Breast milk transfer across the globe

The influence of maternal body composition, socioeconomic status and smoking

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BREAST MILK TRANSFER ACROSS THE GLOBE

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Master thesis

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After 7 years of studying, my life as a student has come to an end with this thesis. I still remember the first day of the master in September 2012, when we were given the assignment to come up with a subject for the master thesis by the end of that week. For me, that was quite an overwhelming start since I just completed two bachelor theses a few weeks before and I didn't felt comfortable with rushing through another thesis. I decided to postpone the writing of the thesis to the next year. In the end, this turned out to be a good decision since the extra time gave me the opportunity to do an internship. Altogether, my two years in the Master Population Studies were quite intense, but above all very inspirational and full of personal development.

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ABSTRACT

Background: In previous studies, variations in breast milk transfer from mother to baby are explained by biological factors only. **Objective**: To gain insight in the biological and context-related maternal factors that influence breast milk transfer. **Methods**: A combined database with data from 16 milk intake studies from 12 countries is used. The association between body composition and breast milk transfer is studied by means of generalized linear models. The effect of socioeconomic status (SES) is assessed using analysis of covariance. Breast milk output is studied on the macro level by calculating interactions between body composition and stage in the nutrition transition, the latter implying changes in diet related to economic development. **Results**: Higher fat mass of the mother results in lower breast milk output, with smoking as an important confounder in this association. Mothers with lower SES levels have a higher breast milk output than mothers with higher SES levels, which is party explained by the lower weight of low SES mothers. When including the nutrition transition in the analysis it appears that, for countries who have not yet undergone the transition, fat mass is positively, instead of negatively, associated with breast milk output. **Conclusion**: Contextual factors influence breast milk transfer, indicated by the effect of smoking and SES on breast milk output, and by the finding that the association between fat mass and breast milk transfer is different for countries that are at the beginning of economic development. Since the underlying processes leading to this associations are mainly biological in nature our findings suggest that both contextual factors and biological factors should be taken into account in future breast milk transfer studies.

***Key words**: breast milk output, body composition, socioeconomic status, smoking, nutrition transition*

Table of Contents

List of tables

List of figures

List of abbreviations

BMI	Body mass index
EBF	Exclusive breastfeeding
DR-NDC	Diet-related non-communicable diseases
FMI	Fat mass index
FFMI	Fat free mass index
GLM	Generalized linear model
GNP	Gross National Product
IAEA	International Atomic Energy Agency
MIMA	Milk Intake Meta-Analysis
NT	Nutrition transition
Part. BF	Partially breastfeeding
Pred. BF	Predominantly breastfeeding
PNG	Papua New Guinea
SES	Socioeconomic status
WHO	World Health Organization

1. Introduction

1.1 Background

According to WHO statistics (2013), nowadays about 38% of the infants from zero to six months old are exclusively breastfed worldwide, meaning that these infants receive nothing but breast milk, not even water. The practice of breastfeeding a newborn has benefits for both the mother and the child. For the mother, breastfeeding reduces for example her risk of getting type 2 diabetes (WHO, 2013). For children up until the age of six months, it is found that being breastfed offers protection against gastrointestinal infections and lowers mortality due to diarrhea. Jones et al. (2003) predicted that the universal coverage of breastfeeding practices could prevent 13% of under-five mortality worldwide. More recently, the WHO estimated that about 800,000 deaths could be prevented among children under five years of age, if all children worldwide would be optimally breastfed in the first 24 months of their live (WHO, 2013). It is argued that breastfeeding can contribute significantly to the infant's health since human milk represents the most complete form of nutrition for them (Jihong Liu, 2006). However, being breastfed is not only beneficial at the very beginning of life. Childhood can be seen as a critical period for setting later health outcomes, also when it comes to the way people are fed during infancy. Breastfeeding has been mentioned as a 'protective factor throughout life', for example in reducing overweight in adulthood, and related to that, reducing the prevalence of cardiovascular diseases (Dietz, 2001, in: Magalhaes et al., 2012, p. 2).

Even though the positive effects of breastfeeding are widely known and reported, dose-response relationships are difficult to make. Unlike other food sources, the actual amount that is consumed by the infant is not directly observable (WHO, 2002, in Da Costa et al, 2010). This means that the effects of breastfeeding on the health condition of the infant are hard to measure as the amount of milk transferred is difficult to quantify. Quantification is important, since the way breastfeeding is influencing health relies to a great extent on the actual amount of milk that the infant has ingested. Besides, quantifying the amount of milk transferred enables researchers to study from what dose onwards the positive effects of breastfeeding start to manifest. Because of the limited ability to observe breast milk transfer, methods have been developed to measure the flow of milk from mother to baby. The conventional method of measuring intake of milk was to weigh the infant before and after each feed, known as the 'test-weighing' technique. This method is time consuming, and it is also found that the procedure of weighing before and after each fed can disturb the normal food pattern. Many infants are also fed during the night which limits the practical use of the test-weigh method (IAEA, 2010).

The introduction of isotope tracer methods, introduced by Coward in the late 1970s, has been an important step forward in measuring human milk intake. In this technique, deuterium-labeled water, also known as 'heavy water', is used to determine the rates of water flux from mother to baby (Coward, 1979). In the early days of this technique, a dose of deuterium-labeled water (^2H_2O) was given to the infant, as it was assumed that breast milk was the only source of water for the baby. Later this was seen as a limitation since babies may also get other fluids in addition to breast milk . Therefore, the technique was revised and a dose of heavy water was given to mother, to be able to determine the actual amount of breast milk that is consumed by the infant (Da Costa et al., 2010). With the deuterium oxide dose-to-the-mother method, a dose of deuterium labeled water is given to the mother at the first day of study. In the following 14 days the disappearance of the isotope from her total body water (sampled from urine, saliva or breast milk) and the appearance of the isotope in the baby (sampled from saliva and urine) is monitored (Da Costa et al., 2010). In practice the method works as follows: on the first day of the study, both the mother and the baby are weighted and baseline saliva samples are taken from the mother and the baby. Then the mother is offered a dose of 30 g (the standardized dose according to IAEA) of deuterium oxide (^2H_2O) through a straw to avoid spillage. The baby is fed as usual by his or her mother and ingests the deuterium labeled water exclusively from breast milk. Samples from the body water of the mother and the baby are taken on several days in the 14-day study period, and are measured on deuterium enrichment by use of infrared spectrometry. After this, the human milk intake and intake of water from other food sources can be calculated using a spreadsheet (IAEA, 2010). The deuterium oxide turnover method has major advantages over the former test-weighing method, since the isotope tracer method could be applied in field conditions and is suitable for large group studies (Da Costa et al., 2010).

Figure 1. Deuterium oxide (^2H_2O) dose-to-the-mother technique (IAEA, 2010, p. 2).

1. Mother drinks ^2H_2O (deuterium labeled water)
2. Baby consumes ^2H_2O in human milk
3. Saliva in both mother and baby is enriched with ^2H_2O

1.2 Academic and societal relevance of study

Since its introduction the deuterium oxide turnover method has been used in different kinds of research, for example to evaluate the effect of introducing solid foods on the consumption of human milk in breastfed babies (Galpin et al., 2007, in: IAEA, 2010), or in policy related research towards the efficacy of nutrition programmes and counseling on lactating mothers (Cissé, 2002, in IAEA, 2010). Many of the studies using the deuterium method focus on the association between maternal body composition and breast milk output, since the technique also allows the estimation of fat mass and fat free mass, in addition to measuring breast milk transfer. The mother's fat free mass is estimated from her total body water, and since it is assumed that the body is composed of fat mass and fat free mass, the amount of fat mass can be obtained by calculating the difference between body weight and fat free mass. By using the deuterium oxide turnover method, both body composition and breast milk output can thus be assessed, making it the ideal method for studying associations between these two. This is reflected in the existing body of literature with studies focusing on this association publishing data from all over the world: for example from rural Pakistan (Nazlee et al., 2011), Kenya (Ettyang et al., 2005) and Mexico (Villalpando et al., 1992), among many different contexts.

What these previous studies have in common is that they: a) focus on the biological factors underlying the association between body composition and breast milk transfer; and b) study the association between body composition and breast milk transfer at one single context.
Breast milk output may be affected by more factors than biological ones alone, and may also be influenced by contextual factors. Thus, it would be interesting to see what happens when contextual determinants are considered in addition to the more biological factors. Besides, comparisons between countries are not made so far, since studies all aim at finding associations within their own dataset, collected at one particular location.

Recently, the MIMA-database (Milk Intake Meta Analysis) was set up, in which all data from the milk-intake studies that were either conducted or supervised by Coward over a 20-year period were combined (Da Costa, 2010). It consists of data from 16 studies from various parts of the world, all using the deuterium oxide turnover method to determine the average daily quantity of breast milk that is transferred from mother to baby. In addition to information about the biological characteristics of the mother, like anthropomorphic measures as weight and height or measures for her body composition, contextual determinants are also part of the database. Thus, by using the MIMA-database, the association between maternal body composition and breast milk output can be studied while including contextual factors, like smoking and socioeconomic status, which potentially act as confounders in this association. Additionally, instead of looking at the data from one particular country, breast milk output can be studied across countries. This affords the opportunity to relate breast milk output to the nutrition transition: the shift in dietary patterns and related diseases that countries undergo when they experience economic growth. By combining data from different countries it is not only the contexts that can be compared, but it also means that breast milk transfer can be studied among subjects with different biological histories.

Gaining more information on the maternal factors that play a role in breast milk transfer is relevant at the societal level as well. Evaluating breast milk output by examining the effect of nutritional status and contextual factors on a mother's capacity for adequate lactation is of particular importance for setting breastfeeding recommendations (Ettyang, 2005). In the long run, obtaining more knowledge about the underlying processes that influence breast milk transfer can help in designing more tailor-made interventions, which might provide successful breastfeeding in particular situations. Given the central importance of breastfeeding in child health (and to some extent in maternal health as well), such interventions are highly useful.

1.3 Objective and research questions

The objective of this study is to gain insight in the biological and context-related maternal factors that determine the quantity of breast milk transfer from mother to baby. The main research question is: *'What maternal characteristics have an influence on breast milk transfer from mother to child?'* . To show the effect of the biological traits of the mother on breast milk transfer the association between maternal body composition and milk output will be described first. Secondly, the socioeconomic status of the mother is taken into account to find out whether socioeconomic status is a factor that influences breast milk transfer. Thirdly, the association between maternal body composition and breast milk transfer will be studied on the macro-level by examining whether there is a difference in this association between countries that are at different stages in the nutrition transition.

The following three sub-questions are formulated:

1. What is the association between maternal body composition and milk transfer when taking into account potential confounding and mediating variables?

2. Is there a difference in milk transfer between mothers of high and low socioeconomic status? And if so, what explains the difference in breast milk output between the groups of high and low socioeconomic position?

3. Is there a difference in the association between maternal body composition and breast milk output between countries that are at different stages in the nutrition transition?

1.4 Structure of the thesis

In the next chapter, the theoretical framework of this research will be described, consisting of an overview of the used theories, a literature review and a conceptual model. Also the hypotheses that arise from the theory and literature will be mentioned here. The third chapter is about the data source and the design of this study. The used methodology will be described here as well. The results of this research will be presented in chapter 4, followed by a discussion of these results in the fifth chapter. This final chapter also includes a general conclusion, recommendation for further research and policy implications.

2. Theoretical framework

In this chapter the theoretical framework will be presented, starting with a description of the three theories that are used. Thereafter, the results of the literature study will be described. Both the theories and the literature review form the basis of the conceptual model, as presented in section 2.4. Hypothesis are formulated in section 2.5. The chapter is finalized by giving definitions of the used concepts.

2.1. Theories from evolutionary biology

Parent –offspring conflict theory

In the previous studies about breastfeeding it is often assumed that milk production of a women is an outcome of biological factors alone. What these current studies about breastfeeding do not take into account is the notion that mothers are able to make sensible choices in their biological and social context with regard to child feeding. The parent-offspring conflict theory, proposed by Wells (2003) includes the evolutionary biological component of behavior. According to Wells, parents make choices on feeding of the offspring on the basis of maximum fitness for their reproductive outcome. This implies that what is beneficial for the mother's fitness is not necessarily beneficial for the offspring too. The notion of parental investment was firstly mentioned by Trivers (1972) who described it as 'any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring' (p. 139). The parent-offspring conflict theory finds its origin in genetics, since parents are -genetically seen- equally related to all their children, meaning that their investment in their offspring should be equal. However, since the offspring is only half (or even less) related to each other they will try to get more investment than their parents intended to provide, resulting in a genetic conflict (Wells, 2003). The mother has to make a choice on what level she wants to provide feeding to her offspring. The choice of the mother regarding giving breastfeeding depends amongst other things on a variety of household characteristics: how many people live in the house, how old are the siblings, how many people take care of the children, does the mother work out of the house? In fact, the behavior of the mother is a reflection of her biological characteristics on the one side and her environment on the other.

Parent-offspring life history theory

The second theory used in this research is also proposed by Wells (2010). His parent-offspring life history theory recognizes the fact that social phenomena are highly relevant in explaining population variability in health. According to this theory, the mother's phenotype (defined as the outcome of the interrelations between genotype (i.e. genetic basis) and the environment) can set the trend or norm for the offspring's growth and health trajectory during gestation and lactation (Wells, 2010). In the parent-offspring life history theory Wells suggests that maternal capital, defined as the 'phenotypic resources enabling investment in the offspring allows for 'effective buffering of the offspring from nutritional perturbations and represents the environmental niche initially occupied by the offspring' (Wells, 2010, p.1). Thus, maternal capital, influenced by the genetic factors as well as by the environment in which the mother lives, enables the mother to invest in her children (for example by giving breastfeeding), and by that, she is 'programming' the growth trajectory of the child. When growth and health trajectories of people from different countries are assessed, it is important to refer to the biological history of a population: disease prevalence, like the prevalence of malaria or other infectious diseases in certain parts of the world may affect its population immune systems or nutritional status. This may in turn affect a mother's maternal capital, resulting in a lower capacity to give adequate lactation to the infant. The link between maternal capital and later health outcome can be shown by the example of India. Diabetes prevalence in India is high and will raise in the future: it is estimated that 55-65% of the population will suffer from this disease by 2025 (Mohan, 2007). The prevalence of low birth weight is also high, which implicates that these babies start with low maternal capital. Growing fast in childhood would mean that the risk of developing degenerative diseases later in life increases, as described by Barker in his theory of fetal origin of disease (1992). However, if they not recuperate from their deprived start in life they will become stunted adults, meaning that they will have low maternal capital, and thus low resources enabling them to breastfeed their children. Low maternal capital cannot be raised within one generation, as it will lead to predisposing to diabetes or overweight. Wells (2010) concludes that accelerating the recovery of maternal capital within generations overloads the metabolic capacity and exacerbates cardiovascular risks, reflected in increased degenerative disease risks in populations undergoing rapid change, for example in the process of urbanization or swift development. Here a link can be made to the nutrition transition theory.

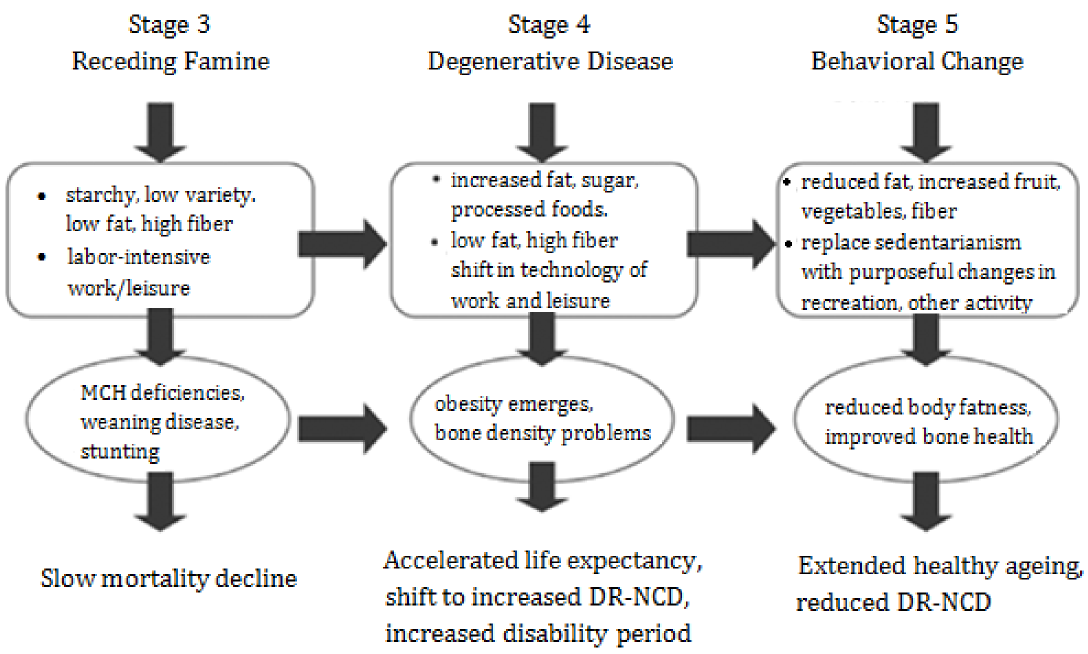
2.2. Demographic theory

Nutrition transition theory

The nutrition transition theory proposed by Popkin (1994) describes the changes in dietary and physical activity patterns of populations over time, resulting in a shift from under-nutrition to over-nutrition, and subsequently to nutrition-related non-communicable diseases like diabetes and cardiovascular diseases, or risk factors like hypertension (Popkin, 2002). These changes in dietary patterns are paralleled by changes in life-style and health status, as well as by major demographic and socioeconomic changes (Popkin, 2004).

According to Popkin (2002), this transition occurs in five stages. The first stage is the stage of the hunter-gatherers with related diets and activity patterns; the diet is varied, low in fat and high in fiber. Physical activity levels are high in this stage because of hunting- and gathering activities. The demographic outcome is low fertility and low life expectancy as well. The second stage (stage of famine) refers to the period where food is scarce and dietary diversity is low and mainly dominated by cereals. Demographic outcomes in this stage are high fertility, high maternal and child mortality and low life expectancy. In the third stage (fig. 1) the famines decline and the diet consists of starchy, fiber-rich food sources. Physical activity levels are still quite high, as people do labor-intensive work at their jobs or at home. The fourth stage (fig. 1) is the stage of the diet-related non-communicable diseases (DR-NCD) as the diet becomes high in fat, high in refined carbohydrates, sugar and cholesterol, and low in fiber. Physical activity is low because of a shift in technology of work and leisure and the prevalence of obesity is high. Mostly the transition from the third stage towards this fourth stage is regarded as *the* nutrition transition. In the fifth and final stage behavioral change takes place due to a desire to prolong health and prevent degenerative diseases. This results in healthier diets with reduced fat and an increased consumption of fruit and vegetables. Also physical activity levels are higher again because sedentariness is replaced with purposeful action. Therefore, body fatness will be reduced, resulting in extended ageing in a healthy way (Popkin, 2002).

Figure 2. Stage 3,4 and 5 of the nutrition transition. Source: Popkin, 2002.



2.3 Literature review

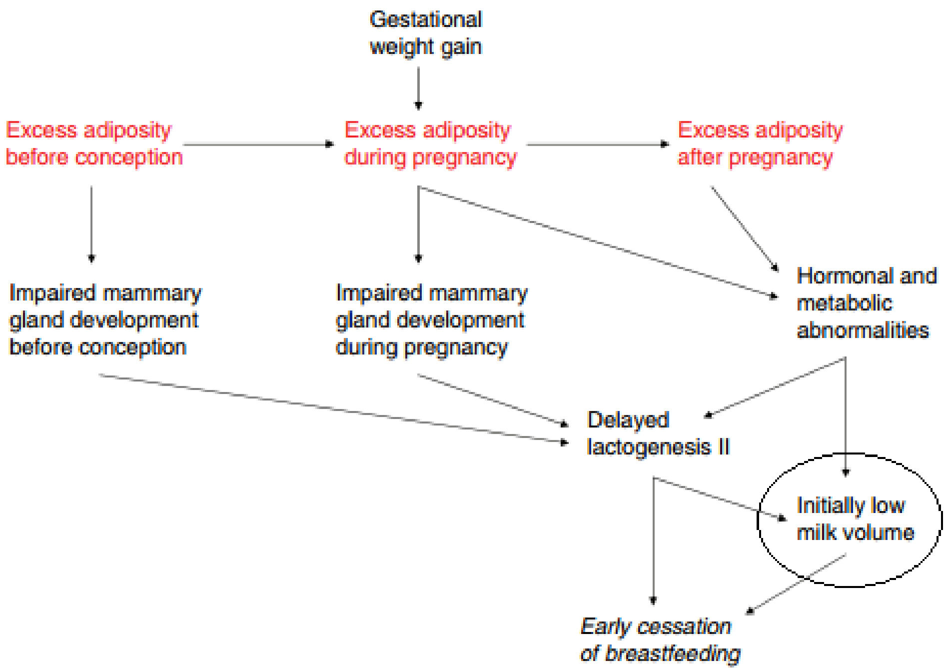
Maternal body composition and breast milk output

It is a common belief that the body composition of a lactating mother affects her breast milk output. There are several studies showing that there is a negative correlation between maternal fat mass and milk output, that is, leaner mothers tend to have significantly more breast milk output, and the other way around: high levels of maternal fat are associated with decreased breast milk transfer (Villalpando et al., 1992; Haisma et al., 2003; Nazlee et al., 2011). Additionally, the study of Nazlee et al. (2011) found that high levels of maternal fat are related to decreased breast milk transfer as well as increased use of other food.

Related to the lower milk volume of mothers with higher fat mass is the finding that overweight and obese mothers are less likely to breastfeed their infants than mothers with a healthy weight (Li, 2005 in Paliy, 2014). This was also found by the study of Rutishauer (1992, in: Hilson et al., 2004), who studied breastfeeding behavior of Australian women. The results showed that among women who were breastfeeding for at least two weeks, those who were overweight (BMI >26 kg/m^2) were 1.5-fold less likely to continue breastfeeding until their infants were 3 months old than were women with a lower BMI. Additionally, it was found that obese women (BMI >29 kg/ m^2) were 3.65-fold less likely to initiate breastfeeding successfully (Hilson et al., 1997, in: Hilson et al., 2004).

The lower breast feeding rates among overweight and obese women may be due to biological and physiological difficulties with initiating breast milk production. Rasmussen (2007) came with a conceptual model to visualize the route between high body fat, and early cessation of breast feeding (fig. 3). According to this model, excess adiposity before or during pregnancy results in impaired mammary gland development. The mechanisms behind this association have not yet been determined though (Rasmussen, 2007). Another potential explanation for low milk volume in obese or overweight mothers lies in the hormonal and metabolic characteristics of fat mass. High fat mass during or after pregnancy may lead to hormonal and metabolic abnormalities, which in turn delays the start of milk secretion (lactogenesis II). This delayed onset of lactation leads to a low milk volume at the very beginning of giving breastfeeding. Eventually, this may cause early cessation of breastfeeding, since the mother may have the perception that her milk volume does not meet the energy requirements of the infant.

Figure. 3 Possible pathway by which maternal obesity could lead to low milk volume and early cessation of breastfeeding. Source: Rasmussen, 2007. Own additions.



Rasmussen (2007) also proposes another route from gestational weight gain towards early cessation of breastfeeding: high maternal body fat may lead to the birth of a large baby, resulting in difficulties with positioning the baby and latching on (Rasmussen, 2007). This, in turn, leads to delayed onset of milk secretion as well, causing the mother to stop giving breastfeeding. A third route proposed by Rasmussen (2007) leading to earlier cessation of breastfeeding in overweight and obese women includes the element of choice. The choice women make with regard to the feeding of their child is affected by different socio-demographic and psychosocial factors. Obese women were less satisfied with their appearance, resulting in a shorter breastfeeding period. This was confirmed by the study of Hauff et al. (2014). They found that overweight and obese women had certain psychosocial characteristics that were associated with lower breastfeeding success. For example, overweight and obese women were less confident than normal weight women in reaching their breastfeeding goals and they reported they had less friends and relatives who were giving breastfeeding to get support from.

However, according to Hilson et al. (2004), the lower breastfeeding success in overweight and obese women is not a matter of psychosocial characteristics, but is caused by biological factors alone. Having a high pre-pregnancy BMI was related to poor breastfeeding outcomes in their study, because of a delayed onset of lactogenesis II. This associations between pre-pregnant BMI and breast feeding outcome was found to be independent from demographic and psychosocial correlates.

Other biological factors that influence breast milk transfer
Besides maternal body composition, there are more factors that have an effect on the quantity of breast milk transferred from mother to baby. One of them is parity, the number of pregnancies carried by the mother to a viable gestational age. The study of Ingram et al. (1999) showed that parity was the most significant factor determining the amount of breast milk transfer at the first week of breastfeeding: multiparous women delivered an average of 142 ml more milk in 24h than primiparous women did. This positive association was also found by Hilson et al. (2004): the milk volume of multiparous women raised faster in the first week than the milk volume of primiparous women. Even though biological factors may cause this differences, it should also be taken into account that some of the multiparous women will have had prior experience with giving breastfeeding, where primiparous women have not. With regard to breastfeeding duration, parity also seems to have a positive influence: in the study by Kristiansen (2010) among Norwegian mothers it was found that the odds of exclusive breast feeding at four months after giving birth increased with increasing numbers of children.

Another factor that should be taken into account when assessing breast milk output is whether the baby receives exclusive breastfeeding (EBF), that is intake of nothing but breast milk, or receives complementary foods in addition to breast milk (partial breastfeeding, PartBF). Early introduction of complementary foods may reduce the level of breast milk output, or it can cause earlier cessation of breastfeeding (WHO, 1998a). The study of Haisma et al. (2003) confirms the association between feeding pattern and breast milk intake. The sample of 70 infants from Pelotas (Brazil) showed that breast milk intake by PartBF infants was significantly lower than HM intake by EBF infants: the level of breast milk intake of PartBF was reduced to 74% compared to the intake by EBF infants. More recently, higher milk intakes among EBF infants were also found by Agne-Djigo et al. (2013) and Wells et al. (2012) in samples from Senegal and Iceland.

The third factor that possibly affects breast milk output is the sex of the baby. In the study by Da Costa et al. (2010) it is found that the factor sex is significantly related to breast milk transfer, with milk intake of males being 5% higher than that of females. Although gender difference in breast milk intake are quite clear, gender is not associated with the magnitude of complementary food intake (Da Costa et al., 2010). The gender difference in milk intake can be due to biological factors, with male infants having a greater lean mass than females throughout infancy, or to behavioral factors, since mothers may have the perception that male infants have higher energy requirements and therefore feed them differently (Da Costa et al., 2010).

The weight of the infant is a factor to consider when analyzing breast milk output: heavier babies tend to consume more milk than small babies. It is suggested in the literature that children who grow more rapidly, thus heavier babies, cry more often from hunger, and will therefore get breastfed more often (Kramer et al., 2007, in: Kramer et al., 2011). On the other hand, mothers may also think that their milk supply is inadequate, resulting in the introduction of complementary foods (Kramer, 2011). The association between weight and milk intake is a difficult one: is it because of their weight that they consume more breast milk, or because they drink more, they have a higher weight? It is often assumed that babies who are exclusively breastfed grow faster than infants who are partially or predominantly breastfed in the first months of life (WHO, 1994). However, previous studies on the association between infant weight and breastfeeding have shown that it is the other way around: infants are more likely to be weaned when they do not grow well. Additionally, these infants are also more likely to change from being exclusively breastfed to being partially breastfed (Martines et al., 1994, in: Victora, 1998, Piwoz et al. 1994 in: Victora, 1998). Studying feeding pattern and growth of a child in the same interval will lead to a bias, wherein breastfed babies grow faster than they actually do (Victora et al., 1998). The phenomenon of reverse causality should thus be considered: it is not because of exclusive breastfeeding that these children grow faster, but because smaller babies are more likely be partially breastfed or not being breastfed at al.

Mothers who smoke are less likely to breastfeed than mothers who do not smoke. Besides, if smoking mothers give breastfeeding, they tend to start to wean their babies earlier than the non-smoking mothers, despite current recommendations on the optimal duration of breastfeeding (Liu et al., 2006). In the literature, there are several explanations for the association between maternal smoking and early weaning. According to Hopkinson et al. (1992, in Mennella et al., 2007) smoking ≥10 cigarettes at a daily basis has a negative effect on the milk production: in their study, the quantity of milk was significantly lower among the smoking mothers, even when controlled for influencing variables like age, parity, birth weight, educational level, prior nursing experience or pumping frequency. It remained unclear in this study what the physiologic basis is for this lower breast milk production of smoking women. The same negative association was found in a study of Vio et al. (1991). In this study among 10 smoking and 10 non-smoking mothers it appeared that the non-smokers had a significant higher milk output (981 g/day compared to 683 g/day). Amir & Donath (2002) suggested that it is likely that the association between smoking and breast feeding duration is not a physiological one, but that psychosocial factors are mainly responsible for the lower breast feeding rates of smoking women. According to them, the variation in breastfeeding rates by smoking women is too wide to assume that smoking has a consistent negative physiologic effect on lactation (Amir and Donath, 2002). Perception can also be a factor in explaining the low breast feeding rate. Smokers are for example more likely to think that their milk supply is inadequate (Hill and Aldag, 1996, in Mennella, 2007). Besides, a lower motivation can play a role in whether the practice of breastfeeding is continued or not. It is also suggested that smoking is associated with a lifestyle in which breastfeeding does not fit (Amir & Donath, 2007). Finally, when mothers smoke ≥5 cigarettes daily, breastfed babies tend to exhibit behaviors like crying or colic, that may promote early weaning (Matheson and Rivrud, 1989, in Mennella, 2007). New evidence however, shows that the lower breast feeding duration among smoking mothers is not merely a reflection of their poor health motivation, but has indeed a physiological basis. The study from Bahadori et al. (2013) shows that high nicotine levels may disrupt prolactin release, a hormone that stimulates breast milk production.

The influence of socioeconomic status on body composition and breastfeeding

The influence of socioeconomic status on body composition is well known: the prevalence of overweight and obesity is considerably higher among low socioeconomic status groups than among people with high socioeconomic background, mainly because of different dietary patterns (Fernandez-Alvira et al., 2013). This negative association was already described in a study from 1981 (Gain et al., 1981, in: Boot et al., 1997), where it was found that children from a lower socioeconomic status had a higher percentage of body fat during adolescence. The effect of socioeconomic status on body composition is straightforward for high income countries, while for countries in transition this association is questioned.

The study by Monteiro et al. (2004a) describes the shift in the academic discourse on the association between socioeconomic status (SES) and obesity. In the literature prior to 1989 it was assumed that obesity in the developing world would be a disease of the higher socioeconomic classes, where obesity rates in high income countries were generally found among the poor. In these studies it was found that people with a higher income in developing countries were more prone to develop overweight and obesity, because these people were the ones that had access to the larger amounts of foods, where the poor had to deal with food scarcity. Besides, the lower economic status group could benefit less from the shift towards technology in work and leisure and was stuck in more labor intensive jobs, which led to a life-style of high energy expenditure. Hence, the higher socioeconomic class was regarded as worse off in terms of the chances for developing obesity.

However, the studies performed between 1989 and 2003 are showing evidence that this pattern is changing. It appears that obesity in the developing world can no longer be considered as a disease solely for the people with high SES, and that the burden of obesity is shifting towards the lower SES groups when the GDP of a country is increasing (Monteiro, 2004a, Popkin and Larsen, 2004). When a country is in transition from a low towards a middle income country, obesity is shifting from high SES to low SES population groups in that country. Possible explanations could be that lack of food and high energy expenditure become less common when a certain level of economic growth has been reached, even among the low SES groups. Besides, the poor may be more constrained in their food choice and unable to resist more obesogenic environments (WHO, 2003). A study by Monteiro et al. (2004b) among 37 countries in various stages of economic development, measured by gross national product (GNP), showed that being of low SES only offers protection against obesity in the low-income countries. For lower- middle-income countries, obesity is already shifting towards the lower SES population groups. The cut-off level where obesity starts to shift towards the poor was found to be at a GNP of US$2500: once a country reaches this level obesity 'starts to fuel health inequalities in the developing world' (Monteiro, 2004b, p. 1181).

With regard to the associations between socioeconomic status and breastfeeding, it is generally acknowledged that SES is positively associated with breastfeeding. Women with higher income and education are related to higher breastfeeding rates than their counterparts. It is assumed that SES is positively associated with the likelihood of searching out information about breastfeeding, and higher SES women may thus have more knowledge about the benefits. (Heck et al., 2006).

2.4 Conceptual model

In the conceptual model, as illustrated below, the factors that influence breast milk transfer are shown as well as the variables that are included in this study. In the first box the socioeconomic factors, like education, and demographic factors like age and parity, are mentioned followed by contextual factors in the second box. Whether the mother smokes or not is an important contextual factor to take into account when considering the association between body composition and breast milk output. In the third box the maternal factors, like body composition, height and weight are mentioned. In the last box the child factors are mentioned. Demographic characteristics of children that may influence their breast milk intake are sex, age, feeding pattern (whether the child receives exclusively breastfeeding or receives some complementary feeding on top of that) and their birth weight and weight during the milk intake analysis. The hierarchical structure of the model shows whether the variables can be considered as possible confounders or mediators. When studying the association between the maternal factors and breast milk transfer the factors above the box with maternal factors should be considered for their confounding effect since they are outside the causal chain. Thus, socioeconomic, demographic and contextual factors should be considered as possible confounders. Child factors are situated between maternal factors and breast milk output, meaning that they are *in*side this causal chain and should be considered for their potential mediating effect.

Figure 4. Conceptual model (based on Haisma, 2004).

1. Socioeconomic and demographic factors (*education, age, parity*)
2. Contextual factors (*smoking*)
3. Maternal factors (*body composition, height, weight*)
4. Child factors (*sex, age, feeding pattern, (birth) weight*)

Outcome: Breast milk transfer from mother to baby

2.5 Hypothesis

On the basis of the above mentioned theories, literature review and conceptual model, some hypothesis can be drawn. With regard to the question whether the body composition of the mother has an effect on her breast milk output, the following hypothesis came up:

1. Women with a higher fat mass tend to transfer less breast milk, therefore it can be hypothesized that body composition is *negatively* related to breast milk transfer.

Breast milk output cannot be explained by the mother's body composition alone, and therefore some potential confounding and mediating variables should be taken into account. For these variables the following associations with breast milk transfer are expected:

2. a. Multiparous women tend to transfer more breast milk than primiparous women, thus parity is positively associated with breast milk output;

b. Smoking mothers tend to have a lower breast milk output than non-smoking mothers, thus smoking is negatively associated with breast milk output;
c. Babies who are being exclusively breastfed have higher breast milk intake than babies who are predominantly/partially breastfed
d. With regard to the sex of the infant: male babies have higher breast milk intake than female babies;
e. Weight of the baby is positively associated with breast milk intake, thus heavier babies tend to drink more breast milk.

With regard to the effect of SES on breast milk output, it is hypothesized that women from a lower socioeconomic status have a lower milk output than high SES women. This effect is either caused by the fact that low SES women have a higher risk of obesity or because they have a lower chance of practicing exclusive breastfeeding, This leads to the following hypothesis:

3. a. Mothers from low SES have a higher risk of obesity, which leads to lower milk output;
   b. Mothers from low SES have a lower chance of practicing exclusive breastfeeding, which leads to lower milk output.

Wells (2003) suggested in his life-history theory that prevalence of certain diseases in different parts of the world may affect a mother's maternal capital. Since maternal capital is related to breast milk output, it is assumed that breast milk output and its associations with body composition are different between countries that are in different stages in the nutrition transition.

4. Since the nutrition transition is accompanied by differences in disease patterns between countries that are in different stages in the transition, breast milk output and its association with body composition is different between countries that are in different stages in the nutrition transition.

2.6 Definition of used concepts

The used concepts in this study are defined below:

Body composition: Body composition is the term used to describe the different components that, when taken together, make up a person's body weight. The following concepts of body composition are used in this research:
(Note: for convenience, weight and height are also mentioned as measures for body composition throughout this thesis, even though they are actually anthropometric measures instead of measures for body composition)

BMI *Body Mass Index:* Body mass index (BMI) is a 'simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2)' (WHO, 2012a, p. 3).

The WHO uses the following cut-off points to classify persons:
Underweight: < 18.5 (kg/m^2);
Normal range: 18.5 – 24.99 (kg/m^2);

Overweight: ≥ 25 (kg/m^2);
Obese: ≥ 30 (kg/m^2).

FFM *Fat free mass (or lean mass):* 'The term used in body composition studies to refer to the part of the body that is not fat. FFM includes water, protein, bone minerals and non-bone minerals. FFM contains 73.2% water in healthy adults' (IAEA, 2010, p. 62). FFM is calculated from total body water divided by a hydration coefficient (Haisma, 2003).

FFMI *Fat free mass index* (FFMI) is an index calculated from fat free mass over height squared (Haisma, 2003).

FM *Fat mass:* calculated from body weight minus fat free mass.

FMI *Fat mass index (*FMI) is an index calculated from fat mass over height squared (Haisma, 2003). Together, FFMI and FMI sum up to BMI.

Breast milk transfer: The volume of human milk transferred from mother to baby, either received in the natural way or by expression of the milk with a pump (WHO, 1991).

Feeding pattern: The way children are fed, in particular whether the infant is being breastfed or not. Breastfeeding is defined as 'the child receives breast milk (direct from the breast or expressed)' (WHO, 1991, p. 8), and can be classified in the following three gradations:

EBF *Exclusive breastfeeding* – 'that is, the infant has received only breast milk from his mother, or a wet nurse, or expressed breast milk and no other liquids with the exception of drops or syrups consisting of vitamins , mineral supplements, or medicine' (WHO, 1991, p. 8).

PBF *Predominantly breastfeeding:* 'The infant's predominant source of nourishment has been breast milk. However, the infant may also have received water or water-based drinks (sweetened or flavored water , teas, infusion, etc.); fruit juice; Oral Rehydration Salts (ORS); drop and syrup forms of vitamins, minerals, and medicines; and folk fluids* (in limited quantities). With the exception of fruit juice and sugar-water, no food based fluid is allowed under this definition' (WHO, 1991, p.8).
** Folk fluids refer to liquids that are used for non-nutritional purposes; oil to relieve constipation, tea for relief colic etc.*

PartBF *Partially breastfeeding:'* Giving a baby some breastfeeds, and some artificial feeds, either (cow's or formula) milk or cereal, or other food' (WHO, 1991, p. 8). Throughout this thesis, predominantly breastfeeding and partially breastfeeding are grouped together and referred to as partially breastfeeding.

Socioeconomic status: Socioeconomic status refers to an 'economic and sociological combined measure of a person's work experience, or social and economic position in relation to others' (Adler & Ostrove, 1999, p. 3). This concept of socioeconomic status can be divided in three parts: economic status, social status and work status. The main indicators are income, education, and occupation of the population (Adler et al., 1994).

3. Data & methods

In this chapter the used data and methodology will be presented. In the first section the data source will be described, followed by a description of study design, study population and operationalization of the variables. In the second part of this chapter the methodology will be addressed. The chapter is finalized by appraising the quality of the data.

3.1 Data source

The data used for this research comes from the Milk Intake Meta-analysis (MIMA) database, which was provided by a collaborative research group with researchers from different disciplines and research institutes (RUG/PRC, MRC-Human Nutrition Research in Cambridge, Institute of Child Health, University College London and the University of Brasilia). It is a collection of data from human milk-intake studies from 12 countries from across 5 continents: Bangladesh, Brazil, Chile, Gambia, Kenya, Malawi, Mexico, Papua New Guinea (PNG), Senegal, UK, USA and Zambia. The research group of Coward who developed the deuterium-oxide turnover method in the late 1970s was leading in the world, and through his position in Cambridge and his secondment at the International Atomic Energy Agency in Vienna, he disseminated this method in developing countries. Analysis of the samples was initially mostly done in Cambridge, and so most data were available from within the group. Nevertheless, all authors were contacted for their consent. The database was completed by doing a literature search in PUBMED. The following search terms were used: 'human milk and deuterium oxide', 'breast milk and deuterium oxide' and 'breastfeeding and stable-isotope' (Da Costa et al., 2010). This literature search yielded twenty studies up to 2007, from which 8 were excluded because they used the dose-to-the-infant method instead of the dose-to-the-mother method, studied cattle instead of human milk, or it was a review. Some of the studies were associated with the samples and countries that were already in the database. The two search methods together yielded 16 useful studies to be included in the database (table 1). It includes the vast majority of the available data worldwide using the dose-to-the-mother deuterium oxide turnover method (Da Costa et al., 2010).

Although all the studies included in the database have in common that they use the dose-to-the-mother deuterium oxide method in measuring breast milk transfer from mother the baby, it appears that these studies are different from each other when it comes to their research design (table 1). With respect to the study type, ten studies are cross-sectional and six are longitudinal in nature. In the longitudinal studies, the breast milk transfer was measured more than once. How often these measurements were done ranges from 2 (Mexico, Balaños et al., 2000) up to 11 times, in a period of 2 years (Papua New Guinea, Orr-Ewing et al., 1986). Secondly, the number of cases varies in the 16 included studies. The study by Butte et al. in the USA (1988) included only 9 cases, where the study in Senegal has 129 unique cases. Because of the repeated measurements in longitudinal studies, the PNG study had the highest number of measurements (n=214) for only 23 cases. Thirdly, the age range of the infants under study has to be considered: the age of the infant ranged from 0.4 – 24 months. However, the majority of the sampled infants were between 2 – 4 months old during the study. The data from infants who are >10 months old comes from PNG and Gambia only.

Table 1. Countries and characteristics of the milk intake studies included in the MIMA-database. Source: Da Costa et al., 2010)

Country	Reference	Type of study	Infants, *n*	Age range or age at follow up *(months)*	Measurements, *n*
UK	Laskey et al., 1991	Cross-sectional	30	1-2	30
UK	Jones, 2003	Cross-sectional	50	1-2	50
UK	YWP study (Laskey, unpublished)	Cross-sectional	33	1-2	33
USA	Butte et al., 1988	Cross-sectional	9	1-6	9
Senegal	Cissé et al., 2002	Cross-sectional	129	2-3	129
Bangladesh	Moore et al., 2007	Cross-sectional	94	2-3	94
Kenya	Ettyang et al., 2005	Cross-sectional	10	2-5	10
Brazil	Haisma et al., 2003	Cross-sectional	70	3-4	70
Brazil	Haisma et al., 2006	Cross-sectional	74	7-8	74
Zambia	Owino et al., 2007	Cross-sectional	52	9	52
Mexico	Balaños et al., 2000	Longitudinal	52	0.5, 3	104
UK	Goldberg et al., 1991	Longitudinal	10	1,2,3	29
Chile	Alvear et al., 2004	Longitudinal	29	1,3,6	78
Gambia	Jarjou et al., 2006	Longitudinal	30	4, 12	60
Malawi	Galpin et al., 2007	Longitudinal	42	6-9	79
Papua New Guinea	Orr-Ewing et al., 1986	Longitudinal	23	0.7, 2, 3, 5, 7, 9, 12, 14, 17, 20, 24	214
Total			737		1115

3.2 Study design

This study is quantitative in nature and is done by use of secondary data, using the MIMA database. The present study can be classified as being both explanatory and exploratory. The first two research questions are considered as being explanatory, since these questions are focusing on explaining variations in breast milk output. The third research question, aiming at looking into breast milk transfer at the macro level is more exploratory in nature, since the link between breast milk output and stage in the nutrition transition is not found in the existing body of literature.

3.3 Study population and sampling

For this research, the population under study are mother-infant dyads. Since the research designs of the included studies in the MIMA database vary, participant recruitment is different for the included studies as well. Women were most often recruited at local maternity hospitals. They had to meet certain criteria to be included in the study, which varied between studies. For example, some studies focused on bone change during lactation and therefore mothers were only included when they had no history of bone disease (Laskey et al., 1998, Jarjou et al., 2006). Overall, women were included when they were healthy, were pregnant or gave birth very recently, and when they were willing to give, or were already giving breastfeeding. In most studies only healthy infants were eligible to take part in the study, meaning that they were excluded when they had too low birth weight (<2500 g), or if there was evidence of a chronic disease. Children who were part of multiple gestation were also excluded. In the longitudinal studies, an additional criteria was that the participants should be available for the duration of the study. From the women and infant pairs who met all of these requirements, the studies used random selection to select the participants. The participants are selected from both rural areas (Bangladesh, Gambia, Kenya, Malawi, PNG) and urban areas (Brazil, UK, USA, Zambia).

As mentioned before, the sample size ranges from n=9 (USA) to n=129 (Senegal). In total, information about 737 mother-infant dyads is available in the database, with 1115 valid milk intake measurements. Originally, 1170 measurements were available, but after a critical review of the values 55 cases were excluded because of measurement errors (Da Costa et al., 2010).

3.4 Operationalization of variables

The variables that are used in this study can be classified as being a dependent, independent or control variable:

Dependent variable

The dependent variable in this research is the quantity of breast milk transfer from mother to baby, measured in g/day.

Independent variables

In this research it will be tested whether the quantity of breast milk transfer is dependent on the body composition of the mother. The body composition of a mother can be measured in different ways, however, the best known and mostly used measure is BMI, a measure which is used by the WHO to classify persons as being under- or overweight, or having a normal weight. Although BMI is a measure that can be obtained easily, using this measure has drawbacks since you get high values for BMI when either having a high weight or being of short stature; and it therefore does not necessarily mean that persons with a high BMI have a high fat mass too. Therefore, fat mass and fat free mass are included, both as absolute value (measured in kg) and as a relative value (fat mass index/fat free mass index, measured as kg/m^2 and fat percentage). Height is also considered as an independent variable, even though it has to be considered as an anthropometric measurement instead of being a measure for body composition. It was stated in Wells' life histories theory that maternal capital is expressed by both fat free mass and height and therefore it might be interesting to look at the effect of height as well. Weight, also being an anthropometric measure, is included as well. To test which of these measurements is explaining the most of the variability in breast milk transfer, all of them will be included as independent variables separately.

Control variables

The relationship between maternal body composition and her breast milk output can be affected by socioeconomic and demographic variables of mother and child. The literature study (section 2.3) revealed these variables and on the basis of availability of variables in the database, a selection of control variables is made (table 2). For the mother, the following variables are included as these may influence her breast milk volume: age, parity and whether she is smoking . For the infants, the following variables should be taken into account: age, sex and weight (both at birth and during study). For the second sub-question, socioeconomic status is included by using years of education the mother has had as a proxy. In the third question, the macro level is included. For this, information on the country of residence of the mother-infant dyads is recoded in the countries' stage in the nutrition transition.

Table 2. Operationalization of used variables in research.

Variable	Operationalization in MIMA database	Measurement scale
Dependent variable		
Breast milk transfer	Breast milk intake by the infant	Continuous, in kg/day
Independent variables		
Maternal body composition	Height	Continuous, in cm
	Weight	Continuous, in kg
	Body mass index	Continuous, in kg/m^2
	Fat mass	Continuous, in kg
	Fat mass index	Continuous, in kg/m^2
	Fat free mass	Continuous, in kg
	Fat free mass index	Continuous, in kg/m^2
	Fat percentage	Continuous, in percentages
Control variables - demographic variables		
Age of the mother	Age of the mother at day 0 of milk intake analysis	Continuous, in years
Parity	Number of children the mother had on day 0 of milk intake analysis	Continuous
Smoking	Does the mother smoke?	Binary, 0=no; 1=yes
Age of infant	Age of the infant at day 0 of milk intake analysis	Continuous, in months
Sex	Sex of the infant	Binary, 1=male; 2=female
Birth weight	Weight of infant at birth	Continuous, in kg
Weight during study	Weight of the infant, average of day 0 and day 14	Continuous, in kg
Feeding pattern	Feeding pattern during study	Binary, 1=EBF; 2= Part.BF&Pred.BF
Socio-economic variables		
Educational level	Number of years of education the mother has had	Continuous, in years
Macro level		
Country	Country under study	Categorical, 1=Brazil; 2=UK; 3=Zambia; 4=Senegal; 5=Bangladesh; 6=Gambia; 7=PNG; 8=Malawi; 9=USA; 10=Chile; 11=Kenya; 12=Mexico
Country recoded in: Nutrition transition	Stage in the nutrition transition	Categorical, 1= Third stage 2=Transition stage 3= Fourth stage

3.5 Missing data

Before the data is analyzed, the data is explored by running descriptive statistics for the variables under study. Firstly, it was checked which variables were present in which study, to get an idea of the missing values in the database. This is done by splitting the database by country and running frequencies. The result of this preliminary data exploration is presented in table 3.

Table 3. Review of available variables by country in the MIMA database.

	Country	Brazil	UK	Zambia	Senegal	Bangladesh	Gambia	PNG	Malawi	USA	Chile	Kenya	Mexico	(n)
Variable	Breast milk output	x	x	x	x	x	x	x	x	x	x	x	x	1115
	Maternal height	x	x	x	x	x	x			x		x	x	735
	Maternal weight	x	x	x	x	x	x		x	x	x	x	x	861
	Maternal BMI	x	x	x	x	x	x			x		x	x	727
	Maternal fat mass	x	x	x	x	x			x		x	x		689
	Maternal fat mass index	x	x	x	x	x						x		528
	Maternal FFM	x	x	x	x	x			x			x		620
	Maternal FFMI	x	x	x	x	x						x		534
	Maternal fat percentage	x	x	x	x	x			x			x		649
	Age of the mother	x	x	x	x	x	x			x	x	x	x	809
	Parity	x	x		x	x	x					x	x	610
	Smoking	x	x										x	318
	Education of mother	x	x		x	x								400
	Age infant at start study	x	x	x	x	x	x	x	x	x	x	x	x	1115
	Sex of infant	x	x	x	x	x	x	x	x	x	x	x	x	1106
	Birth weight	x	x	x	x	x		x				x	x	580
	Weight during study	x	x	x	x	x	x	x	x	x	x	x	x	1103
	Feeding pattern	x	x	x	x	x	x		x	x				679

As can be seen from the table, the only two variables with no missing values are breast milk output (dependent variable) and age of the infant at the start of the study. All other variables have missing values; ranging from a negligible 0.8% for sex of the infant, to a substantial percentage of 64.7% for the smoking variable. The studies from Brazil and the UK are the only two studies having information for all the mentioned variables. Studies from Senegal and Bangladesh do miss information on smoking but are nevertheless highly useful. The study from Papua New Guinea did not take maternal body composition into account and was therefore excluded from the further analysis. The total database consists of 901 valid milk-intake measurements after excluding PNG.

To deal with the missing data in the database, it is decided to use a method called complete-case-analysis. In this method, only cases with complete information will be included, meaning that they have information on all the variables of interest. To make the best use of the available data, separate databases for each of the research questions will be used. In table 4, a description of these databases is given. The first research question is divided into three parts, since the smoking variable is only present in two studies. Including this variable restricts the analysis to only 219 cases and by separating the analysis for maternal factors and child factors, the 445 observations for the child factors could be included in the analysis. For the second research question, all cases that had information on the socioeconomic status of the mother were selected. This variable was only present in the studies from Bangladesh, Brazil, Senegal and the UK. Besides, potential explanatory variables had to be present in all cases. The result is a database consisting of 364 cases. The third research question aims at comparing the association between body composition and breast milk output across stages in the nutrition transition. For each case, the country of residence of the mother was known. However, the number of cases (434) was restricted by the fact that all measures of body composition (weight, height, BMI, fat mass, FMI, fat free mass, FFMI, fat percentage) had to be present to be sure that the comparisons are made on the basis of the same cases.

Table 4. Description of database by research question.

Research question		**Variables included**	**(*n*)**	**Countries included**
1 – Association between maternal body composition and breast milk intake	Including maternal factors	Maternal body composition, measures, maternal demographic factors (age, parity, smoking)	219	Brazil, UK
	Including child factors	Maternal body composition measures, child demographic variables (feeding pattern, birth weight)	445	Bangladesh, Brazil, Senegal, UK, Zambia
	Maternal and child factors combined	All variables mentioned above	219	Brazil, UK
2 – Difference in milk intake between low and high SES	Analysis of covariance	SES Potential explanatory variables	364	Bangladesh, Brazil, Senegal, UK
3- Differences in association between body composition and breast milk output across countries	Interaction between body composition and a countries' stage in the NT	Stage in the nutrition transition, maternal body composition measures	434	Bangladesh, Brazil, Kenya, Senegal, UK

3.6 Methodology

Question 1: Testing the association between body composition and breast milk output
Since both the dependent and independent variable are continuous variables, linear regression would be the method of first choice when considering the appropriate statistical technique. However, when linear regression would be performed here, one assumption would be seriously violated: the independency of observations. Since a part of the MIMA-database is longitudinal in nature and consists of repeated measurement for each mother-child pair, some observations cannot be considered as being independent from each other. To take these repeated measurements into account in the analysis, a Generalized Linear Model (GLM) is used. With this method, it is possible to correct for within-cluster correlation (with respondents as clusters, having one or more measurements). By using the option *robust cluster,* standard errors are corrected by cluster. In STATA, these clusters can be specified by including the identification

numbers of the respondent, and therefore statistical analysis for the first research question are done in STATA (version 13) instead of SPSS.

The independent variables for the first research question are various measures for body composition (weight, height, BMI, fat mass, FMI, fat free mass, FFMI and fat percentage). Since these measures are quite similar, the variables will probably take over each other's effect when they are included in the model at once. To test for correlation between the measures, correlations are calculated for those measures. It was found that the variables for measuring body composition are all significantly related to each other. To avoid biased results because of collinearity, the variables will be entered into separate models.

Confounding and mediating variables

The conceptual model (p. 12) is used to differentiate between potential confounding and mediating variables. Confounding variables have to meet the following criteria: they have to be associated with the outcome variable ($p<0.10$), they have to be associated with the exposure variable ($p<0.10$) (here maternal body composition), the variable has to lay *outside* the causal chain, and, most importantly, including the variable in the model must change the association between the exposure and outcome variable with more than 10% (Rothman & Greenland, 1998). Mediating variables have to meet the same criteria as set above, but are situated *inside* the causal chain. Thus, the variables that are higher in the hierarchy than maternal body composition as shown in the conceptual model are considered as potential confounders, where the variables that are shown in between body composition and breast milk output are considered as mediators. This means that smoking, age and parity are tested for their confounding effect, and birth weight and feeding pattern are tested for their mediating effect.

Age and sex of the infant belong to a different category of control variables. In the conceptual model they appear between maternal factors and breast milk output, meaning that they should be considered as potential mediators. However, they cannot be in the causal chain between body composition and breast milk output, since body composition will not affect the sex and age of an infant. However, since they are able to modify to association between body composition and breast milk output they are considered as effect modifiers. The sex of the infant is not found to be associated with breast milk output, meaning that there is no need to control for sex in the following analysis. The age of the infant during the milk intake measurement is an important variable when considering the effect of maternal body composition on breast milk output, since the effect of age on breast milk output is not equal among all age categories: in the first few months of life, the older the baby gets, the more milk it consumes. However, after a certain age (around 6 - 7 months) infants start to receive solid foods and the effect of age on human milk intake then becomes a negative one, with older babies drinking less milk. Since age is significantly related to breast milk output, there has to be controlled for the modifying effect of age in the further analysis; all further analysis are therefore adjusted for infant age.

The weight of the baby during the study was considered as an important control variable, and should therefore be included in the analysis. However, age and weight are significantly related to each other, leading to a bias in the results because of collinearity. Since all cases have information on age, it is decided to keep the 'weight during study' variable out in the further analysis.

Question 2: Testing the effect of socioeconomic status on breast milk output
The second research question aims at investigating whether there is a difference in breast milk transfer between women of high and low socioeconomic status. First, a t-test for independent samples will be conducted to check whether there is a difference in breast milk output between women of high and low SES. When the t-test shows a significant difference between both groups, analysis of covariance will be used to find out what causes this difference. In the database used for this question, no repeated measurements were present, thus it is assumed that the observations are independent from each other.

To define high and low socioeconomic status, the number of years of education the mother has had is taken as a proxy. The cut-off values for high and low SES with respect to the number of years of education were different for the different countries included in the database (Brazil, Bangladesh, Senegal and UK). For Brazil, women with <4 years of education were considered as low SES, and women with >8 years were categorized in the high SES group (Haisma, 2004). It was assumed that these values could also be used for Bangladesh. The Senegalese mothers all had zero or one year of education, and therefore they were all considered as being from low SES. The UK mothers had either 11 years of education or 17 years or higher, and since at least 12 years are necessary to complete the compulsory education, women with 11 years of education are considered as being from low SES.

The hierarchy of the variables used in this study, shown in the conceptual model from chapter 2 (see p. 12), is used as the basis for the statistical analysis. Socioeconomic status is at the top of this model, and therefore contextual factors, maternal factors and child factors should be considered as potential mediators in the association between socioeconomic status and breast milk output. Firstly, to test whether the continuous variables have a mediating effect on breast milk output, Pearson's correlation coefficient is used. For the dichotomous variables, a t-test for independent samples is used to check whether breast milk volume is different between categories. Those variables that are associated with breast milk output at the $p<0.10$ level are then entered in the model of covariance one by one. Instead of the usual significant level of $p<0.05$, a level of $p<0.10$ is used here to be sure that no variables are missed that could explain (part of) the difference in milk output between high and low socioeconomic groups. By running models of covariance with each of these variables, and with SES as fixed factor, it is tested whether the difference in breast milk output between the two SES groups changes by >10% (indicated by a change in the β-level). After this, a model is built with those variables that substantially changed the difference in breast milk volume between the women of high and low socioeconomic status, by making combinations of the explanatory variables. The final model is chosen by the level of the R^2 value; the model with the highest explanatory power is defined as the final model. The analysis as described above will be done in SPSS (version 22).

Question 4: Studying breast milk output on the macro level: including the nutrition transition
The third research question aims at answering the question whether there is a difference in the association between maternal body composition and breast milk output between countries that are at different stages in the nutrition transition. Again, no repeated measures were present in this database. Firstly, the countries have to be assigned to a certain stage in the nutrition transition. Because complete case analysis is used, only five countries were selected in the database that is used for this question (Bangladesh, Brazil., Kenya, Senegal and UK). The following categorization is made of the basis of literature:

Third stage (stage of receding famines):
Bangladesh, Kenya, Senegal (Abrahams et al., 2011).

Transition stage (transition from third towards fourth stage) :
Brazil (Popkin et al., 2012).

Fourth stage (stage of degenerative and nutrition related diseases):
UK (Popkin, 2011).

To test whether there is a difference in milk output between these three groups, an ANOVA test is conducted, with breast milk output being the dependent variable, and stage in the nutrition transition being the comparing factor. Subsequently, to test whether the association between body composition and breast milk output is different for countries in different stages in the nutrition transition, an interaction term between body composition and stage in the nutrition transition is created. Since the 'stage in nutrition transition' variable is divided in three categories, two dummy variables are made, with the fourth stage being the reference category. Then, the interaction terms are included in a linear model in which the main effects (body composition and stage in the NT) are added as well. The obtained parameter values will be exported to excel to calculate the interaction outcomes and to create graphs.

3.7 Reflection on data quality

Before the data were analyzed, it was checked for inconsistent values by running descriptive statistics for all included variables. Data inconsistencies for several cases were found, like negative birth weights, implausible high birth weights, and maternal weights between zero and one. An overview of the found inconsistencies was made and sent to the data organizer of the MIMA research group. Some of the problems were found to be due to wrong variable labels. The low values for maternal weight were a calculation mistake and by multiplying the numbers by 100 the correct values were obtained. Some cases in the database showed a negative fat mass of the mother, which was due to a problem with the determination of the maternal sample. These values were excluded. The incorrect data were corrected before starting the analysis. The second issue regarding the quality of this database is the size of the sample: 901 valid milk intake measurements were included. Even though this number of observations is high when compared to other milk intake studies, the sample size might be too low as they are used to compare populations. Besides, the samples taken from the different countries may not be representative for the populations of that countries, because in all studies samples come from one particular location. This implies that the results should be interpreted with great caution since they may not reflect the real situation in the included countries.

4. Results

In this chapter, the results of the statistical analyses are shown. It starts with a general description of the data to explore the dataset. Section 4.2 aims at answering the first sub-question; here the effect of maternal body composition on breast milk output is tested by means of a generalized linear model. In the subsequent section (4.3) the effect of socioeconomic status of the mother on breast milk output is examined. In the last section (4.3) breast milk transfer is studied on the macro level by exploring whether the effect of body composition on breast milk transfer is equal among countries in different stages of the nutrition transition.

4.1 General description of data

First of all, the data is explored by obtaining descriptive statistics for each of the variables. For the continuous variables, the mean, minimum and maximum value and the standard deviation (SD) are shown in table 5. The table is based on the dataset with the most observations that is used in this study (n=445). Since this is the database used to determine whether the child factors have mediating effects on the association between body composition and breast milk output, there are some missing values for maternal age, parity and years of education the mother has had.

Table 5. Descriptive statistics for continuous variables.

	Mean	**Min.**	**Max.**	**SD***
Milk output (g/day)	793.48	5.07	1670.00	238.37
Maternal weight (kg)	59.37	30.50	110.00	13.34
Maternal height (cm)	158.86	135.00	179.00	8.35
Maternal BMI (kg/m^2)	23.38	15.60	42.62	4.20
Maternal fat mass (kg)	18.17	2.48	53.65	9.05
Maternal fat mass index (kg/m^2)	7.09	0.93	21.03	3.30
Maternal fat-free mass (kg)	41.40	20.69	66.54	6.81
Maternal fat-free mass index (kg/m^2)	16.36	8.84	24.81	2.07
Maternal fat percentage	29.19	4.76	58.11	9.20
Maternal age (years)	28.80	16	47	6.00
Parity	2.55	1	11	1.71
Years of education	6.40	0	20	1.76
Infant age (months)	4.41	1	9	2.55
Birth weight (kg)	3.17	1.48	4.96	0.52

*SD = standard deviation; *n*=445

The average breast milk output is 793 g/day, having a relatively broad range: from a minimum of only 5 g/day to a maximum of 1670 g/day. The weight of the mothers included in the studies ranges from 30 kg to 110 kg. This minimum value for weight is rather low, but is not implausible since the corresponding height for this person is low as well. The range in fat mass of the mother is quite wide as well, with a minimum of 2.5 kg and a maximum around 54 kg. The low values for fat mass (and to some extent for FMI) can be due to some spillage of the deuterium oxide dose given to the mother, leading to an underestimation of her total body water, and by that an overestimation of her fat free mass. The high values for fat free mass (and fat free mass index) can thus be explained through the same mechanism. However, since the values are not outliers (±5 SD) they are kept in the database for further analysis. The mean fat percentage is 29.19%, ranging from a minimum value of 4.76% to a maximum of about 58%. With respect to the demographic characteristics of the mothers that are included in the milk intake studies, it appears that their average age is 28 years, with the youngest mother being 16 and the oldest 47. For some, the current child under study is their first, and have a parity of 1. The maximum number of children the women has had is 11. On average, the parity level for the mothers in the database is 2.55. Their number of years of education ranges from 0 to 20, where the women who had 20 years of education are all from the UK. The average number of years of education is 6.4. The age of the infants under study ranges from 1 month to 9 months. The average age of the infants is around 4.5 months. Their birth weight is quite diverse, with 1.5 kg as being the minimum and about 5 kg being the maximum.

With respect to the categorical variables (results not shown), there are slightly more children who are being partially of predominantly breastfed (53.5% vs 46.5% exclusively breastfed children). Whether the mother smokes or not is known for 319 cases, and only 11% of them does smoke.

4.2 Testing association between body composition and breast milk transfer

The association between body composition and breast milk transfer will be tested by means of three sub-questions. First, this relationship will be tested when taking into account maternal factors to check whether these factors act as confounders in the association between body composition on breast milk output. Next, child factors will be tested for their mediating effect. Finally the maternal and child factors will be included together in the model.

4.2.1 Part I: maternal factors

In the first part the question that will be answered is: What is the association between maternal body composition and milk transfer when taking into account potential confounding maternal factors? To get an idea of the association between body composition and breast milk output without taking into account confounding factors, bivariate analyses are done first. In table 6, the results of these bivariate analyses are shown.

Table 6. Bivariate associations between body composition and breast milk output (adjusted for age of infant during milk intake measurement).

	β	SE (robust)	z-score	p-value
Maternal weight	-3.01	1.45	-2.07*	0.039
Maternal height	1.57	2.45	0.64	0.523
Maternal BMI	-9.58	4.39	-2.18*	0.029
Maternal fat free mass	-1.99	3.49	-0.57	0.569
Maternal FFMI	-9.51	10.48	-0.91	0.364
Maternal fat mass	-4.79	2.05	-2.33*	0.020
Maternal FMI	-13.25	5.82	-2.28*	0.023
Maternal fat percentage	-5.67	2.34	-2.42*	0.015

*significant at the $p<0.05$ level; *n*=219

Table 6 shows that the variables maternal weight, BMI, fat mass, FMI and fat percent are all significantly related to breast milk output at the $p<0.05$ level. As expected, they have a *negative* effect on breast milk output, which means that a higher weight, BMI, fat mass, FMI or fat percentage is related to a reduction in breast milk transfer. The β values can be interpreted as follows: one kg increase in weight results in a decrease in breast milk transfer by 3 g/day; for BMI, one unit (kg/m^2) increase means a decrease of milk output by 9.6 g/day; one kg increase in fat mass reduces the amount of transferred milk by 4.8 g/day; one unit increase in FMI lowers milk volume by 13.25 g/day and for one percentage point increase in fat percentage, milk transfer is decreased by 5.7 g/day. Maternal height, fat free mass and fat mass are not associated with breast milk output, meaning that any increase or decrease for these variables does not lead to changes in breast milk output.

Next, potential confounders will be considered. The results of the bivariate association between age, parity, smoking, and breast milk output is presented in table 7. The table shows that maternal age and parity are not related to breast milk transfer. To check whether these variables could be potential confounders of the association between body composition and breast milk transfer, they are added separately to the multivariate analyses for testing association between body composition and breast milk transfer (see appendix 1 for results). From these tables, it appears that including maternal age and parity in the model, the association between body composition and breast milk output does not result in a >10% change in coefficient for the body composition variables, suggesting that they have no effect on the association between body composition and breast milk output. Therefore, they will not be included in the final model.

Table 7. Bivariate associations between potential confounding variables and breast milk output (adjusted for age of infant during milk intake measurement).

Variable	**β**	**SE (robust)**	**z-score**	**p-value**
Maternal age	0.64	3.039	0.21	0.833
Parity	-4.66	16.60	-0.28	0.779
Smoking (non-smoking=ref.)	-106.63	72.09	-1.48	0.139

n=219

Even though smoking is not related to breast milk transfer as such (p=0.139) in the bivariate analysis, including it in the analyses of the association between body composition and breast milk transfer changes the coefficient of the body composition measures by more than 10% (table 8). When smoking is included in the model, one kg increase in weight results in a reduction in milk transfer of 3.3 g/day, whereas this reduction was 3 g/day when smoking was not in the model. This is also the case for the other body composition variables. Smoking has the strongest effect on the association between fat percentage and milk output: where one percentage point increase in fat percentage resulted in a decrease in milk output of 5.7 g/day in the bivariate model, in the multivariate model with smoking the amount lowers with 7.1 g/day; a decrease of more than 25%. When looking at the effect of smoking itself, smoking is borderline significant in the models with maternal weight and BMI, but becomes significant at the $p<0.05$ level when fat mass, FMI or fat percentage are taken into account. In these models, smoking lowers the breast milk output substantially by 139 g/day, 141 g/day and 156 g/day, respectively.

Table 8. Multivariate associations (GLM) for testing the effect of body composition on breast milk output, including smoking as a potential confounder (adjusted for age of infant).

Variable	β	Robust SE	z-score	p-value
Maternal weight	-3.32	1.39	-2.39*	0.017
Smoking (non-smoking = ref.)	-121.40	69.54	-1.75	0.081
Maternal BMI	-10.61	4.23	-2.51*	0.012
Smoking	-126.81	69.43	-1.83	0.068
Maternal fat mass	-5.56	1.95	-2.86**	0.004
Smoking	-136.37	69.34	-1.97*	0.049
Maternal FMI	-15.56	5.60	-2.78**	0.005
Smoking	-140.86	69.39	-2.03*	0.042
Maternal fat percentage	-7.12	2.25	-3.17**	0.002
Smoking	-156.70	69.39	-2.26*	0.024

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*=219

This table clearly shows that smoking is an important confounder in the association between body composition and breast milk output, in such a way that smoking increases the (negative) effect of body composition on breast milk transfer. Thus, when a mother smokes, an increase in her weight, BMI, fat mass, FMI or fat percentage will result in a greater decrease in the amount of milk transferred than when she would not smoke.

4.2.2 Part II: child factors

After testing the effect of the maternal factors, the effect of child factors (birth weight and feeding pattern) on the association between body composition and breast milk output will be analyzed. Since for this question a database with more observations can be used (n=445), the bivariate analyses for testing the effect of body composition on milk transfer are shown again in table 9. Even though the values for β are somewhat different, the results of the bivariate analyses in this different part of the database show the same pattern as in table 1; maternal weight, BMI, fat mass, FMI and fat percentage are still negatively related to breast milk transfer to a significant extent (p<0.01). The effect on milk transfer ranges from a decrease by 2.5 g/day for one kg increase in weight to a decrease by 12.5 g/day for one unit (kg/m^2) increase in FMI. Maternal height, fat free mass and FFMI are not related to breast milk output, as was also shown before.

Table 9. Bivariate associations between body composition and breast milk output (adjusted for infant age).

Variable	β	Robust SE	z-score	p-value
Maternal weight	-2.43	0.77	-3.16**	0.002
Maternal height	-0.08	1.11	-0.07	0.943
Maternal BMI	-9.19	2.77	-3.32**	0.001
Maternal fat free mass	-1.54	1.57	-0.97	0.333
Maternal FFMI	-5.65	5.69	-0.99	0.321
Maternal fat mass	-4.35	1.22	-3.56**	<0.0005
Maternal FMI	-12.50	3.62	-3.45**	0.001
Maternal fat percentage	-4.16	1.24	-3.35**	0.001

** significant at the p<0.01 level; *n*=445

In table 10 the results of the bivariate analysis for the child factors are shown. Feeding pattern is associated to breast milk output to a significant extent: children who are partially or predominantly breastfed consume 70 g/day less breast milk than children who receive exclusive breastfeeding (p=0.002). Birth weight is not related to breast milk intake (p=0.307).

Table 10. Bivariate analysis for testing effect of potential mediating child factors on breast milk output (age adjusted).

	β	**Robust SE**	**z-score**	**p-value**
Birth weight	22.08617	21.62198	1.02	0.307
Feeding pattern *(EBF = ref.)*	-70.2135	22.93555	-3.06	0.002

n=445

Even though birth weight is not associated with breast milk output in the bivariate analysis, including birth weight in the multivariate models (see appendix 2) changes the effect of body composition on milk output by more than 10%; when looking at weight, one kg increase in weight results in a decrease in breast milk output of 2.4g/day in the bivariate analysis (table 9), where the decrease is 3.4g/day in the multivariate model. In the models for the other four measures of body composition the same effect is shown. The same holds for feeding pattern: although the effect of feeding pattern on the association between body composition and breast milk output is less strong for feeding pattern than it is for birth weight, including feeding pattern increases the effect of body composition on milk transfer by slightly more than 10%. Birth weight and feeding pattern can thus both be considered as important mediators in the association between body composition and breast milk transfer. In table 11 the results of the multivariate analyses with both mediators included together are shown for the five separate body composition models.

Table 11 shows that body composition is again significantly related to breast milk transfer in all five models (p<0.0005). Birth weight is associated with breast milk output as well, even though it was not associated with breast milk output in the bivariate analysis. The association is positive, meaning that a higher birth weight leads to a higher milk output. The effect of birth weight is strongest in the weight model: one kg increase in birth weight leads to an increase in milk output of 60.3 g/day. Feeding pattern is associated with breast milk transfer as well: partially breastfed children receive significantly less milk than those who are exclusively breastfed (with weight, p=0.006, with BMI, fat mass and FMI, p=0.004, with fat percentage, p=0.003). For feeding pattern the effect is strongest in the fat percentage model: here partially breastfed children receive 67 g/day less milk than breastfed infants.

When birth weight and feeding pattern are both included at once, the effect of body composition on breast milk transfer becomes stronger as compared to the bivariate associations between body composition and breast milk transfer (see table 9). Where one kg increase in weight results in a decrease in milk transfer of 2.4 g/day when no mediating factors are included, this reduction is more than 3 g/day when birth weight and feeding pattern both are taken into account (see table 11). The same holds for BMI (from -9.19 g/day to -10.12 g/day), fat mass (from -4.35 to -5.11 g/day), FMI (from -12.50 to -13.8 g/day) and fat percentage (from -4.16 to -4.56). The effect is strongest for weight, where the inclusion of birth weight and feeding pattern lead to a change of 28% in the effect of weight on breast milk transfer.

Table 11. Multivariate analysis (GLM) to test the association between body composition and breast milk output, including potential mediating factors (adjusted for age of the infant).

Variable	β	Robust SE	z-score	p-value
Maternal weight	-3.14	.82	-3.83**	<0.0005
Birth weight	60.27	22.17	2.72**	0.007
Feeding pattern (EBF = ref.)	-61.55	22.45	-2.74**	0.006
Maternal BMI	-10.12	2.82	-3.58**	<0.0005
Birth weight	48.82	20.87	2.34*	0.019
Feeding pattern (EBF = ref.)	-63.55	22.27	-2.85**	0.004
Maternal fat mass	-5.12	1.28	-4.00**	<0.0005
Birth weight	56.60	21.57	2.62**	0.009
Feeding pattern (EBF = ref.)	-63.24	22.23	-2.84**	0.004
Maternal FMI	-13.85	3.70	-3.74**	<0.0005
Birth weight	50.23	21.07	2.38*	0.017
Feeding pattern (EBF = ref.)	-64.65	22.18	-2.91**	0.004
Maternal fat percent	-4.56	1.23	-3.70**	<0.0005
Birth weight	45.89	21.35	2.15*	0.032
Feeding pattern (EBF = ref.)	-67.19	22.33	-3.01*	0.003

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*=445

Thus, birth weight and feeding pattern can both be regarded as mediating factors in the association between maternal body composition and breast milk output. Having a higher weight, BMI, fat mass (index) or fat percentage leads to a lower breast milk output, and this association becomes even stronger through birth weight and feeding pattern – a higher fat mass affects birth weight and feeding pattern to such an extent that breast milk output becomes even lower.

4.2.3 Part III: maternal and child factors combined

The results of the previous analyses have shown that for the maternal factors, smoking is an important confounder in the association between body composition and breast milk transfer, whereas parity and maternal age seem to have no effect on breast milk output itself and on the association between body composition and breast milk output. For the child factors, birth weight and feeding pattern were both found to have a mediating effect on the association between body composition and breast milk transfer. The question then is how these three variables affect the association between body composition and breast milk transfer when adding them to the model at once. These results are shown in table 12.

Table 12. Multivariate analyses for testing effect of body composition on breast milk output, including all confounding and mediating factors identified (adjusted for infant age).

Variable	β	Robust SE	z-score	p-value
Maternal weight	-3.91	1.41	-2.77**	0.006
Smoking (non-smoking = ref.)	-98.05	69.58	-1.41	0.159
Birth weight	86.69	36.48	2.38*	0.017
Feeding pattern (EBF = ref)	-54.47	33.99	-1.60	0.109
Maternal BMI	-10.73	4.261	-2.52**	0.012
Smoking (non-smoking = ref.)	-103.75	69.97	-1.48	0.138
Birth weight	74.05	36.16	2.05*	0.041
Feeding pattern (EBF = ref)	-51.267	33.81	-1.52	0.129
Maternal fat mass	-5.87	1.96	-3.00**	0.003
Smoking (non-smoking = ref.)	-113.67	69.58	-1.63	0.102
Birth weight	79.12	36.44	2.17*	0.030
Feeding pattern (EBF = ref)	-52.53	33.86	-1.55	0.121
Maternal FMI	-15.51	5.58	-2.78**	0.005
Smoking (non-smoking = ref.)	-140.86	69.39	-2.03*	0.042
Birth weight	72.16	36.30	1.99*	0.047
Feeding pattern (EBF = ref)	-51.38	33.84	-1.52	0.129
Maternal fat percentage	-7.029	2.18	-3.22**	0.001
Smoking (non-smoking = ref.)	-132.92	70.08	-1.90	0.058
Birth weight	69.08	37.10	1.86	0.063
Feeding pattern (EBF = ref)	-53.92	34.13	-1.58	0.114

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*= 219

Again, body composition is associated with breast milk output in all five models. Birth weight is significantly related to breast milk input in four of the five models; in the fat percentage model birth weight is no longer related to breast milk transfer (p=0.063). To be sure that this effect is due to the inclusion of smoking, instead of being caused by the use of a different database, the multivariate analysis with child factors (table 11) is repeated with the smaller database (n=219, see appendix 3 for results). In this comparison model, birth weight is significantly associated with breast milk output, even in the fat percentage model. Thus, this change in effect can be attributed to the inclusion of smoking. For feeding pattern the same result is found: whether children are exclusively or partially breastfed is no longer related to more or less breast milk output when smoking is in the model (with ranging significance levels of p=0.109 to p=0.129), where it was borderline significant (p<0.1) in de models without smoking (appendix 3).

Smoking is only related to breast milk transfer in the FMI model. Here, a smoking mother tends to transfer 140 g/day less milk than a non-smoking mother.

When looking at the effect of including smoking, birth weight and feeding at once on the association between body composition and breast milk output, it can be seen from table 12 that including these variables together strengthens the negative association between body composition and breast milk transfer even more than in the separate models for maternal and child factors. The effect of one kg weight gain on breast milk transfer is increased from -3 g/day in the bivariate analyses to around -4g/day in the final model (a decrease of 33%). The same holds for BMI, fat mass, FMI and fat percentage, although to a somewhat smaller extent.

Overall, it can thus be stated that body composition is associated with breast milk transfer, since weight, BMI, fat mass, FMI and fat percentage were all significantly related to breast milk transfer in a negative direction, meaning that having a higher weight or proportion of fat results in a reduction of the amount of milk that is transferred from mother to baby. Smoking is an important confounder, whereas birth weight and feeding pattern are important mediators. Including these variables in the models makes the negative effect of body composition on breast milk transfer even more pronounced. Smoking seems to be most important. It does not only affect the association between body composition and breast milk transfer, including smoking in the overall model also eliminates the significant association between feeding pattern and breast milk output.

Height, fat free mass and fat mass index were not found to be related to breast milk output in the bivariate analyses. Including smoking, birth weight and feeding pattern to these models did not lead to a change in these insignificant associations. Thus, body composition is associated with breast milk output only when considering fat mass, either absolute (fat mass,) or relative (weight, BMI, FMI and fat percentage).

4.3. The effect of SES on breast milk output

In this section the association between socioeconomic status (SES) and breast milk output will be examined. Is there a difference in breast milk output between women from high and low SES? If so, what causes this difference? The results of the analysis of covariance are shown below. It has to be mentioned that the analysis done in this section are based on a different part of database than previous analyses since SES is only available for part of the cases (*n*=364).

4.3.1 Difference between high and low SES

Table 13 shows the difference between high and low SES groups with regard to breast milk output, maternal body composition, and maternal and child demographic characteristics. P-values are based on t-tests for independent samples to examine whether the difference between both groups is significant.

Table 13. Breast milk output and potential explanatory variables by SES.

	Low SES *(n=211)*	**High SES** *(n=153)*	**P-value**
Milk output (g/day)	833.6 (226.9)*	750.4 (237.8)	0.001
Maternal weight (kg)	56.6 (12.3)	60.6 (12.8)	0.003
Maternal height (cm)	158.8 (8.5)	159.0 (7.8)	0.816
Maternal BMI (kg/m^2)	22.3 (4.0)	23.8 (4.1)	0.001
Maternal fat mass (kg)	15.1 (8.2)	20.2 (8.5)	<0.0005
Maternal FMI (kg/m^2)	5.9 (3.1)	7.9 (3.1)	<0.0005
Maternal fat-free mass (kg)	42.6 (7.2)	40.3 (6.1)	0.002
Maternal FFMI (kg/m^2)	16.8 (2.5)	15.8 (1.8)	<0.0005
Maternal fat percentage	25.3 (8.5)	32.2 (8.2)	<0.0005
Maternal age (years)	28.5 (6.5)	28.5 (5.6)	0.992
Parity	3.5 (1.99)	1.9 (1.17)	<0.0005
Age at start study (months)	4.0 (1.9)	4.4 (2.3)	0.048
Birth weight (kg)	3.1 (0.5)	3.2 (0.5)	0.012
Sex ratio (male/female)	105/106	79/74	0.725
Feeding pattern (EBF/non-EBF)	99/112	82/71	0.210

*standard deviation is given between brackets

Interestingly, the milk output by mothers from low socioeconomic status is significantly higher than the output from high SES mothers (low SES, 833.6 g/day; high SES, 750.4 g/day, p=0.001). With respect to the measures of body composition of both groups, there were no significant differences in their height, but low SES mothers had a significant lower weight (low SES, 56.6 kg; high SES 60.6 kg; p=0.003). The BMI of the low SES mothers was also significantly lower than the BMI of the high SES women. The same holds for the variables fat mass, FMI and percentage fat, where low SES mothers have significantly lower values than high SES mothers (p<0.0005). For fat free mass opposite associations were found, Lower SES mothers have a significant higher fat free mass (low SES, 42.6 kg; high SES 40.3 kg; p=0.002). Related to that, low SES mothers have a significantly higher FFMI (low SES, 16.8; high SES, 15.8, p<0.0005).

The average age of the mothers is equal among both groups (28.5 years both). The number of children they have had is significantly lower for the high SES group: these mothers have on average 1.9 children, where the low SES mothers have 3.5 children (p<0.0005). With regard to the child-related variables shown in table 13 the differences between low SES and high SES groups are less pronounced. Sex ratio and feeding pattern are both not significantly different between both groups. There is a small difference in birth weight (3.1. kg for low SES versus 3.2 for high SES, p = 0.012) and in age of the children when the breast milk measurements were done: children from low SES mothers were on average 4 months old, those from high SES 4.4 months (p=0.048).

4.3.2 Correlation with breast milk transfer

The first step in building a model of covariance is to explore bivariate associations between all potential explanatory variables mentioned above and breast milk output. For the continuous variables, the results of the partial correlations (Pearson's r) are shown in table 14. For the categorical variables, a t-test is used to examine whether breast milk transfer is significantly different between the defined categories. The results of t-tests are shown in table 15.

The results of the partial correlations between the continuous variables and breast milk transfer (table 14) show quite similar results to the correlations that were obtained in order to answer the first research question. Maternal height, fat free mass and FFMI are all not related to breast milk output. Weight, BMI, fat mass, FMI and fat percentage are all correlated to breast milk transfer to a negative extent. The variable maternal age shows different results, which is significantly associated with breast milk output (p=0.032), which is in contrast to the previous analysis. Parity and birth weight are both not related to breast milk output (p=0.709, p=0.838).

Table 14. Partial correlation (Pearson's r) of the association of breast milk intake and potential confounding and mediating factors (adjusted for age of infant).

Variable	*r*	p-value
Maternal weight	-0.145**	0.006
Maternal height	-0.015	0.774
Maternal body mass index	-0.147**	0.006
Maternal fat mass	-0.160**	0.002
Maternal FMI	-0.163**	0.002
Maternal fat free mass	-0.015	0.781
Maternal FFMI	0.051	0.336
Mother percentage fat	-0.158**	0.003
Maternal age	-0.113*	0.032
Parity	0.015	0.709
Birth weight	0.011	0.838

* *Correlation is significant on the p<0.05 level, **correlation is significant on the 0.01 level;, n=364*

T*able 15. Associations (t-tests) between categorical variables and breast milk transfer*

Variable	t-test score	p-value
Sex of infant	2.252*	0.025
Feeding pattern	3.078	0.002

* *Correlation is significant on the p<0.05 level, n=364*

Table 15 shows that sex of the infant and feeding pattern are both significantly related to the amount of milk transferred from mother to baby (p=0.025, p=0.002 respectively).

Those continuous and dichotomous variables that are significantly associated with breast milk intake at the $p<0.01$ level are now entered in the model of covariance one by one.

4.3.3 Building a model of covariance

In the model of covariance (table 16) the effect of the included variables on the difference in milk output between the groups of high and low SES is shown. When no potentially explanatory variables are included, the difference in milk output between high and low SES is around 70 g/day. Since high SES is the reference, this means that low SES mothers give on average 70 g/day more milk than high SES mothers. Including weight as a potential explanatory variable lowers this difference with almost 10 g/day. For BMI the difference decreases even more: including this variable lowers the difference between groups with 13 g/day. When fat mass is included, the difference in milk intake reduces from 70 g/day to 50 g/day. Maternal FMI shows a somewhat smaller decrease: including these variables decreases the difference between groups to 52 g/day. The largest decrease is seen when fat percentage is included: milk intake from lower SES is then only 46 g/day higher for low SES mothers as compared to high SES mothers.

The other potential explanatory variables that are included (maternal age, sex of infant and feeding pattern) are not changing the difference between low and high SES mothers by more than 10%. When maternal age is included, the difference decreases with less than 1 g/day, which is not enough to be considered as an explanatory variable. The changes in β level of the SES variable when sex and feeding pattern are included are too low as well. Differences in maternal age, infant sex and feeding pattern between high and low socioeconomic status are too low (as already indicated in table 13)and cannot account for the difference in milk output between low and high SES mothers.

Normally, variables that are shown to decrease the difference between groups by more than 10%, are then entered in the model in combinations with each other to examine which of the combinations explains the difference between the two groups best. However, the only variables that meet this condition are body composition measures and it doesn't make sense to include them together in a model. Thus, from the models presented in table 16, the final model has to be chosen. When looking at the values of R^2 it seems that maternal weight explains the most difference in milk output between low and high SES best. When including weight, 11.5% of the difference between groups is explained: low SES mothers have a higher milk output because of their lower weight as compared to high SES women. For the other measures of body composition, this explanatory power ranges from 10.9% (for BMI) to 11.2% (fat mass). However, since the difference in milk transfer between SES groups is still significant in the weight model ($p=0.011$), not all of the difference in milk transfer between high and low SES mothers is explained by the lower weight of the low SES mothers. Other factors are important when aiming to explain the effect of SES. What these factors are, cannot be revealed by this research, since all the other potential explanatory variables are either not related to breast milk output, or including them in the model of covariance does not result in a >10% change in the difference in milk output between high and low SES groups.

Table 16. Analysis of covariance of the association between breast milk transfer and SES (adjusted for age of infant).

Variable	**High-low SES (B)**	**95% CI**		**p-value**	**R^2**
		Lower bound	**Upper bound**		
SES (high = ref)	69.707	22.81	116,60	0.004	0.101
SES Maternal weight	61.08 -2.23	13.95 -4.056	108.21 -0.39	0.011 0.017	0.115
SES Maternal BMI	57.64 -6.91	9.84 -12.73	105.44 -1.10	0.018 0.020	0.109
SES Maternal fat mass	50.19 -3.36	1.12 -6.15	99.27 -0.57	0.045 0.018	0.112
SES Maternal FMI	52.34 -9.05	3.10 -16.62	101.57 -1.47	0.037 0.019	0.111
SES Maternal fat percentage	46.42 -3.01	-4.41 -5.79	97.25 -0.23	0.073 0.034	0.110
SES Maternal age	68.94 -4.15	22.14 -7.92	115.74 -0.39	0.004 0.031	0.112
SES Sex of the infant (male = ref.)	70.95 -49.46	24.26 -95.37	117.64 -3.58	0.003 0.035	0.112
SES Feeding pattern (EBF = ref.)	74.56 -50.41	27.65 -98.15	121.47 -2.67	0.002 0.039	0.112

n = 364

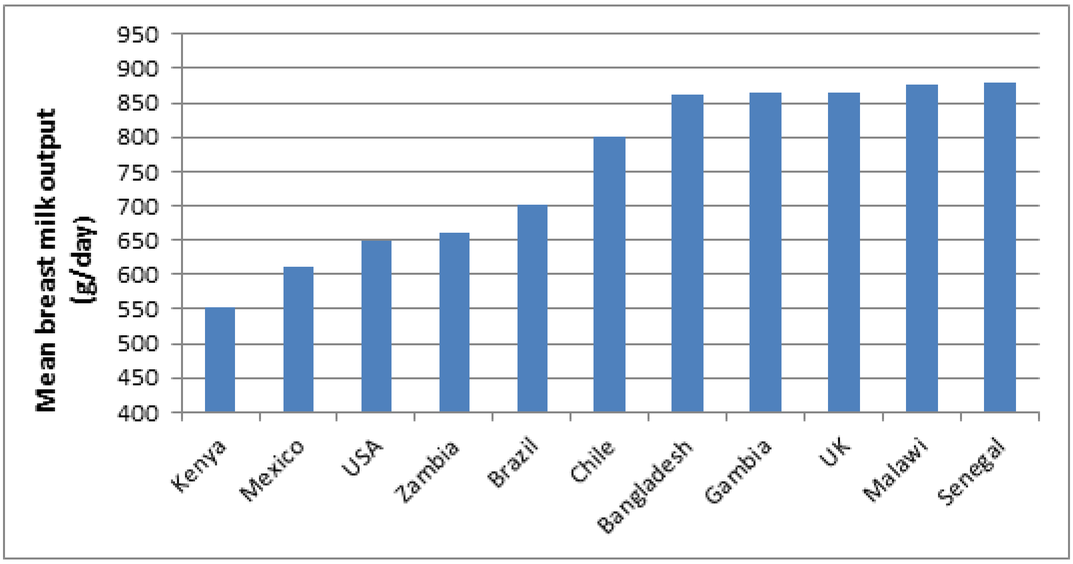
It would be interesting to test for the effect of smoking on the diverse models presented in table 16, since prevalence of smoking is significantly higher in the low SES group (36,4% vs. 7.2% in high SES, p<0.0005). Besides, smoking is significantly related to breast milk output (p=0.022). However, since the data availability for smoking is limited, a different dataset is needed to study the effect of smoking here (n=169). In this smaller dataset there is no significant difference in milk output between the groups of high and low SES (p=0.257). Thus, including smoking in a model where there is no difference between groups in the first place would not make sense at all.

4.4. Including the country level: the effect of a country's stage in the nutrition transition on breast milk output

In this section breast milk output is studied on a macro level. It will be questioned whether a country's stage in the nutrition transition is related to the amount of breast milk that is transferred. Furthermore it is analyzed whether there is a difference in the association between maternal body composition and breast milk transfer between countries that are at different stages in the nutrition transition.

Figure 5 shows the mean output (g/day) per country, to get an idea of the diversity in breast milk output. Kenya appears to have the lowest breast milk transfer: 552 g/day, whereas Senegal shows to have the highest breast milk output (878 g/day).

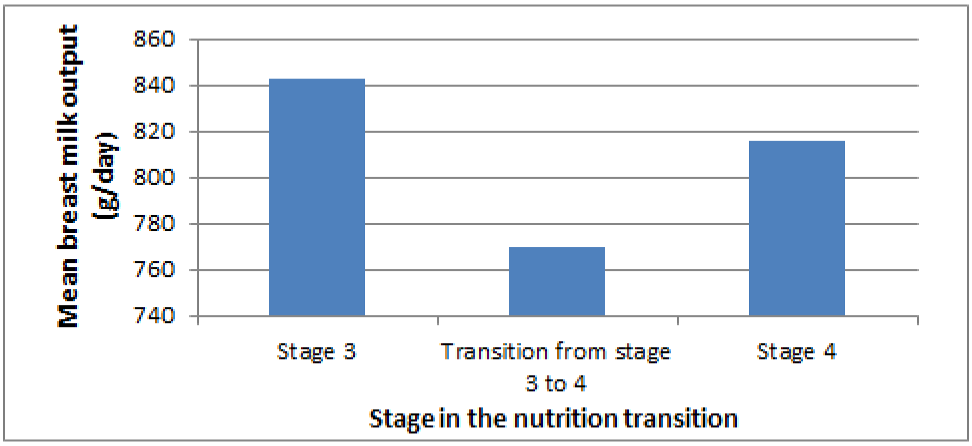
Figure 5. Mean breast milk output (g/day) by country (adjusted for age of infants) (n=901).



Note: the graph is not adjusted for feeding pattern.

Although figure 5 gives a first idea of the differences between countries with respect to the mean breast milk output, the results may be more insightful when the countries are grouped according to their stage in the nutrition transition, since the stage in the nutrition transition gives a better indication of the context wherein the mother lives; countries that are in different stages in this transition are characterized by differences in nutritional status of the population, diets and disease patterns. In figure 6 the mean outputs by stage in the nutrition transition are shown. The highest amount of breast milk transfer is seen in the third stage (stage of receding famines) (836 g/day), which is close to the result for fourth stage countries (stage of degenerative diseases) (823 g/day). In transition (from third stage to fourth stage) countries, the breast milk output is about 100 g/day lower. The results of the ANOVA test show that the milk output in transition countries is significantly lower than in both third stage and fourth stage countries ($p<0.0005$).

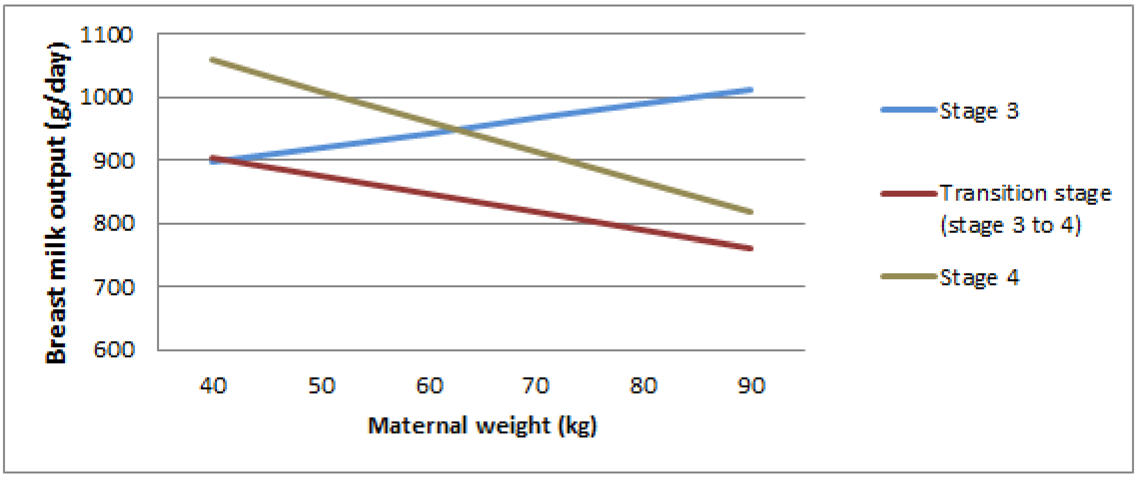
Figure 6. Mean breast milk output (g/day) by stage in the nutrition transition (adjusted for infant age). (n=434)



Body composition was found to be significantly related to breast milk output in the previous analyses, and it might be interesting to check whether the effect of body composition is the same for each of the defined stages. In the figures 4-8 the interaction effect of body composition by stage in the nutrition transition on breast milk transfer is shown for each of the body composition measures separately (weight, BMI, fat mass, FMI and fat percentage respectively). These five figures all show a similar pattern: the effect of weight, BMI, fat mass, FMI and fat percentage on breast milk output is negative for countries who are currently in the fourth stage of the nutrition transition, or who are moving from the third stage into the fourth stage; for countries in the third stage, however, this effect is the opposite: increasing weight, BMI, fat mass or FMI result in more breast milk output.

When looking at the graph for maternal weight (fig. 7), a clear cross-over interaction between the lines representing third stage and fourth stage countries can be noticed. The significance levels (table 16) of the interaction model tells us that the association between weight on breast milk output in third stage countries is significantly different from this association in fourth stage countries (p=0.016). The association between weight and breast milk output in transition countries shows a similar downward pattern as fourth stage countries. Transition countries and fourth stage countries are not significantly different from each other with regard to the association between weight and breast milk output.

Figure 7. Association between maternal weight (kg) and breast milk output by stage in the nutrition transition (adjusted for infant age).



Similar patterns can be observed from the figures 8-10: the effects of BMI, fat mass and FMI on breast milk transfer are significantly different for third stage countries than for fourth stage countries (BMI, p=0.004; fat mass, p=0.015; FMI, p=0.019), in such a way that for third stage countries increasing levels of BMI, fat mass or FMI area associated with more breast milk output, where in stage four countries increasing levels of these body composition measures are related to a lower breast milk output. Again, there is no significant difference in the effect of BMI, fat mass or FMI on milk output between transition countries and fourth stage countries (BMI, p=0.249; fat mass, p=0.225; FMI, p=0.129). The figure for fat percentage (fig. 11) shows a somewhat different picture: the effect of fat percentage on breast milk output in third stage countries is declining slowly, meaning that a higher fat percentage does not lead to more milk output, but to a small decrease. However, the interaction is still found to be significant (p=0.022), meaning that the effect of fat percentage in third stage countries is still different from the effect shown in fourth stage countries.

Figure 8. Association between maternal BMI (kg/m²) and breast milk output by stage in the nutrition transition(adjusted for infant age).

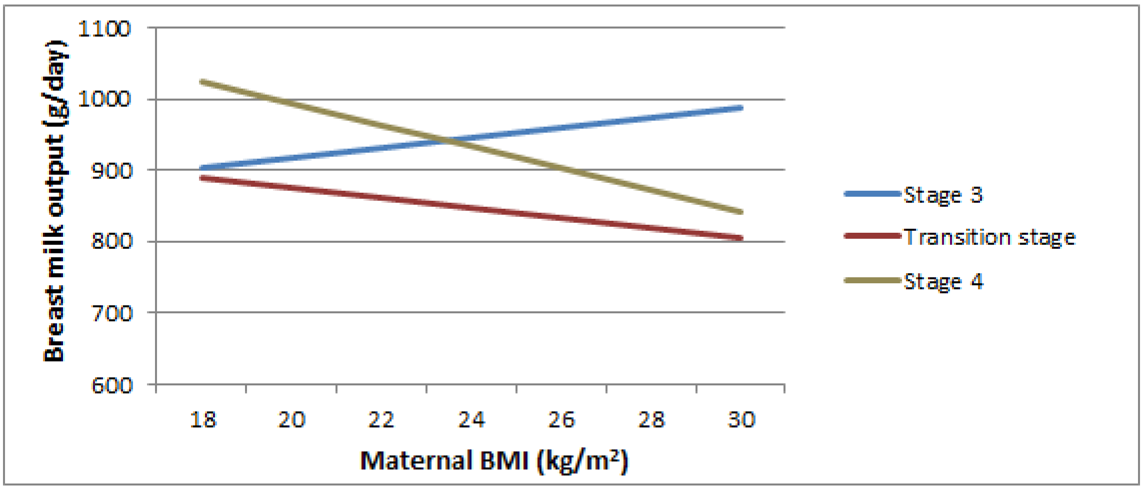


Figure 9. Association between maternal fat mass and breast milk output by stage in the nutrition transition (adjusted for infant age).

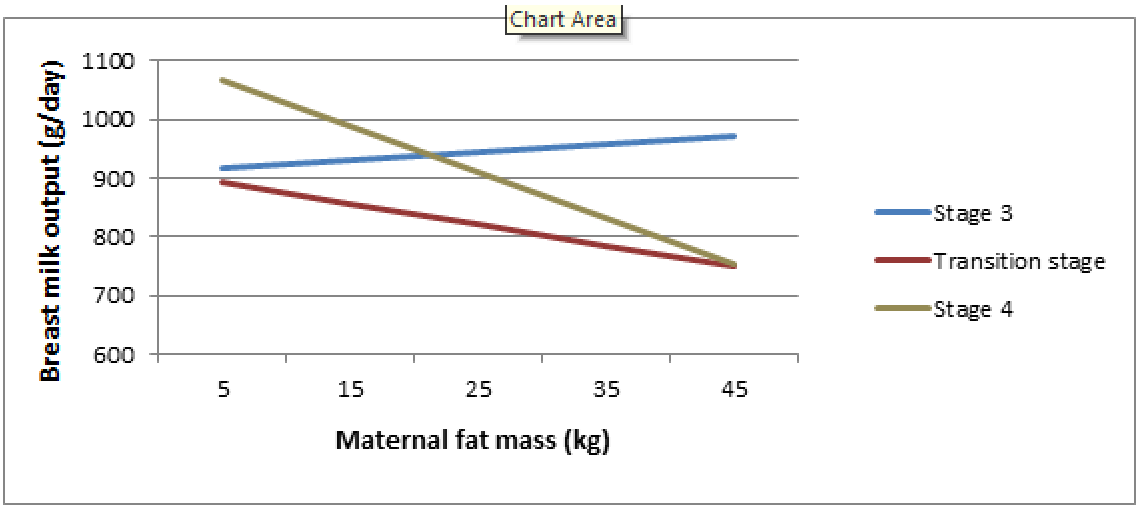


Figure 10. Association between maternal FMI (kg/m²) and breast milk output by stage in the nutrition transition (adjusted for infant age).

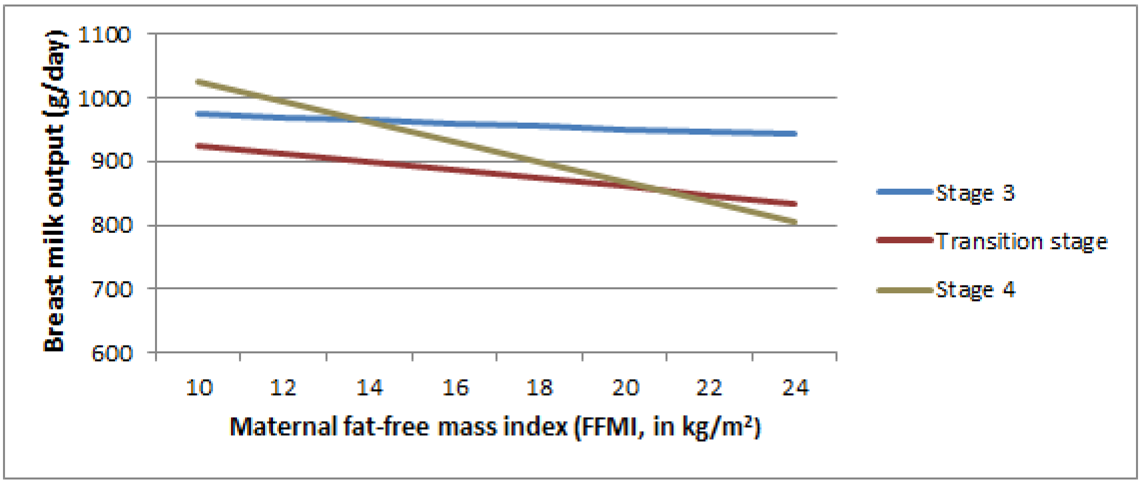
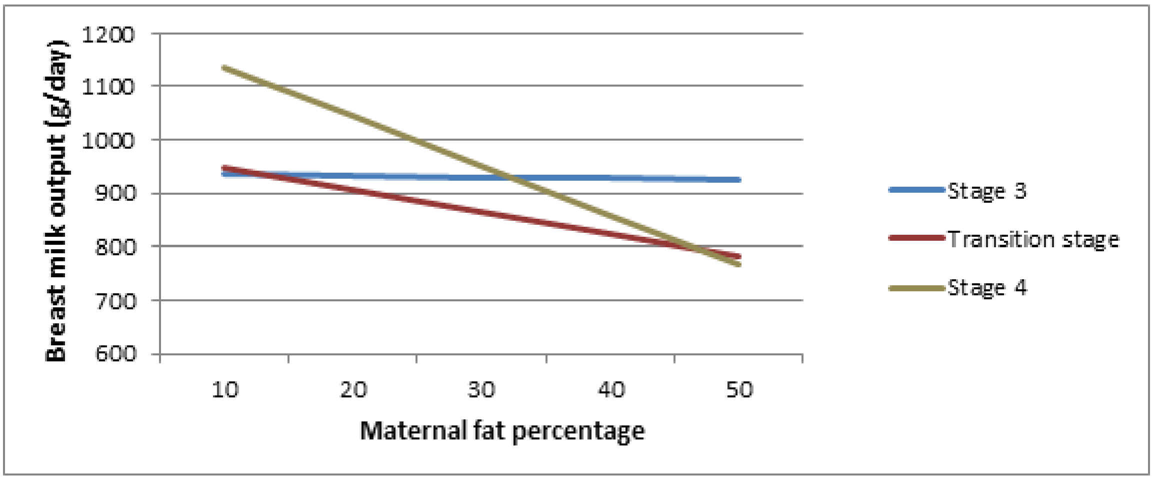


Figure 11. Association between maternal fat percentage and breast milk output by stage in the nutrition transition (adjusted for infant age).



In the previous analyses from section 4.2 and 4.3, maternal height, fat free mass and fat free mass index were not found to be associated with breast milk transfer, neither in the bivariate analysis nor when potential confounders and mediators were included. However, since the graphs above have shown that the effect of body composition on breast milk output might be different among countries that have or have not gone through the nutrition transition, it might be interesting to see whether this is also the case when maternal height, fat free mass or fat free mass index are taken into account. The results of the interactions are shown in figures 12-14, separate for height, fat free mass and fat free mass index. The graphs show a much less consistent pattern than was seen with the fat mass figures, since height and fat free mass are not associated with breast milk output in the first place (height, p=0.699; fat free mass, p=0.404; FFMI, p=0.275)(table 16). Additionally, the interaction parameters show that there is no clear interaction effect between both height and stage in the nutrition, and fat free mass and stage in the nutrition transition.

Figure 12. *Association between maternal height (cm) and breast milk output by stage in the nutrition transition (adjusted for infant age).*

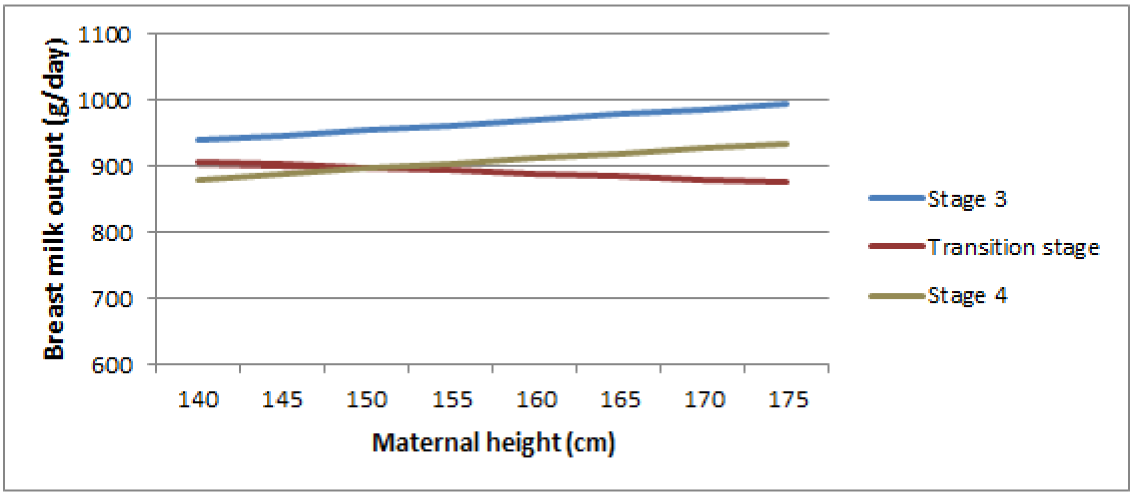


Figure 13. *Association between maternal fat free mass (kg) and breast milk output by stage in the nutrition transition (adjusted for infant age).*

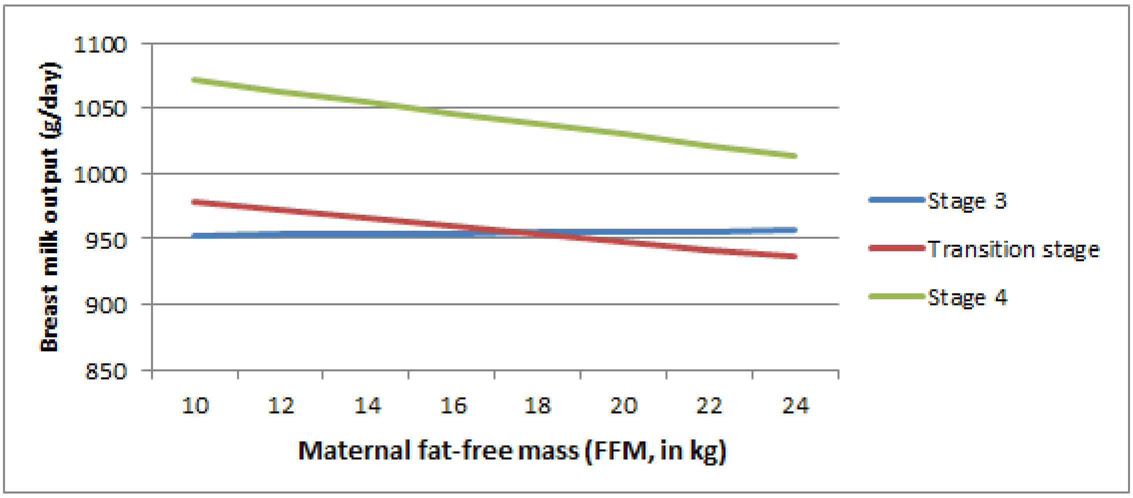


Figure 14. *Association between maternal FFMI (kg/m²) and breast milk output by stage in the nutrition transition (adjusted for infant age).*

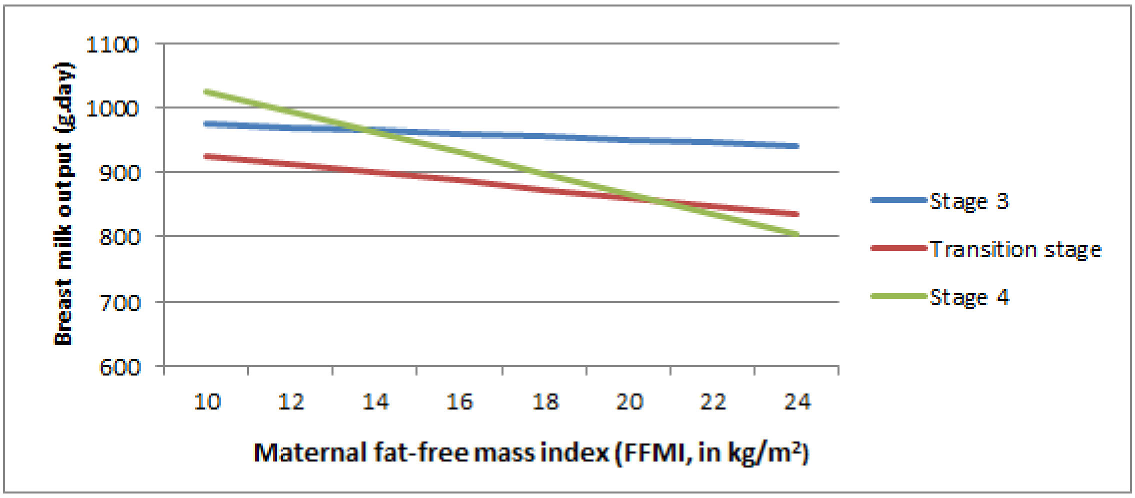


Table 16. Parameter values of interaction between body composition measures and stage in the nutrition transition (adjusted for infant age).

Variable	β	Std. error	t	p-value
Weight	-4.83	1.997	-2.42*	0.016
Third stage	-442.56	166.284	-2.66**	0.008
Transition stage	-234.18	183.086	-1.28	0.202
Weight x third stage	7.07	2.503	2.82**	0.005
Weight x transition stage	1.99	2.594	0.77	0.443
(fourth stage = ref.)				
BMI	-15.16	5.71	-2.65**	0.008
Third stage	-519.21	186.85	-2.78**	0.006
Transition stage	-282.72	186.32	-1.52	0.130
BMI x third stage	22.15	7.72	2.87**	0.004
BMI x transition stage	8.18	7.09	1.154	0.249
Fat mass	-7.84	2.74	-2.860**	0.004
Third stage	-195.64	83.31	-2.348*	0.019
Transition stage	-184.80	96.66	-1.912	0.057
Fat mass x third stage	9.22	3.7	2.450*	0.015
Fat mass x transition stage	4.24	3.490	1.216	0.225
FMI	-23.26	7.75	-3.00**	0.003
Third stage	-196.86	85.27	-2.31*	0.021
Transition stage	-199.90	97.38	-2.05*	0.041
FMI x third stage	24.34	10.34	2.36*	0.019
FMI x transition stage	14.43	9.48	1.52	0.129
Fat percentage	-9.26	3.37	-2.74**	0.006
Third stage	-288.03	129.00	-2.23*	0.026
Transition stage	-240.88	148.26	-1.63	0.105
Fat % x third stage	8.91	3.88	2.30*	0.022
Fat % x transition stage	5.14	4.05	1.27	0.205
Height	1.55	4.00	0.39	0.699
Third stage	52.14	720.50	0.07	0.942
Transition stage	363.62	831.05	0.44	0.662
Height x third stage	0.04	4.37	0.01	0.992
Height x transition stage	-2.41	5,07	-0.48	0.634
Fat free mass	-4.19	5.01	-0.84	0.404
Third stage	-163.14	246.14	-0.66	0.508
Transition stage	-105.95	272.69	-0.39	0.698
FFM x third stage	4.44	5.41	0.82	0.412
FFM x transition stage	1.22	6.07	0.20	0.841
FFMI	-15.69	14.34	-1,09	0.275
Third stage	-184.44	265.17	-0.70	0.487
Transition stage	-189.75	280.57	-0.68	0.499
FFMI x third stage	13.43	15.94	0.84	0.400
FFMI x transition stage	9.15	16.86	0.54	0.588

* *Correlation is significant on the p<0.05 level,* ***Correlation is significant on the p<0.01 level, n=434*

4. Results

The results of this third research question show that the effect of body composition on breast milk transfer is not equal among the three defined stages of the nutrition transition for weight, BMI, fat mass or FMI. Where body composition is negatively related to breast milk transfer in countries in transition or countries that finished this transition, increasing weight (or BMI, fat mass and FMI) results in an increase in breast milk transfer for countries that are have not yet undergone the transition. Shifting development with related shifting patterns in nutrition and disease has not only implications for breast milk transfer as such, but also has an effect on the underlying mechanisms that lead to variations in breast milk output.

5. Discussion

This study aimed at gaining insight in the maternal factors that determine the quantity of breast milk transfer from mother to baby. With this objective in mind, the following three sub-questions were formulated: 1. What is the association between maternal body composition and milk transfer when taking into account potential confounding and mediating variables?; 2. Is there a difference in milk transfer between mothers of high and low socioeconomic status? And if so, what explains the difference in breast milk output between the groups of high and low socioeconomic position?; 3. Is there a difference in the association between maternal body composition and breast milk output between countries that are at different stages in the nutrition transition?

It was found that body composition was negatively associated with breast milk transfer only when a measure of fat mass was taken into account. For height and fat free mass, no association was found. With regard to potential confounders and mediators, smoking was found to be an important confounder and birth weight and feeding pattern were both mediators in the association between body composition and breast milk transfer. Secondly, it was found that the socioeconomic status of the mother was associated with breast milk output as well, with low SES mothers having a significant higher breast milk output than high SES mothers. Thirdly, interactions between body composition and stage in the nutrition transition showed that the association between body composition on breast milk output was significantly different for third stage countries, as compared to transition or fourth stage countries.

With regard to the first research question, the results showed that maternal weight, BMI and fat mass, FMI and fat percentage were all negatively associated with breast milk output, whereas fat free mass was not associated with breast milk output. The possibility of reverse causality, when a lower breast milk output would lead to a higher fat mass was considered, but because of the limited time frame in which breastfeeding takes place this would probably not be the case. The association between body composition and breast milk output have been studied in different contexts and the results of these studies are in line with the present findings (e.g. Villalpando et al., 1992, Ettyang et al., 2005). Several explanations for the found association exist. Rasmussen (2007) proposed that having a high fat mass before or during pregnancy results in impaired mammary gland development, which in turn leads to delayed lactogenesis II. Having low milk output at the very beginning of giving breastfeeding may cause early cessation of breastfeeding, since mothers may perceive their milk output as being too low for the infant to meet its energy requirements. Being overweight after pregnancy may lead to hormonal abnormalities, leading to low milk volume as well (Rasmussen, 2007). This was also mentioned by Akre (1989, in: Amir & Donath, 2007), who found that adipose tissue (fat mass) acts as a reservoir for certain hormones (progesterone), leading to higher progesterone levels in obese and overweight women. For lactogenesis II to start, progesterone levels have to drop, but the high levels of this hormone in women with a high fat mass, disrupts this process of milk production, leading to a lower breast milk output. According to Hauff et al. (2014) the explanation for the association between fat mass and breast milk output may be sought in psychosocial factors instead of biological factors as described above. They found that women with a higher pre-pregnancy BMI are, despite their intentions, associated with poor breast feeding outcomes because they have a lower confidence in successful breast feeding. However, not all studies found the inverse association between fat mass and milk output. In the study among mothers from rural northern Thailand, a significant association between the nutritional status of the mother and her breast milk output was absent (Imong et al., 1989). This may be due to the fact that the variation in BMI values were too low to find an association, however, it was also suggested that the malnourished mothers that were studied may indicate that lactation may be resistant to poor maternal nutrition, which is found by others as well (WHO, 1985, in: Imong et al., 1989, Prentice, 1983, in: Imong et al., 1989)

Smoking was found to be an important confounder, strengthening the negative association between body composition and breast milk output. Since lower SES women are more likely to smoke than higher SES women, it is possible that the smoking variable acts as a proxy for measuring SES instead of measuring the effect of smoking versus non-smoking. To test this hypothesis, the education variable was added to the smoking models. Since the parameter values for smoking do not change when adding the education variable as proxy for SES, it can be assumed that smoking does not measure SES, but indeed measures the effect smoking. Thus, smoking has an effect on breast milk output, independent of SES**.** The central importance of smoking in breast milk transfer is highlighted in several studies (Vio et al., 1992, Mennela et al., 2007). According to Hopkinson (1992, in: Mennella, 2007), the negative association of smoking on breast transfer is partly due to the lower prolactin levels that are present in smoking women during pregnancy. These hormones are necessary for milk production to start. Additionally to the effect of smoking on breast milk volume, smoking is also associated with earlier weaning (Mennella et al., 2007), suggesting that smokers tend to have a certain lifestyle in which the practice of breastfeeding does not fit. Hopkinson et al. (1992, in Mennella et al., 2007) also found that mothers who smoke are less likely to breastfeed, regardless of their social group or educational level.

While smoking was found to be a confounder, birth weight and feeding pattern had a mediating effect. According to Rasmussen (2007), mothers with a high fat mass have a higher chance of getting a large baby, leading to problems with positioning the baby and latching on, which then leads to a lower breast milk output. However, in this study it was found that birth weight is positively associated with breast milk output, meaning that a higher birth weight leads to more breast milk output. Since maternal fat mass is not found to be associated with birth weight in previous studies (Farah et al., 2011, Kent et al., 2013), the explanations behind the mediating effect of birth weight on the association between fat mass and breast milk output remains unclear. The mediating effect of feeding pattern can be explained by the fact that overweight and obese mothers are more likely to partially breastfeed than to practice exclusive breastfeeding, resulting in a lower breast milk output (Hauff et al., 2014). With regard to the birth weight of the infants, it has to be mentioned that some of the infants had a low birth weight of less than 2500g. Where most studies included in the database only included children with a normal birth weight (>2500g), this was not done in the study from Bangladesh. These infants (20) may be either premature or small for gestational age and it could be speculated that for these infants, different associations with breast milk output arise. To test this speculation, the analyses that included birth weight were repeated, excluding the cases with low birth weight. It turned out that excluding those cases did not lead to different results and thus, including the low birth weight cases did not bias the results.

The second research question aimed at answering the question whether there is a difference in milk output between women from high and low socioeconomic status. Women from low SES had a significant higher breast milk output, which could be partly explained by their weight, with low SES mothers having a significantly lower weight than high SES women. However, after including weight as an explanatory variable, the difference in milk output between the two groups was still significant, meaning that not all of the differences could be explained by weight alone. Even though the effect of SES on the practice of giving breast feeding in general is thoroughly studied, the link of SES with the amount of milk that is transferred to the infant has not yet been elucidated. Studies including SES focus for example on the lower breast feeding rates among lower SES women (Heck et al., 2006) and the shorter duration of practice breast feeding among those lower SES subjects (Flacking, 2007). These studies have in common that they associate low SES with lower breastfeeding success. The findings of the present study however, show a more positive picture, with lower SES mothers transferring significantly more breast milk to their infants.

In the third sub-question, the association between body composition and breast milk output is studied on the macro level, aiming at exploring whether this association differs between countries which are at different stages in the nutrition transition. Even though this question might be more exploratory in nature, the analyses provided promising results. Women from countries in transition appeared to have a significant lower breast milk output than women from third stage (stage of receding famines) or fourth stage (stage of degenerative diseases) countries. Interactions between body composition and the country's stage in the nutrition transition revealed that the association between body composition and breast milk output does not show a similar pattern among countries that are at different stages in the nutrition transition. For the third stage countries included in this study the association between body composition and breast milk output was positive, meaning that a higher weight, BMI, fat mass or fat mass index resulted in a higher breast milk output. For transition and fourth stage countries, however, this association was negative. An increasing weight, BMI, fat mass or fat mass index resulted in a decreasing milk output. When interactions were added to the models for height and fat free mass, third stage countries did not differ significantly from transition and fourth stage countries on the association between body composition and breast milk output.

However, it can be questioned to what extent this finding can be generalized, since the third stage and fourth stage are based on one country each (Brazil as country in transition and UK as fourth stage country). Besides, rural and urban areas within the same country might be at different stages in the nutrition transition, as mentioned by Abrahams et al. (2011). By allocating countries to one certain stage, this potential diversity within a country is being ignored. Further research into the underlying mechanisms that causes a difference in the association between fat mass and breast milk output for third stage countries is necessary, since the present study lacks the necessary explanatory variables for all included countries. Both physiological and socio-cultural factors should be included. Economic variables, like GDP, could be included as well to test if they contribute to the difference breast milk output and differences in the association between fat mass and breast milk output.

The results as described above confirm the proposed hypothesis partially. As was expected, maternal fat mass is negatively related to breast milk output, that is, increasing fat mass results in decreasing breast milk transfer. Various body composition measures were tested for their effect on breast milk output and it turned out that weight, BMI, fat mass, fat mass index and fat percentage are all negatively related to breast milk. Fat mass, and not fat free mass, is thus related to breast milk output and explanations for this might be found in physiological factors (hormone levels) or in more psychosocial factors (breastfeeding behavior). For smoking, the hypothesis can be confirmed as well. In the model where smoking was found to be related to breast milk output, it was shown that smoking can lower breast milk transfer to a substantial amount of around 140 g/day. Smoking is negatively related to breast milk output either because of the deteriorating effect of nicotine, or because of life style-related factors. The hypothesis with regard to the effect of birth weight and feeding pattern on breast milk output are supported by this study too: a higher birth weight is associated with a higher breast milk output. Since the model is adjusted for the age of the infant at time of the milk intake measurement this means that even when there is controlled for the current age of the infants (and thus weight in a sense, since these two variables are highly collinear) their birth weight is still an important predictor for milk output. As expected, exclusively breastfed babies do consume more human milk than partially breastfed babies. The fourth hypothesis was confirmed as well: the association between body composition and breast milk output is indeed different between countries that are in different stages of the nutrition transition, as was assumed on the basis of the life-history theory. However, the present study could not reveal the underlying mechanisms for this difference since explanatory variables were missing.

For sex and parity, associations with breast milk output were not found. With regard to parity, it was expected that having more children would lead to more breast milk output, either because of biological factors, or because multiparous women might have more experience in giving breastfeeding. Ingram et al. (1999) found that parity is no longer associated with breast milk output from four weeks after giving birth onwards, since infant-related factors, like the infants weight at that time is gaining influence. Since the majority of infants included in the MIMA database were older than four weeks when their milk intake was measured, this could be a valid explanation for the fact that an association between parity and breast milk output is not found in this dataset. Unfortunately, the effect of the weight of the infant during study could not be taken into account in the analyses, since collinearity would bias the results when age and weight were included together.

Contrary to the expectations, lower SES mothers had a higher breast milk output than high SES mothers, while it was hypothesized that low SES mothers would have a lower milk output since they are associated with a higher risk of obesity. However, it turned out that the opposite held true , since higher breast milk output in the group of low SES could partly be explained by the lower weight of the women in this group. A potentially explanation of this opposed finding is the fact that the assumption of women from lower SES being more prone to overweight or obesity, even in developing countries, is mainly based on findings from lower-middle-income countries (Monteiro, 2004b). For low income countries, this shift in patterns of overweight and obesity towards the poor might not have taken place yet. Since a large part of mothers in the low SES group came from a low income country (Senegal) this might explain the lower weight in the lower SES group.

According to the parent-offspring conflict theory of Wells (2003) mothers have to make choices with regard to the feeding of their infants. The extent to which the mother is willing to invest in new offspring depends, amongst other things, on the number of babies she already had and whether there is conflict between older siblings and the new offspring. Thus, parity seems to be an important factor in explaining variations in breast milk output. In this study however, parity is not related to breast milk output. The breastfeeding behavior of the mother is also a reflection of her environment in addition to parity; household characteristics, like how many people care for how many young. and whether caretakers are involved in other activities than running the household have to be considered when studying the breastfeeding behavior of the mother. The only household characteristic that is available in the database is information on whether the mother works out of the house or not. Running a t-test shows that milk output does not differ significantly between mothers who work outside the house and those who do not. Thus, whether the mother works out of the house is not related to breast milk transfer, at least not in the Brazilian context. Since the work variable was only available in the study from Brazil, this variable is not included in previous analysis. The results found here may therefore not me representative for all the included cases.

In addition, the biological history of a population has to be taken into account when feeding practices are studied. In the life-history theory of Wells (2010) it is proposed that the prevalence of certain diseases in different parts of the world leads to variations in immune systems and nutritional status of the population. These factors have an effect on the resources that are available to a mother which enables her to give breastfeeding to her infant, known as maternal somatic capital. According to Wells (2010) this maternal capital is reflected in, amongst others things, a mother's height and lean mass. These two variables were tested for their effect on breast milk output, but were not found to be associated with breast milk output.

Even though the concept of maternal capital does not seem to affect breast milk intake as expected, the biological life history theory may be used in finding an explanation for the fact that the association between fat mass and breast milk is a positive one in third stage countries, where this association is negative for transition stage and fourth stage countries. In the third stage of the transition, underweight is more prevalent in society, and any increase in a person's weight or fat mass from underweight to normal weight may lead to an increase in their breast milk output. In transition and fourth stage countries, overweight and obesity becomes more widespread, and nutrition related diseases are no longer restricted to the richer part of society but shift towards the poor as well. Increasing fat mass may lead to a lower breast milk output in this context. The different circumstances in which mothers live thus might lead to differences in their metabolic system resulting in the deviant association between body composition and breast milk output for mothers residing in third stage countries. However, this outcome results from a very preliminary study and further research is needed to search for a more exact explanation why this inverse association does exist.

Doing research with the use of database that combines data from various studies has both its strengths and limitations. In this case, the database gave the opportunity to study breast milk transfer among mothers from different parts of the world, and thus, with different biological histories. Since data came from different countries, breast milk transfer could be studied on the macro level by referring to the country's stage in the nutrition stage, giving a new dimension to the existing body of research in breast feeding studies.

However, using a database that contains data from different sources also has its limitations. The main problem that arose during the analysis was the fact that not all variables that could potentially explain the variation in breast milk output were included in each of the studies that were part of the database. Particularly, the lack of data on smoking imposed restrictions on the analysis. From the first research question, smoking turned out to be an important confounder in the association between body composition and breast milk output. It would therefore be interesting to include smoking in the analysis of covariance, but because of the limited presence of a smoking variable restricted the possibility to assess whether smoking could explain (part of) the difference between high and low SES. The same goes for the third sub-question: since smoking is only included in the studies from Brazil and UK, data on smoking for women from third stage countries is missing. Since some studies lack information on certain variables, the missing observations are not completely missing at random but are missing by country. Thus, by using complete-case analysis certain countries were excluded when they lacked information on the included variables. In the second research question for example, it was found that low SES women had a significant higher breast milk output than high SES women. This finding was extracted from a database including data from Brazil, Bangladesh, Senegal and UK, but when the effect of smoking was taken into account, data from Senegal and the UK had to be excluded completely. Since many of the low SES mothers came from Senegal, the association between SES and breast milk output changed completely when this country was excluded. Now breast milk output was not significantly different between women from high and low SES. This example shows that complete-case analysis has drawbacks, and may lead to biased results.

For this database, a linear mixed model would be the method of first choice. By using linear mixed models, analyses can be controlled for both the dependency of the repeated measurements as well as the effect of clustered data on the country level. A multiple imputation technique could then be used to deal with the missing data. However, because of the complexity of this method and because of time constraints, linear mixed models and multiple imputation were not used in this study. Instead, generalized linear models were used to be able to control for the dependency of the repeated measurements by correcting the standard error for the clustering around individuals with more than one measurement. Even though there was controlled for dependency of the measurements, this methods disregards the potential effect of clustering on the country level. To deal with the missing data, it was decided to use a method called complete case analysis, where different databases for different questions are used. For this method no missing cases for each of the included variables are allowed, thus different databases were used for different sub-questions to make the best use of the available data. The main implication of using this method is that for these different databases results might turn out differently. This is also the case in the present study. In the model based on 219 cases, in which maternal factors were tested for their confounding effect, maternal age had no effect on breast milk output. However, in the analysis regarding the second sub-question consisting of 364 cases, maternal age did have a significant association with breast milk output.

5.1 Conclusion

On the basis of the findings as described above the following conclusions can be made; 1. Maternal body composition is related to breast milk output, in such a way that a higher fat mass leads to lower breast milk output. For smoking mothers this association is even stronger. Birth weight and exclusive breastfeeding are important mediators and do also reinforce this association; 2. Mothers from low SES tend to transfer more breast milk to their infants than women from high SES, which can be partly explained by the lower weight of low SES mothers. 3. The association between body composition and breast milk output is different for countries that are not yet undergoing the nutrition transition, than the countries that are currently in the transition, or have already experienced the transition from high prevalence of malnutrition and stunting towards increasing prevalence of obesity and diet related non-communicable diseases.

These findings give insight in the complex processes that underlie breast milk transfer and contribute to the existing knowledge by providing a more context-related perspective, both by including socioeconomic status, and by studying breast milk output on the macro level by taking the stage in the nutrition transition into account. Even though this research has shown that context-related factors are important to take into account when studying breast milk transfer, indicated by the significant effect of smoking and socioeconomic status on breast milk transfer, contextual factors cannot explain variations in breast milk transfer alone, since many of the underlying processes are biological in nature, as was elaborated on in the discussion. Studying breast milk output at the macro level by including the nutrition transition revealed interesting results although the concept of the nutrition transition is more descriptive than explanatory. With the life-history theory, a theory from evolutionary biology, a part of the differences in associations may be explained. Thus, context is important to acknowledge in breast milk transfer studies, but their effect should not be considered as being completely separate from biological factors.

5.2 Recommendations for further research

Although this study reported some interesting findings about the maternal factors that could play a role in breast milk output, further research is needed to reveal the complete process of breast milk transfer from mother to baby. The database, with information on breast milk output and related factors from 12 countries has high potential for explaining variations in breast milk transfer. To make the best use of the available data, a linear mixed model is recommended to be able to reflect the hierarchical basis of the data, with multiple milk output measures per mother, and mothers being clustered in countries. Furthermore, the database could be expanded with studies from different countries to be able to test whether the found interactions between body composition and stage in the nutrition will persist if more countries are included. Additional data from Colombia, UK and Iceland are already available and will be added to the current database.

When new studies are done in the field of breast milk transfer it is recommended to include contextual factors in addition to biological factors, to get the best understanding of the causes of variations in breast milk output. The number of years of education the mother has had should be included to be able to get a measure of her socioeconomic status. Smoking was shown to be an important confounder and should thus be included in future studies. More environmental factors, like crowding and the work status of the mother could be taken into account to gain knowledge about the underlying processes leading to variations in breast milk output.

5.3 Policy recommendations

Recently, the central importance of the nutritional status of the mother in improving infant and young child feeding has gained attention. It became apparent that a child is completely dependent on the mother in the most important and vulnerable period of its life – from conception to 6 months of age (in the ideal case of practicing exclusive breastfeeding) (Mason et al., 2014). In the WHO 'Comprehensive implementation plan on infant and young child nutrition' (WHO, 2012b) maternal nutrition is set as one of the key priorities, and this gave rise to various programs and policies to protect women's nutritional status in low- and middle income countries. However, these programs have the primary aim to reduce malnutrition, and their interventions therefore focus on the supplementation of micronutrients and providing balanced protein energy supplements. For low income countries this approach might be appropriate, since in these areas malnutrition is widespread and still of major concern. However, for middle income countries the rate of overweight and obese women is rising, even among the poor.

5. Discussion

Thus, even though maternal nutritional status has begun to be highlighted in policies and interventions directed at infant health, their focus on malnutrition of mothers disregards the fact that overweight or obesity might have negative consequences for breast milk transfer too, as was revealed in this study. The link between increasing maternal fat mass and its negative effect on breast milk output is not made in any policy. This study has also revealed the importance of smoking in breast milk transfer. Smoking is no longer contraindicated with breastfeeding, as is mentioned in breastfeeding policies from 2008 (Bogen, 2008), meaning that mothers can give breastfeeding when they smoke. It is assumed that the positive effect of receiving breast milk outweigh the negative effects of nicotine exposure. On the contrary, since it is known that smoking reduces milk volume, women are encouraged to stop or reduce smoking in other policies. Smoking policies are thus not that straightforward when it comes to encouraging or discouraging breastfeeding.

Given the pivotal role of breastfeeding in infant health, the rising prevalence of smoking among women, and the fact that overweight and obesity become more dominant among women in their reproductive ages all over the world (Rasmussen, 2007), it is clear that interventions are needed to address these challenges.

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APPENDICES

Appendix 1

Multivariate tests (GLM) for testing association between body composition and breast milk output, including maternal factors (age and parity)
(adjusted for infant age)

Maternal age

	β	Robust SE	z-score	p-value
Maternal weight	-3.16	1.48	-2.13*	0.033
Maternal age	1.82	3.12	0.58	0.560

*significant at the p<0.05 level; *n*=219

	β	Robust SE	z-score	p-value
Maternal BMI	-10.09	4.46	-2.26*	0.024
Maternal age	2.08	3.13	0.66	0.506

*significant at the p<0.05 level; *n*=219

	β	Robust SE	z-score	p-value
Maternal fat mass	-5.0	2.07	-2.42*	0.015
Maternal age	1.96	3.07	0.64	0.523

*significant at the p<0.05 level; *n*=219

	β	Robust SE	z-score	p-value
Maternal FMI	-13.90	5.85	-2.38*	0.018
Maternal age	2.08	3.07	0.68	0.498

*significant at the p<0.05 level; *n*=219

	β	Robust SE	z-score	p-value
Maternal fat percentage	-5.921	2.34	-2.53*	0.011
Maternal age	2.02	3.01	0.67	0.502

*significant at the p<0.05 level; *n*=219

Parity

	β	Robust SE	z-score	p-value
Maternal weight	-3.10	1.45	-2.13*	0.033
Parity	3.38	16.92	0.20	0.842

*significant at the $p<0.05$ level; *n*=219

	β	Robust SE	z-score	p-value
Maternal BMI	-10.24	4.53	-2.26*	0.024
Parity	7.13	17.39	0.41	0.682

*significant at the $p<0.05$ level; *n*=219

	β	Robust SE	z-score	p-value
Maternal fat mass	-4.98	2.036	-2.45*	0.014
Parity	4.96	17.04	0.29	0.771

*significant at the $p<0.05$ level; *n*=219

	β	Robust SE	z-score	p-value
Maternal FMI	-14.00	5.84	-2.40*	0.017
Parity	6.68	17.30	0.39	0.699

*significant at the $p<0.05$ level; *n*=219

	β	Robust SE	z-score	p-value
Maternal fat percentage	-5.83	2.35	-2.48*	0.013
Parity	4.13	17.19	0.24	0.810

*significant at the $p<0.05$ level; *n*=219

Appendix 2

Multivariate tests (GLM) for association between body composition and milk intake, taking into account child factors
(adjusted for age of the infant at milk intake measurement)

Birth weight

	β	Robust SE	z-score	p-value
Maternal weight	-3.44	.83	-4.15**	<0.0005
Birth weight	60.00	22.59	2.66**	0.008

** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal BMI	-10.82	2.88	-3.75**	<0.0005
Birth weight	46.67	21.29	2.19*	0.028

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal fat mass	-5.44	1.30	-4.18**	<0.0005
Birth weight	54.83	22.06	2.49*	0.013

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal FMI	-14.59	3.78	-3.86**	<0.0005
Birth weight	47.72	21.58	2.21*	0.027

*significant at the p<0.05 level; ** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal fat percentage	-4.76	1.26	-3.77**	<0.0005
Birth weight	42.79	21.95	1.95	0.051

** significant at the p<0.01 level; *n*= 445

Feeding pattern

	β	Robust SE	z-score	p-value
Maternal weight	-2.12	.76	-2.80**	0.005
Feeding pattern (EBF = ref.)	-61.27	22.67	-2.70**	0.007

** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal BMI	-8.47	2.71	-3.11**	0.002
Feeding pattern (EBF = ref.)	-61.58	22.41	-2.75**	0.006

** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal fat mass	-4.00	1.20	-3.34**	0.001
Feeding pattern (EBF = ref.)	-61.42	22.42	-2.74**	0.006

** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal FMI	-13.68	3.54	-3.30**	0.001
Feeding pattern (EBF = ref.)	-62.32	22.33	-2.79**	0.005

** significant at the p<0.01 level; *n*=445

	β	Robust SE	z-score	p-value
Maternal fat percentage	-4.93	1.21	-3.24**	0.001
Feeding pattern (EBF = ref.)	-64.61	22.45	-2.88**	0.004

** significant at the p<0.01 level; *n*=445

Appendix 3

Multivariate analyses to test associationbetween body composition and breast milk output, inclusing potential mediating child factors, using a smaller database (n=219)
(For comparison with table 12, p. 30)

Variable	β	Robust SE	z-score	p-value
Maternal weight	-3.67	1.478	-2.49*	0.013
Birth weight	90.65	36.59	2.48*	0.013
Feeding pattern (EBF = ref)	-63.10	34.61	-1.82	0.068
Maternal BMI	-9.85	4.41	-2.24*	0.025
Birth weight	78.85	36.34	2.17*	0.030
Feeding pattern (EBF = ref)	-60.74	34.42	-1.76	0.078
Maternal fat mass	-5.23	2.05	-2.55*	0.011
Birth weight	83.56	36.69	2.28*	0.023
Feeding pattern (EBF = ref)	-62.84	34.60	-1.82	0.069
Maternal FMI	-13.53	5.76	-2.35*	0.019
Birth weight	77.40	36.64	2.11*	0.035
Feeding pattern (EBF = ref)	-62.26	34.58	-1.80	0.072
Maternal fat percentage	-5.80	2.24	-2.58*	0.010
Birth weight	75.12	37.53	2.00*	0.045
Feeding pattern (EBF = ref)	-65.77	34.86	-1.89	0.059

*significant at the $p<0.05$ level; *n*=219