Initiation and establishment of lactation in women who have delivered pre-term infants: Changes in milk composition and the fortification of mother's milk for pre-term infants.

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I would also like to thank you for inviting me to speak at the Japan Academy of Neo-Natal Nursing Congress. It's indeed an honor. Today I want to talk to you about the initiation of the establishment of lactation in women who delivered pre-term infants as well as women who delivered term infants. And also look at the changes in milk composition and the fortification of mother's milk for pre-term infants.

History of breastfeeding in Australia

But first I wanted to mention that I started off at the University of Sydney and then traveled across Australia to the University of Western Australia in Perth. Traveling across to Perth through the central part of Australia, the roads are very long and very straight. This particular section of road is straight for 160 km. So there's not one bend in the road. I traveled across from Sydney to Perth in 1972. Perth is on the Indian Ocean and if we look from the center of Perth down the Swan River we can see the University of Western Australia and the King Edward Memorial Hospital for Women, where I also work, is situated about 10 minutes drive from the University of Western. The department of bio-chemistry is on the fore shore of the Swan River situated near the middle of on the campus of the University of Western Australia. King Edward Memorial Hospital for Women is the only tertiary care hospital in Western Australia. So all the high risk pregnancies give birth in this hospital. In fact, 95% of all the high risk pregnancies are born in this hospital and the hospital has about 1000 pre-term births a year.

Women travel very great distances to this hospital. If a woman has a high-risk pregnancy in Kununurra in Western Australia, she has to travel a distance equivalent to traveling from Norway to Spain to get to King Edward Memorial Hospital. The Australian aboriginals were the first inhabitants of Australia and Donald Thompson, the anthropologist who studied the aboriginals up at Cape York in 1928, Arnhem Land in Northern Australia in 1935 and in the Great Sandy Desert in Western Australia in 1957. Donald Thompson commented that the aboriginal babies in the Great Sandy Desert were some of the most obese babies he had ever seen and these babies were fully breastfeed. This is an extremely arid environment and we would have great difficulty surviving for more than five days in this area but the aboriginal women obviously produced plenty of milk for their babies under these harsh conditions

The obese babies didn't grow into obese adults. In fact the adults had a very good stature. So why were the babies obese? Well, up north in the desert, the nights are very cold and they needed the heat of their mother to keep them warm and perhaps also the high level of body fat to insulate their bodies against the cold nights. So co-sleeping (the mother sleeping with her baby) was very important for the aboriginal mothers and babies.

So James McKenna in the USA has found also that co-sleeping in western societies is very important and close sleeping together with breast feeding integrated, mutually reinforcing mechanism.

The aboriginal mothers breast feed their children until they were 6 years old and it was rare to see more

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than one baby under three years of age in a camp. This was because the long period of breastfeeding, suppressed fertility in the aboriginal mothers. The aboriginal children learned from osmosis learning, that is, by mimicking the adult behavior. So they learned their dances and their hunting skills by following and imitating what their parents or their grandparents were doing.

In the ILCA Conference in Sydney in 2003. Aboriginal dancers opened the Conference and a little girl was on stage learning the dance by trying to do what the adults were doing (she was osmosis learning). Towards the end of the dance the lactation consultants were invited on stage and they did some osmosis learning as well as they danced with the aboriginal mothers. The aboriginal girls learn to breast feed in a similar way. Thompson observed "little mothers [young girls], each with a mud baby and clay breasts hung from the neck at play after the arrival of a new baby in the camp". So the aboriginal girls learn to breast feed at quite a young age.

In Australia we had specially selected emigrants from Britain who came to Australia in 1788 and set up a penal colony as convicts. All states of Australia were first settle by convicts, with the exception of South Australia when free settlers settled Adelaide. Because of the long voyage from Europe free emigrants had to have very clean conditions on the ship and go food or they died. So the free emigrants coming to Adelaide learned good hygiene and about good food,

But at that time there were high death rates of babies in the summer in Australia when they were feed anything other than breast milk. So the government recruited the women who had learned hygiene and food on their boats coming out to Australia to set up child health nurses to advise mothers on how to care for their babies. They recommended breastfeeding until 10 to 12 months and then weaning gradually. But if the breast feeding period fell at the height of summer, then weaning could be delayed for a month or two and then the baby weaned slowly. So at this timed it was recommended that babies could be fully breastfed up to almost 12 months of age. But of course at this time, the infant formula began to replace breastfeeding. So we had the pocket wet nurse (the baby feeding bottle) replacing breastfeeding and breastfeeding rates declined during the middle of the last century in Australia, as in the USA and in Europe.

In Australia, the Australian Breast Feeding Association commenced in 1964 with 6 women starting the association with the idea of helping other mother' s breastfeed and now there are 1400 Australian Breast Feeding Association (ABA) counselors who are assisting mothers to breast feed their babies. A mother to mother help system. As the result of the formation of this association, breastfeeding rates increased and now in Perth, 93% of mothers begin to breastfeed and approximately 65 - 70% of these mothers are still breastfeeding at six months. In addition to the ABA counselors, there are more than 1800 lactation consultants in Australia; almost 1 lactation consultant for every 140 babies born in Australia.

The change that took place in 1972 was very interesting because up until that time, women who were of high socio-economic status employed wet nurses to breastfeed their babies. So it was very rare for women of high status in Europe to feed their babies themselves. With the increase of breastfeeding in 1972, it was women of high socio- economic status who were the successful breast feeders. So it was a complete turn around from the poor mothers who were the successfully breastfeeding 100 years ago to the women of high socio-economic status who are now successfully breastfeeding their babies. So we need to remember that these women have only been successful breast feeders for a little over one generation.

We have a breastfeeding challenge for Japan. Australian Breast Feeding Association had a challenge of having the most number of mother's together breastfeeding their babies at the one time. In 2001 in Western Australia, we had 438 mothers all breastfeeding their babies at the one time but Sydney had about 100 more. In 2003 Adelaide had 750 mothers together at the one time, breastfeeding their babies and currently this still stands as the record. So the challenge for Japan is to have more than 750 mothers breast feeding at the one time.

Importance of Breastfeeding

The Barker hypothesis states that at baby is programmed for life, that is, how the fetus is nourished in the womb during fetal life is related to diseases that people get in old age. This hypothesis evolved from studies that were carried on in Preston in the UK where they had good records of the babies at birth some 70 years ago and then they were able to relate birth characteristics to health outcomes of people as they became older. While fetal nourishment is important, the nourishment of the baby immediately after birth is also very important.

In Australia we have the dietary guidelines for Australian and one of the guidelines is to encourage and support breastfeeding **(http://www.nhmrc.gov.au/publications/synopses/dietsyn.htm)**. Australia, as far as I'm aware, is the only country that includes breastfeeding in their dietary guidelines. Yet, of course breastfeeding is the first food that babies should be given.

Heinz Ltd make an infant formula in Australia and they've written a newsletter where they compare feeding infant formula to feeding human milk to infants and they state if you feed infant formula, the babies are 2.8 times more likely to get necrotizing endrocolitus, 14 times more likely to get diarrhea, 3.2 times more likely to get septosemia and so it goes on. Even diseases of young adults are more likely to occur if they were fed formula when they were babies. So, this is an infant formula company stating that feeding formula is a significant health risk.

If you look at the composition of human milk is of a completely different composition to cows milk or wolf milk. So each milk is designed for that species young. Of course you might say from knowledge of the legion of Romulus and Remus that wolf's milk is suitable for babies, however, in fact the legion is probably incorrect. The Latin word for she wolf is Lupra and Lupra is also the term used for a female prostitute. So it's much more probably that they were fed by a prostitute rather than by a wolf.

Breast Growth and Development during Pregnancy

The human breast develops extensively during puberty and this is unusual as in most other mammals as there's not a lot of breast growth at puberty, so women tend to be different in this respect. But breast growth also occurs during pregnancy. So we have mammogenesis, that is, growth of the breast secretory during pregnancy. In addition, lactogenesis I, the development of the synthesis of specific milk components such as casein, lactose and alpha lactalbumin, also occurs during pregnancy. So, mammogenisis is the growth of the mammary secretury tissue. Lactogenisis I is the differentiation of the lactocytes (mammary secretory epithelial cells) into functional cells, the lactocytes that synthesize the unique milk constituents such as lactose.

Hytten in 1954 tried to measure the growth of the breast during pregnancy by water displacement. He put a dome over the breast and filled it with water to find out how much of the volume of the dome was displaced by the breast. He was able to show that the breast grew during pregnancy, but that it was quite variable between mothers. We were interested in measuring the volume of mother's breasts and in Western Australia we have a lot of minerals and they measure the volume of the mineral stockpiles by stereo photography. We thought that my work for measuring breast volume. So we used two cameras to take stereo images of the mothers breasts and we could actually measure their volume. But then we developed what we called a computer breast measurement (CBM) system that worked a little differently, it projected strips onto the mother's breast and we looked at this distortion of the curvature of the strips. Once we have the image of the strips on the breast we can compute a digitized image of the breast and then calculate the volume of the mother's breast.

We then showed that if we measured the amount of milk that was removed from the breast by the baby, it was closely related to the decrease of the volume of the mother's breast. So there's a very good relationship between amount of milk removed by the baby and the decrease in the volume of the mothers breast measured by the CBM. So we can see the breast decreasing volume at a breastfeed, then increasing volume as more

milk was synthesized in-between breastfeeds and then decreasing again at the next breastfeed. So, in this case we could show that the mother was making 14 ml of milk per hour in-between the breastfeeds. In addition, we could also measure the amount of milk that was in the mother's breast. We used the computerized breast measurement system to measure the short-term rates of milk synthesis as well as the storage capacity of the breast. We can measure how full the breast is at any time and we can measure the relative breast growth.

It has been shown in rats, that estrogen, progesterone and growth hormone and adrenal steroids to get duct growth in the mammary gland and lactogenesis I. Full milk secretion is achieved by removing the estrogen and progesterone by retaining prolactin and the adrenal steroids. Although it is impossible to do these types of studies in women, we can look at the differences in the size of the breast from pre-conception through pregnancy.

Following mothers from pre-conception through their pregnancies, we can show the differences of the rates of growth of the mother's breast. Some mother's breast grew early pregnancy and then stopped growing. Other mother's breast grew gradually throughout pregnancy and some mothers have very little growth throughout pregnancy. So there's very big variation between mothers. If we try to relate that to the hormones that are involved, we find that the increase in the size of the breast growth is related to the human placental lactogen in the blood. So the women with higher levels of placental lactogen show higher breast growth.

To look at the increase in the function of the breast, we look at the 24-hour output of lactose in urine during pregnancy. If lactose is being made in the breast, it can be absorbed into the blood and since it not metabolized in the blood, it is excreted in the urine. So when we get an increase in lactose in the urine, we know there been an increase in the synthesis of lactose in the breast. Again, we can see big variation between mothers in lactose output in their urine. A mother, who had very little breast growth, also had very low lactose in her urine.

When we compared these changes to the changes in hormones in the mother's blood we found that the increase in lactose in urine was related to the levels of prolactin in the mother's blood. So lactogenisis 1, the functional capacity of the breast, was related to increases in prolactin in mother's blood. So in summary for a pregnancy, breast growth or mammomegenisis was related to placental lacotgen in the blood whereas the development of breast function (lactogenesis I) was related to prolactin in the blood.

Lactogenesis 2 and the initiation of lactation after birth

Lactogenesis 2 occurs around the time of parturition. Lactogenesis 2 is the initiation of copious milk production, that is, it is when milk production switches on after birth. The first studies looking at lactogenisis 2 were carried out in rats. We found that you could detect an increase in the synthesis of lactose in the glands. You can get an increase in lactose in milk and you can also get an increase in the amount of lactose in the glandular tissue. These are all potential markers of lactogenisis 2. If we then compare that to what' s happening in women, women notice milk coming in about 2 to 3 days after birth, some earlier, some later. We didn't find much difference between women who delivered vaginally and those who delivered by caesarian section. Although currently in the USA they now find that the initiation of lactation is delayed in women who have caesarian section delivery.

There is a very rapid increase in milk production after birth in breastfeeding mothers in and in women who express (pump) their milk. This rapid increase in milk production is termed lactogenesis 2. Of course this can be associated with engorgement that is one of the problems that can occur after birth. Engorgement can be extremely painful for mothers and we really don't have good treatment for breast engorgement as yet. This is one of the areas that should be more fully investigated.

Milk also changes in composition from colostrum that is often yellowish and thick, to established lactation where at the beginning of a breastfeed the milk has a low fat content and at the end of a breastfeed it has a much higher fat content. The reason why lactose is interesting to measure in the milk is because as lactose is synthesized, water is drawn into the cell and into milk

to maintain the osmotic pressure. So if more lactose is synthesized, more water is transferred into the milk. Thus lactose in a good measure of what's happening as milk production increases.

There is an increase in the concentration of lactose in the mother's milk after birth and this is coupled to an increase in milk volume. We can also measure other changes in the mothers milk. The milk colostrum is thick, high in protein and as lactation starts, the protein contents falls. Again the increase in lactose reflects the increase in milk volume. The sodium content of milk decreases as lactation starts and the citrate level increases. So total protein, citrate, sodium and lactose are all useful markers for the initiation of lactation in women.

For about the first 30 hours after birth, the lactose levels are similar to the levels that occurred during pregnancy. Then from 30 to 40 hours, there is a rapid increase in lactose that marks the initiation of lactogenesis 2. We found that there was no difference in lactogenesis 2 between vaginal delivery and caesarian section mothers, lactose increased in a similar way in both modes of delivery.

Various hormones that could be involved in controlling lactogenisis 2, progesterone, estrogen, cortico-steroids placental lactogen and insulin. Studies in rats showed that it was the fall in progesterone that triggered the initiation of lactation. So progesterone inhibited milk synthesis during pregnancy and when it falls, it triggers the initiation of lactation. In rats, it has been shown that cortico-steroid hormones are also required, because if they the cortico-steroid hormones are removed very little lactose is made compared to controls rats.

Studies also carried out in sheep were able to show there if estrogen was administered after late gestation caesarean section, the increase in lactose still occurred. But if gave progesterone was administered no increase in lactose occurred. These studies showed that progesterone fall was the trigger for the initiation of lactation (lactogenesis 2).

In women, progesterone increases during pregnancy, but then the progesterone falls occurs after birth. In most other species, progesterone falls before birth, and lactogenesis 2 begins at birth. But in women, progesterone falls after birth and a there is a similar relationship between the fall in progesterone and the rise in lactose.

So it is clear that it is the withdrawal of progesterone that is the trigger for the initiation of lactation (lactogenesis 2) in women. But unlike other animals, this fall occurs after birth. Women, who have retained placental fragments that are viable, have no increase in lactose in their milk. When the fragments were removed, an initiation of lactation occurs. Again, we think that it's a progesterone production that's inhibiting lactation during this period.

In women who do not breastfeed their babies there is an increase and then a decrease in the lactose synthesis in the milk. If bromocryptine (a drug that inhibits prolactin secretion) is administered, the initiation of lactation is inhibited. So prolacton, like cortisol is required for lactogenisis 2.

In diabetic women there is a delay in the initiation of lactation and all the animal studies show that insulin is also required for initiation of lactation. This delay could be related to the level of glucose control that is exercised in women immediately after birth. It may be controlling the blood glucose level, but there may not be sufficient insulin to initiate lactation. But the bottom line is that insulin dependent diabetic mothers require more support from the nursing staff and lactation consultants to establish breastfeeding after birth because they're going to have more difficulty with the initiation of lactation. But if they're given support, they can breastfeed very successfully.

Initiation of lactation will occur even when the baby does not suckle at the breast, but the breast milk must be removed to establish the lactation. Thus sucking is important for the establishment of lactation once lactogenesis 2 has been trigger. So in summary, the trigger for initiating lactation is the withdrawal of progesterone, but prolactin, insulin and cortisol must be present.

Lactogenesis 2 can be inhibited by retained placenta because of elevated progesterone, if the milk

is not removed from the breast, then factors in the milk inhibit further milk secretion - called autocrine inhibition. Lactogenesis 2 can be delayed in insulin dependent diabetic mothers. Recent studies from America now show that the initiation of lactation is delayed in obese women and that could be related to progesterone withdrawal. If the mothers are given hormonal therapies, such as progesterone or estrogen, before lactogenesis 2 is established milk production may be inhibited. In addition, we should always be aware that there is a possibility that anesthetics used at delivery could inhibit lactation.

Lactogenesis 2 in Mothers who deliver pre-term

Often babies born pre-term are unable to breastfeed and therefore the normal stimulus for the establishment of lactogenesis 2 in their mothers is not available. Comparison of the analysis of the concentration of compounds in the milk of pre-term and full-term women at five days post-partum shows that the mean concentration of total protein, lactose and sodium in milk are somewhat similar between term and preterm woman. But what is very clear is that there is much more variation between mothers of pre-term babies, than there is between mothers of term-babies.

So we analyzed this variation and found that it was due to abnormal levels in some of the mothers. Most of the mothers had normal protein concentrations in their milk at 5 days post-partum, but some (14%) still had high protein. Whereas about a third of the pre-term mothers (36%) had low lactose and 32% had low citrate in their milk, 55% had high sodium in their milk. So the mothers with abnormal, protein, lactose, citrate or sodium had not fully initiated their lactation. We concluded that the initiation of their lactation was compromised, that is it had not completely switched on at 5 days after birth.

The amount of milk that was produced at day 5 showed that the pre-term mothers that had normal milk composition had the highest milk production. Those that had one component abnormal, say lactose or citrate, they gave much less milk, those who had two abnormal produced even less milk and those with the concentration of three of the components abnormal gave very little milk. Thus the more components that were abnormal in the milk at day 5, the lower the milk production.

Pre-term birth can occur as early as 22 week of gestation and at this time breast growth (mammogenesis) has been completed in most of the mothers, although some mothers who delivered at term had significant breast growth after 22 weeks. However, if we look at lactogenesis 1 we see that in a number of mothers lactogenisis 1 is just starting at this early pre-term period. So it is possible that the functional development of the breast may be impaired in women who deliver pre-term babies. In fact we find that milk production is less in women who have very pre-term babies. Therefore, the mothers who have 22 - 23 week babies have more difficulty getting normal milk production than mothers who deliver at 32 week.

The compromised initiation of lactation in pre-term mothers could be caused by factors such as retain placenta, type 1 diabetes or ineffective removal of milk, that delay the lactogenesis 2 in term mothers. Effective removal of milk after birth is more important in pre-term mothers because they may have difficulty either hand expressing or using a breast pump. If the milk that is synthesized is not removed, then further milk synthesis is inhibited. In Australia, mothers are more successful when they use an electric breast pump than when they hand express. When mothers are given betamethozone to mature the fetus in a high-risk pregnancy, it may prematurely initiate lactation, but we're not sure whether or not this has a bad effect on lactogenesis 2 after the mother delivers. We have studies in sheep that suggests it could be a problem.

Depo-Provera given in the peri-natal period could also be a problem as it may alter the normal pattern of progesterone withdrawal after birth. Furthermore, there may be inadequate stimulation and a failure to achieve milk ejection in the emotional environment of pre-term delivery. Again we have the anesthetic agents use at delivery could be a problem. Thus it is to be expected that a pre-term mother will find it more difficult to initiate her lactation than term mothers. In conclusion, clinically, the delay and onset of lactogenisis 2 should be considered a possible risk factor for the neonatal management of human lactation.

Control of milk production in established Lactation

Many mothers in Australia work while they're breastfeeding and the babies are rather keen to see mum come back from work so they can get a breastfeed. So the question is how does the mother regulate her milk production to the changing needs a baby who is breastfed on demand?

Milk production from one to 6 months of lactation is very constant, with an average of about 750-800 ml per day. However, there is quite a big variation between mothers; some mothers will produce much higher volumes of milk, while other mothers lower amounts of milk when they are exclusively breastfeeding their babies. For individual mothers the range can be from as little as 500ml per day to as much as 1200ml per day. Strangely this variation in milk production is not closely related to differences in the rate of growth of the mother's babies.

Initially it was thought that the nourishment of the mother was very important, so milk production in traditional societies was compared with milk production in developed countries and the researchers were pretty amazed to find that the average milk production in developed countries, where mothers were on poor diets, was very similar to the amount of milk produced by women who were on good diets in developing countries. So it became clear that the actual diet of the mother wasn't an important determinant of the amount of milk that she produced. This is in agreement with the early observations of James (1912) in Australia who said, "there is no special food for the production of breast milk, that which is best for the general health of the mother is best for the child" and I think that recommendation still holds today. The mother should eat healthy food and should just eat to her appetite.

It was found in the early 1970's that when a baby breastfeeds, the prolactin concentration in the mothers blood increases to reach a peak about 45 minutes after the start of the feed and then decreases over the next 90 minutes. Back in the 1970s when the prolactin studies were first carried out; it was thought that the prolactin release was the factor that it was determining the amount of milk that the mother produced. However, when we looked at the prolactin release from one month to 6 months of lactation, we found that it decreased. The basal levels decreased, the peak levels decreased, so that at 6 months, the mother had much less prolactin released than she did at 1 month. However, there was no difference in the mother's milk production from 1 month to 6 months. Therefore, although prolactin is absolutely necessary for milk production, but it does not determine how much milk is produced by the normal breastfeeding mother.

Two different drugs (domperidone, drug of choice, and metoclopramide) to increase prolactin secretion in mothers who have low milk production, but I think it is important that the prolactin levels of these mothers should first be tested by measuring the basal prolactin level just before a breastfeed and the peak levels 45 minutes after the start of the breast feed to determine whether or not the mother's blood is deficient in prolactin before drugs to stimulate prolactin are administered. These drugs are being given in Australia and the USA without ever measuring the prolactin levels to see if they are abnormal. This would not be an acceptable practice with any other endocrine therapy.

Since the mothers diet and prolactin do not appear to control the mother's milk production in normal healthy women in established lactation, other mechanisms have been investigated. It is now clear that with exclusively breastfeed babies, it is the baby's appetite that regulates its milk intake. Thus the mother has to regulate her supply to the baby's needs. The mechanism to be An autocrine control mechanism appear to regulate the mothers milk synthesis to the amount of milk the baby removes from the breast, that is, as the baby feeds from the breast, it removes milk and that allows more milk to be synthesized. To study how this regulation occurs, it is not sufficient to just measure the amount of milk produced over a 24 hour period, it really doesn't tell us how the baby is regulating it's intake from breastfeed to breastfeed.

For example three mothers can be producing 800 grams of milk per 24 hours, however if you look at the intakes of each baby one baby may take in 800 grams per 24 hours with only 5 or 6 breastfeeds while another

baby may take in the same amount of milk but have much more frequent but smaller breast feeds. So, you can have the same milk intake but the baby takes that milk in very different ways. It is really important to realize that the babies are drinking to appetite; they can take large quantities of milk or small quantities of milk, depending on what their appetite dictates. So it's very important to realize that appetite control work extremely well in breastfed babies, that is, the baby is controlling its milk intake and thereby regulating its energy intake.

Again, we can use this computerized breast measurement system to see what was occurring at this time. We're able to measure the changes in the mother' s breast volume before and after each breastfeed over a complete 24-hour period. At some time during the day we found that the mother's breast was at its largest volume and at another time during the day we found the breast at it's smallest volume. For example the breast of one mother (with large breasts of about 2000ml in volume) were at their smallest volume in the evening. Then her baby slept for a long period over night and her breast filled up with milk so that early the next morning her breasts were at their largest volume. The difference between the lowest volume and the highest volume we call the storage capacity of the breast.

Furthermore, at any particular time during the day, we can look at how full a breast is and know how much of the available milk is present in the breast, so this case, the degree of fullness is 30%. It's 30% full at the beginning of the third feed of the day. It is important to calculate the degree of fullness when we want to know how much milk is in the mother's breast. You can see very clearly here that the baby has stopped breastfeeding at this point in time when there is still 300 ml of milk available in the mother's breast. Therefore it is the baby's appetite that is determining how much milk it takes in at any one time and the baby rarely takes all the available milk at any particular breastfeed. I have discussed these concepts in more detail in a CD that is available from Medela so if you want more detailed explanation of these measurements, it can be found in that CD.

If we look at how the mother was synthesizing her milk, and we look at the rate of synthesis of milk, that is, the increase in breast volume between breastfeeds divided by the time interval between the feeds, and we see that when the breast is full of milk, the synthesis rate is low and when the breast is drained of milk, the synthesis rate is high. This has led to the study of inhibiting factors (Feedback Inhibitor of Lactation - FIL) that is in milk that when the milk accumulates in the breast, FIL switches off milk synthesis - an autocrine control mechanism.

But not all mothers follow this pattern and if we look at a mother with much smaller breast, this is, about 800 ml in size, her breasts can't store as much milk and so the baby has to drink more frequently over the day to get the same amount of milk. So if a mother has larger breast and a large storage capacity, then she has more flexibility over her breastfeeding schedule. If a mother has smaller capacity breast, then the baby can still get the same amount of milk, but it has to drink more frequently over the day and in this case, the rates of synthesis remain more constant.

We conclude from the studies with the computerized breast measurement system, that milk intake depends on the infants appetite, that the storage capacity - the amount of milk the mother can contain in her breast determines the flexibility in the frequency of breastfeeds, that the short term rate of milk synthesis is controlled locally within the breasts and this is an autocrine control mechanism that inhibits milk synthesis when the breast is full.

It's actually a little more complicated than that, because the whole of the breast doesn't drain uniformly. We find that some lobules empty and other can remain full of milk. The lobules that have been emptied of milk go into rapid synthesis of casein and other milk proteins. So we can get differential rates of synthesis within the breast itself. It is thought that when the breast is drained of milk, there are high rates of alpha lactalbumin and casein. As the breast fills with milk, protein synthesis slows but the breast continues to make lactoferrin. But as the breast fills up, the autocrine control inhibits further synthesis, so feed back inhibitor of lactation (FIL) inhibit further synthesis. When the baby feeds, the milk is removed. The inhibitor effect is taken away and rapid milk synthesis can proceed.

We can also have alveoli that are not being emptied

and these will either regress if they are not emptied over several breast feeds, or they can be recruited back into production if more milk is required. It is quite a clever system.

When I was born back in 1941, the instructions to my mother were to feed to a schedule - feed at 6 am in the morning, then about 9:30, again at 2:00 in the afternoon, at 6 in the afternoon and then around 9:30 at night. Then the baby was supposed to have an uninterrupted night sleep from 9:30pm through to 6:00 am. Apparently I couldn't read this because I didn' t tend to have this uninterrupted night sleep. But you can predict the problem with this schedule, as mothers with small storage capacity would be disadvantaged over the long uninterrupted sleep period. This was the case, as studies carried out at that time showed that women who had small breast were much less successful at breastfeeding than women who had larger breasts. The reason why women with small breast were less successful at breastfeeding was because they were told that they should not breastfeed their babies at night. So they had a very long period at night where they were not removing milk from their breast and so they were not making more milk. Women with larger breast could store more milk in their breast so they could get away with this long night period. Today we encourage mothers to feed on demand and the mothers with smaller breast can produce as much milk as those with larger breast but their babies feed more frequently. Now generally in Perth we see mothers with smaller doing better than those with the larger breast. Breast size is not related to how much milk the mother can produce.

Now looking at the pre-term mother, and one of the models for looking at prematurity is the Australian marsupial, in this case the Tammar wallaby. Macropods such as the kangaroo and the tammar wallaby are born very immature, and grows from a body weight of 30 grams to 750 grams during its breastfeeding (lactation) period. Kevin Nicholas did a very clever study where in one group of tammar wallabies he followed them during normal development from 60 days to about 300 days and monitored their growth and development.

Another group of mothers, at 60 days then he replaced the joey with another 60 day old joey, so at each of these time points, the mother went back to feeding a little 60 day old baby until he got up to 134 days, and then he left this 60 day baby on the mother so she could rear it through to weaning.

There was no difference in the growth rates of the young between the two groups although the foster young were about 50 days younger from the controlled young. Therefore, this means that it was really the changes in the composition and quantity of the mother' s milk that determined the growth of the young, not the age of the young.

Returning to the pre-term baby, we know that the mother's milk is the best for the term baby, but there has been no selection for milk composition for pre-term babies because in the past they did not survive.

If we look at mothers who delivered prematurely, they do seem to produce a different composition of milk, for example the protein levels appear to be higher. But even so, the nutritional requirements of the pre-term baby are greater than the nutritional requirements of the term baby. Therefore it is necessary to fortify the breastmilk to meet the nutritional needs of the pre-term baby. However there is also large variation in milk composition between mothers and also during lactation. Therefore, it is necessary to measure the composition of the individual mother's milk so that it can be fortified to meet the correct nutritional needs of her baby.

Term mothers also show great variation in milk composition, for example some mothers can have twice as much fat in their milk as other mother. Currently in Australia we do not measure the composition of the pre-term mother's milk and therefore we make the erroneous assumption that all pre-term mothers produce milk of the same composition and then add a fortifier accordingly. I don't think that is really a good way to do it. We should measure the composition of the mother's milk.

If we look at the fat content of the mother's milk, we can see that from the beginning of expressing the milk to the end of expressing the milk, we get an increase in the fat content. It is quite a marked increase in fat from beginning to the end of either a breastfeed or breast expression with a breast pump. Again, the

fat content also varies between mothers and the fat is important for the energy content of milk, among other things. If particular energy content is required for a pre-term baby, we really do need to know what the energy content of the mother's milk is to be able to ensure that the baby receives the correct energy intake.

Paula Meier at the Rush Hospital in Chicago has used this change in the fat content of the milk to modify mother's milk for the pre-term baby. The mother's in this hospital actually measure the fat content of the milk using the creamatocrit technique. They monitor the fat content of their milk at intervals as they express their milk with a hospital grade electric breast pump and then and then select milk that has the composition of fat that gives the right energy value for the baby. Normally the hospital does this when the pre-term baby's weight starts to falter and they feed this high fat milk for up to a couple of weeks. One of the neonatologists working at the Rush Hospital said that this high fat milk was the rocket fuel for pre-term babies.

The fatty acid composition of human milk is different to that of other mammals in that it contains high level of the polyunsaturated fatty acid; particularly those that are important for brain development. On the other hand cow's milk is almost devoid of long chain polyunsaturated fatty acids. Unfortified formula also does not contain these important fatty acids.

There is enormous brain growth in the human baby from birth to one year of age. In fact, the brain more than doubles in size from birth to one year of age. It not only doubles in size, but there's very complex development occurring with the formation of an amazing network of dendritic connection to the brain cells (the neuron). These connections transmit information to the neurons and establish the neural network of the brain for its subsequent development. That is why the polyunsaturated fatty acid DHA is very important because it is important in forming the lipid that insulates dendridic connection.

Protein is also important. The protein content and the composition of proteins present in breastmilk are quite different in amounts to those present in cow's milk. Casein for example is only 2.5 grams per liter in human milk, whereas it is 26 grams per liter in cow's milk. Human milk has higher lactoferrin, alpha lactalbumin, secretory IgA, land lysozyme than cow's milk. Furthermore, the amount of protein in breastmilk is very low, about 9.4 grams per liter compared to 33 grams per liter in cow's milk. I think that this means that the actual amino acid composition of the protein in human milk is very important because the baby is really on a very low protein diet, this only about 5.6% of the baby's total energy intake comes from protein.

If we look at infant formulas either whey dominated or casein dominated formula basically most of the essential amino acids in the whey dominated formula are lower than what the baby gets from breast milk and similarly in a casein dominated formula, most of them are lower and some of them are very much lower than what the baby would obtain from breastmilk. The only way to get over this problem is actually to give the baby a lot higher level of protein in the formula than what it would get from breast milk, and I'm not sure that that's good for babies.

To overcome this problem in pre-term babies, we are taking the mother's original milk, concentrating the protein in the milk by removing the non-protein part of the milk and adding the concentrated protein back to the mother's milk so that we are in fact fortifying the mother's milk with her own milk protein and we also increase the energy content of her milk by adding back some of the fat.

Breast Anatomy

Milk ejection can be studied using ultrasound imaging, as there is an increase in the diameter of the milk duct as milk ejection occurs. Since oxytocin causes the contraction of the myoepithelial cells in both breasts at the same time, the baby can suckle on one breast while the other breast is used to monitor milk ejection.

To our amazement when we started looking at milk ejection with ultrasound, we found that the breast did not look like the diagrams that we saw in the text books which turned out to be based on the dissections of Sir Ashley Coopers 1840. He injected wax into the breasts of lactating women who had died and then made wax models of the breasts and produced beautiful diagrams of the structure of the mother's breast.

Current diagrams of the breast have large lactiferous sinuses located underneath the areola area. The sercretry tissue positioned back towards the chest wall. We did not see any lactiferous sinuses under the areola area. The milk ducts were small and the same diameter throughout their length and sometimes increasing in diameter where they branched. Indeed they branched very early underneath the nipple and well within the areola area. Whereas the textbooks report 15 to 20 openings on the nipple, we only found an average of nine openings to each nipple.

As we go back towards the chest from the nipple, we get relatively more fatty tissue and less secretery tissue. Women with smaller breast basically have all secretery tissue and that is why they can lactate very well.

Milk Ejection

When oxytocin is release, both breast respond at the same time so it is not uncommon to have the leakage of milk from the breast that is not being fed from. Normally without milk ejection, we get very little milk from the mother, either breastfeeding or if the mother' s pumping her breast with a breast pump. We would estimate that on average usually less than 5-10 grams of milk can be obtained prior to milk ejection.

If we have either the baby or an electric pump on one breast, we can monitor milk ejection by ultrasound on the other breast. On ultrasound you can see the duct opening up as milk ejection occurs and you can see the milk flowing towards the nipple. Remember the milk is not being removed, so it flows towards the nipples, expands the ducts and flow starts to slow down.

If we look at the same breast 20 seconds later, the expanded duct is now contracting and the milk is flowing back into the smaller ducts of the breast. So after milk ejection, if the baby does not remove the milk it does not stay near the nipple but flows back into the breast tissue.

As a baby feeds and stimulates milk ejection, the duct diameter increases and then decreases as the milk flows back into the breast and that occurs over a period of about 2 minutes. If we pump the mothers breast and measure the increase in diameter in the milk duct in the breast that is being pumped, we see that the increase in diameter is closely related in an increase in the availability of milk from the breast. When the mother has a second milk ejection, milk flow increases again and the milk duct expands. Thus milk ejection increases the availability of milk to either the baby or to an electric breast pump.

Breast pump function

When the baby suckles at the breast, if we watch it's movements while suckling, it first sucks quite fast as it goes to the breast, then when milk ejection occurs you see the slower suck, swallow, breath pattern with intermittent pauses. We wanted to see if we could incorporate a stimulation pattern into breast pump function. We tested different patterns for stimulating milk ejection, assessed the mother's responses to the comfort of these patterns and measure the time taken to milk ejection.

We used ultrasound to detect milk ejection because some mothers don't sense milk ejections, and then we measured the amount of milk that the mother produced up to the first minute after milk ejection. This was done with an experimental breast pump that was driven by a computer and then we could record exactly what it was doing on another computer.

We investigated stimulation patterns that varied both in frequency (up to about 125 cycles per minute) and the level of vacuum that was applied. These patterns were compared to the conventional classic pattern that has 45 cycles per minute and the vacuum set to comfort. So we found that the time to milk ejection was the fastest when the baby was feeding at the breast and the classic breast pump pattern took longer to get milk ejection than our faster stimulatory patterns. In addition more successful milk ejections were obtained with the stimulatory patterns. So we concluded that there was an advantage in using a faster stimulatory pattern.

Mothers also have multiple milk ejections. So we were just stimulating to induce the first milk ejection. Mothers can have up to eight milk ejections during a breastfeed but on average 2.2 milk ejections during

an average 11-minute breastfeed. When we used the conventional Classic breast pump, 91% of the mothers had multiple milk ejections. When we first stimulated using the new Symphony breast pump, 100% of the mothers had multiple milk ejections. Although we didn't use the stimulation pattern after the initial milk ejection occurred with the Symphony breast pump, all of the mothers had multiple milk ejections. So we concluded that the baby was best at getting milk ejections, the conventional pumps were quite good, but the faster patterns were actually better.

Having a pause in the stimulatory pattern did not enhance milk expression and it did not depend on the amount vacuum that was applied to the mother's breast. Mother could just adjust the vacuum to comfort and that was quite ideal.

To investigate the removal of milk from the breast after milk ejection, we did 24 hour milk productions by weighing the baby before and after feeding and then we used an experimental computer controlled breast pump and collected fractions of milk every 30 seconds during a 5 minute pumping session. We then measured the fat content on the milk using the creamatocrit system. We knew that if the fat is high in the milk, the mother has drained her breast of milk. If the fat is low, then the mother's breast is quite full with milk.

Einar Egnel did the first good studies on breast pumps and published his work in the Journal of Swedish Medical Association in 1956. Found that three phases of the vacuum cycle were important. Phase A was the increased the vacuum on the mother's breast, Phase B was the decreased the vacuum, and Phase C was a pause with zero vacuum. It is very important to have the pause because this allows blood to continue to flow through the mother's breast and prevents it from becoming bruised and painful. He also found that there should be a maximum of vacuum of 250 mm of Hg and that 47 cycles per minutes was ideal.

From 1956 onwards, there had been very little research on breast pump design. Then, at the University of Western Australia, Medela asked us if we would have a look at breast pump design and so this is Donna Ramsay and Jackie Kent and Leon Mitoulas carried out studies on the development of the patterns for the new breast pump. And of course we couldn't do without the mothers and their babies. We tested 6 different patterns of applying the vacuum to the mother's breast. The conventional Classic pattern, one with a holding phase in it, and patterns that held the vacuum at three different levels following the peak vacuum.

We were able to work out how much milk was in the mother's breast from looking at changes in the fat content of the milk. For example, if a mother had a storage capacity of 100 ml and she was 55% full, we could calculate that she had 55 ml of available milk in her breast. The volume of milk removed by the pump, divided by the volume of milk that was available provided a measure of the efficiency of the pumping patterns.

When we looked at the first 2 minutes of pumping pattern 3 (with a longer hold of the vacuum) was more effective. And it also used the least vacuum, which to us looked to be good. But when we asked the mothers, they felt this pattern was quite uncomfortable, but they found pattern 2 to be much, much more comfortable. Since it was nearly as effective as three, overall, we decided that pattern 2 was the best pattern. In addition from our observations of the vacuum level that the mothers applied to their breast, we were quite confident that the mother should express using a comfortable vacuum setting as her pumping efficiency would not be greatly improved to setting the highest tolerable vacuum.

We concluded from these studies that milk ejection is essential for both effective breastfeeding and for the expression of breast milk. Multiple milk ejections occurred during breastfeeding and also during breast expression and mothers rarely sensed the milk ejections that occurred after the first milk ejection. Babies rarely remove all the milk that's available in the mother's breast at a breastfeed, showing that the babies are regulating their intake of milk according to their appetite. When expressing, strong vacuums does not insure complete drainage of the breast. Therefore the vacuum should be set so it is not uncomfortable. If the breast pump that the mothers is uncomfortable the mother should be encouraged to try a different brand of electric breast pump as subtle differences in the vacuum curve can influence to comfort in individual mothers. We recommend choosing a comfortable vacuum and

continue expression while milk is flowing and draining the breast at least once daily. This is following more what the baby does, rather than what's conveniently done in breast pumping or hand expression.

Stimulation with a pattern with a faster frequency is more effective in stimulating milk ejection. Effective expression of breast milk with an electric breast pump depends on the shape of the vacuum curve. So what we have recommended is a combined curve with a fast frequency curve initially to elicit milk ejections and then pattern 2 which we found to be efficient in removing breastmilk and very comfortable for the mother to use.

These studies have led to the development of the Medela Symphony breast pump with both stimulation and expression vacuum pattern. A computer card controls the vacuum patterns in this pump so they can be easily reprogrammed if we find better ways of expressing the mother's milk.

In Australia, most women prefer to use electric breast pump, rather than to hand express their breast milk, basically because it's convenient and it saves time because they can pump both breasts at the same time. Ten years ago most women would have been either hand expressing or using a hand breast pump. So a very big change has occurred over recent times in Australia.

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