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The association between breastfeeding during late pregnancy and the occurrence of small for gestational age and prolonged active phase of labor among Peruvian women

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ABSTRACT

Maternal nutritional status is considered an important risk factor for the progression of labor and birth weight. Poor birth outcomes, such as experiencing a prolonged labor or being small for gestational age, represent a risk factor for maternal as well as infant morbidity and mortality. Understanding the factors that may affect maternal nutritional status is necessary. The objective of the present study was to examine the association between the practice of breastfeeding during late pregnancy and the risk of having a prolonged active phase of labor (PAPL) or giving birth to a small for gestational age (SGA) baby.

A case-control study was conducted with two matched controls for each case of SGA or PAPL. Between March 2006 and April 2007 three Peruvian hospitals were visited daily. Mothers who met the inclusion criteria were interviewed and information regarding maternal as well as newborn characteristics was extracted from their clinical charts. Hair samples for zinc analysis were collected on a subsample of mothers. The association between the overlap of breastfeeding during late pregnancy and the risk of SGA or PAPL was tested with conditional logistic regression.

No differences were found in the proportion of mothers who breastfed during late pregnancy between the cases of SGA or PAPL and their respective controls. In addition, there was no difference in maternal hair zinc concentration between cases and controls.

Our findings do not support the hypothesis that the practice of breastfeeding during late pregnancy represents a risk for having a small for gestational age infant or experiencing a prolonged active phase of labor. However, research to confirm these results is needed since negative birth and postnatal consequences have been previously associated with this practice.

CHAPTER 1: GENERAL INTRODUCTION

Maternal nutritional status is an important determinant of the progression of labor and birth outcomes (1). Pregnant women are among the demographic groups with a highest rate of nutritional deficiencies (2-4) even though physiological adjustments, like increased absorption of nutrients, help compensate for elevated requirements. Practices that represent additional stress for pregnant women can place them even at a higher risk for nutritional deficiencies. One example of additional stress, the practice of breastfeeding during pregnancy, implies a higher demand of nutrients since there is the dual cost of fetal development and the production of milk. This practice is not uncommon; data from the National Health and Nutrition Examination Survey (NHANES) III showed that 5.1% of American lactating women were pregnant concurrently (5). A study with Peruvian women showed that 10% of pregnant women who had children under 4 years old were also breastfeeding during pregnancy (6). This latter study also showed that after the first month of life, infants who were born from mothers who breastfed during the last trimester of pregnancy gained 125 g less than infants who were born from mothers who did not breastfeed at all during pregnancy.

A poor birth outcome such as small for gestational age (SGA) not only increases the risk of infant morbidity and mortality but also can increase the risk of obesity, coronary heart disease and diabetes in adult life (7-9). Due to the dearth of information regarding gestational age there are no estimations of the rates of SGA, but it is estimated that 15.5 % of all births worldwide are born with low birth weight (10). In addition, it is estimated that 529,000 women die annually of causes related to complications during the pregnancy, labor,

childbirth and postpartum period (11). Two of the most common causes of maternal death are severe bleeding and sepsis, which frequently are associated with complications during labor.

Prevention of SGA and labor complications such as a prolonged labor can play an essential role for improving maternal as well as infant health. Therefore, it is important to examine maternal practices, such as breastfeeding during pregnancy, which may represent a risk for the mother and the infant.

Thesis Organization

The present thesis starts with a general introduction followed by a thorough literature review relevant to the study. In addition, detailed information of the methodology used has been included as a separate chapter followed by two manuscripts prepared to be sent to scientific journals. The last part includes a chapter with the general conclusions, followed by the list of the references used for the first three chapters and the appendices with additional results from the study and an example of the instruments used for the data collection.

CHAPTER 2: LITERATURE REVIEW

Birth weight

Birth weight is measured during the first hour after birth, before the postnatal weight loss occurs and it can be expressed in pounds or kilograms (12). Although there is still some debate about the classification and diagnoses of growth-retarded newborns, the World Health Organization (WHO) has set cut-offs to categorize low birth weight. Low birth weight (LBW) has been defined as weight at birth less than 2500 g. Although in the past newborns who weighted less than 2500 were considered as premature babies, further experience showed that many of the infants born with less than 2500 g were not premature babies. Thus, officially since 1982 LBW refers to infants born weighing less than 2500 g regardless of gestational age (12). This cut-off has been based on epidemiological observations that infants weighing less than 2500 g are approximately 20 times more at risk to die than heavier babies (10).

LBW, which is the classification more often used, since gestational age is not frequently available, still represents a public health problem in many countries. It has been estimated that 15.5 % of all births worldwide are born with low birth weight, from which more than 95 % are born in developing countries (10). It is clear that LBW represents a risk factor for infants' health, which is why one of the major goals of the United Nations regarding infant health is to reduce LBW incidence at least one third in the present decade (10). An estimation of the LBW rates worldwide is shown on Table 1.

Table 1. Percentage and number of LBW infants by UNICEF regions

	Estimated % of LBW infants	% of births not weighed	Number of LBW infants (in millions)
Sub-Saharan Africa	14	65	4
Eastern and Southern Africa	14	63	2
Western and Central Africa	15	66	2
Middle East and North Africa	15	60	1
South Asia	28	74	11
East Asia and Pacific	8	30	3
Latin America and Caribbean	9	17	1
CEE/CIS	7	21	0.5
Industrialized countries	7	--	0.8
Developing countries	16	58	20
Least developed countries	19	68	5
World	16	--	21

UNICEF 2000

Besides LBW, small for gestational age (SGA) is another classification of birth outcomes regarding weight which is also important in order to identified newborns at risk. SGA is recommended to be used instead of LBW classification, every time the information of gestational age is available in order to differentiate low birth weight for gestational age from premature newborns. The Latin-American Center of Perinatology and Human Development (CLAP) defines SGA when the birth weight is less than the 10 percentile for the gestational age of the newborn (13). CLAP elaborated a table reference of percentiles of weight according to gestational age with a sample of 14814 newborns from Brasil, Uruguay and Argentina (13).

Birth outcomes such as LBW or SGA not only represents an important neonatal feature since it represents a significant risk factor for infant morbidity and mortality (10, 12); but also birth weight is an important characteristic of pregnancy outcome , given that it reflects the intrauterine environment.

Factors associated with birth weight

The increased risk for LBW associated with maternal factors such as physiological and environmental has been reported by many studies. These characteristics have been grouped as follow:

Sociodemographic Among these factors age is one of the first maternal characteristics that has been confirmed as an important risk factor. In 1997, Reichman et al. reported on a multivariate analysis from New Jersey data that both mothers, younger than 15 and older than 40 y, were at higher risk of having LBW deliveries than 25-29-year-olds (14). Later Lasker et al. found a similar trend on a study of 5,528 deliveries in Pensylvania, although the cut off for the younger group at highest risk was women younger than 20 years (15).

Other characteristics such as maternal education and marital status, which are interrelated with socioeconomic level, have also been described as important factors associated to poor birth outcomes (12, 16-19). Bobak et al. reported a difference of 322 g in mean birth weight between infants born to mothers with primary and university education; when researchers explored the influence of marital status, they found a difference of 232 g between infants born to married and single mothers, (18). A consistent finding was shown by Barbieri et al. who reported that among women who had vaginal delivery, the adjusted risk of LBW was higher for live births of non-cohabiting mothers (19). Reime et al. suggested that

the association between marital status and low birth weight could be because pregnant women who are single could have an inadequate prenatal care, they are also more likely to be nulliparous, and are under more psychological stress related with their single condition (20).

In relation to ethnicity, Lasker et al. reported that looking at most reported risk factors associated with LBW deliveries, the occurrence of this factors increased more the risk of LBW among Latin and African American mothers than for white mothers (15); however as reported by Valero de Bernabe et al., it is difficult to attribute these differences only to ethnicity, since race it is commonly interrelated with environmental factors which are complicated to isolate (12).

Obstetrical history The first factor reported in this group is parity. Studies have been shown that mothers who deliver the first child as well as mothers who deliver the fourth or more, are at higher risk of having LBW babies (12, 15, 21, 22). Furthermore, another factor that has been found to increase the risk of LBW is the interpregnancy interval. In 1992, Mavalancar reported that a short interpregnancy interval was associated with an increased risk of preterm delivery while primiparus women had increased risk of term LBW (21). Later, Zhu et al. described in a study of 173, 205 birth certificates that newborns who were conceived less than 6 months after a live birth, were at higher risk of LBW, preterm birth and SGA (23). Short interpregnancy interval has not only been linked to increased risk of the event of LBW or SGA but, it also has been associated with a risk of LBW repetition (24). An epidemiological case-control study conducted in Chile showed that women who delivered LBW babies were more likely to have an history of a previous LBW (25). Consistent with this is a study by Abdulrazzaq et al. who studied obstetrical risk factors on indigenous

population of United Arabian and found that mothers of LBW infants had a significantly higher number of previous LBW deliveries (26).

Risk of the current pregnancy One of the most important factors related to birth outcomes is the nutritional status of pregnant women during the pregnancy period, even important from the moment that they become pregnant. Studies on different populations, either from developing countries or developed countries, have shown a significant association between the prepregnancy weight and LBW (24, 25, 27). Furthermore, another important maternal risk factor reported is low pregnancy weight gain. It is not necessary for a women to have both of these two mentioned factors to represent a risk, each one separately is considered as a significant risk for LBW (22, 25). Strauss et al. reported on two cohorts of approximately 5000 mothers each, that low weight gain in one of the trimesters of pregnancy , could also represent risk for LBW; they found that mothers who had low weight gained either during the second or the third trimester of pregnancy, were two times more at risk of LBW (28).

Besides the direct associations found between pregnancy weight gain and birth weight, the weight gain during pregnancy is also a good indicator of the maternal nutritional status, which also represents an important factor associated with birth weight (12, 15). Poor maternal nutritional status is of concern due to that either before or during pregnancy maternal status can be associated to LBW cases (1). In many developing countries not only pregnant women, but also childbearing-age women regularly do not meet their nutritional requirements, therefore one is likely to find a significant number of women who become pregnant without being physically and nutritionally prepared. A good illustration of this problem is the prevalence of iron deficiency; even in a developed country like the US a prevalence of 12% iron deficiency has been reported on women of child-bearing age (2). As

mentioned before this problem becomes more of concern in developing countries. Jieng et al. reported on a study of 1165 Nepali pregnant women of whom 40% were iron deficient and 20% were deficient in more than one nutrient (3).

Requirements become very high for many nutrients during pregnancy, which are difficult to meet with dietary sources. Consequently it is not surprising to see that the rates of nutritional deficiency are high in pregnant women, placing this group of population at higher health risk. Ramos Hernandez et al. has reported an inadequate nutritional status in more than 50% of pregnant women in a peri-urban area of Mexico City who had a poor variety in dietary intake, as well as inadequacies in mineral and vitamin intake such as iron, folate and zinc (4). In addition, according to Caufield et al. 82% of pregnant women worldwide likely have inadequate usual intakes of zinc (29).

Besides poor dietary intake, maternal nutritional status can be affected by other factors; for instance anything that represents an additional physiological stress for the mother could account for an increased nutritional requirement. An example of additional physiological stress is the practice of breastfeeding during pregnancy, which might be thought as an influential factor on maternal nutritional status, as will be discussed later.

In addition to mother's nutritional status, other risk factors related to maternal behavior during pregnancy have been reported to be associated with birth weight. The first and most consistent factor found in many epidemiological studies is the practice of smoking during pregnancy (10, 12, 30).

Finally, even though most of studies published regarding factors associated with birth weight have approached LBW as the birth outcome, the same factors have been reported to be associated with the occurrence of SGA (31). Gao et al. reported results from a cohort of

1398 infants. Maternal smoking during the third trimester of pregnancy significantly increased the risk of having a SGA newborn (32). Although reports about passive smoking are not consistent, Han et al. lately have reported on a case control study that passive smoking during the second and third trimester of pregnancy was significantly associated with an increased risk of SGA (33). Other maternal practices such as alcohol or drug consumption during pregnancy have been also cited as risk factors for SGA (10, 12)

The role of maternal zinc status on birth weight

Zinc is an essential mineral that is found in almost all the compartments of the human body. It plays an important role in more than 100 different enzymes. Among the most important roles of zinc are: immune function, neurobehavioral changes, growth and development, and maternal health and pregnancy outcome (Figure 1) (34).

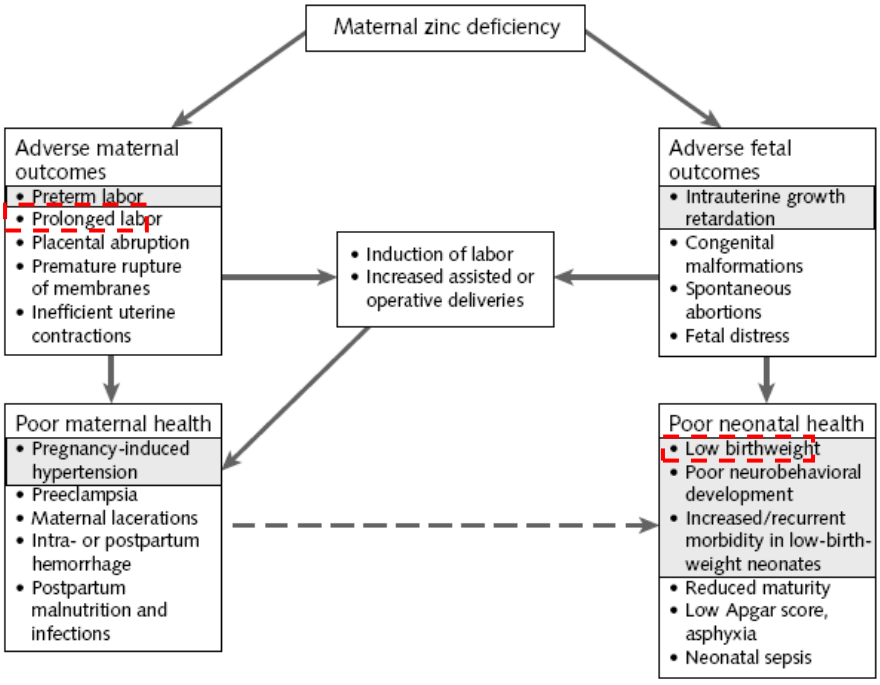

Figure 1. Influence of zinc status on maternal and infant's health

One of the first observations that suggested the association between maternal zinc status and birth outcomes was that poor pregnancy outcomes were found in women with acrodermitis enteropathica (35), a disorder that results in the inability to absorb adequate amounts of zinc from the diet. Although the association between maternal zinc status and birth outcomes is still subject of studies, researchers have reported some evidence of a positive association between maternal zinc status and birth weight (36, 37). Neggers et al. reported an 8-fold higher prevalence of low birth weight associated to mothers with serum zinc concentration in the lowest quartile compared with the highest quartile (38). A similar association, although not conclusive because of the small sample, were reported by De Jong et al. (39). In addition, a study conducted in Tanzania (2005) reported that mothers with low zinc levels were two and half time more at risk of having an infant weighing $\leq$ 2000 g compared to those with normal zinc levels (40). Studies that looked at the maternal zinc intake during pregnancy have also reported an association with LBW. Scholl et al. studied a cohort of 818 pregnant women and found that low zinc intake was associated with increased risk of LBW and when the same group compared women with moderate zinc intake vs. women with low zinc intake, the latter group doubled their risk of having a LBW newborn. (41).

Regarding zinc status in SGA newborns, Krebs et al. studied preterm newborns between 32 and 34 weeks of gestational age and found a smaller exchangeable zinc pool (which is the combined pools of zinc in the body that exchange with zinc in plasma) in SGA comparing with adequate for gestational age (AGA) newborns (42).

In addition, some studies that have looked at the effect of maternal zinc supplementation on birth outcomes, have described an increment on birth weight associated

with the use of zinc supplements by pregnant women. Goldenberg et al. conducted a supplementation trial with women who had a low plasma zinc level during early pregnancy; this group reported a significant difference in birth weight between infants who were born from women who received zinc supplements compared to infants who were born from women who did not; although this difference was significant only in women with a body mass index (weight in kg/square of height in meters) less than 26 kg/m^2 (43). On the other hand there have been some studies that have not shown a positive effect of zinc supplementation on birth outcomes (44, 45).

Regarding contradictory findings on supplementation trials, Pérez-Escamilla and Pollitt (46) proposed that the lack of significant effect on birth weight could be due to the short period of maternal supplementation; these researchers analyzed the study conducted by Villar and Rivera (47) where undernourished mothers from rural Guatemalan villages were divided into three treatment groups and a control group; one group received supplements during the previous two pregnancies and lactation, the other group received supplements during the previous pregnancy and lactation, and the third group that received supplements only during the latest pregnancy. This study showed a statistically significant difference on birth weight between supplemented and control mothers only in the group that received the supplements during two pregnancies and lactation. Therefore, Pérez-Escamilla and Pollit suggested that perhaps undernourished mothers need longer periods of supplementation to replenish their nutritional reserves, before showing an effect on birth outcomes (46). In addition, other studies where no association between zinc supplementation and birth weight was found, reported that one of the reasons for the lack of effect is that mothers who received the supplements could have been also deficient in other nutrients that could limited fetal

growth (48). In fact, the deficiency of more than one micronutrient in pregnant women has been already reported, especially in developing countries. A study conducted in India looked at multiple micronutrient deficiencies among pregnant women. The women were screened for 6 micronutrients; more than 50% of the pregnant women were deficient in at least two micronutrients (zinc and iron), around 25% were deficient in three (zinc, magnesium and iron) and even almost 10% were deficient in four micronutrients (zinc, magnesium, iron and folic acid) (49). Following this publication Jiang et al. reported that approximately 18% of pregnant women of a rural area of Nepal were deficient in at least five micronutrients (3).

Even when, as just showed, several studies have looked at the association between maternal zinc status and birth outcomes, the mechanism whereby zinc deficiency affects birth weight is not known yet. It has been suggested that this association is the result the negative effect of zinc deficiency over metabolic functions causing abnormal synthesis of nucleic acids and proteins, impaired cellular growth, excessive cell death, among others (35). It has been also reported by Simmer et al. that zinc depletion may contribute to intrauterine growth retardation by affecting placental and/or umbilical prostaglandins production (50), but more research is needed to confirm this association.

Consequences of being small for gestational age

Short-term consequences

One of the most important concerns about being born SGA is the higher risk of infant morbidity and mortality that it represents. Martinez et al. reported on a cohort of 12,311 Spanish infants. The mortality rate in SGA newborns was 5.75 times higher than AGA newborns (defined as birth weight between the 10^{th} and 90^{th} percentile for gestational age).

They also found in the same study that the proportion of congenital malformations, hypocalcemia and hypoglycemia was increased in SGA (51). Consistent findings have been reported previously by Gortnel et al., who besides increased mortality risk also reported an increased risk for developing chronic lung disease in SGA newborns (52). Even premature babies have shown different neonatal outcomes according to birth weight. Sharma et al. compared short-term consequences on SGA and AGA premature babies and reported an increased risk of mortality and chronic lung disease in SGA premature babies (53).

Long term consequences

It is been more than 10 years since Baker and colleagues introduced for the first time the term fetal origins of adult disease, after they found an association between deprived living conditions of mothers and an increased risk of stroke in their offspring, suggesting a long term effect of nutrition deprivation during early life (54). Later, Baker reported that the impairment of fetal growth was associated with an increased risk of obesity, coronary heart disease and diabetes in adult life (55). Since then, many epidemiological studies have reported consistent findings (7-9), for which this area of research have received more attention this last decade.

What at the beginning was called "fetal programming" referring to fetal adaptative responses to a detrimental intrauterine environment (54) now after several studies, especially in animals, is called "developmental plasticity", defined as "the phenomenon by which one genotype can give rise to a range of different physiological or morphological states in response to different environmental conditions during development" (56). There are changes in phenotype development by the fetus in order to adapt to an adverse environment which help the fetus to survive. At the same time these changes might have long term consequences

that seem to be more evident when a period of impaired fetal growth is followed by a period of faster neonatal catch-up growth.

The mechanism involved in these long term consequences is not clear yet, but some hypotheses have been postulated, such as the reduced number of cells in some organs as a consequence of undernutrition (55) or the alteration of hormonal systems as a result of the exposure to an adverse intrauterine environment, which implicates changes in hormone secretion or tissue sensitivity (57).

Birth weight and obesity The prevalence of obesity has increased not only in developed countries, but also in developing countries. The increased risk that obesity represents for several chronic diseases has located obesity as focus of many epidemiological studies. Some of these epidemiological studies that looked at the origins and the risk factors associated with obesity have found an association between LBW or SGA and obesity, especially with central obesity (7-9, 56). Consistent findings have been reported by Rassmussen et al. on a cohort of young men where those who were born SGA showed a significant higher fat mass and abdominal fat/total fat mass proportion compared with those who were born with normal birth weight (defined as birth weight between the 50^{th} and 90^{th} percentile for gestational age) (58). It has been observed that these associations between SGA and adiposity are facilitated by a faster catch up weight gain. Ibanez et al. studied body composition change in the first years of life of SGA and AGA and found that even when both groups gained similar weight between 2 – 3 years, children who were SGA gained more abdominal fat and body adiposity and less lean mass (59). In addition, Loos et al. who studied young female and male twins found differences in body composition between the lighter and the heavier twin where the lighter twin showed less lean mass and more subcutaneous fat than the heavier twin (60, 61).

Birth weight and chronic diseases After the findings presented by Barker about the association of birth weight with chronic diseases in adult life, many investigations have been conducted with consistent results. Insulin resistance, an important component of the pathology of diabetes, has been investigated in different populations. Yajnik et al. examined children at 8 years of age and found that children who were born with the lowest birth weight and had a faster catch up, having grown the biggest in the group, presented the highest insulin resistance and central adiposity (62). Soto and Mericq looked at changes occurred in 1 year old infants and found higher levels of fasting insulin in infants who were born SGA and had a faster catch up compared to SGA without catch up growth or AGA infants (63). In 2000 Lawlor studied a cohort of 5,793 subjects who were born between 1950 and 1956 and found an increased risk of diabetes in the subjects who were born with lower weight (64).

A follow up study of 3641 men in Finland on coronary heart disease showed high death rates among the subjects who had LBW and had an average or above average body mass index at 11 years of age (65). Lawlor et al. reported later in a cohort of post menopausal women, an inverse association between birth weight and coronary heart disease (66). It is noteworthy that consistent trends have been reported by the same group and other researchers (67, 68).

Birth weight and neurodevelopment In addition to these physical consequences discussed above, neurodevelopmental consequences have been reported as well. Van Wassenaer reviewed results from different cohort studies and found consistent trend about this association. One study showed that SGA babies more often made late entry at secondary school; another group who examined subjects at 26 years of age found that those who were born SGA less often performed professional work (69). Learning difficulties present at a

higher rate in SGA than in AGA. Similar findings were reported by Chaudari et al. who examined academic performance of 12 year old children born with less than 2000 g and found that it was significantly lower than the performance of same age children who were born with more than 2500 g (70).

Scientific evidence is consistent about these different associations but the understanding of the mechanisms involved in the physiological as well as neurodevelopmental consequences of LBW or SGA are still under investigation.

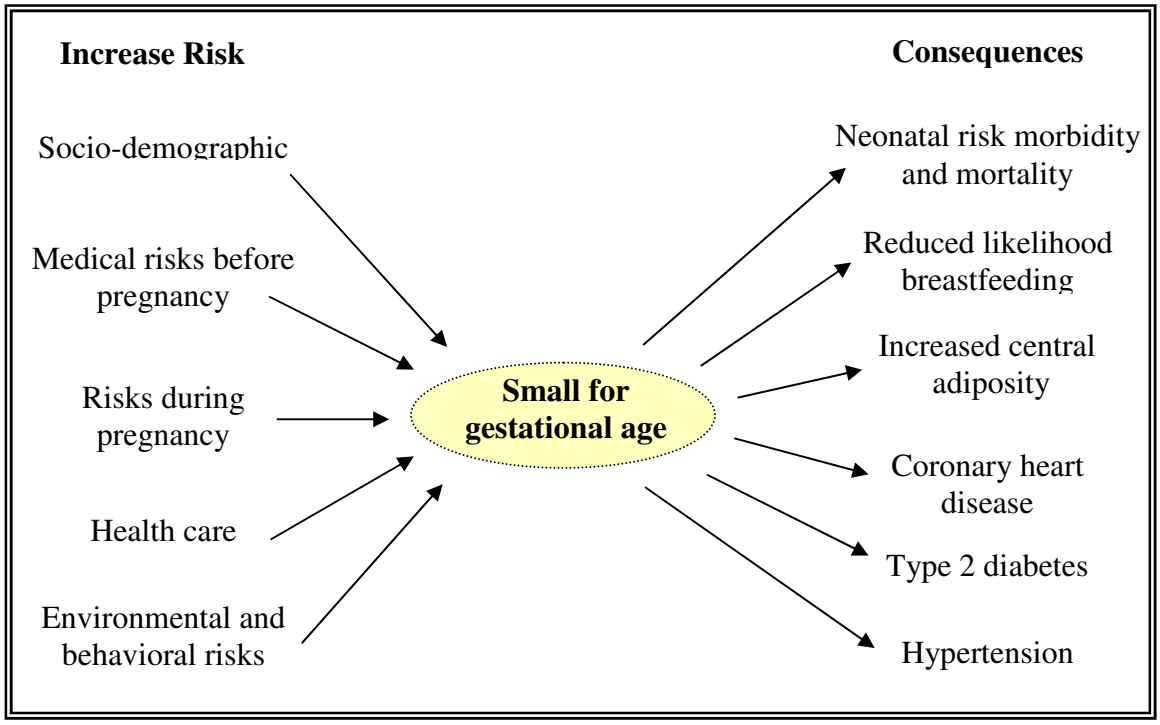

Figure 2. Risk factors associated to small for gestational age

Breastfeeding-pregnancy overlap

Contrary to what is assumed about the practice of breastfeeding during pregnancy, this is not an uncommon practice. Data from the National Health and Nutrition Examination Survey (NHANES) III showed that 5.1% of American lactating women were at the same

time pregnant (5). This practice is even more common in developing countries; Merchant et al. reported on a nutrition-supplementation trial conducted on rural Guatemalan women that 50.2% of the participants with a child < 7 years were breastfeeding during pregnancy (71). In addition, Ramachandran has stated that around 30% of pregnancies in India occur in lactating women (72). While there are some mothers who wean their offspring when they find out about the new pregnancy, there are mothers who continue breastfeeding until giving birth. Marquis et al. on a study of Peruvian pregnant women reported the practice of breastfeeding during the last trimester of pregnancy in 10% of the women with a child less than 4 years old (6). According to Moscone and Monroe the most stated reason given by mothers to continue breastfeeding is the security and comfort of the nursing child (73), therefore it is expected to continue finding the practice of breastfeeding during pregnancy in different populations.

Breastfeeding-pregnancy overlap and birth weight Most of the studies regarding the effects of breastfeeding during pregnancy started in animals, with especial interest from dairy companies on its association with changes in the quality of milk as well as the milk yield. Since the 1950's until now, effects on the milk yield as well as the milk composition has been reported on studies of pregnant cows milked before parturition (74-76); most of these effects have been found when the milking continues through late pregnancy. Among the consequences of this practice Roche in 2003 reported a reduced milk, protein and fat yield during late pregnancy on cows milked during pregnancy compared with their not pregnant twins (76).

At present there are just a few studies on humans that have looked at the practice of breastfeeding during pregnancy. The results of these studies suggest a negative effect on the birth outcomes, pregnant women's health, or newborn's growth. To our knowledge, the first

study that looked at the effect of breastfeeding pregnancy overlap on fetal growth was conducted by Merchant et al. in 1990 where they found a non-significant 57 g decrease in birth weight when they compared babies who were born from mothers who overlapped and mothers who did not (71). A few years later, Siega-Riz and Adair reported on a cohort study of Filipino pregnant women that lactation into the third trimester of pregnancy had a significant negative effect on maternal pregnancy weight gain, estimating that a women with the overlap into this period would gain 1.84 Kg less than a women with an overlap only in the first trimester (77). Regarding mother's weight Ramachandran pointed out, from investigations conducted by the National Institute of Nutrition in India, that mothers who became pregnant while breastfeeding weighed less during all the trimesters of pregnancy, compared to mothers who became pregnant while not breastfeeding (72) .

It is noteworthy that low weight gain, as was mentioned previously, and especially in the third trimester of pregnancy significantly increases the risk of low birth weight (78). Finally, Marquis et al. observed in a study of Peruvian pregnant women that after the first month of life, infants who were born from mothers who breastfed during pregnancy gained 125 g less than infants who were born from mothers who did not, in addition these infants were also 5 times more likely to have a cough for at least 7 days (6). Until now, there is still a gap in the knowledge of possible effects of breastfeeding during pregnancy on the newborn's and mother's health.

Zinc during pregnancy and lactation

Zinc requirements are increased approximately 40-50% for lactating and pregnant women compared with women of the same age (Table 2); The increased requirements in

these two physiological states are due to the zinc needed for synthesis of new tissue, transfer of zinc to the fetus, and the cost of the milk production.

Several studies have looked at the adjustments of zinc homeostasis that occur in pregnant or lactating women in order to face the additional demands of zinc during these physiological stages. The principal adjustment reported is the increment of fractional zinc absorption (FZA). Fung et al. reported significant increased FZA when they compared preconception with the lactation period, although a not significant change was reported during the pregnancy period (79). Similar findings have been shown later by Sian et al. where lactating women had 71% higher FZA during lactation comparing with their preconception period (80). In 2005, Donangelo et al. reported on a longitudinal study of women with marginal zinc intake that FZA increased significantly during pregnancy and lactation (81). Until now there has been no study that has looked at zinc homeostasis in women who breastfeed during pregnancy, however it would be expected that when the additional requirements of zinc due to lactation are added to those for pregnancy, women with poor quality diets, even with increased FZA, will not be able to meet these requirements. Hambidge conducted a study of women on their third trimester of pregnancy; these women showed an improvement in zinc absorption but they did not meet the physiological requirements due to their low zinc intake (82). The estimation that 82% of pregnant women worldwide have inadequate zinc intake may explain why, even when there are physiological adaptations of zinc absorption, high rates of zinc deficiency are reported in pregnant or lactating women. Not only in developing countries a high prevalence of zinc deficiency (>60%) among pregnant women has been reported (3); but also in developed

countries. Data extracted from NHANES III showed that approximately only 52% of lactating women and 59% of pregnant women had an adequate zinc intake (5).

Table 2. Recommended dietary intake of Zinc in pregnant and lactating women.

Age	Women status		
	Non - Pregnant	Pregnant	Lactating
14-18	9 mg	12 mg	13 mg
19 -30y	8 mg	11 mg	12 mg
31 - 50y	8 mg	11 mg	12 mg

Source: Institute of Medicine: "Dietary Reference Intakes 2001" (83)

Pregnancy and childbirth

The experience of pregnancy and childbirth represents a special period in women's lives, but unfortunately sometimes it can become life threatening with the presence of complications, when these complications are not treated adequately. It is estimated that 529,000 women die annually of causes related to complications during pregnancy, labor, childbirth and postpartum period (11). The distribution of the maternal deaths around the world shows that only 1% is represented by developed countries and that of the 20 countries with the highest rate of maternal mortality 19 are in Africa, showing once more the higher risk at what mothers in developing countries are exposed (11). It has been reported that two of the most common causes of maternal death are severe bleeding and sepsis; complications during labor are most of the times associated with these outcomes, but, unfortunately there is not much information about its prevalence worldwide. Prevention of labor complications or early detection with an adequate management could help to reduce maternal mortality, so the

understanding of the factors related to these complications is needed in order to prevent them.

Dystocic labor

Dystocic labor, which indicates difficult labor, is characterized by abnormally slow progress of labor due to any complication or circumstance that interferes with the vaginal delivery (84). Around the late1950's Friedman observed the pattern of labor and divided its progress in two stages:

First stage composed by two phases:

- Latent phase initiates when the mother identifies regular contractions and ends when mother has between 4 and 5 cm of dilatation. A prolonged latent phase was defined by Friedman as greater than 20 hours for nulliparous and 14 hour for multiparous (84). Later WHO defined a prolonged latent phase as cervix not dilated more than 4 cm after 8 hours of regular contractions (85).
- Active phase initiates when the mother experiences a dilatation between 4 - 5 cm and ends when a dilatation of 10 cm is reached. Friedman defined slow dilatation progress as a prolonged active phase when less than 1.2 cm or 1.5 cm of dilatation per hour was showed by nulliparous or multiparous respectively (84). According to WHO and CLAP prolonged active phase is defined as cervical dilatation's curve moves to the alert line of the partograph and crosses it to the right (86, 87).

Second stage It begins when the cervical dilatation is complete and ends with the delivery of the baby. The average time of the second stage is approximately 50 minutes for nulliparous and 20 minutes for multiparous.

Complications that interfere with the normal progression of labor have been categorized as follow:

1. *Abnormalities of the power* includes problems related to uterine contractility and maternal expulsive effort.
2. *Abnormalities involving the passenger* includes problems related to the position or development of the fetus.

   Abnormalities of the passage includes problems with the pelvis (small pelvis for the size of the baby).

Partograph and Prolonged labor

Since Friedman described the sigmoid curve pattern that cervix dilatation follows when graphing against the time (Figure 3), different investigators have developed partographs looking for a standard pattern to evaluate labor progress. In the 1990's CLAP developed a partograph, based on the study of the labor of 1188 women, where a dilatation progress at the 10 percentile represents the alert line; the cervix dilatation would be graphed starting at the active phase according to mother's parity, position and the condition of the membrane (87); In addition, in 1994 a working group organized by WHO, reviewed different partographs and developed a standard one with an alert line that represents the cervix dilatation expected during the active phase, which should not be slower than 1cm/hour and the action line that is placed four hours to the right of the alert line, which represents the time where special actions on the labor management should be considered by the health workers (85). The promotion of the use of the partograph by the health professionals is based on the key role that this tool plays in the early diagnosis of labor complications (85). However, until

now there was not total agreement about the standards that a partograph should follow and the time frame to be considered normal for cervical dilatation (88). Some studies have suggested that the action line of the WHO partograph is not accurate for the prevention of labor complications. Khan and Rizvi reported an increased risk of scar rupture just after 2 hours of crossing the alert line (89), on the other hand Lavander et al. reported no difference between maternal or neonatal outcomes of mothers with labors that were followed with a partograph with a 2 hour action line to the right of the alert line comparing with mothers who were followed with a partograph with a 4 hour action line (90).

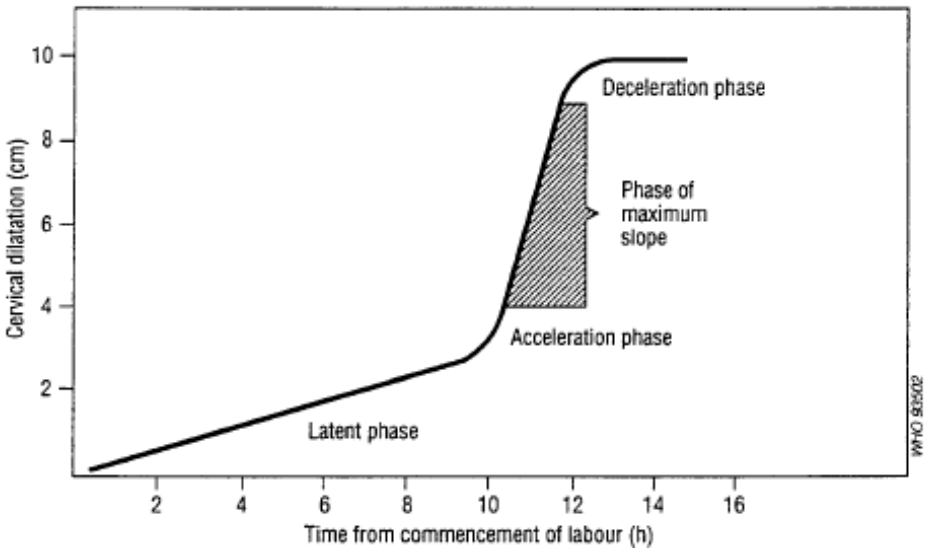

Figure 3. Friedman's curve of labor progress (85)

Nowadays, the problems associated with prolonged labor are not only related with an increased risk of perinatal morbidity or mortality, but also there is a psychological implication as reported by Nysted et al. on a case-control study; mothers that experienced a prolonged labor reported more often the childbirth as a negative experience than normal labor women and in some cases they even said that this experience will mark them for life (91). Perinatal mortality and rates of cesarean section can be reduced when there is an early

detection of labor complications as well as an adequate management of this complication, which can be achieved with an adequate use of the partograph (85). In addition, some studies have suggested that an active management of labor, which involves early amniotomy, 2-hourly vaginal examination and the use of oxytocin for slow progress in labor, could decrease the risk of prolonged labor as well as decrease the rate of cesarean sections (92, 93).

Zinc and labor

The association between zinc status and labor has been studied both in animals and humans. Dura-Trave et al. conducted an experimental study, where one group of rats received a zinc-deficient diet and the other group received a normal diet and they reported that the group placed in the zinc-deficient diet showed a prolonged labor compared to the normal diet group (94). The same group of researchers reported on a study on 336 pregnant women, significant association between plasma zinc concentration and duration of the active period of delivery; women who had an active phase of labor longer than 10 hours showed lower plasmatic zinc levels than women with periods of 10 or less hours, they also found a significant association between plasmatic zinc levels and the final mode of delivery (95). Consistent findings were described by Lazebnik et al. who studied 279 pregnant women and found that lower plasma zinc levels was significantly associated with labor complications such as a labor longer than 20 hours, a second stage of labor longer than 2.5 hours; they also observed a higher rate of premature rupture of membranes among the women with lower alkaline phosphatase levels (96). In addition, Simmer et al. have reported that low zinc content in leucocytes was associated with an alteration in their production of prostaglandins, especially $F_{2\alpha}$ (50). Prostaglandin $F_{2\alpha}$ is a prostaglandin that mediates the induction of an

enzyme important to establishing the estrogen dominance over the uterus, which makes this tissue sensitive to the contractile stimulation, an event that is essential for labor (97). In addition, Sirkoski et al. suggested subnormal zinc content in pregnancy may be a causative factor in premature rupture of membranes at term (98).

Summary

It has been estimated that infants who weight less than 2500 g (definition of LBW) are approximately 20 times more at risk to die than heavier babies. In order to differentiate LBW babies from premature babies, the scientific community has recommended classifying the birth weight according to the gestational age of the newborn, every time that this information is available. The low birth weight for gestational age, better known as small for gestational age (SGA), is defined as weight less than the 10^{th} percentile of the reference population.

Among the main factors reported to represent a risk factor for SGA are:

- Sociodemographic factors such as maternal age, maternal education, marital status.
- Obstetrical history such as parity and interpregnancy interval.
- Current pregnancy factors such as maternal nutritional status, low pregnancy weight gain.

The interest in this birth outcome is not only related to the immediate risk that it represents for infants' health, but also on its long term consequences that have been associated as result of a phenomenon called "fetal programming" or " developmental plasticity". SGA has been reported to increase the risk of chronic diseases later in life, such

as diabetes, coronary heart disease and increased central adiposity. It is noteworthy that neurodevelopment consequences has been associated to poor birth outcomes as well.

Since maternal nutritional status has been reported to play a key role on birth outcomes, additional physiological conditions that could affect maternal nutritional status would be expected to also represent a risk for birth outcomes. A practice that can affect maternal physiological status and is not as uncommon as it is thought is the breastfeeding during pregnancy. This overlap could represent a risk for maternal nutritional status, since it increases requirements, making even more difficult for the pregnant women to meet their nutritional needs. Although just a couple of human studies have looked at this practice, a negative effect on maternal weight gain during pregnancy as well as on infant weight gain after the first month of life have been reported. To our knowledge there is still a gap on information regarding the association of this practice and birth outcomes.

A nutrient that has been associated to birth weight due to its important role in growth and development is zinc. In addition, this mineral has been also associated with the progress of labor; researchers have reported significant differences on the duration of the active phase of labor associated to plasmatic zinc levels.

The progress of labor is very important since complications during this process represent a risk not only for the fetus, but also for the mother's life. It has been reported that two of the most common causes of maternal death are severe bleeding and sepsis, which are complications that mainly take place during labor. Perinatal mortality and rates of cesarean section can be reduced when there is an early detection of labor complications as well as an adequate management of this complication; these can be achieved with an adequate use of

the partograph. A partograph is a graphic representation that allows a close monitoring of the progress of mothers' cervix dilatation during labor.

It is important the prevention of risk factors associated to childbirth complications or poor birth outcomes in order to reduce detrimental consequences that can threat infant as well maternal health.

CHAPTER 3: METHODS

Justification of the Study

The practice of breastfeeding during pregnancy has been reported to occur both in developing or developed countries, but until now no evidence based recommendation has been formulated by health professionals about this practice due to the lack of information available. There have been some suggestions of possible negative effects of this practice on birth weight as well as pregnancy weight gain and infant health during the first month of life, yet conclusive evidence is not yet available. Therefore, it is important to determine the effects of this practice, in order to develop adequate recommendations for the community.

Study aim

1. Determine the association of lactation-pregnancy overlap and the occurrence of low birth weight for gestational age births.

   Hypotheses 1a. An overlap increases the risk of low birth weight for gestational age births.

2. Determine the association of a lactation-pregnancy overlap on the occurrence of prolonged active phase of labor.

   Hypotheses 2a. An overlap increases the risk of prolonged active phase of labor.

3. Determine whether maternal zinc status is associated with a lactation-pregnancy overlap.

   Hypotheses 3a. An overlap of lactation and pregnancy is associated with decreased maternal zinc status.

4. Determine whether maternal zinc status is associated with the occurrence of low birth weight for gestational age and prolonged active phase of labor.

   Hypotheses 4a. The risk of prolonged active phase of labor is increased with decreased maternal zinc status.

   Hypotheses 4b. The risk of low birth weight for gestational age is increased with decreased maternal zinc status.

Study site

Peru is the third largest country in South America (Figure 1). The current population of Peru is 27,219, 264 (99). According to its physiographic characteristics Peru is divided in 3 major regions: the coastal region, the highlands and the rainforest. The study was conducted in the City of Lima, capital of Peru. Lima is located on the coastal region with a total of 6, 954, 583 of inhabitants (99).

The study was conducted in coordination with the Instituto de Investigación Nutricional, a Peruvian non-governmental organization with more than 30 years of research experience in the field of nutrition.

Figure 1. Map of Peru with its physiographic regions and its location in South America

Hospitals The three hospitals where the study was conducted are among the hospitals with the highest rates of attended births in Lima. The three hospitals were located in 3 of the 43 districts in Lima (Figure 2):

- Maria Auxiliadora Hospital, with a total of 7,453 births on 2006; it is located in the district of San Juan de Miraflores (# 29 on Figure 2)
- San Bartolome Mother-Child National Teaching Hospital, with a total of 7,637 births on 2006; it is located in the district of Cercado de Lima (# 1 on Figure 2)
- Hipolito Unanue Hospital, with a total of 9,378 births on 2006; it is located in the district of El Agustino (# 10 on Figure 2)

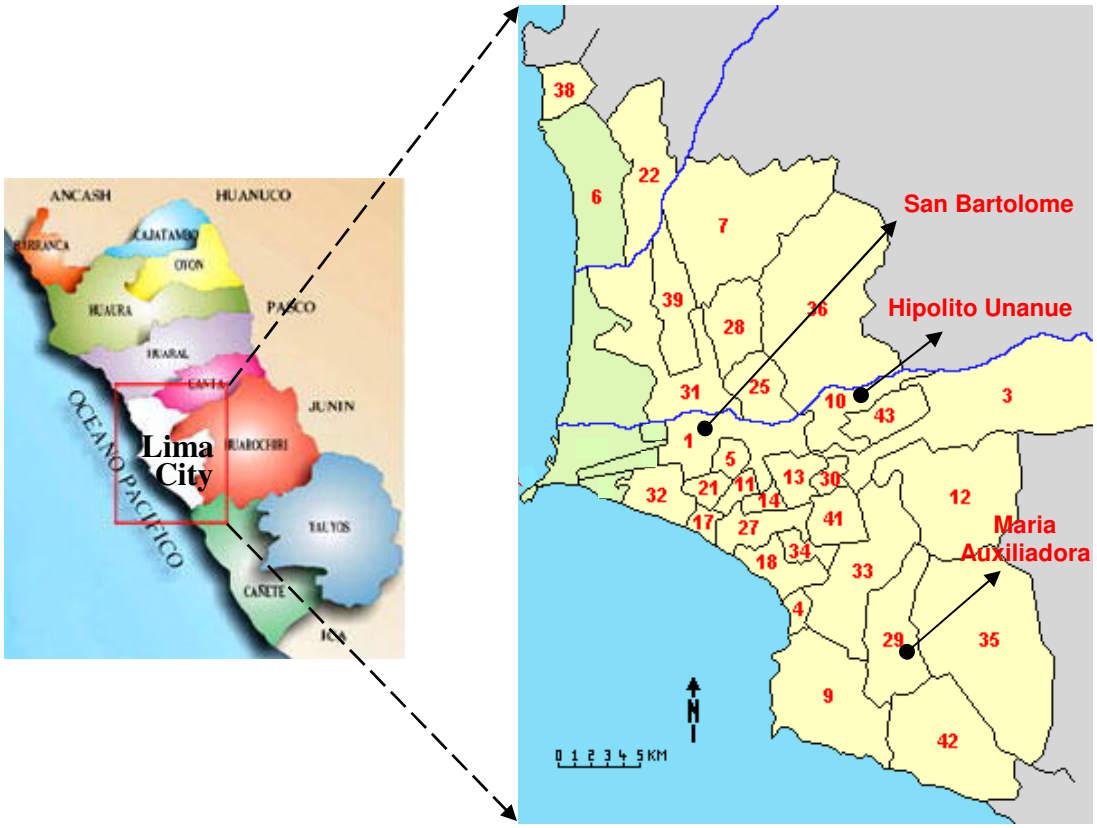

Figure 2. Location of hospitals in Lima city

Study design

This was a case-control study designed to have two matched controls for each case. In addition we had a nested case-control in this study designed to have one control matched to one case for the samples collected for the analysis of zinc status.

Ethical approval

The study was reviewed and received the ethical approval from five institutions: the Institutional Review Board of Iowa State University and the ethical committees of The Instituto de Investigación Nutricional and the three hospitals where the study was conducted.

Study population

The participants were mothers ≥ 18 years old who gave birth at any of the three hospitals and met the inclusion criteria for the study:

1) Mother agreed to participate.

2) Labor started spontaneously.

3) Newborn is a single, live birth.

4) Newborn has a sibling <4 y living with the family and who was breastfed at birth.

Sample size

The calculations for the sample size (Table 1) were made considering two controls per case, a two-tailed test, significance level of 0.05 and power of 80%. Considering the probability of lactation-pregnancy overlap among the cases and the controls for each study, the odds ratios used for the calculation were from Marquis' preliminary data that documented the incidence of LBW and dystocic labor.

Table 1. Estimations of sample size for aims 1 and 2

Outcome	Odds ratio for overlap	Probability of overlap		Sample size	
		Cases	Controls	Cases	Controls
Small for gestational age	1.6	14.6%	9.67%	367	734
Prolonged labor	1.9	13.0%	3.9%	170	340

The calculations for the sample size (Table 2) were based on zinc values reported from previous studies and Marquis' preliminary data.

Table 2. Estimations of sample size for aims 3 and 4

Outcome	Published zinc values	Mean group difference	Sample per group
Zinc status[1]	Overlap: 2.89 +/- 0.21 mg/L No overlap: 3.13 +/- 0.35 mg/L	8%	23
Small for gestational age[2]	LBW: 62.34 +/- 33.0 µg/dL Normal: 89.5 +/- 51.4 µg/dL	30%	40
Prolonged labor[3]	Dystocic: 65 +/- 21 µg/dL Normal: 83 +/- 17.0 µg/dL	22%	21

[1]Marquis unpublished results; [2]Bogden et al. 1978; [3]Durá-Trave 1984.

Definitions of cases and controls

The cases were mothers who delivered low birth weight for gestational age babies or mothers with prolonged active phase of labor.

Low birth weight for gestational age (SGA) A case was defined using the same criteria used by the Latin-American Center of Perinatology and Human Development (CLAP) which defines SGA as birth weight less than the 10 percentile for the gestational age of the newborn.

The controls of SGA cases were defined as newborns with a birth weight over the 50 percentile and under the 90 percentile according to their gestational age.

The reference used to classify the cases and controls was the table of weight for gestational age elaborated by CLAP (appendix D). In order to explore the interactions of breastfeeding – pregnancy overlap during the third trimester of pregnancy, where the highest amount of

weight is gained by the fetus, newborns with a gestational age < 33 weeks at birth were not included, as well as post mature newborns (gestational age > 41 weeks).

Besides the inclusion criteria mentioned previously, the controls were matched to the cases according to hospital of delivery, gestational age and interpregnancy interval. The interpregnancy interval was divided in 3 groups:

- 9 - 21 months
- 22 – 34 months
- 35 – 47 months

Prolonged active phase of labor (PAPL) A case was defined as mother with a cervical dilatation curve that crossed the alert line to the right on the partograph. The partograph used by the three hospitals to control mother's labor was the one developed by CLAP (appendix E).

The controls of PAPL were defined as mothers with cervical dilatation curve that did not cross the alert line on the partograph, without receiving any medication to improve the progress of dilatation.

In addition to the same inclusion criteria, controls were matched to cases according to the initial examination of cervical dilatation; the initial report of cervical dilatation of the control had to be at least at the same initial dilatation reported on the case (i.e. if the initial point on the curve of the case was at 5 cm of dilatation, the control had to have the initial point also at not higher than 5 cm of dilatation). The controls were matched also according to the hospital of delivery and interpregnancy intervals, having the same grouping like the study of SGA.

Lactation-pregnancy overlap To compare the zinc status between mothers who overlap and did not overlap, a case of lactation-pregnancy overlap was defined as mother who continued breastfeeding her child < than 4 years, during the third trimester (from 7^{th} to 9^{th} month) of pregnancy. A control was defined as mother who did not breastfeed her child < than 4 years while pregnant. Here, cases were also matched to controls according to hospital of delivery and interpregnancy intervals.

Data collection

Each hospital was visited every day by a trained health professional who screened all the births that occurred after the last visit. Each birth screened according to the inclusion criteria was reported on an enrollment form. Once a possible case or control for the SGA or PAPL study was identified, the mother was approached, at least 6 hours after delivery, by the health professional who invited her to participate in the study. In order to be able to match the controls to their respective cases, the health professional identified the case first.

After the mother agreed to participate and the informed consent form (appendix C) was signed, the health professional interviewed the mother using the tools (appendix B) developed for collecting information regarding:

- Sociodemographic.
- Breastfeeding-lactation overlap.
- Food frequency intake during pregnancy.

After the interview, the health professional reviewed the mother's and newborn's clinical chart to extract information regarding:

- Newborn (e.g., weight, length, gestational age, Apgar scores)

- Labor, for the study of PAPL (e.g., vital functions, cervical dilatation vs. time, use of oxytocin, state of membranes, obstetrical procedures)
- Obstetrical records (e.g., # of deliveries, type of previous deliveries, weight gained during pregnancy, hemoglobin).

The gestational age used, as first option, was the one calculated with the last date of menstruation (when mother was sure of this date) which was confirmed with postnatal age calculated with a clinical-neurological examination of the newborn (Capurro's method), looking for a consistent calculated age (difference $\leq$ than 2 weeks between the two methods). Additionally, among the cases and controls enrolled for the studies of SGA and PAPL, the health professionals were also identifying the cases and controls of lactation-pregnancy overlap. Once a mother was identified as a possible participant for the comparison of zinc status (aim 3 and 4), she was also invited to participate in that part of the study, in addition to the interview.

Zinc status Since mothers were enrolled and interviewed at the hospitals some hours after delivery, the sample collected for the zinc analysis was hair, because blood was not going to be a reliable assessment due to the mothers' physiological changes after delivery.

Hair samples were collected from the occipital portion of the head and as close as possible to the scalp with stainless steel scissors using a stainless steel scissors. For the analysis, the proximal 2.0 cm (cut in a < 1cm length pieces) were retained in a small polyethylene bottle that was previously washed with a special detergent and EDTA to ensure its zinc-free condition. Hair samples were taken, at room temperature, to the laboratory at the IIN for the analysis by Atomic Absorption Spectrophotometry (100).

Cross Sectional study

Since we were using the hair samples to compare the zinc status between the cases and controls during late pregnancy, we planned to explore if there was an association between zinc status determined in blood taken at the 7th month of pregnancy and zinc status determined in hair samples taken during the 48 hours after delivery.

Study site The study was conducted in Canto Grande, located in San Juan de Lurigancho, district of Lima, where the IIN has been carrying out field research for more than 20 years and currently is conducting other studies.

Study population The participants were pregnant women $\geq$ 18 y in the 7th month of pregnancy, who agreed to participate and who were having a single pregnancy.

Data collection Mothers in their 7th month of pregnancy were identified and visited at home by field workers of the IIN. After mothers signed their informed consent form field workers set an appointment with each one to visit the IIN's office located in Canto Grande where blood samples were going to be taken by a health professional. Overnight fasting blood samples were taken by venipuncture using stainless steel needles and trace-element free evacuated tubes (LH-Metall-Analytik: SARSTEDT Monovette). After the blood sample was taken it was left to settle for 30 minutes after what it was centrifuged at 7000 rpm for 10 minutes. Plasma was separated and stored in 2.5 ml mineral-free vials and stored at -20 ^{o}C.

After delivery the same mothers were asked for hair samples. Hair samples were collected from the occipital portion of the head and as close as possible to the scalp with stainless steel scissors. The hair closest to the scalp was retained for the zinc analysis. The length of the sample represented the hair growth between the date when the blood sample was taken and date of the delivery. This length was calculated considering an average hair

growth of 1 cm per month. Hair samples were collected in the same type of small bottles as the case-control studies.

Plasma analysis The sample was diluted 5 times with water type I. The analysis was conducted against zinc standards prepared in 5% glycerol to approximate the characteristic viscosity of the sample diluted. The samples and the standards were read in the spectrophotometer, using zinc lamps at the wave of 214 nm. Reference material (NIST - 1577b bovine liver certified by the National Institute of Standards and Technology, Gaithersburg, MD) was prepared by the laboratory following the same process used for the plasma samples. This material was used to monitor the precision of the analysis.

Human hair analysis

Each sample (approximately 0.1g of weight) was mixed to insure homogeneity; then it was washed in a 150 ml containing 70 ml of a 1% solution of non-ionic detergent, by agitating on a mechanical mixer for 30 minutes at room temperature. After that, the sample was transferred to a polyethylene filter crucible and rinsed with a total of 500 ml of Type I ultra pure water. Following, the hair was dried overnight at 100°C, weighed and transferred to a 10 ml Erlenmeyer flask. Dry weight had to be about 0.1 g. Nitric acid (3 ml) was added and it was allowed to react at room temperature overnight. The digested product was warmed to 100°C for 3 hr after what 0.5 ml of $HCLO_4$ was added and heated at 200°C until dense white fumes were evolved. The solution had to be water clear. Final volume was made up to 5 ml with Type I ultra pure water.

Analysis by the atomic absorption spectroscopy The standards for Zn were prepared by diluting the stock standard solution with Type I ultra pure water. The standards and blank

solution were prepared using the same acid levels used for samples preparation. In addition, a certified reference hair was obtained from the National Institute for Environmental Studies (certified reference material No 13, Tsukuba, Ibaraki) in order to control analytical accuracy. This certified reference hair followed the same procedure used for the hair samples.

The samples were read in the atomic absorption spectrophotometer with a wave length of 214 nm, using the zinc lamp. Each sample followed a complete reading cycle twice. The zinc results were obtained by linear regression of the standard curve and expressed as µg/g.

CHAPTER 4: AN OVERLPAP OF BREASTFEEDING DURING LATE PREGNANCY DOES NOT INCREASE THE RISK FOR SMALL FOR GESTATIONAL AGE AMONG PERUVIAN WOMEN

A paper to be submitted to Pediatrics

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Abstract

Objective The purpose of this study was to investigate if there was an association between the practice of breastfeeding during late pregnancy (third trimester) and the risk of having a small for gestational age (SGA) infant.

Participants and methods A case-control study was conducted with two matched controls for each case. Between March 2006 and April 2007 three Peruvian hospitals were visited daily. A case of SGA was defined as birth weight bellow the 10th percentile for gestational age; a control was defined as birth weight over the 50th and under the 90th. Mothers who met the inclusion criteria were interviewed and information regarding maternal as well as newborn's characteristics was taken from their clinical charts. Hair samples for zinc analysis were collected on a sub sample of mothers. The association between the overlap of breastfeeding during late pregnancy and the risk of SGA was tested with conditional logistic regression.

Results 78 cases and 150 controls were included in the analysis. An overlap of breastfeeding during late pregnancy was not associated with an increased risk of being SGA; 14 controls

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vs. 3 cases had an overlap; odds ratio = 0.41, 95% CI= 0.11, 1.47). Compared to the controls, mothers in the SGA group were less likely to be married ($p = 0.042$), gained less weight during pregnancy ($p = 0.004$), were more likely to have a female child ($p = 0.001$), and had a previous child born with lower weight ($p < 0.000$). There was no group difference in maternal hair zinc concentration (cases = 168.43 $\pm$ 30.41 µg/g vs. controls = 163.90 $\pm$ 30.41 µg/g; $p = 0.568$).

Conclusions Our results do not support the hypothesis that breastfeeding during pregnancy increases the risk for SGA. Health professionals should be cautious about recommending an overlap in late pregnancy due to other possible negative consequences of this practice that have been previously reported.

Key words: breastfeeding, pregnancy, small for gestational age, maternal weight gain
Abbreviations: SGA- Small for gestational age, CLAP - Centro Latinoamericano de Perinatología y Desarrollo Humano.

Introduction

Low birth weight for gestational age, commonly referred to as small for gestational age (SGA), not only represents a significant risk factor for infant morbidity and mortality (1, 2) but also it has been associated with long-term consequences such as central obesity (3, 4) higher levels of fasting insulin (5) and coronary heart disease (6). Birth weight is an important pregnancy outcome that reflects the intrauterine environment. This environment can be influenced by poor maternal nutritional status, representing a risk factor for SGA (1, 7, 8).

In many developed and developing countries pregnant women regularly do not meet their nutritional requirements. Caufield et al. estimated that around 82% of pregnant women

worldwide do not have an adequate intake of zinc (9). Jieng et al. reported on a study of 1165 Nepali pregnant women that 40% were iron deficient and 20% were deficient in more than one nutrient (10). Requirements become very high for many nutrients during pregnancy, which are difficult to meet with dietary sources. Consequently, it is not surprising to see that the rates of nutritional deficiency are high in this group.

Besides poor dietary intake, maternal nutritional status can be affected by other factors, including additional physiological stresses that may increase nutritional requirements and place some women at even higher risk of nutritional deficiencies during pregnancy. One example of such a physiological stress is the practice of breastfeeding during pregnancy, a behavior that in many areas of the world is not an uncommon practice. Data from the National Health and Nutrition Examination Survey III showed that 5.1% of lactating U.S. women was pregnant (11). Merchant et al. reported on a nutrition-supplementation trial conducted among Guatemalan women. Among these rural women, 50.2% of the participants who had a child < 7 years were breastfeeding during their current pregnancy (12). In addition, Ramachandran found that around 30% of pregnancies in India occurred in lactating women (13). Recently, Marquis et al. reported the practice of breastfeeding during the last trimester of pregnancy in 10% of the Peruvian women who had a child under 4 years of age (14).

We hypothesized that the practice of breastfeeding until late pregnancy (the last trimester of pregnancy) would increase the risk of having a SGA infant and that zinc status among mothers with SGA infants would be lower than zinc status of mothers with normal birth weight infants.

Participants and methods

Study site

The study was conducted at three government hospitals in Lima, Peru: Maria Auxiliadora Hospital (southern Lima), San Bartolome Mother-Child National Teaching Hospital (north-central Lima), and Hipolito Unanue Hospital (east-central Lima). The hospitals were chosen because each had a high rate of attended births and an overlap of breastfeeding during late pregnancy was common among prenatal clinic clients.

Study design

This was a case control study designed to have two matched controls for each case. In addition, we incorporated a nested case-control with one control matched to one case for the analysis of maternal zinc status.

Ethical approval

The study was reviewed and received the ethical approval from the Institutional Review Board of Iowa State University and the ethic committees of the Instituto de Investigación Nutricional (IIN) and the three collaborating hospitals.

Participants

The participants were mothers at least 18 years of age who gave birth at any of the three hospitals and met the inclusion criteria for the study:

1) labor started spontaneously;

2) newborn was a single, live birth; and

3) newborn has a sibling <4 y living with the family and who was breastfed at birth.

Definition of cases and controls A case was a SGA newborn defined as having a birth weight less than the 10^{th} percentile for gestational age, using the criteria of the Latin-

American Center of Perinatology and Human Development (CLAP) (15). A control was defined as newborn with a birth weight over the 50th percentile and under the 90th percentile for gestational age. In addition, controls were matched to the cases according to hospital of delivery, gestational age and interpregnancy interval (time between delivery of last child and birth date of newborn). The interpregnancy interval was categorized into 3 levels: 9 - 21 months, 22 – 34 months, 35 – 47 months.

To examine the effect of breastfeeding pregnancy overlap during late pregnancy when the greatest amount of weight is gained by the fetus, newborns with a gestational age < 33 weeks at birth were not included. Post-mature newborns (gestational age > 41 weeks) were also excluded.

Data collection

Each hospital was visited every day by a trained health professional who screened all the births that occurred since the last visit. To be able to match the controls to their respective cases, the health professional identified a case first. Mothers were approached for enrollment at least 6 hours after delivery. Participants were interviewed about their sociodemographic characteristics, breastfeeding-pregnancy overlap practice and food frequency intake during pregnancy. After the interview, the health professional reviewed the mother's and newborn's clinical chart to extract information regarding maternal (e.g., prenatal control, obstetrical records, hemoglobin) and newborn (e.g., birth weight, length, Apgar scores) characteristics. Birth weight was measured using a digital calibrated scale with a capacity of 22 kg and a precision of 5 g that was provided to each hospital. Gestational age was calculated using the last date of menstrual period. In cases where the mother was not

sure about this date we compared this gestational age to the one calculated with Capurro's method (16); if the difference between the two methods was greater than two weeks, the mother was not considered for enrollment.

A subsample of mothers provided hair samples for zinc analysis. Hair samples were collected from the occipital portion of the head and as close as possible to the scalp using stainless steel scissors. For the analysis, the proximal 2.0 cm (cut in < 1 cm length pieces) were retained in a small polyethylene bottle. Hair samples were taken, at room temperature, to the laboratory for the analysis by the atomic absorption spectrophotometer (17). A certified reference hair was used from the National Institute for Environmental Studies (certified reference material No 13, Tsukuba, Ibaraki) to assure quality control.

Data analysis

Comparisons between cases and controls for continuous variables were made using analysis of variance, with the matched case-controls treated as blocks. Comparisons between case and controls for categorical variables were made using Pearson χ^2. A conditional logistical regression was used to explore the risk of breastfeeding during late pregnancy on the occurrence of SGA. This is a model used for matched case-control designs, where a relationship between the binary outcome of interest (SGA) and a binary predictive factor (breastfeeding during late pregnancy) was tested. This is a model used for matched case-control designs, where a relationship between the binary outcome of interest (PAPL) and a binary predictive factor (breastfeeding during late pregnancy) was tested. The analyses were conducted using SYSTAT version 9.0 (SPSS, Chicago, IL). Statistical significance was set at $p < 0.05$.

Results

Study participants

Between March 2006 and April 2007 a total of 21,534 deliveries were screened (**Figure 1**). The general inclusion criteria was met by 2587 mothers; of the 1250 women who met the criteria for cases or controls, 261 mothers were invited to participate. Fourteen women refused. Most of the women who met the inclusion criteria but did not participate in the study were women who were not randomly selected as controls. A total of 95 cases and 150 controls were enrolled. Of the 95 cases of SGA, 17 were excluded from the analysis due to the lack of any matched control. A total of 78 cases and 150 controls were included in the analysis.

A subsample of 74 women provided hair samples for zinc analysis. Fourteen cases were excluded due to the lack of a matched control, thus the maternal hair zinc concentration of 30 cases and 30 controls were analyzed.

Maternal sociodemographic characteristics

There were few differences in the sociodemographic characteristics between SGA and non-SGA mothers (**Table 1**). Mothers of SGA newborns were about a year and a half younger ($p = 0.046$). They were also less likely to be married and about twice as the proportion of women were single women as compared to the control group ($p = 0.042$). No differences were found in maternal housing characteristics (**Table 2**).

Maternal practice of breastfeeding during late pregnancy

A total of 42.5% of mothers reported that they breastfed during the current pregnancy; 18.9% of the women continued breastfeeding during the second trimester and 7.5% still continued during the third trimester. Fourteen non- SGA mothers overlapped

breastfeeding during late pregnancy vs. only 3 SGA mothers (**Table 3**); the average frequency of breastfeeding episodes per day was similar between cases and controls (2.7 ± 2.1 vs. 2.9 ± 1.8) An odds ratio of 0.41 was found for the risk of breastfeeding during late pregnancy for the occurrence of SGA.

In addition, many women reported receiving recommendations regarding breastfeeding during pregnancy; a significantly higher proportion of mothers from the control group received recommendations from relatives compared to case mothers (**Table 4**).

Newborn's characteristics

The average gestational age in the cases as well as in the controls was 38.7 ± 0.2 weeks. Besides the expected difference in birth weight between cases and controls, a significant difference was observed in the newborn's length, where controls were approximately 3.5 cm longer than cases ($p < 0.000$) (**Table 5)**. A significant difference was also found in the distribution of females to males; around 67% of the cases were female vs. 44% in the control group.

Maternal obstetrics and health characteristics

There were no differences between cases and controls regarding maternal health behaviors during the current pregnancy, such as use of medicines, smoking, or supplement use (**Table 6**).

There were significant group differences in anthropometric measurements of the mother and her previous child. The birth weight of the last child in the control group was around 500 g higher than among the cases ($p < 0.000$) (**Table 7**). A significant difference in maternal weight gained during the current pregnancy was also observed, with a higher weight

gain in the control group *(p* = 0.004). Mothers in the control group were also taller than mothers in the case group (*p* = 0.007).

Maternal food frequency intake

Although a wide range on monthly food frequency intake was observed on most food, mothers from the control group consumed more meat, such as beef or pork *(p* = 0.030) and chicken liver (*p* = 0.046), approximately two times more per month (data not shown).

Maternal zinc status

Maternal hair zinc did not differ between women who gave birth to a SGA baby and women with a normal birth weight baby (cases = 168.43 $\pm$ 30.41 µg/g vs. controls = 163.90 $\pm$ 30.41 µg/g; *p* = 0.568).

The zinc concentration of the certified reference material, used to control the quality of the analysis, was 172 $\pm$ 11 µg/g (National Institute for Environmental Studies) and the mean value obtained in our laboratory was 170.0 $\pm$ 3.2 µg/g.

Discussion

To our knowledge this is the first study that has looked at the association between the practice of breastfeeding during late pregnancy and SGA. Contrary to what we hypothesized, our results showed that this practice did not increase the risk of SGA. The proportion of mothers in the case group who reported to breastfeed during late pregnancy was very low compared to the average found in the present study and that reported previously among Peruvian women (14). Since mothers were interviewed after delivery, they were aware of their birth outcomes which could have influenced maternal response regarding breastfeeding during pregnancy. This feeding behavior is discouraged in the Peruvian culture and this may

have lead to a biased reporting, with higher denial among mothers delivering an SGA baby. The only other study that examined the association between birth weight and a breastfeeding-pregnancy overlap (at any trimester) reported a non-significant +57g difference in birth weight in infants who were born to mothers who had not overlap compared to those with an overlap (12). In addition, Moscone et al. studied this practice in 57 women who breastfed during pregnancy, with 43% overlapping until delivery and reported that the average birth weight in this group was normal (3.43 Kg) (18).

These previously published studies together with the present results showing a more than two-fold greater proportion of mothers who breastfed during late pregnancy among the controls compared to the cases, suggest that there are maternal physiological adaptations that enable adequate fetal growth (19). More research is needed to understand these physiological adaptations and their effect on maternal body stores.

We found differences between SGA cases and their controls on some important maternal characteristics, including maternal height, weight gain during pregnancy and previous children's birth weights. These results are consistent with reported risk factors associated with low birth weight (20-23). Analysis of interactions involving factors that may modify the effect of a breastfeeding-pregnancy overlap on the risk of SGA was not feasible due to the limited sample size.

We did not find a significant difference in maternal hair zinc concentrations between SGA cases and controls. This finding is in contrast to previous studies (24-26), that used other indicators to evaluate maternal zinc status (including serum, plasma and leucocytes). The inconsistent result with other studies could be due to 1) the use of a different biological indicator; 2) the small sample size and wide range of zinc hair concentrations that limited our

ability to capture differences between the SGA cases and controls; and 3) changes in zinc homeostasis such as increase on fractional zinc absorption and intestinal conservation, which occur in lactating as well as pregnant women (27-29).

Conclusion

In conclusion, in our population an overlap of breastfeeding during late pregnancy did not represent a risk for SGA. However, health professionals should be cautious about recommending this practice as negative postnatal consequences have been associated with this practice. More longitudinal research is recommended in this area, to understand the maternal physiological adaptations behind the practice of breastfeeding during pregnancy and to further explore its short as well as long-term postnatal consequences.

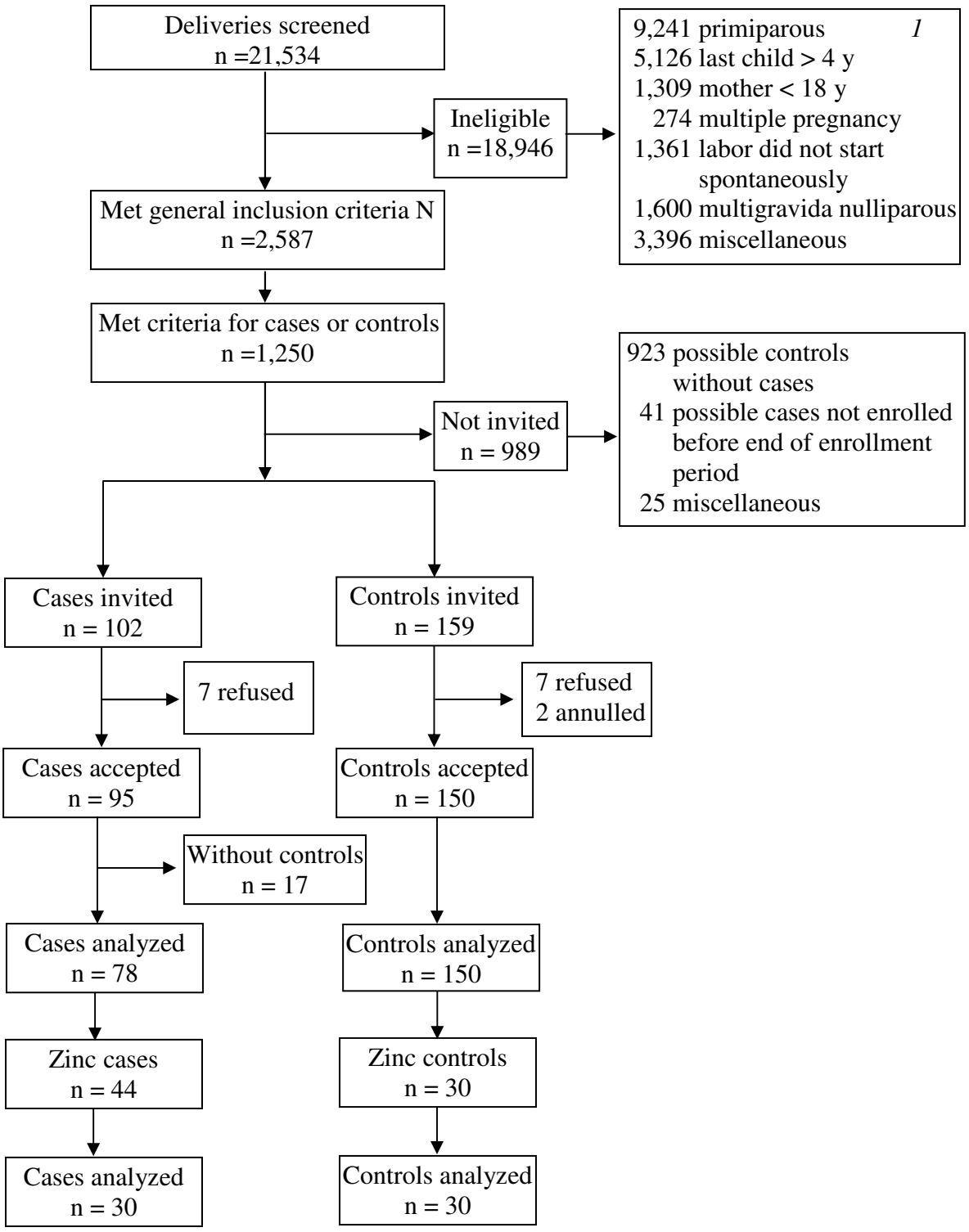

Figure 1. Flow of participants enrollment showing detailed numbers of subjects who met and did not met the general as well as the specific case-control criteria. [1] Some mothers did not meet more than one general criteria, therefore the sum is > 18946.

Table 1. Maternal sociodemographic characteristics by presence of SGA

	SGA n = 78 n (%)	Non-SGA n = 150 n (%)	$p^{\dagger}$
Age, y *	26.2 ± 5.9	27.8 ± 6.0	0.046
Place of birth			0.264
Lima City	35 (44.9)	81 (54.0)	
Lima (mountain)	14 (5.1)	2 (1.3)	
Coast	8 (10.3)	16 (10.7)	
Mountain	23 (29.5)	43 (28.7)	
Rainforest	7 (8.9)	7 (4.7)	
Other	1 (1.3)	0 (0.0)	
Unknown	0 (0.0)	1 (0.6)	
Time living in Lima, y *	17.7 ± 12.1	19.8 ± 12.2	0.214
Educational status			0.246
Not completed elementary school	5 (6.4)	10 (6.7)	
Completed elementary school	6 (7.7)	10 (6.7)	
Not completed high school	23 (29.5)	37 (24.7)	
Completed high school	30 (38.5)	68 (45.3)	
Not completed technical college	9 (11.5)	5 (3.3)	
Completed technical college	3 (3.8)	14 (9.3)	
Not completed college	1 (1.3)	3 (2.0)	
Completed college	1 (1.3)	3 (2.0)	
Marital Status			0.042
Married	10 (12.8)	40 (26.7)	
Live together	58 (74.4)	98 (65.3)	
Single	9 (11.5)	8 (5.3)	
Divorced	0 (0.0)	0 (0.0)	
Separated	1 (1.3)	4 (2.7)	
Working status			0.497
Yes	13 (16.7)	20 (13.3)	
No	65 (83.3)	130 (86.7)	

* mean ± SD

† Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
SGA: small for gestational age, < 10th percentile birth weight
Non-SGA: 50th percentile > birth weight < 90th

Table 2. Household characteristics of SGA cases and their controls

	SGA n = 78 n (%)		Non-SGA n = 150 n (%)		*p**
Housing					0.358
Own	7	(9.0)	25	(16.7)	
Rented	25	(30.1)	39	(26.0)	
Relative's	44	(56.4)	80	(53.3)	
Other	2	(2.5)	6	(4.0)	
Housing main material					0.580
Bricks and cement	52	(66.7)	107	(71.4)	
Bricks	0	(0.0)	2	(1.3)	
Wood	14	(17.9)	27	(18.0)	
Straw mats	4	(5.1)	5	(3.3)	
Others	8	(10.3)	9	(6.0)	
Housing source of light					0.552
House connection	62	(79.5)	128	(85.3)	
Neighbors	14	(17.9)	18	(12.0)	
Lamp	0	(0.0)	1	(0.7)	
Candles	2	(2.6)	3	(2.0)	
Housing source of water					0.791
Potable water	62	(79.5)	115	(76.7)	
Public sink	3	(3.9)	3	(2.0)	
Tanker	10	(12.8)	23	(15.3)	
Neighbors	3	(3.8)	8	(5.3)	
Well	0	(0.0)	1	(0.7)	
Housing fuel used for cooking					0.213
Gas	60	(76.9)	125	(83.4)	
Kerosene	8	(10.3)	18	(12.0)	
Electricity	1	(1.3)	0	(0.0)	
Wood	6	(7.7)	5	(3.3)	
Others	3	(3.8)	2	(1.3)	
Place used as a bathroom					0.791
Toilet	59	(75.6)	118	(78.7)	
Outhouse with drain	2	(2.6)	4	(2.7)	
Outhouse without drain	12	(15.4)	23	(15.3)	
Open land	3	(3.8)	4	(2.7)	
Others	2	(2.6)	1	(0.6)	

* Pearson X^2 was used for testing significant difference of proportions.
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 3. Maternal practice of breastfeeding during pregnancy by presence of SGA

	SGA n = 78 n (%)	Non-SGA n = 150 n (%)
Breastfeeding during late pregnancy		
Yes	3 (3.8)	14 (9.3)
No	75 (96.2)	136 (90.7)

	Odds Ratio	95% CI*	
		Lower	Upper
Risk of breastfeeding during late pregnancy on the occurrence of SGA	0.41	0.11	1.47

* Conditional logistic regression
SGA: small for gestational age, < 10th percentile birth weight
Non-SGA: 50th percentile > birth weight < 90th

Table 4. Maternal exposure to recommendations regarding breastfeeding during pregnancy by presence of SGA

	SGA n = 78 n (%)	Non-SGA n = 150 n (%)	*p*†
From a health professional			0.468
Yes	13 (16.7)	31 (20.7)	
No	65 (83.3)	119 (79.3)	
From a relative			0.000
Yes	12 (15.4)	61 (40.7)	
No	66 (84.6)	89 (59.3)	
From a friend			
Yes	13 (16.7)	26 (17.3)	0.899
No	65 (83.3)	124 (82.7)	

* mean ± SD
† Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
SGA: small for gestational age, < 10th percentile birth weight
Non-SGA: 50th percentile > birth weight < 90th

Table 5. Newborn characteristics of SGA cases and their controls

	SGA n = 78 n (%)	Non-SGA n = 150 n (%)	*P**
Newborn's sex			0.001
Masculine	26 (33.3)	84 (56.0)	
Feminine	52 (66.7)	66 (44.0)	
Newborn's weight, g	2529.1 ± 173.9	3534.2 ± 175.5	0.000
Newborn's length, cm	46.9 ± 1.4	50.5 ± 1.4	0.000
Newborn's Apgar scores			
1 minute	8.0 ± 1.0	8.1 ± 1.0	0.497
5 minutes	8.9 ± 0.5	9.0 ± 0.5	0.402
Newborn in incubator			0.194
Yes	4 (5.1)	3 (2.0)	
No	74 (94.9)	147 (98.0)	
Newborn pathology			0.168
Yes	5 (6.4)	4 (2.7)	
No	73 (93.6)	146 (97.3)	
Newborn use of oxygen			0.145
Yes	6 (7.7)	5 (3.3)	
No	72 (92.3)	145 (96.7)	

* Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 6. Maternal health behavior during current pregnancy by presence of SGA

	SGA n = 78 n (%)	Non-SGA n = 150 n (%)	*p**
Use of medicines			
Antibiotics			0.524
Yes	25 (32.0)	42 (28.0)	
No	53 (68.0)	102 (72.0)	
Vaginal suppositories			0.200
Yes	22 (28.2)	55 (36.7)	
No	56 (71.8)	95 (63.3)	
Smoking			0.194
Yes	4 (5.1)	3 (2.0)	
No	74 (94.8)	147 (98.0)	
Passive smoking			0.197
Yes	13 (16.7)	16 (10.7)	
No	65 (83.3)	134 (89.3)	
Supplement use			0.461
Yes	66 (84.6)	121 (80.7)	
No	12 (15.4)	29 (19.3)	
Zinc			0.402
Yes	14 (17.9)	34 (22.7)	
No	64 (82.1)	114 (76.0)	
Unknown	0 (0.0)	2 (1.3)	
Iron			0.512
Yes	64 (82.1)	117 (78.0)	
No	14 (17.9)	31 (20.7)	
Unknown	0 (0.0)	2 (1.3)	
Calcium			0.566
Yes	22 (28.2)	39 (26.0)	
No	56 (71.8)	109 (72.7)	
Unknown	0 (0.0)	2 (1.3)	
Folic Acid			0.108
Yes	21 (26.9)	58 (38.7)	
No	57 (73.1)	90 (60.0)	
Unknown	0 (0.0)	2 (1.3)	

* Pearson X^2 was used for testing significant difference of proportions.
SGA: small for gestational age, < 10th percentile birth weight
Non-SGA: 50th percentile > birth weight < 90th

Table 7. Maternal antecedents and health characteristics by presence of SGA

	SGA n = 78 mean ± SD	Non-SGA n = 150 mean ± SD	*P* **
Last child's age, mo	29.3 ± 3.3	29.0 ± 3.3	0.567
Last child's birth weight, g *	2859.7 ± 526.6	3309.3 ± 531.3	0.000
Last child's sex†			0.196
Masculine	45 (57.7)	73 (48.7)	
Feminine	33 (42.3)	77 (51.3)	
Last child's age when weaned, mo ‡	14.3 ± 7.2	16.5 ± 7.3	0.033
Number of pregnancies, #	2.0 ± 1.5	2.4 ± 1.5	0.088
Number of deliveries, #	1.8 ± 1.3	2.0 ± 1.3	0.256
Weight gained during pregnancy, kg §	8.6 ± 5.2	11.7 ± 5.0	0.004
Maternal height, cm ‖	151.8 ± 6.2	154.2 ± 6.2	0.007
Maternal hemoglobin, g/dL ¶	11.0 ± 1.2	11.2 ± 1.2	0.177
Maternal preeclampsia†			0.124
Yes	7 (9.0)	6 (4.0)	
No	71 (91.0)	144 (96.0)	

* cases n = 70 controls n = 139
† n (%) cases
‡cases n = 76 controls n = 139 (2 cases and 11 controls breastfed until delivery)
§ cases = 42 controls = 87
‖ cases n = 77 controls = 146
¶ cases n = 62 controls n = 134; Hb values available were from different trimesters: cases (1^{st} = 0%, 2^{nd} = 27.4%, 3^{rd} = 72.6%) controls (1^{st} = 9%, 2^{nd} = 29.8%, 3^{rd} = 61.2%)
** Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

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CHAPTER 5: THE OVERLAP OF BREASTFEEDING DURING LATE PREGNANCY IS NOT ASSOCIATED WITH PROLONGED ACTIVE PHASE OF LABOR AMONG PERUVIAN WOMEN

A paper to be submitted to The Journal of American Clinical Nutrition

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Abstract

Background The practice of breastfeeding during pregnancy has been reported in developing as well as developed countries. This practice implies a dual physiological stress that may affect maternal nutritional status and pregnancy outcomes.

Objective We hypothesized that the practice of breastfeeding until late pregnancy would be associated with an increased risk of experiencing a prolonged active phase of labor (PAPL) and mothers with PAPL would have a lower zinc status than mothers with normal progression of labor.

Design A case-control study was designed and two matched controls (n = 345) for each case (n = 176) were enrolled. In addition, we had a nested case-control study designed with one control (n = 32) matched to one case (n = 32) for the analysis of maternal hair as an indicator of zinc status.

Results There was no group difference in the proportion of mothers who breastfed during late pregnancy (7.95% of cases and 7.83% of controls); the odds ratio was 1.02 (95% CI= 0.51, 2.04) for breastfeeding during late pregnancy with the occurrence of PAPL. In addition,

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no difference between PAPL cases and the controls was found for maternal hair zinc concentrations (165.94 ± 36.14 µg/g in cases and 155.75 ± 36.14 µg/g in controls; $p = 0.268$).

Conclusion Our findings do not support the hypothesis that the practice of breastfeeding during late pregnancy represents a risk for prolonged active phase of labor. More research on other birth and postnatal outcomes are needed to fully understand any potential risks associated with this practice.

Key words: breastfeeding, pregnancy, prolonged active phase of labor.

Introduction

It is estimated that 529,000 women die annually from causes related to complications during pregnancy, labor, childbirth, and the postpartum period (1). Two of the most common causes of maternal death, hemorrhaging and sepsis, are often associated with complications during labor. Prevention of labor complications or early detection with adequate management will reduce maternal mortality; therefore, understanding the factors that relate to labor complications is a maternal health priority.

The association between maternal nutritional status and labor complications has been reported previously (2). For example, anemia has been linked to prolonged labor (3). Experimental animal studies have reported prolonged labor in rats on a zinc-deficient diet compared to those on a normal diet (4). In addition, observational studies of women have shown associations between plasma zinc levels and the duration of the active period of delivery (5, 6).

Several studies have examined the adjustments of zinc homeostasis that occur in pregnant or lactating women in response to the additional demands of these physiological stages (7-9). However, there is a dearth of information on the physiological implications of breastfeeding during late pregnancy, when a woman would be exposed to a double physiological stress; which is of concern due to the high rates of micronutrient deficiencies as well as deficient nutrient intake frequently reported among pregnant and lactating women in developing countries (10,11) as well as in developed countries, like the United States where only 52% of lactating women and 59% of pregnant women have an adequate zinc intake (12). It is estimated that worldwide 82% of pregnant women have inadequate zinc intakes (13).

While many mothers wean their children when they become pregnant again, other mothers chose to continue breastfeeding for part or all of the pregnancy. This practice has been reported not only in developing countries (14, 15) but also in the United States (12). In Peru, a recent study reported that 10% of the women with a child less than 4 years old breastfed during the last trimester of pregnancy (16). According to Moscone and Monroe the most common reason to continue breastfeeding that was given by mothers was the security and comfort of the nursing child (17).

We hypothesized that the practice of breastfeeding through late pregnancy would increase the risk of having a prolonged active phase of labor (PAPL) and that maternal zinc status among mothers with a PAPL would be lower than maternal zinc status among mothers with a normal active phase of labor.

Subjects and methods

Study site

The study was conducted at three government hospitals in Lima, Peru: Maria Auxiliadora Hospital (southern Lima), San Bartolome Mother-Child National Teaching Hospital (north-central Lima), and Hipolito Unanue Hospital (east-central Lima). The hospitals were chosen because each had a high rate of attended births and an overlap of breastfeeding during late pregnancy was common among prenatal clinic clients.

Study design

This was a case-control study designed to have two matched controls for each case. In addition, we had a nested case-control study designed to have one control matched to one case for the analysis of maternal zinc status.

Ethical approval

The study was reviewed and received the ethical approval from the Institutional Review Board of Iowa State University and the ethical committees from the Instituto de Investigacion Nutricional (IIN) and the three hospitals where the study was conducted.

Subjects

The participants were mothers > 18 years old who gave birth at any of the three hospitals and met the inclusion criteria for the study:

1) labor started spontaneously with cephalic presentation;

2) newborn was a single, live birth;

3) newborn had a sibling <4 y of age, lived with the family, and was breastfed at birth; and

4) mother was without a diagnosis of mechanic dystocia (e.i. small pelvis for birth size).

Definition of cases and controls A case was defined as a mother with a cervical dilatation curve that crossed the alert line to the right on the partograph. The partograph used by the three hospitals to control progression of labor was developed by the Centro Latinoamericano de Perinatología y Desarrollo Humano (CLAP) (Latin-American Center of Perinatology and Human Development) (18).

A control for PAPL was defined as a mother with a cervical dilatation curve that did not cross the alert line on the partograph and did not receiving any drug intervention to improve the progress of dilatation. Controls were matched to cases according to the initial examination of cervical dilatation:, the initial report of cervical dilatation of the control had to be at least at the same initial dilatation reported on the case (e.g., if the initial point on the partograph curve for the case was 5 cm of dilatation, then the control had to have the initial point at not higher than 5 cm of dilatation). In addition, controls were matched by hospital of delivery and interpregnancy intervals (9 - 21 months, 22 – 34 months, and 35 – 47 months).

Data collection

Each hospital was visited by a trained health professional who screened the births daily. To be able to match the controls to their respective cases, the health professional identified the case first. Mothers were approached for enrollment at least 6 hours after delivery. Participants were interviewed about their sociodemographic characteristics, breastfeeding-lactation overlap practice and food frequency intake during pregnancy. After the interview, the health professional reviewed the mother's and newborn's clinical chart to extract information regarding obstetrical records, prenatal control, labor characteristics, newborn's birth weight, length, and Apgar score. A subsample of women provided hair

samples for zinc analysis. The hair samples were collected from the occipital region, as close as possible to the scalp using stainless steel scissors. For the analysis, the proximal 2.0 cm (cut in a < 1cm length pieces) were retained in a small polyethylene bottle. Hair samples were taken, at ambient temperature, to the laboratory for the analysis by atomic absorption spectrophotometry (19). A certified reference material (hair) from the National Institute for Environmental Studies (certified reference material No 13, Tsukuba, Ibaraki) was used in each batch analysis, for quality control.

Data analysis

Comparisons between PAPL cases and controls for continuous variables were made using analysis of variance with the matched case-controls treated as blocks. Comparisons between cases and controls for categorical variables were made using Pearson χ^2. A conditional logistical regression was used to examine the risk of breastfeeding during late pregnancy associated with the occurrence of PAPL. The analyses were conducted using SYSTAT version 9.0 (SPSS, Chicago, IL).

Results

Study participants

Between March 2006 and April 2007 a total of 21,534 deliveries were screened (**Figure 1**), of whom 2,587 mothers met the general inclusion criteria, and 1,175 met the criteria as cases or controls. A total of 550 mothers were invited to participate; 18 women refused (4 cases and 14 controls). Most of the women who met the inclusion criteria but did not participate in the study were women who were not randomly selected as controls. Four mothers who accepted to participate were not included in the study because of inconsistent

information on the clinical chart (n=2) and intervention with oxytocin during labor (n = 2). A total of 183 cases and 345 controls were enrolled.

From the 183 cases of PAPL 7 were excluded from the analysis due to the lack of matched controls. A total of 176 cases and 345 controls were included in the analysis.

A subsample of 65 women provided hair samples for zinc analysis. One cases was excluded due to the lack of a matched control, thus the maternal hair zinc concentration of 32 cases and 32 controls were analyzed.

Maternal sociodemographic characteristics

PAPL cases did not differ from controls in maternal sociodemographic or housing characteristics, with the exception of maternal work (**Table 1**). A higher proportion of PAPL mothers worked until delivery as compared to the cases ($p < 0.05$).

Maternal breastfeeding practices during late pregnancy

A total of 38.2% of mothers reported that they breastfed during the current pregnancy; 16.3% of the women continued breastfeeding during the second trimester and 7.9% still continued during the third trimester. A similar proportion of PAPL and control mothers breastfed during late pregnancy (7.95% and 7.83%, respectively) (**Table 2**); the average frequency of breastfeeding episodes per day was similar between cases and controls (3.4 ± 1.9 vs. 3.0 ± 2.1). An odds ratio of around one was found for the risk of breastfeeding during late pregnancy with the occurrence of PAPL.

There were no differences between cases and controls on maternal exposure to recommendations regarding breastfeeding during pregnancy (**Table 3**).

Maternal health antecedents

No differences were found between cases and controls regarding maternal practices during the current pregnancy such as use of medicines, smoking or supplement use (**Table 4**).

Mothers in both groups showed similar anthropometric as well as obstetric characteristics (**Table 5**). Although not reaching significance in this study, PAPL may be associated with having had a previous cesarean section (27.7 % PAPL cases vs. 20.6 % controls; $p = 0.114$).

Obstetrics procedures during labor

There was no difference in the proportion of mothers who arrived at the hospital with a spontaneous rupture of membranes (**Table 6**), but a difference was found in the proportion of mothers who experienced an artificial rupture during labor. Around 56% of the mothers in the control group remained with intact membranes until the delivery of the baby. Oxytocin was used on 34.7% PAPL mothers. In addition, nine deliveries (5.1%) in the case group ended in cesarean section. Although there was a tendency for a higher proportion of episiotomies to be performed on PAPL mothers (16% cases vs. 15.4% controls, $p = 0.054$), there was still a significantly higher proportion of mothers in this group who presented tears (40.91% cases vs. 30.14% controls; $p = 0.014$).

Newborn characteristics

There were significant group differences in newborn anthropometric measures, including birth weight ($p = 0.001$) (**Table 7**); newborns in the PAPL group were around two days older and were smaller than the controls ($p \leq 0.020$). There were no differences between

the groups regarding Apgar scores or medical interventions at birth, such as use of oxygen or an incubator.

Maternal food frequency intake

Food intake was similar between groups with only one exception. PAPL mothers reported consuming fish on average one time more per month (6.94 times PAPL vs. 5.97 controls; $p = 0.016$). There was a wide range of values for monthly food frequency intakes on most of foods (data not shown).

Maternal zinc status

No group differences were found in maternal hair zinc concentrations. PAPL cases had on average 165.94 $\pm$ 36.14 µg/g and the controls had 155.75 $\pm$ 36.14 µg/g ($p = 0.268$).

The zinc concentration of the certified reference material, used to control the quality of the analysis, was 172 $\pm$ 11 µg/g (National Institute for Environmental Studies) and the mean value obtained in our laboratory was 170.0 $\pm$ 3.2 µg/g.

Discussion

Contrary to what we hypothesized, breastfed during late pregnancy was not associated with an increase in the active phase of labor. To our knowledge this is the first study designed to examine the association between the practice of breastfeeding during pregnancy and the occurrence of a prolonged active phase of labor. Our results are not consistent with Marquis et al. previous work where they reported a higher incidence of dystocic labor among mothers who overlapped breastfeeding during late pregnancy compared to mothers who did not breastfeed at all during their pregnancy (16). The inconsistent results may be due in part to the inclusion of both mothers with dystocic labor

due to mechanical problems (i.e., cephalopelvic disproportion) as well as mothers who had a dystocic labor due to a delay on cervical dilatation, the outcome examined in the present study. In addition, the absence of an association could be due to a minimal nutritional stress of the feeding practice as both the case and control mothers reported a low frequency of breastfeeding events per day during late pregnancy.

Around 8% of our study population reported the practice of breastfeeding during late pregnancy which although it is 20% less than what Marquis et al. reported in their Lima sample (16), it is a substantial number of pregnant women. Our study mothers came from many different districts of Lima while the previous study enrolled mothers living in only from one district, suggesting that breastfeeding practices vary by mothers' regional backgrounds, economic status, or other cultural factors.

Unlike previous reports (5, 6, 20), we did not find significant differences in maternal hair zinc concentrations between the PAPL cases and their controls. In earlier studies of PAPL, zinc concentrations were evaluated using maternal plasma or serum. Hair can be considered as a long-term indicator of zinc status. Any difference in maternal zinc status between our PAPL cases and their controls that occurred close to the delivery date, would not be detectable in our hair samples.

Birth weight and gestational age have been reported to influence dilatation curves (21), so we are not sure if this could have influenced our classification of cases and controls since small statistical significant differences were found between these two groups on birth weight and gestational age. Since we relayed the diagnosis of a case or control on reports made by the hospitals' health professionals, we tried to minimize possible bias by matching cases and controls by hospitals.

In conclusion, our findings do not support the hypothesis that the practice of breastfeeding during late pregnancy represents a risk for a prolonged active phase of labor. Given that the practice of breastfeeding during late pregnancy is still occurring frequently, additional research is needed to determine the consequences, if any, for the mother and infant. Health professionals need evidence-based recommendations to optimize maternal and child health.

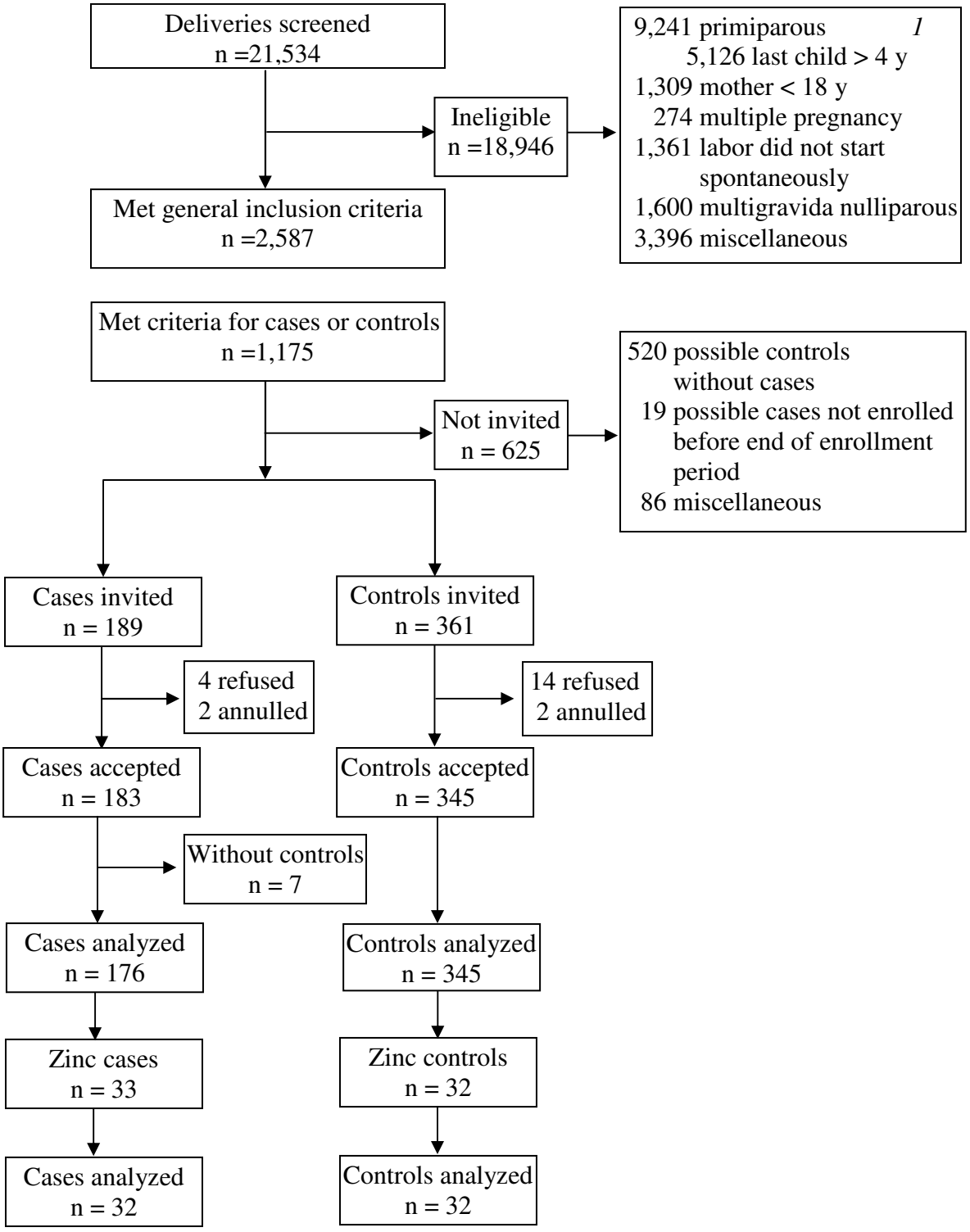

Figure 1. Flow of participants enrollment showing detailed numbers of subjects who met and did not met the general as well as the specific case-control criteria. [1] Some mothers did not meet more than one general criteria, therefore the sum is > 18946.

Table 1. Maternal sociodemographic characteristics by presence of PAPL

	PAPL n = 176 n (%)	Non-PAPL n = 345 n (%)	*p* [2]
Age, y [1]	27.0 ± 5.2	27.7 ± 5.2	0.151
Place of birth			0.911
Lima	98 (55.7)	183 (53.0)	
Lima province	6 (3.4)	9 (2.6)	
Coast	15 (8.5)	32 (9.3)	
Mountain	48 (27.3)	988 (28.4)	
Rainforest	9 (5.1)	23 (6.7)	
Time living in Lima, y [1]	19.9 ± 9.8	19.8 ± 9.8	0.923
Educational status			0.143
Illiterate	0 (0.0)	1 (0.3)	
Not completed elementary school	14 (8.0)	17 (4.9)	
Completed elementary school	6 (3.4)	20 (5.8)	
Not completed high school	32 (18.2)	88 (25.5)	
Completed high school	95 (54.0)	161 (46.7)	
Not completed technical college	13 (7.4)	16 (4.7)	
Completed technical college	9 (5.1)	28 (8.1)	
Not completed college	5 (2.8)	6 (1.7)	
Completed college	2 (1.1)	8 (2.3)	
Marital Status			0.975
Married	41 (23.3)	78 (22.6)	
Live together	126 (71.6)	246 (71.3)	
Single	6 (3.4)	14 (4.1)	
Separated	3 (1.7)	7 (2.0)	
Working status until delivery			0.047
Yes	34 (19.3)	44 (12.7)	
No	142 (80.7)	301 (87.3)	
Housing			0.199
Own	18 (10.2)	45 (13.1)	
Rented	39 (22.2)	99 (28.7)	
Relative's	114 (64.8)	195 (56.5)	
Other	5 (2.8)	6 (1.7)	

[1] mean ± SD

[2] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.

PAPL: prolonged active phase of labor

Table 2. Maternal breastfeeding practices by presence of PAPL

	PAPL n = 176 n (%)	Non-PAPL n = 345 n (%)		
Breastfeeding during late pregnancy	14 (7.9)	27 (7.8)		
	162 (92.1)	318 (92.2)		
	Odds Ratio	**95 % CI[1]**		
		Lower	Upper	
Risk of breastfeeding during late pregnancy on the occurrence of PAPL	1.02	0.51	2.04	

[1] Conditional logistic regression conducted for the Odds Ratio
PAPL: prolonged active phase of labor

Table 3. Maternal breastfeeding antecedents by presence of PAPL

	PAPL n = 176 n (%)	Non-PAPL n = 345 n (%)	*P*[3]
Maternal contact with recommendations about breastfeeding during pregnancy			
From a health professional			0.361
Yes	32 (18.2)	52 (15.1)	
No	144 (81.8)	293 (84.9)	
From a relative			0.675
Yes	65 (36.9)	121 (35.1)	
No	111 (63.1)	224 (64.9)	
From a friend			0.152
Yes	23 (13.1)	62 (18.0)	
No	153 (86.9)	283 (82.0)	
Last child's age when weaned, mo [1,2]	16.3 ± 7.5	15.8 ± 7.4	0.460

[1] mean ± SD
[2] cases n = 165 controls n = 325
[3] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
PAPL: prolonged active phase of labor

Table 4. Maternal health behavior during current pregnancy by presence of PAP L

	PAPL n = 176 n (%)		Non-PAPL n = 345 n (%)		*P* [1]
Use of medicines					
Antibiotics					0.415
Yes	55	(31.2)	96	(27.8)	
No	121	(68.8)	249	(72.2)	
Vaginal suppositories					0.541
Yes	55	(31.2)	117	(33.9)	
No	121	(68.8)	228	(66.1)	
Smoking					0.512
Yes	7	(4.0)	10	(2.9)	
No	169	(96.0)	335	(97.1)	
Passive smoking					0.718
Yes	21	(11.9)	45	(13.0)	
No	155	(88.1)	300	(87.0)	
Use of supplements					0.596
Yes	153	(86.9)	294	(85.2)	
No	23	(13.1)	51	(14.8)	
Zinc					0.534
Yes	43	(24.5)	73	(21.1)	
No	131	(74.4)	270	(78.3)	
Unknown	2	(1.1)	2	(0.6)	
Iron					0.300
Yes	151	(85.8)	290	(84.1)	
No	24	(13.6)	55	(15.9)	
Unknown	1	(0.6)	0	(0.0)	
Calcium					0.484
Yes	56	(31.8)	96	(27.8)	
No	118	(67.1)	247	(71.6)	
Unknown	2	(1.1)	2	(0.6)	
Folic Acid					0.734
Yes	72	(40.9)	136	(39.4)	
No	102	(58.0)	207	(60.0)	
Unknown	2	(1.1)	2	(0.6)	

[1] Pearson X^2 was used for testing significant difference of proportions
PAPL: prolonged active phase of labor

Table 5. Maternal antecedents and health characteristics by presence of PAPL

	PAPL n = 176 mean ± SD	**Non-PAPL** n = 345 mean ± SD	***P*** [5]
Last child's age, mo	30.6 ± 3.6	30.9 ± 3.7	0.440
Number of pregnancies, #	2.0 ± 1.3	2.2 ± 1.3	0.037
Number of deliveries, #	1.7 ± 1.1	1.9 ± 1.1	0.127
Weight gained during pregnancy, kg [1]	11.1 ± 4.3	11.3 ± 4.2	0.726
Maternal height, cm [2]	153.4 ± 5.6	154.2 ± 5.6	0.114
Maternal hemoglobin, g/dL [3]	11.1 ± 1.3	11.2 ± 1.3	0.246
Maternal preeclampsia [4]			0.970
Yes	5 (2.8)	10 (2.9)	
No	171 (97.2)	335 (97.1)	
Maternal previous cesarean [4]			0.114
Yes	47 (27.7)	71 (20.6)	
No	129 (73.3)	274 (79.4)	

[1] cases n = 111 controls n = 219

[2] cases n = 174 controls n = 340

[3] cases n = 156 controls n = 313; Hb values available were from different trimesters: cases (1st = 6.4%, 2nd = 30.8%, 3rd = 62.8%) controls (1st = 4.8%, 2nd = 27.5%, 3rd = 67.7%)

[4] n (%)

[5] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.

PAPL: prolonged active phase of labor

Table 6. Obstetrics procedures during labor by presence of PAPL

	PAPL n = 176 n (%)		Non-PAPL n = 345 n (%)		*P* [2]
Maternal membranes at the first clinical report					
Intact	154	(87.5)	289	(83.8)	0.259
Broken	22	(12.5)	56	(16.2)	
Maternal membranes during labor					
Artificial rupture	85	(48.3)	38	(11.0)	0.000
Intact until last cm of dilatation	38	(21.6)	194	(56.3)	
Spontaneously broken	50	(28.4)	105	(30.4)	
Broken but unknown artificial or spontaneous	3	(1.7)	8	(2.3)	
Use of oxytocin in cases					
Yes	61	(34.7)	0	(0.0)	
No	115	(65.3)	345	(100)	
Use of forceps					0.987
Yes	1	(0.6)	2	(0.6)	
No	175	(99.4)	343	(99.4)	
Delivery ended in cesarean					0.000
Yes	9	(5.1)	0	(0.0)	
No	167	(94.9)	345	(100)	
Maternal episiotomy					0.054
Yes	39	(22.2)	53	(15.4)	
No	137	(77.8)	292	(84.6)	
Maternal tears					0.014
Yes	72	(40.9)	104	(30.1)	
No	104	(59.1)	241	(69.9)	
Maternal examinations at hospital, # [1]	9.9 $\pm$ 3.1		5.6 $\pm$ 3.1		0.000

[1] mean $\pm$ SD, number of examinations at the hospital during labor.
[2] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
PAPL: prolonged active phase of labor

Table 7. Newborn characteristics of PAPL cases and their controls

	PAPL n = 176 n (%)	Non-PAPL n = 345 n (%)	*P* [2]
Newborn's sex			0.061
Masculine	102 (58)	170 (49.3)	
Feminine	74 (42.0)	175 (50.7)	
Newborn's weight, g [1]	3416.8 ± 388.6	3299.2 ± 390.6	0.001
Newborn's length, cm [1]	50.5 ± 2.2	50.0 ± 2.3	0.014
Newborn's gestational age, wk [1]	39.2 ± 1.3	38.9 ± 1.3	0.020
Newborn's apgar			
1 minute	8.1 ± 0.7	8.1 ± 0.7	0.203
5 minutes	9.0 ± 0.3	9.0 ± 0.3	0.581
Newborn in incubator			0.559
Yes	5 (2.8)	7 (2.0)	
No	171 (97.2)	338 (98.0)	
Newborn pathology			0.906
Yes	7 (4.0)	13 (3.8)	
No	169 (96.0)	332 (96.2)	
Newborn use of oxygen			0.965
Yes	7 (4.0)	14 (4.1)	
No	169 (96.0)	331 (95.9)	

[1] mean + SD

[2] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.

PAPL: prolonged active phase of labor

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CHAPTER 6: GENERAL CONCLUSIONS

The findings in the population studied demonstrate that the practice of breastfeeding during late pregnancy does not represent a risk for having a small for gestational age baby or experiencing a prolonged active phase of labor. Maternal physiological adaptations may occur to compensate for the extra physiological stress that this practice represents for the mother. More research is needed to examine these adaptations and the possible short-term as well as long-term implications of the adaptations on maternal, toddler, and infant health.

No correlation between zinc concentration in plasma and zinc concentration in hair was found (data shown in appendix A). This absence of an association suggests that hair zinc concentrations may be a poor indicator of maternal nutritional status and could explain the non-significant results we found when we compared maternal hair zinc concentrations between cases and controls of small-for-gestational age and prolonged active phase of labor. Previous studies that reported an association between maternal zinc status and these birth outcomes used mainly plasma or serum as an indicator of zinc status.

To our knowledge this is the first study that has examined birth outcomes such as small-for-gestational age and prolonged active phase of labor, in association to the practice of breastfeeding during late pregnancy. Recommendations for health professionals require confirmatory studies before drawing conclusions about birth risks associated with this practice.

Recommendations for future research

Future studies that involve collecting information from clinical charts should consider employing trained health professionals to work in the hospitals and report the information

needed on the clinical charts. In the present study we relied on the clinical information collected by the hospitals' own health professionals and we had to exclude the enrollment of some participants due to the lack of consistent information on the clinical charts.

Longitudinal studies are recommended for a more in-depth study of the practice of breastfeeding during pregnancy since a better understanding of maternal physiological adaptations to this practice and its possible consequences is required. Moreover, since we have shown that this practice is still frequent among pregnant women, health professionals need to be prepared with evidence-based recommendations for women who choose to continue breastfeeding during their pregnancy.

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APPENDIX A. ADDITIONAL TABLES

Table 1. Maternal hair zinc concentration by the presence of BDP

	BDP n = 23 mean ± SD	**Non-BDP** n = 23 mean ± SD	***p****
Maternal zinc in hair (ug/g)	160.57 ± 29.42	162.13 ± 29.42	0.858

* ANOVA was conducted for continue variables with the matched case-controls treated as blocks
BDP: breastfeeding until the last trimester of pregnancy

Table 2. Correlation between maternal zinc in plasma and zinc in hair

	Plasma n = 29 mean ± SD µg/dL	**Hair** n = 29 mean ± SD µg/g	Correlation	***p****
Maternal zinc in hair (ug/g)	60.5 ± 8.3	153.48 ± 52.5	-0.113	0.559

* Bonferroni probabilities
The zinc concentration of the certified reference material, used to control the quality of the plasma analysis, was 127 ± 16 µg/dL (NIST) and the mean value obtained at the laboratory was 124 ± 3.8 µg/dL.

Table 3. Household appliances of SGA and their controls

	SGA n = 78 n (%)		Non-SGA n = 150 n (%)		*P* *
Appliances at home					
Refrigerator					0.007
Yes	15	(19.2)	55	(36.7)	
No	63	(80.8)	95	(63.3)	
Blender					0.009
Yes	31	(39.7)	87	(58.0)	
No	47	(60.3)	63	(42.0)	
Television					0.951
Yes	70	(89.7)	135	(90.0)	
No	8	(10.3)	15	(10.0)	
Radio					0.707
Yes	49	(65.8)	98	(65.3)	
No	29	(37.2)	52	(34.7)	
Microwave oven					0.188
Yes	2	(2.6)	10	(6.7)	
No	76	(97.4)	140	(93.3)	
Washing machine					0.188
Yes	2	(2.6)	10	(6.7)	
No	76	(97.4)	140	(93.3)	
Sewing machine					0.955
Yes	3	(3.8)	6	(4.0)	
No	75	(97.2)	144	(96.0)	
Computer					0.259
Yes	1	(1.3)	6	(4.0)	
No	77	(98.7)	144	(96.0)	
Stereo					0.385
Yes	11	(14.1)	28	(18.7)	
No	67	(85.9)	122	(81.3)	

* Pearson X^2 was used for testing significant difference of proportions
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 4. Household members and food expenses by presence of SGA

	SGA n = 78 mean ± SD	**Non-SGA** n = 150 mean ± SD	***p*** †
Family members living at home, #	4.9 ± 0.3	5.1 ± 0.2	0.526
Family members sleeping /room, #	3.1 ± 0.1	3.0 ± 0.1	0.492
Money spent weekly on food per family member, S/.*	20.8 ± 0.9	21.9 ± 0.7	0.323

* 1 $ = 3.35 S/.

† ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.

SGA: small for gestational age, < 10^{th} percentile birth weight

Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 5. Maternal self reported diseases of SGA and their controls

	SGA n = 78 n (%)		Non-SGA n = 150 n (%)		*P* *
Diabetes					0.470
Before current pregnancy	0	0.0	0	0.0	
During current pregnancy	0	0.0	1	(0.7)	
Never	78	(100)	149	(99.3)	
High pressure					0.780
Before current pregnancy	5	(6.4)	10	(6.7)	
During current pregnancy	6	(7.7)	8	(5.3)	
Never	67	(85.9)	132	(88.0)	
Asthma					0.641
Before current pregnancy	5	(6.4)	6	(4.0)	
During current pregnancy	1	(1.3)	1	(0.7)	
Never	72	(92.3)	143	(95.3)	
Gastritis					
Before current pregnancy	18	(23.1)	24	(16.0)	0.380
During current pregnancy	4	(5.1)	11	(7.3)	
Never	56	(71.8)	115	(76.7)	
Vaginitis					0.089
Before current pregnancy	7	(9.0)	28	(18.7)	
During current pregnancy	48	(61.5)	91	(60.7)	
Never	23	(29.5)	31	(20.6)	
UTI					0.018
Before current pregnancy	10	(12.8)	41	(27.3)	
During current pregnancy	35	(44.9)	45	(30.0)	
Never	33	(45.31)	64	(42.7)	
Pneumonia					0.693
Before current pregnancy	4	(5.1)	6	(4.0)	
During current pregnancy	0	0.0	0	0.0	
Never	7	(94.9)	144	(96.0)	

* Pearson X^2 was used for testing significant difference of proportions
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 5. Maternal self reported diseases of SGA and their controls (continue)

	SGA n = 78 n (%)		Non-SGA n = 150 n (%)		*P* *
Tuberculosis					0.086
Before current pregnancy	5	(6.4)	3	(2.0)	
During current pregnancy	0	(0.0)	0	(0.0)	
Never	73	(93.6)	147	(98.0)	
Edema					0.077
Before current pregnancy	3	(3.8)	20	(13.3)	
During current pregnancy	24	(30.8)	40	(26.7)	
Never	51	(65.4)	90	(60.0)	
Hepatitis					0.359
Before current pregnancy	1	(1.3)	5	(3.3)	
During current pregnancy	0	(0.0)	0	(0.0)	
Never	77	(98.7)	145	(96.7)	
Typhoid					0.842
Before current pregnancy	3	(3.8)	5	(3.3)	
During current pregnancy	0	(0.0)	0	(0.0)	
Never	75	(96.2)	145	(96.7)	
Morning sickness					0.605
Before current pregnancy	8	(10.3)	20	(13.3)	
During current pregnancy	16	(20.5)	24	(16.0)	
Never	54	(69.2)	106	(70.7)	
Preeclampsia					0.551
Before current pregnancy	6	(7.7)	13	(8.7)	
During current pregnancy	5	(6.4)	5	(3.3)	
Never	67	(85.9)	132	(88.0)	
Threat of abortion					0.727
Before current pregnancy	12	(15.4)	28	(18.7)	
During current pregnancy	10	(12.8)	22	(14.6)	
Never	56	(71.8)	100	(66.7)	

* Pearson X^2 was used for testing significant difference of proportions
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 6. Maternal food intake during pregnancy of SGA and their controls

	SGA n = 78 mean ± SD	Non-SGA n = 150 mean ± SD	$p^{\ddagger}$
Monthly food frequency intake*			
Chicken	12.7 ± 5.9	11.9 ± 5.9	0.325
Fish	6.7 ± 4.7	6.4 ± 4.8	0.637
Other meat (beef, pork)	3.9 ± 4.4	5.3 ± 4.5	0.030
Chicken liver	5.3 ± 8.0	7.6 ± 8.1	0.046
Beef liver	1.7 ± 2.7	2.0 ± 2.7	0.491
Other organ meat	2.4 ± 2.6	2.8 ± 2.6	0.254
Shellfish	0.9 ± 2.8	1.3 ± 2.8	0.227
Dairy products (milk, cheese, yogurt)	22.5 ± 9.0	22.7 ± 9.1	0.878
Eggs	12.2 ± 7.3	13.1 ± 7.4	0.387
Beans	8.9 ± 4.2	8.6 ± 4.3	0.660
Quinua/wheat	2.2 ± 2.9	2.8 ± 2.9	0.190
Rice	26.1 ± 4.6	26.9 ± 4.7	0.234
Pasta	4.3 ± 2.5	3.6 ± 2.6	0.068
Bread	26.9 ± 6.6	28.1 ± 6.7	0.169
Vegetables	11.6 ± 8.9	12.6 ± 9.0	0.430
Potato, cassava, sweet potato	22.5 ± 8.3	22.7 ± 8.4	0.889
Fruits	24.6 ± 8.1	25.0 ± 8.1	0.712
Number of meals + snacks per day†	4.19 ± 0.9	4.12 ± 0.9	0.603

* Food consumption: means of number of times per month.
† cases n = 77
‡ ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
SGA: small for gestational age, < 10^{th} percentile birth weight
Non-SGA: 50^{th} percentile > birth weight < 90^{th}

Table 7. Household characteristics of PAPL and their controls

	PAPL n =176		Non-PAPL n = 347		*P* [1]
	n	(%)	n	(%)	
Housing main material					0.425
Bricks and cement	121	(68.8)	258	(74.8)	
Bricks	2	(1.1)	4	(1.2)	
Wood	30	(17.0)	43	(12.5)	
straw mats	3	(1.7)	10	(2.9)	
Others	20	(11.4)	30	(8.7)	
Housing source of light					0.322
House connection	149	(84.7)	299	(86.7)	
Neighbor's	24	(13.6)	35	(10.1)	
Candles	3	(1.7)	11	(3.2)	
Housing Source of water					0.560
Potable water	139	(79.0)	277	(80.3)	
Public sink	7	(4.0)	9	(2.6)	
Tanker	18	(10.2)	39	(11.3)	
Neighbors	8	(4.6)	15	(4.4)	
Well	2	(1.1)	5	(1.4)	
Others	2	(1.1)	0	(0.0)	
Place use as a bathroom					0.102
Toilet	140	(79.6)	286	(82.9)	
Outhouse with drain	12	(6.8)	7	(2.0)	
Outhouse without drain	20	(11.4)	42	(12.2)	
Open land	2	(1.1)	4	(1.2)	
Others	2	(1.1)	6	(1.7)	
Fuel used for cooking					0.074
Gas	142	(80.7)	283	(81.4)	
Kerosene	24	(13.7)	54	(15.7)	
Wood	5	(2.8)	9	(2.6)	
Others	5	(2.8)	1	(0.3)	

[1] Pearson X^2 was used for testing significant difference of proportions
PAPL: prolonged active phase of labor

Table 8. Household appliances of PAPL and their controls

	PAPL n =176 n (%)		Non-PAPL n = 347 n (%)		*P* [1]
Appliances at home					
Refrigerator					0.418
Yes	52	(29.5)	114	(33.0)	
No	124	(70.5)	231	(67.0)	
Blender					0.995
Yes	98	(55.7)	192	(55.7)	
No	78	(44.3)	153	(44.3)	
Television					0.560
Yes	160	(90.9)	308	(89.3)	
No	16	(9.1)	37	(10.7)	
Radio					0.605
Yes	103	(58.5)	210	(60.9)	
No	73	(41.5)	135	(39.1)	
Microwave oven					0.934
Yes	10	(5.7)	19	(5.5)	
No	166	(94.3)	326	(94.5)	
Washing machine					0.055
Yes	8	(4.5)	32	(9.3)	
No	168	(95.5)	313	(90.7)	
Sewing machine					0.263
Yes	5	(2.8)	17	(4.9)	
No	171	(97.2)	328	(95.1)	
Computer					0.699
Yes	5	(2.8)	12	(4.5)	
No	171	(97.2)	333	(96.5)	
Stereo					0.276
Yes	33	(18.7)	79	(22.9)	
No	143	(81.3)	266	(77.1)	

[1] Pearson X^2 was used for testing significant difference of proportions
PAPL: prolonged active phase of labor

Table 9. Household members and food expenses by presence of PAPL

	PAPL n = 176 mean ± SD	Non-PAPL n = 345 mean ± SD	*P* [2]
Number of family members living at home	4.8 ± 2.2	4.7 ± 2.2	0.792
Number of family members sleeping in the same room	3.2 ± 1.3	3.3 ± 1.3	0.460
Money spend weekly on food per family member, S/. [1]	23.1 ± 8.4	22.9 ± 8.4	0.858

[1] controls n = 344
[2] 1 $ = 3.35 S/.
[3] ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks
PAPL: prolonged active phase of labor

Table 10. Maternal and newborn vital functions during labor of PAPL and their controls

	PAPL n = 176 mean ± SD	Non-PAPL n = 345 mean ± SD	*P* [5]
Maternal pulse [1]			
Maximum	85.1 ± 6.5	83.2 ± 6.5	0.002
Minimum	75.6 ± 5.7	76.8 ± 5.7	0.029
Maternal systolic arterial pressure [2]			
Maximum	114.8 ± 11.2	111.6 ± 11.3	0.002
Minimum	101.6 ± 8.9	103.8 ± 8.9	0.010
Maternal diastolic arterial pressure [2]			
Maximum	72.7 ± 8.5	71.7 ± 8.6	0.221
Minimum	63.3 ± 7.3	64.3 ± 7.4	0.142
Fetal cardiac frequency [3]			
Maximum	148.7 ± 4.8	146.8 ± 4.8	0.000
Minimum	136.8 ± 4.8	138.3 ± 4.8	0.001
Estimated maternal bleeding [4]	283.8 ± 136.0	276.5 ± 137.0	0.577

[1] controls n = 343
[2] controls n = 344
[3] cases n = 175 controls n = 344
[4] cases n = 166 controls n = 325
[5] Pearson X^2 was used for testing significant difference of proportions and ANOVA was conducted for the comparison of means with the matched case-controls treated as blocks.
PAPL: prolonged active phase of labor.

Table 11. Maternal self reported diseases of PAPL and their controls

	PAPL n = 176 n (%)		Non-PAPL n = 345 n (%)		*P* [1]
Diabetes					0.140
Before current pregnancy	1	(0.6)	0	(0.0)	
During current pregnancy	1	(0.6)	0	(0.0)	
Never	174	(98.8)	345	(100)	
High pressure					0.601
Before current pregnancy	16	(9.1)	23	(6.7)	
During current pregnancy	7	(4.0)	13	(3.8)	
Never	153	(86.9)	309	(89.6)	
Asthma					0.350
Before current pregnancy	2	(1.1)	11	(3.2)	
During current pregnancy	3	(1.7)	7	(2.0)	
Never	171	(97.2)	327	(94.8)	
Gastritis					0.723
Before current pregnancy	29	(16.5)	48	(13.9)	
During current pregnancy	13	(7.4)	28	(8.1)	
Never	134	(76.1)	269	(78.0)	
Vaginitis					0.770
Before current pregnancy	26	(14.8)	55	(15.9)	
During current pregnancy	99	(56.2)	200	(58.0)	
Never	51	(29.0)	90	(26.1)	
UTI					0.194
Before current pregnancy	42	(23.9)	72	(20.9)	
During current pregnancy	69	(30.2)	117	(33.9)	
Never	65	(36.9)	156	(45.2)	
Pneumonia					0.722
Before current pregnancy	1	(0.6)	3	(0.9)	
During current pregnancy	0	(0.0)	1	(0.3)	
Never	175	(99.4)	341	(98.8)	

[1] Pearson X^2 was used for testing significant difference of proportions
PAPL: prolonged active phase of labor

Table 11. Maternal self reported diseases of PAPL and their controls (continue)

	PAPL n = 176 n (%)		Non-PAPL n = 347 n (%)		*P* [1]
Tuberculosis					0.723
Before current pregnancy	6	(3.4)	14	(4.1)	
During current pregnancy	0	(0.0)	1	(0.3)	
Never	170	(96.6)	330	(95.6)	
Edema					0.157
Before current pregnancy	24	(13.6)	29	(8.4)	
During current pregnancy	45	(25.6)	87	(25.2)	
Never	107	(60.8)	229	(66.4)	
Hepatitis					0.605
Before current pregnancy	6	(3.4)	9	(2.6)	
During current pregnancy	0	(0.0)	0	(0.0)	
Never	170	(96.6)	336	(97.4)	
Typhoid					0.291
Before current pregnancy	4	(2.7)	14	(4.1)	
During current pregnancy	0	(0.0)	0	(0.0)	
Never	172	(97.7)	331	(95.9)	
Morning sickness					0.308
Before current pregnancy	27	(15.3)	48	(13.9)	
During current pregnancy	32	(18.2)	47	(13.6)	
Never	117	(66.5)	250	(72.5)	
Threat of abortion					0.194
Before current pregnancy	24	(13.7)	50	(14.5)	
During current pregnancy	14	(7.9)	45	(13.0)	
Never	138	(78.4)	250	(72.5)	

[1] Pearson X^2 was used for testing significant difference of proportions
PAPL: prolonged active phase of labor

Table 12. Maternal food intake during pregnancy of PAPL and their controls

Food	PAPL n = 176 mean ± SD	Non-PAPL n = 344 mean ± SD	*P* [5]
Monthly food frequency intake			
Chicken	12.4 ± 5.8	12.5 ± 5.8	0.813
Fish	6.9 ± 4.2	6.0 ± 4.2	0.016
Other meat (beef, pork) [2]	5.1 ± 4.2	5.1 ± 4.2	0.990
Chicken liver [2]	7.2 ± 7.0	6.2 ± 7.0	0.113
Beef liver	1.8 ± 2.5	1.8 ± 2.5	0.999
Other organ meat	2.5 ± 2.4	2.7 ± 2.4	0.210
Shellfish [3]	1.3 ± 2.2	1.0 ± 2.2	0.162
Dairy products (milk, cheese, yogurt)	22.3 ± 8.9	23.4 ± 8.9	0.178
Eggs	12.6 ± 7.8	13.1 ± 7.8	0.503
Beans	8.9 ± 4.4	8.8 ± 4.4	0.798
Quinua/wheat	2.4 ± 2.9	2.6 ± 2.9	0.401
Rice	27.0 ± 3.6	27.0 ± 3.6	0.897
Pasta	3.7 ± 2.2	3.7 ± 2.2	0.896
Bread	27.0 ± 6.7	27.9 ± 6.8	0.188
Vegetables	12.0 ± 8.0	13.0 ± 8.0	0.177
Potato, cassava, sweet potato	22.4 ± 8.2	22.7 ± 8.3	0.707
Fruits	24.7 ± 8.0	24.9 ± 8.0	0.813
Meals + snacks per day [4]	4.1 ± 0.9	4.13 ± 0.9	0.860

[1] Food consumption: means of number of times per month

[2] controls n = 343

[3] cases n = 175

[4] controls n = 342

[5] ANOVA was conducted for the comparison of means with the matched case-controls treated as Controls.

PAPL: prolonged active phase of labor

APPENDIX B. SURVEY FORMATS

Nº C.C. [ ][ ][ ][ ][ ] Date [ ][ ][ ] DD MM YY

Maternal Records

Interviewer: _____ Code [ ][ ][ ][ ] Hospital

1. LWGA [ ] _____ [ ][ ][ ][ ]
2. PREM [ ] _____ [ ][ ][ ][ ]
3. DAP [ ] _____ [ ][ ][ ][ ]

1. Last child's age [ ] years [ ] months Date of birth [ ][ ][ ]

1a. Sex: M___ F___ 1b. Name: _____

2. How is your last child doing?

3. How have the last child reacted about your pregnancy?

4. Are you currently breastfeeding your last child? [ ] 1 = yes 2 = no

if the answer is no go to Nº 5

4a. Over the last week, how often? day [ ] night [ ] total [ ]

4b. For how long will you continue breastfeeding? [ ][ ] _____

4c. What is the main reason for your decision to continue breastfeeding?

_____

_____

4d. Who do you think had the most influence in your decision to continue breastfeeding during pregnancy? [ ][ ]

a. Myself
b. Mother
c. Child
d. Husband
d. Relative
e. Friend
f. Health professional
g. Other

5. If not breasfeeding, how old was your last child when weaned? [ ][ ] months

5a. Overlap during pregnancy? [ ] 1 = yes 2 = no 5b. Until what month? [ ]

Code ☐☐☐☐
Hospital

5c. What was the main reason for your decision to not continue to breastfeed? ☐☐ ☐☐

_____

_____

5d. How often did you used to breastfeed your child at the time of weaning? day ☐ night ☐ total ☐
(Ask if mother overlaped during the third trimester of pregnancy)

5e. Who do you think had the most influence in your decision to not continue to breastfeed? ☐☐

a. Myself
b. Mother
c. Child
d. Husband
d. Relative
e. Friend
f. Health professional
g. Other

6 Have you ever had any advice from a health professional regarding breastfeeding during pregnancy?

☐ 1 = yes 2 = no

6a. What did they tell you? _____

6b. Did you follow the recommendation? ☐ 1 = yes 2 = no

6c. What do you think of the recommendation?

_____

_____

7. Have you ever had any advice from a relative regarding breastfeeding during pregnancy?

☐ 1 = yes 2 = no

7a. What did they tell you? _____

7b. Did you follow the recommendation? ☐ 1 = yes 2 = no

7c. What do you think of the recommendation?

_____

Code [ ][ ][ ][ ]
Hospital

8. Have you ever had any advice from a friend regarding breastfeeding during pregnancy?

[ ] 1 = yes 2 = no

8a. What did they tell you? _____

8b. Did you follow the recommendation? [ ] 1 = yes 2 = no

8c. What do you think of the recommendation?

_____

9 Have you ever heard or seen through mass media any advice regarding breastfeeding during pregnancy?

9a. What did you read or hear? _____

10. Do you have or have had any of the following diseases?

Enfermedad	yes no	Before or during
Diabetes		
High pressure		
Asthma		
Gastritis		
Vaginitis		
UTI		
Pneumonia		
TB		
Edema		

Enfermedad	yes no	Before or during
Hepatitis		
Typhoid		
Morning sickness		
Preeclampsia		
Threat of abortion		
Cancer		
Epilepsy		
Others:		

88 = Not applicable

Code ☐☐☐☐
Hospital

11. Have you used medicaments during pregnancy? ☐ 1 = yes 2 = no

11a. Which ones? _____ _____

_____ _____

12. Do you smoke ? ☐ 1 = yes 2 = no

12a. For how long have you smoked? ☐☐ _____

12b. How many cigarrettes do you smoke per month? ☐☐

12c. How many days per month? ☐☐

13. If not smoking, have you ever smoked? ☐ 1 = yes 2 = no

13a. How long ago did you quit smoking? ☐☐ _____

14. During your pregnancy, did you live or work with somebody who smoked? ☐ 1 = yes 2 = no

14b. Have they smoked in your presence? ☐ 1 = yes 2 = no

15. Do you take any vitamins or minerals? ☐ 1 = yes 2 = no

Which ones? _____ How often? _____

_____ How often? _____

_____ How often? _____

16. What recommendation would you give to a mother who is breastfeeding and becomes pregnant?

_____

_____

Nº C.C. | | | | | | | Date | | | | DD MM YY

Sociodemographic Survey

Interviewer: _____ Code | | | | | Hospital

Mother status

1. Where were you born ? _____ | |

2. Mother´s age: | | Date of birth | | | | |

3. How long have you been living in Lima? | | _____

4. In what district do you live? _____

5. What is your educational status? ____ ____ | |

   a. Not completed elementary school
   b. Completed elementary school
   c. Not completed high school
   d. Completed high school
   e. Not completed technical college
   f. Completed technical college
   g. Not completed college
   h. Completed college

6. What is your marital status? _____

   a. Married c. Single e. Separated
   b. Live together d. Divorced f. Widowed

7. Do you work? | | 1 = yes 2 = no

   7a. Inside / outside the home? _____ | | 1= inside 2=outside

   7b. What kind of work do you do? _____ | |

   7c. Self-employed or salaried? _____ | | 1= self-employed 2= salaried

   7d. What is your monthly income? | | | | |

   7e. Your contract is: | | 1= short term (≤ 6 months) 2= long term (> 6 months)

Code ☐☐☐☐
Hospital

8. Have you worked during this last year? ☐ 1 = yes 2 = no

8a. How long ago did you stop working? ☐☐ months

8b. How much was your income? ☐☐☐☐

9. Do you have health insurance? ☐ 1 = yes 2 = no

9a. What type? ☐☐

1. ESSALUD
2. private
3. SIS
4. Other

10. What is your husband´s job? ☐☐

House

1. The house where you live is: ☐☐
   1. Own
   2. Rented
   3. Relative's
   4. Other

2. What material is your house contructed of? ☐☐
   1. Bricks and cement
   2. Bricks
   3. Wood
   4. straw mats
   5. others

3. How do you light your house? ☐

4. From where do you get the water to cook and to wash? ☐

5. Where do you go to the bathroom (defecate) ☐

6. What kind of fuel do you use for cooking?

1 Gas
2 Kerosene
3 Electricity
4 Wood
6 others

Code ☐☐☐☐
Hospital

7. What appliances do you have in your house?

1. Refrigerator	4. Radio	7. Sewing machine
2. Blender	5. Microwave oven	8. Computer
3. Television	6. Washing machine	9. Stereo

8. How many members does your family have? ☐☐
members that share from the same familiar pot

Children ☐ Adolescents ☐ (12 - 17y.) Adults ☐ (> 18y.)

9. How many rooms does your home have? ☐☐
Exclude bathroom and kitchen

8a. How many rooms are used for sleeping? ☐☐

10. How many of your family members work? ☐☐

11. How many of your family members have a steady job? ☐☐
with contract > 6 months

12. How much per week does your family spend on food? ☐☐☐

Nº C.C. | | | | | | | Date | | | | DD MM YY

Maternal Information From Clinical Chart

Interviewer: _____ Code | | | | | Hospital

1. Age | | | years Date of birth | | | |

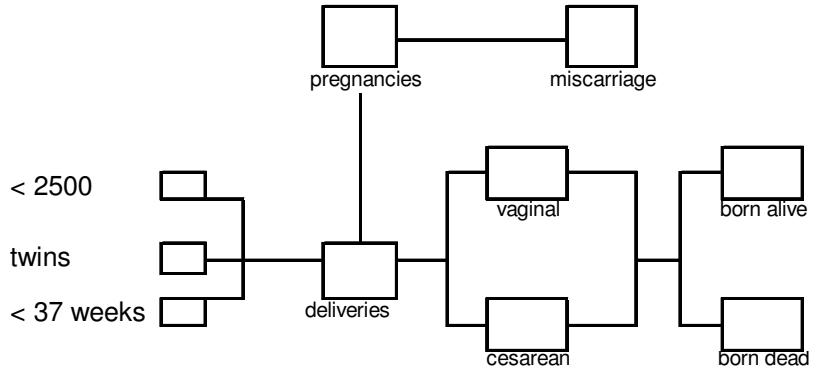
2. Height ____ ____ ____ . ____ cm

3. Weight before pregnancy ____ ____ ____ . ____ Kg.

4. Weight gained during pregnancy ____ ____ ____ . ____ Kg.

Date of control	Weight Kg	Gestational age weeks

5. Pregnancies



Code ☐☐☐☐
Hospital

6. Last child´s age ☐ years ☐ months Date of birth ☐☐☐

7. Hemoglobin ___ ___ . ___ Date ____ / ____ / ____

___ ___ . ___ Date ____ / ____ / ____

8. HIV status Positive ☐ Negative ☐ Not done ☐

9. Preeclamsia ☐ 1 = yes 2 = no

Labor Observations

Bleeding´s calculation ___ ___ ___ ___ ml

Membranes broken ☐ complete ☐

Placenta´s weight ___ ___ ___ g

Spontaneous delivery ☐ 1 = yes 2 = no

Procedures: Forceps ☐ 1 = yes 2 = no

Vacuum ☐ 1 = yes 2 = no

Cesarean ☐ 1 = yes 2 = no

Mechonial liquid ☐ 1 = yes 2 = no

No CC NB | | | | | | | Date | | | | DD MM YY

Newborn's Information

Interviewer: _____ Code | | | | |

No CC mother | | | | | | |

1. Date of birth: | | | | DD MM YY Time: ___ ___ : ___ ___ 2. Sex: M __ F__

3. Gestational age: ____ ____ weeks 3a.Method used to calculate: _____

4. Weight ____ ____ ____ ____ g 5. Length or height ____ ____ cm.

6. Head circunference ____ ____ . ___ cm.

7. Torax circunference ____ ____ . ___ cm.

8. Apgar 1 min. ___ 5 min. ____

Procedures

1. Incubator | | 1 =yes 2=no

2. Nasogastric tube | | 1 =yes 2=no

3. Oxygen | | 1 =yes 2=no

4. Use of medication | | 1 =yes 2=no

Medication	Reason

5. Pathology | | 1 =yes 2=no _____

6. Malformations | | 1 =yes 2=no _____

Observations: _____

Nº C.C. | | | | | | | Date | | | |
DD MM YY

Labor Information

Interviewer: _____ Code | | | | |
Hospital

HOSPITALIZATION

1. Admittance Date: ____ / ____ / ____ Time : ___ ___ : ___ ___

2. Examinations during labor

Date	Time	Contractions			FHR	Pulse	BP
		Freq.	Intens.	Durat.			
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						
/ /	:						

Code [ ][ ][ ][ ]
Hospital

LABOR

1	Mother´s position at labor	Vertical	☐	Horizontal	☐
2	Membranes	Intact	☐	Broken	☐
3	Type of presentation	right	☐	left	☐

First stage

1 Latent phase: Reported on C.C.: ☐ 1 =yes 2=no

Date start: ____ / ____ / ____

Time start: ___ ___ : ___ ___

Time end: ___ ___ : ___ ___

Total duration: ___ ___

Use of oxytocin: ☐ 1 =yes 2=no

Use began at dilatation: 1 2 3

Dilution: 0.5% 1% 2%

Drops: _____ _____ _____

Time: _____ _____ _____

2 Active phase: Date start: ____ / ____ / ____

Dilatation cm	Hour begins	Hour ends	Maximum Time programmed (min)	Time real (min)
	:	:		
	:	:		
	:	:		
	:	:		
	:	:		
	:	:		
Total				

Code [ ][ ][ ][ ]
Hospital

2a. Use of oxytocin: [ ] 1 =yes 2=no Use began at dilatation: 1 2 3

Dilution: 0.5% 1% 2%

Drops: _____ _____ _____

Time: _____ _____ _____

Observations: _____

_____

2b. Artificial rupture of membranes: [ ] 1 =yes 2=no Dilatation: 4 5 6 7 8 9

Observations: _____

_____

Second stage: Expulsion

Real time longer than programmed time [ ] 1 =yes 2=no Time in excess: ___ ___ min.

Spontaneous: [ ] 1 =yes 2=no

Procedures: Forceps [ ] 1 =yes 2=no

Vacuum [ ] 1 =yes 2=no

Cesarean [ ] 1 =yes 2=no

Observations: _____

_____

Third stage: Childbirth

1. Estimate of bleeding: ___ ___ ___ ___ ml.

2. Placenta and umbilical cordon:

2a. Placenta's weight ___ ___ ___ g

Code | | | | | |
Hospital

2b. Placenta´s morphology Complete cotyledons ☐ 1 =yes 2=no

Type of insertion _____

Complete membranes ☐ 1 =yes 2=no

2c. Umbilical cord Length ___ ___ cm

3. Mechonial liquid ☐ 1 =yes 2=no

Obstetric interventions

1. Episiotomy ☐ 1 =yes 2=no

2. Tears ☐ 1 =yes 2=no ID IID IIID

3. Medication ☐ 1 =yes 2=no

type: _____ Time of use _____

_____ _____

Observations : _____

_____

Labor complications

☐ 1 =yes 2=no

Type of complication	Moment of complication

Observations : _____

Nº C.C. | | | | | | |

DD MM YY | | | |

Intake Frequency

Interviewer: _____ Code | | | | |

1. Have you changed your diet during pregnancy? [ ] 1 = yes 2 = no

2. At what moment? [ ]

   1. 1º trimester
   2. 2º trimester
   3. 3º trimester
   4. 1º y 2º trimester
   5. 2º y 3º trimester
   6. All pregnancy

2a. What changed?

_____

_____

3. How many times per day did you used to eat? _____

   How many where snacks? _____

Food	Times per week							Times month	Times pregnan.	Never
	1	2	3	4	5	6	7			
Chicken										
Fish										
Other meat (beef, pork)										
Chicken liver										
Beef liver										
Other organ meat										
Shellfish										
Dairy products (milk, cheese, yogurt)										
Eggs										
Beans										
Quinua/wheat										

Code | | | | |

Food	Times per week							Times month	Times pregnan.	Never
	1	2	3	4	5	6	7			
Rice										
Pasta										
Bread										
Vegetables (not in soup or stew)										
Potato, cassava, sweet potato										
Fruits										

Observations: _____

_____

APPENDIX C. INFORMED CONSENT FORMS

INFORMED CONSENT DOCUMENT

Study of the practices during pregnancy among women who live in Lima.

Investigators:
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Instituto de Investigación Nutricional
Av. La Molina 1885 – La Molina; Teléfono 349-6023.

This study is conducted by Iowa State University (USA) in coordination with the Nutrition Reasearch Institute (PERU) and the Hospital María Auxiliadora, Hospital Hipólito Unánue and Hospital Nacional Docente Madre Niño San Bartolomé. Please take your time in deciding if you would like to participate. Please feel free to ask any question.

Introduction
The Instituto de Investigación Nutricional is an Institution that has been working for more than 30 years in different areas of our country through projects of health and nutrition. In this opportunity we are obtaining information about feeding practices of pregnant women with their children.

What is the study about?
The purpose of this study is to examine the way that pregnant women care for their children so that we can develop sound recommendations to improve children's lives.

Who can participate in the study?
Women who gave birth in this hospital and have a child younger than 4 years old can participate.

What I will be asked for if my child and I participate in the study?
If you agree to participate in this study, your participation will involve one interview of approximately 30 minutes with our worker. In this interview you will be asked about characteristics of yourself and your household, your health, obstetric history, your diet and feeding practices with your youngest child, before you gave birth. When you are interviewed, you may skip any question that you do not wish to answer or that makes you feel uncomfortable. Additional information about you and your baby, such as obstetrical evaluation after admission, your delivery, your weight and height, newborn's length and weight, will be taken from you and your baby's clinical chart. Finally, if you are chosen (at random), we will ask you for a small
hair sample from you and your newborn as well as a sample of your breast milk (less than a tablespoon), to assess zinc status. If you agree, the results from the test will be given to the hospital to be posted in your clinical chart, so you can have access to them.

Are there any risks for me or my baby if we participate in the study?
There are no risks of participate in this study, since we will just obtain the necessary information from your answers, from your clinical record and if the case from your breast milk sample and hair.

Is there any benefit for participating?
There will not be direct benefits for you, but the information gained in this study would benefit society by providing valuable information for health professionals about pregnant women and their toddlers so they could develop accurate recommendations.

Can I withdraw at any moment?
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled, including services in this hospital.

Confidentiality. Who will know about my identity or my child in the project?
Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available.
To ensure confidentiality to the extent permitted by law, the following measures will be taken, each participant will be assigned a unique code that will be used instead of the name. Only investigators and supervisor in charge of the study will have access to records linking participants' names and codes. Files linking participants' name with a unique code will be destroyed at the end of the project. If the results are published, your identity will remain confidential.

Who should I call if I have questions about the project?
You are encouraged to ask questions at any time during this study. For further information about the study contact Lic. Rossina Pareja 3496023 rossipt@iastate.edu (PERU), Dr. Mary Penny 3496023 mpenny@iin.sld.pe (PERU), Dr. Grace Marquis (515) 294-9231 gmarquis@iastate.edu (USA). If you have any questions about the rights of research

subjects or research-related injury, please contact the Instituto de Investigación Nutricional's ethics committee, Dr. Enrique Morales 3496023 (Perú) or the Human Subjects Research Office, 1138 Pearson Hall, (515) 294-4566 austingr@iastate.edu (USA) or the Research Compliance Officer, Office of Research Compliance, 1138 Pearson Hall, (515) 294-3115; dament@iastate.edu (USA).

Voluntary statement of informed consent form

Your signature indicates that you voluntarily agree to participate in this study, that you authorize us to extract some information from your clinical chart, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Participant's Name (printed) _____

_____ _____

(Participant's Signature/thumbprint) (Date)

Your signature below indicates that you voluntarily agree to allow your infant also to participate in this study.

Infant's Name (printed) _____

_____ _____

(Mother's Signature/thumbprint) (Date)

Investigator statement

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

INFORMED CONSENT DOCUMENT

Study of the practices during pregnancy among women who live in Lima.

(Pre-post delivery cross-sectional study)

Investigators:
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This study is conducted by Iowa State University (USA) in coordination with the Nutrition Research Institute (PERU). Please take your time in deciding if you would like to participate. Please feel free to ask any question.

Introduction
The Instituto de Investigación Nutricional is an institution that has been working for more than 30 years in different areas of our country through projects of health and nutrition. In this opportunity we are obtaining information about the zinc values in pregnant women.

What is the study about?
The present is part of an study that currently is being conducted at 3 hospitals in Lima Metroplolitana, where we are taking hair samples from mothers who give birth and have a child younger than 4 years, to evaluate their zinc nutritional status. The zinc is a very important mineral for all stages of life, especially at times when rapid growth takes place, such as during pregnancy, where the formation and growth of a new human being takes place. Therefore the purpose of this study is to explore if the values of zinc obtained from hair samples taken after delivery reflect or are associated with the values of zinc obtained from blood during the last trimester of pregnancy.

Who can participate in the study?
Women who are in the 7^{th} month of pregnancy and have child younger than 4 years old can participate.

What I will be asked for if I participate in the study?
If you agree to participate in this study, a fieldworker will visit you at home on two occasions, the first during your 7th month of pregnancy. At that time you will be asked for a blood sample of approximately 7ml. (less than a tablespoon). The second visit will be after you give birth when you will be asked for a hair sample (a small lock of hair). The results of the tests will be given to you when they are ready.

Are there any risks for me if I participate in the study?
There are no risks for participating in this study. The taking of blood sample may cause minor localized discomfort.

Is there any benefit for participating?
A benefit for you will be that you will know your zinc nutritional status; additionally we shall give you your iron nutritional status and your blood type. Besides that, the information gained in this study will benefit society by providing valuable information to evaluate the zinc nutritional status in pregnant women.

Can I withdraw at any moment?
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide not to participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

Confidentiality. Who will know about my identity in the project?
Any information that you give us about you or your baby will be confidential and kept it in a locked cabinet at the IIN. To ensure confidentiality to the extent permitted by law, the following measures will be taken: each participant will be assigned a unique code that will be used instead of the name. Only investigators and supervisors in charge of the study will have access to records linking participants' names and codes. Files linking participants' name with a unique code will be destroyed at the end of the project. If the results are published, your identity will remain confidential.

Who should I call if I have questions about the project?
You are encouraged to ask questions at any time during this study. For further information about the study contact Lic. Rossina Pareja 3496023 rossipt@iastate.edu (PERU), Dr. Mary Penny 3496023 mpenny@iin.sld.pe (PERU), Dr. Grace Marquis (001-515) 294-9231 gmarquis@iastate.edu (USA). If you have any questions about the rights of research subjects or research-related injury, please contact the Instituto de Investigación Nutricional's ethics committee, Dr. Enrique Morales 3496023 (Perú) or the Human Subjects Research Office, 1138 Pearson Hall, (001-515) 294-4566 austingr@iastate.edu (USA) or the Research Compliance Officer, Office of Research Compliance, 1138 Pearson Hall, (001-515) 294-3115; dament@iastate.edu (USA).

Voluntary statement of informed consent form

Your signature indicates that you voluntarily agree to participate in this study, that you authorize us to extract some information from your clinical chart, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Participant's Name (printed) _____

_____ _____

(Participant's Signature/thumbprint) (Date)

Investigator statement

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

APPENDIX D. TABLE OF BIRTH WEIGHT FOR GESTATIONAL AGE

PESO NEONATAL (en gramos) EN FUNCION DE LA EDAD GESTACIONAL

PERCENTILES

SEMANAS	10	50	90
26	564	821	1155
27	617	957	1346
28	703	1113	1552
29	843	1276	1800
30	1004	1460	1999
31	1161	1642	2196
32	1304	1842	2373
33	1507	2066	2592
34	1772	2322	2901
35	2055	2611	3206
36	2324	2888	3513
37	2529	3090	3690
38	2696	3230	3826
39	2816	3333	3906
40	2916	3430	4003

APPENDIX E. PARTOGRAPH