

The data gathered from your newborn's medical record as well as the results associated with your newborn's hearing test will be kept in a secure manner. The only information taken from the newborn's medical record will be concerning the presence of severe jaundice (bilirubin concentration or phototherapy use). The paper documents will be kept in a key-locked filing cabinet at the *Institut de recherche de l'Hôpital Montfort*. Access to the *Institut de recherche de l'Hôpital Montfort* is restricted and only authorized research personnel who have a magnetic access card can enter. Only research personnel authorized

by the principal investigator will have access to this magnetic access card and key to the filing cabinet. The information obtained will be transferred to a secure database on the server of *Hôpital Montfort* and compiled in a password-protected computer. Ten years after the end of the study, the paper documents of the study will be destroyed in a secure manner and the electronic files will be erased.

10. CONFIDENTIALITY AND ANONYMITY

You have the researcher's assurance that any information shared with her team will be kept strictly confidential except when required by law. The information will be used only for analysis to determine if iron status during pregnancy influences hearing functions in the newborn, while respecting confidentiality. The details identifying your newborn will be separated from the research data, which will be coded. However, it is possible that research records identifying your newborn will be examined, in the presence of the researcher, by a representative of the organization sponsoring or funding the research (*Institut de recherche de l'Hôpital Montfort*) and the Research Ethics Board for research control purposes. However, you are assured that no record identifying your newborn, by name or initials or with your contact information, will be permitted to leave the secured research database of the *Hôpital Montfort*. Anonymity is guaranteed in that any and all identifying details will be replaced by a code.

The information identifying your newborn will be entered in a folder separate from the other data in the secured database. Access to this information will be limited to members of the research team and used only to contact you.

11. VOLUNTARY PARTICIPATION

Your authorization for your newborn's participation in the study is voluntary You are free to withdraw him/her at any time or to refuse that your newborn undergoes the hearing test, without exposing him/herself to any negative consequences. If you choose to withdraw him/her from the study, the data gathered up to that time will be deleted. You will be informed at the appropriate time if any new information arises that might affect your interest in continuing to participate in the study.

12. REIMBURESEMENT/COMPENSATION

Parking costs associated with your travel, if applicable, will be reimbursed and a $20 gift-certificate will be offered to you.

13. NOTIFICATION OF RESULTS

The results of this study may be published in scientific journals or presented at conferences. You can choose, in the indicated portion of the questionnaire that you will complete at the meeting, if you wish to receive the scientific publications that follow this project.

An interpretation of your newborn hearing test results will be sent to you by email or mail, according to your preference, if you wish so. Please indicate by which means you would like to receive the results, as appropriate:

I want to receive the information by:

☐ Email:_____

☐ Mail (address):

_____

_____

_____

☐ I do not want to receive the information directly. I want to be informed only by my family doctor if the results are not normal.

In the case that a valid and significant scientific result that concerns your child's health is found (e.g. abnormal hearing test results) for which preventative measures or a treatment is available (e.g. a follow-up with a specialist), your family doctor will be informed.

I understand that the communication of this information has certain risks for me and my family such as anxiety and stress (ex. Learning that my child has hearing problems).

14. CIVIL LIABILITY

Your consent to involve your newborn in this study does not affect your right to seek legal recourse in any matter whatsoever. If your participation causes you any prejudice, you reserve the right to take any legal recourse against the various research partners.

15. CONTACTS

For all additional information concerning this study, you can contact our research team by telephone or email.

613-746-4621, ext 6403
feraudition@montfort.on.ca

For all information on the ethical aspects of this research, you can contact the Hôpital Montfort Research Ethics Board, 745-A Montreal Road, suite 102, Ottawa, Ontario by telephone at 613-746-4621, extension 2221 or by email at ethique@montfort.on.ca.

CONSENT

Acceptance: You accept that your newborn participates in this research conducted by Professor Bénédicte Fontaine-Bisson.

Consent for the newborn's participation

I was explained all relevant aspects of the research and my questions were answered to my satisfaction. I was informed that my newborn's participation to this project is voluntary and can cease at any time without any form of penalty. I was given enough time to discuss with my family about the nature and involvement of my newborn in this project. I authorize the archives service to give to the research team only the information from my newborn's medical record mentioned in point 9 of this current form (data conservation). I can withdraw my newborn from this project for reasons that I do not have to justify.

There are two copies of the consent form, one of which you may keep.

_____

Name (please print) of the newborn's mother (participant)

_____

Signature of newborn's mother (participant)

Date : ____/____/____
Day Month Year

_____

Name (please print) of the researcher or the representative

_____

Signature of the researcher or the representative

Date : ____/____/____
Day Month Year

For all information on the ethical aspects of this optional part of the research, I can contact the Hôpital Montfort Research Ethics Board, 745-A Montreal Road, suite 102, Ottawa, Ontario by telephone 613-746-4621, extension 2221 or by email at ethique@montfort.on.ca

Last update : January 25, 2017

Appendix D: Participants' General Questionnaire

Hôpital Montfort

Institut de recherche

Identification number : _____

GENERAL QUESTIONNAIRE

(To be completed by the mother)

1) Today's date: _____
Day / Month / Year

2) Your birthday date: _____
Day / Month / Year

3) Place of residence (circle): Ottawa Gatineau Other_____

4) Did you give birth at the *Hôpital Montfort*?

YES _____ NO_____

5) To how many children did you give birth in your life? _____

6) Indicate their date of birth (use the space beside if necessary):

Not applicable ☐

First child_____

Second child_____

Third child _____

Fourth child _____

Day / Month / Year

7) Please check the highest level of education completed by parents.

Mother		**Other parent or legal guardian**	
Primary school		Primary school	
High School		High School	
College education		College education	
University education		University education	
Undergraduate studies		Undergraduate studies	
Graduate studies (ex. M.Sc., Ph.D.)		Graduate studies (ex. M.Sc. or Ph.D.)	
Other diplomas or certificates		Other diplomas or certificates	

1

8) Circle the corresponding family income category.

Between $10 000 – 39 999
Between $40 000 – 59 999
Between $60 000 – 99 999
Between $100 000 -150 000
>150 000

9) Have you taken iron-containing supplements during your pregnancy?

YES _____ NO_____

10) Have you taken prescribed iron-containing supplements because of anemia during your pregnancy?

YES _____ NO_____

11) If you took iron-containing supplements, please check (√) the period of consumption and indicate the brand for each type of supplements consumed.

Type of supplement	**Period of consumption**		
	1st trimester	2nd trimester	3rd trimester
Prenatal multivitamin (ex. Materna, Pregvit, Centrum Prenatal, Kirkland, Life, Personnel…) ➢ Specify the brand _____			
Regular multivitamin ➢ Specify the brand _____			
Iron supplement ➢ Specify the brand _____ ➢ Specify the dose (if known) _____mg			

12) On average, at which frequency did you take your supplements (ex. daily, 3x per week,…)?

_____

13) Have you suffered from anemia due to insufficient iron intake during your pregnancy?

YES _____ NO_____

14) Have you ever suffered from anemia due to insufficient iron intake during your life?

YES _____ NO_____

15) The following questions are related to your general health satus.

	YES	NO
• Do you have diabetes (excluding gestational diabetes)?	○	○
• **If so**, circle the type : Type 1 / Type 2		
• Have you suffered from gestational diabetes during your pregnancy?	○	○
• Have you received a diagnosis of hypertension during your pregnancy?	○	○
• Have you suffered from pre-eclampsia or eclampsia during your pregnancy?	○	○
• Have you consumed drugs and/or illicit substances during your pregnancy? (ex. alcohol, cigarettes)	○	○
• **If so**, please indicate the **type** of substance and the **frequency** of consumption. _____		
• Have you suffered from an infection or condition listed below during your pregnancy? Rubella, chicken pox, zona, toxoplasmosis, herpes simplex, cytomegalovirus septicemia, syphilis or tuberculosis.	○	○
• **If so**, indicate which one(s) : _____		

16) Do you breastfeed your baby (check below √)?

- Yes (exclusive breastfeeding) ____
- Partially (breastfeeding and formula) ____
- No (formula) ____

- If you answered ***partially*** to the previous question, please estimate the percentage of breastmilk and formula received by your baby.

  _____ % from breast milk
  _____ % from formula

17) By which type have you delivered your baby (circle)?

Vaginal_____ Cesarean section _____

18) Are you **vegetarian**, meaning that you consume some type of animal products (ex. fish, eggs, milk, etc.) in your diet?

YES _____ NO_____

19) Are you **vegan**; meaning that you exclude all forms of animal products from your diet?

YES _____ NO_____

**The following questions are related to your baby*

20) Your baby was born at how many weeks? _____ weeks

21) What was your baby's weight at birth (circle the units)?

_____lb ou kg

21) The following questions are related to your baby's health.

	OUI	NON
• Since birth, has your baby suffered from an infection?	○	○
• **If so**, please indicate which ones : _____		
❖ See examples on page 3.		
• Does your baby have a rare genetic disease (ex. Down syndrom, Usher syndrom, Pendred, Waardenburg…)?	○	○
• Was your baby admitted to the intensive care unit?	○	○
• Did you baby have jaundice?	○	○
• **If so**, was your baby placed under a UV lamp (phototherapy)?	○	○

22) Would you like to be informed of publications associated with this research project (please check)?

YES _____ NO_____

We thank you very much for your participation in this study!

Appendix E: Pamphlet for Dietary Guidelines for the First Year of Life

Summary of a joint statement of Health Canada, Canadian Paediatric Society, Dietitians of Canada and Breastfeeding Committee for Canada.

Dietary guidelines for the first year of life

From birth to 6 months

- Exclusive breastfeeding for the first six months is recognized as the normal and best nutrition standard for infants (see tips at the back of this page).
    - Give a vitamin D supplement of 400 IU daily for breastfed infants.
    - Avoid giving any other food or liquid (including water and juice).

- For non-breastfed infants, cow milk-based infant formula (unless otherwise prescribed by your physician) should be safely prepared and served (see tips at the back of this page).
    - Do not prepare home-made formula with evaporated milk, regular cow or goat milk, soy, rice or other cereal-based beverages.

From 6 to 12 months

- Breastfeeding is encouraged up to two years or longer.
    - Continue to give a vitamin D supplement of 400 IU daily for breastfed infants.
    - From 6-12 months, breastfeeding (or formula feeding) should cover at least half of their energy needs.

- From **6 to 9 months**, gradually introduce up to 2-3 meals and 1-2 snacks per day depending on your infant's appetite.
    - The first complementary food offered should include iron-rich meat (meat, poultry, fish*), meat alternatives (ex. eggs, tofu, legumes) and iron-fortified infant cereals.
        - *Avoid fish containing higher concentrations of mercury (ex. fresh/frozen tuna, shark, swordfish, marlin, orange roughly and escolar).
    - A variety of other foods from the family menu (with little or no added sugar or salt) such as vegetables, fruit, milk products (ex. cheese and yogurt) can be introduced with no particular order as long as the texture and size is adapted to your infant.
    - Serve iron-containing foods two or more times per day; and meat, poultry or their alternatives every day.
    - Avoid commercial infant foods with added sugar or salt.
    - Be responsive to their hunger/satiety cues and promote self-feeding with finger foods.
    - Lumpy texture should be served at no later than **9 months**.

- From **9 to 12 months,** pasteurized homogenized (3.25%) cow's milk can be introduced along with iron-rich foods (no more than 750 mL per day). A variety of textures should be offered by 1 year of age.
- It is better to avoid juice before 1 year of age, but water can be offered in an open cup (to help skill development).
- For more dietary information **after 1 year of age**, please consult the 2nd link below (Health Canada website).

http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/recom/index-eng.php#share
http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/recom/recom-6-24-months-6-24-mois-eng.php

Useful tips for successful breastfeeding

- Breastfeed your baby on demand.
  - Your baby knows best when he/she is hungry.
  - Breastfeed at least 8 times per day (approximately every 1-3 hours).
- Watch for early signs of hungriness (do not wait until your baby cries):
  - Suckling movements with the mouth, hands in the face/mouth, head movement while opening the mouth, head turning towards your breast.
- Breastfeed in a comfortable position
- Ensure a good latch:
  - Wide-open mouth, chin touching your breast with curled lower lip.
- Breastfeeding should not hurt.
  - If so or if you have questions, consult an expert to receive help (see resources below).

For more information, support and access to drop-in breastfeeding clinics:

- Gatineau: http://www.nroutaouais.ca/nourri-lait/
- Ottawa: http://www.ottawabreastfeeds.ca/
- Ontario: http://ontariobreastfeeds.ca/about-us and http://www.beststart.org/resources/breastfeeding/

Useful tips for safe preparation of infant formula

- Liquid infant formula (ready-to-eat and from concentrate) are sterilized, but powdered infant formula are not necessarily sterile.
- To prepare powdered/concentrated formula:
  - Wash your hands with hot water and soap.
  - All feeding equipment (bottle, nipple, lid, spoon) should be sterilized in boiling water for 2 minutes.
  - Use tap or bottled water boiled for 2 minutes and cooled at body/room temperature.
  - Do not use carbonated or mineral water.
- Any prepared formula can be kept in the refrigerator no more than 24 hours, however, it is recommended to feed your infant immediately after preparation.
- Any prepared formula should be used within 2 hours from the start of a feed.
  - Discard all leftovers.

For more information:

- http://healthycanadians.gc.ca/eating-nutrition/healthy-eating-saine-alimentation/safety-salubrite/milk-lait/formula-nourrisson-eng.php
- http://en.beststart.org/

Appendix F: Sample Menu: what you can offer within the first year of life

Summary of a joint statement of Health Canada, Canadian Paediatric Society, Dietitians of Canada and Breastfeeding Committee for Canada.

Sample menu: What you can offer a seven-month old infant	
Time of day	**What you can offer**
Early morning and on cue at any time	• Breastfeeding
Morning	• Breastfeeding • Iron-fortified infant cereal • Mashed strawberries or other soft fruit
Snack	• Whole grain toast, cut into small pieces or strips
Midday	• Breastfeeding • Iron-fortified infant cereal • Hard-boiled egg, mashed, minced or grated • Cooked and mashed sweet potato or other vegetable
Snack	• Unsweetened stewed prunes, pureed
Early evening	• Breastfeeding • Ground or finely minced plain, dark chicken or other meat • Cooked and mashed broccoli or other vegetable
Evening and nighttime	• Breastfeeding

Sample menus: What you can offer an 11-month old infant	
Time of day	**What you can offer**
Early morning and on cue	• Breastfeeding
Morning feedings	• Breastfeeding • Iron-fortified infant cereal • Apple sauce Or: • Breastfeeding • Iron-fortified infant cereal • Strawberries, chopped Or: • Breastfeeding • Iron-fortified infant cereal • Kiwi, chopped
Snacks	• Whole grain bread, cut into strips, with soft margarine • Unsweetened stewed prunes, pureed Or: • Unsweetened o-shaped oat cereal • Blueberries thawed from frozen Or: • Whole grain and fruit muffin • Carrot, grated
Midday feedings	• Breastfeeding • Chicken, chopped • Steamed brown rice • Cooked broccoli, chopped

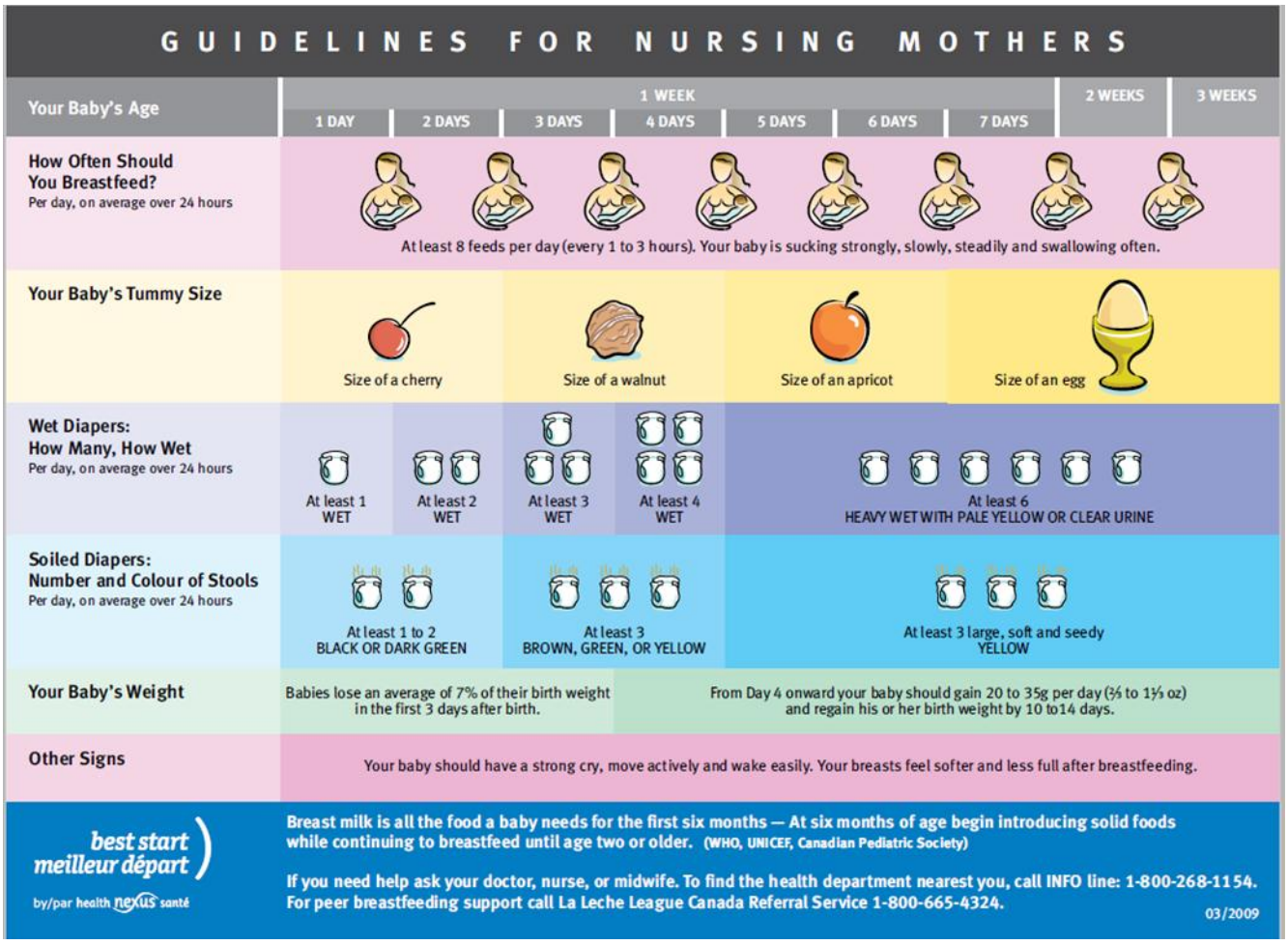
http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/recom/recom-6-24-months-6-24-mois-eng.php

Summary of a joint statement of Health Canada, Canadian Paediatric Society, Dietitians of Canada and Breastfeedin Committee for Canada.

Sample menus: What you can offer an 11-month old infant	
Time of day	**What you can offer**
	Or: • Breastfeeding • Canned salmon, mashed • Sweet potato, mashed • Green peas, cooked soft, mashed Or: • Breastfeeding • Roast turkey leg, chopped • Whole grain bread, cut into strips • Squash, mashed
Snacks	• Cheddar cheese, shredded • Whole wheat pita, cut into small strips Or: • Hard-boiled egg, chopped • Whole grain bread, cut into strips Or: • Soft tofu, mashed • Blueberries, cut in halves • Unsalted crackers
Early evening feedings	• Breastfeeding •

http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/recom/recom-6-24-months-6-24-mois-eng.php

Appendix G: Pamphlets of Guidelines for Nursing Mothers



Appendix H: Pamphlet for Child Language Development

Working together as a team

For the best results, you will need to commit time every day to follow through with the plan that you and your speech language pathologist have put into place. If time is a problem, talk to your speech language pathologist about how to provide the best support possible for your child.

When you and your family become actively involved, you can help your child develop the language skills needed to reach his or her full potential.

For information on local speech and language programs or hearing program please visit ontario.ca/earlychildhood and click on Speech and Language or Hearing.

Preschool Speech and Language Program
Infant Hearing Program

Helping your child learn language

To order by phone:
1-800-668-9938
TTY: 1-800-268-7095

Or visit: www.serviceontario.ca/publications
Publication # 019536

Learn more: www.ontario.ca/children

Ce document est également disponible en français.

Ontario

Helping your child learn

Ontario's Preschool Speech and Language Program and Infant Hearing Program provide support as you begin to help your child develop his or her language skills.

Your involvement is essential in helping your child learn language. Children learn best through relationships with the important people in their lives. As a parent, you are your child's best teacher because you know your child better than anyone else. You are in the best position to help him or her learn how to communicate.

What is language development?

Language development is a continuous process. A baby begins to use language with babbles and coos, then starts imitating words and understanding what they mean. Gradually, a child starts to string words together and moves from simple to more complex conversations.

A child has a language delay or disorder if he or she has difficulty understanding words or putting them together to communicate his or her needs and ideas.

Professional help

If you suspect your child's language is delayed, it is important to seek professional help immediately.

Although you are the most important person in your child's language development, you will not be alone. You will be part of a team with a speech language pathologist.

These professionals help to identify, assess and treat language disorders and to support language development. You may also work closely with other professionals, including communications assistants, American Sign Language consultants, auditory verbal therapists and early childhood educators, to support you and your child.

These professionals will show you how to communicate and play with your child at home to encourage and support your child's language development.

Your family's role

The Preschool Speech and Language and Infant Hearing programs rely on the involvement of a child's family as the key to improving his or her language skills.

A family's lifestyle, customs and environment all come into play as children learn about their world, the people in their lives and how to communicate. So, as a parent, it is your job to provide the professionals with valuable information about your child's strengths and needs. That way, you can help to develop goals to support your child's language development that will work for your family.

Finding the right approach for your family

There are various approaches to helping children learn. For example, a speech language pathologist may work with you to develop a program to follow at home or arrange for you to join regular get-togethers with other parents and children.

The speech language pathologist's first step is to assess your child's speech and language abilities. With your help, he or she will set goals and discuss how you can best work together. The speech language pathologist will then schedule time to work with your child and give you support through at least one of these:

- parent education workshops
- small group sessions
- consultation
- a home program.

Sessions with professionals will involve you, other family members and child care providers, so everyone can learn ways to help support your child's development during daily activities. You will receive helpful strategies and materials including, for example, suggestions for books to read and games to play at home. You may also be asked to videotape your child's progress, keep a journal or fill out questionnaires about your child's development.