Predictors of Milk Production in Lactating Women

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PREDICTORS OF MILK PRODUCTION
IN LACTATING WOMEN

by

SHIRLEY WHITELEY

A thesis submitted for the degree of Master of Philosophy
in the Open University Biology Faculty

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ABSTRACT

The aim of this study is to determine whether breast milk intake by the newborn infant is predicted by the growth in the mother's breast volume during pregnancy. The relationship between breast milk intake, suckling time, birth weight and maternal breast volume were measured and also other measures of fat accumulation.

Volumetric breast measurements were made on primiparous and multiparous women at 3 and 8 months pregnant and at 7 days post partum using adaptations of the methods of Hytten (1954) and Milligan et al. (1975). The mother's body mass index and the upper arm, forearm, thigh and calf circumferences were measured during pregnancy and post partum. The infant's spontaneous milk intake was measured on days 8 and 9 post partum by weighing the infant before and after each feed over 48 hours, using a Sartorius 3865 MP portable Top Pan Balance fitted with a baby weighing pan and coupled to a Sartorius Universal Printer.

Although milk intake was reliably predicted by suckling time, as shown in previous studies, there was no significant relationship between breast enlargement and milk intake, contrary to the finding of Hytten (1954). The discrepancy is probably attributable to his use of breast pumping to measure breast milk production. In the study repeated in this thesis, the infant's natural milk intake was measured, and was found not to be related to the mother's breast enlargement during pregnancy. Enlargement of limb girth was detected only for the thigh; that of the arm did not change.
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CHAPTER 1

INTRODUCTION

Mammals are a unique class that suckle their young on milk produced by the mammary glands of the female. The mammary glands are usually situated ventrally and paired but vary in position from the axilla to inguinal region, depending upon the mother's lifestyle and whether the young are cached, carried or are followers. In ungulates, the mammae are largely or entirely inguinal. The number of glands, in each species, has evolved to accommodate approximately the average number of offspring born at one birth. One pair of pectoral mammae occur in primates and a few others such as elephants and sea cows. Primate mammae appear flat although the nipples may be elongated, except in humans where permanent breast enlargement occurs in females, at puberty.

1.1 Breast development

Breast development is reviewed in detail by Cowie and Tindal (1971). Mammogenesis is dependent upon hormonal stimulation and is discernible very early in foetal life as ridges of ectoderm on either side of the ventral midline from the axilla to inguinal region. Local cell proliferation forms mammary buds, initially similar in both sexes, their number and position differ according to species.

Much of the research on the development of mammary glands has been done on laboratory rodents and farm animals. In foetal mice, the ectoderm sinks into the underlying dermis to form the primary mammary cord, the precursor of the duct system. A teat is formed in the female but teat formation in the male is suppressed. In man, further development occurs in the fifth month of gestation when the bud spreads and a depression appears on its surface. At the same time, the deeper layers proliferate forming secondary buds and solid cellular cords which expand at their extremities and become surrounded with concentric layers of mesenchyme.
By the time the human foetus is 200 mm in length the cords bifurcate developing lumen and ducts and the nipple area is evident. At term the ducts have branched, become separated by septa and embedded in adipose tissue. Canalisation of the cords is all but complete and the nipple depression opens as a duct onto the skin surface.

Growth is limited to the duct system in species with short reproductive cycles, e.g. mice and rats, but in those with longer cycles, including humans and other primates, further growth and branching of ducts results in the formation of lobules. The nature of the stroma also differs between the species, in those with inguinal mammae, mice, rats and ruminants, it is mostly adipose tissue but is both connective and adipose tissue in humans and others with pectoral mammae. In some species, there is little or no adipose tissue around the mammary glands.

Post-natally, mammary growth differs between species. In humans, growth is isometric with a quiescent stage of glandular development. In boys no further development occurs, but in girls allometric growth begins at puberty about 2 years before the onset of ovulation when both glandular and adipose tissue increase, forming paired breasts. Lactiferous ducts proliferate with the formation of lobules of branching ducts. The ends of some of the ducts form small alveoli which later become the main secretory components of the mammary gland. Adipose tissue becomes deposited and the gland assumes the rounded adult shape. Further development of the duct system and alveoli occur during pregnancy in most mammals. However, in most mammals, it is only during pregnancy that branching of the duct system and lobulo-alveolar development occurs, causing temporary enlargement of the mammary glands which return to the pre-pregnancy, resting state at the end of lactation. The mammary gland becomes a dense mass of alveolar lobules separated by connective tissue septa which is interspersed with adipose tissue. This change also takes place during human pregnancy, when extension and branching of the duct system occurs and immature lobules are discernible by the tenth week. Formation of the alveolar tissue occurs early in pregnancy and Neville (1983) stated that human breasts are capable of milk secretion beginning sometime in the mid-trimester when fast droplets are present in the alveolar cells. The pattern of growth is similar in other mammals but on differing time scales e.g. in
Rhesus monkeys there is extensive lobulo-alveolar development by the third month of a five and a half month pregnancy and in goats there is no definite glandular development until the second half of pregnancy. In humans, cell division accelerates in early lactation, only diminishing when peak milk yield is attained, or when weaning begins. In rats, sows, dairy cows and Rhesus monkeys, there is evidence for milk production just before parturition. In humans, the alveoli contain colostrum during the third trimester of pregnancy even though milk does not flow freely until day 3 or 4 post partum. A number of hormones are required to promote mammary growth. They are essentially similar in many mammal species but may work in varying combinations (Robinson, 1992). In humans from birth to puberty oestrogen is the hormone controlling the small amount of growth of the lactiferous ducts. At puberty oestrogens, progesterone and corticosteroids induce duct and alveolar growth to a limited degree. During pregnancy these hormones are active in the hypertrophy of both lactiferous ducts and the alveolar system, insulin and prolactin may also be involved. During mammalian pregnancy additional factors from the feto-placental unit also influences mammary growth, by supplementing the small amount of prolactin and growth hormone normally supplied by the anterior pituitary gland of the mother.

There are theories to account for permanent enlargement of the human breast. Morris (1967) suggested that enlarged breasts evolved with bipedalism in early humans and mimicked the fleshy buttocks of the presenting female when copulation became ventral-ventral. Halliday (1980) put forward the theory that human breasts have developed a secondary function in pair bonding, stimulating and maintaining the sexual interest of the male where estrus is not overt. Gallup (1982) dismissed Morris's (1967) theory, pointing out that buttocks are enlarged musculature required for maintenance of an upright posture and that prior to bipedalism pre-human forms, like other primates probably did not have fleshy, rounded buttocks. Gallup (1982) favoured the theory that enlarged breasts were a reliable cue to sex difference and that enlarged breasts on an otherwise slender body signalled the sexual maturity and healthy nutritional status of the human female, in a species in which, in contrast to other primates, ovulation was not overt. Low et al. (1987) suggested that the size of the breast might advertise its storage capacity,
a theory already dismissed by Minchin (1985) who pointed out that in humans milk storage, even for a short time, leads to reduced milk production, no matter what the breast size. However Low et al. (1987) further argued that mimicry of large storage capacity was a possibility. Another theory put forward by Morris (1967) and reiterated by Anderson (1988) was that permanently enlarged breasts may have evolved with bipedal gait and hairlessness in humans, and be associated with sex differences in adipose tissue distribution generally. Anderson (1988) further suggested that large and flexible breasts were easily accessible to the infant as it rode on its mother's hips. However in the absence of pertinent fossil evidence we do not know why, or when, permanently enlarged female breasts evolved, or whether the feature is in fact associated with hidden ovulation.

1.2 Why breast feed?

Breast feeding is an integral part of the reproductive process and is beneficial to the human infant in a number of ways:

1). Breast milk provides a complete food for the neonate.

2). Breast milk supplies the infant's total fluid requirement. The fluid intake of a healthy infant depends upon the concentration of the milk, the amount the infant consumes and the ambient temperature and humidity of the infant's environment (Martines et al. 1992). Martines et al. showed that infants who consume enough breast milk to satisfy their energy needs take in more fluid than they actually require even in hot, dry environments. Breast milk contains low concentrations of sodium, chloride, potassium and nitrogen so that very small amounts of fluid are needed to eliminate waste products.

3). Breast milk carries specific and non-specific immunoglobulins active against infection in the neonate (Labbok 1985). In areas where sanitation and clean water supplies are inadequate unsupplemented breast feeding protects the infant from prevalent endemic diseases such as diarrhoea. A study by Howie et al. in Dundee (1990) showed that babies who were breast fed
for the first 13 weeks of life had a marked reduction of gastro-intestinal illness in their first year.

4). Breast feeding inhibits ovulation in the mother and helps to space out pregnancies (Short 1984). Although lactation may not provide 100% protection among women who have been well nourished from birth, in women whose food consumption is habitually at or below subsistence level for most of their lives, lactation provides a high degree of contraceptive protection. In 1988 the Bellagio Consensus Conference proposed guidelines that became the basis for Lactational Amenorrhoea Method (LAM) of contraception. Pregnancy is rare among breast feeding women with LAM. Kennedy and Visness (1992) using a worldwide sample of women calculated 98% protection up to 6 months post partum for women who were fully breast feeding. Perez et al. (1992) assessed this method with over 400 women in Chile and also found that it was a viable introductory method for breast feeding women, reporting the incidence of pregnancy as 0.45% over a 6 month trial period. Perez et al. (1992) stressed that mothers must be wholly breast feeding and should be encouraged to continue night feeds. In traditional societies such as Australian Aborigines and Kalahari Bushmen habitually breast feed infants for up to four years and do not conceive again during that period demonstrating the effectiveness of lactation as a contraceptive; but if a suckling child dies, the mother is soon pregnant again (Short 1984). Thus breast feeding can be a method of regulating population growth.

5). A recently published paper by Barker (1990) suggests that men who were totally breast fed as babies and who had an above average weight gain in their first year, irrespective of their birth weight, are less likely to suffer ischaemic heart disease in adult life than men who were exclusively bottle fed. This retrospective study indicates that it is weight gain on breast milk that is the crucial factor.

Although breast feeding is an integral part of the reproductive process lactational failure can occur. The most common reason given by mothers for this apparent failure is 'lack of milk'. Factors contributing to early weaning include lack of information and counselling, lack of milk, fear of lack of milk, maternal fatigue and physical complaints related to the breast (Neifert et al. 1985). Some of these problems are preventable or can be managed but Neifert et al. (1985) were
concerned that, where basic anatomical problems occur, they should be recognised by clinicians and women counselled accordingly. Neifert et al. (1985), demonstrated that, even in highly motivated women, insufficient glandular tissue was the cause of lactational failure in three cases that they studied. Neifert et al. (1985) examined three women who presented with unilaterally underdeveloped breasts. The subjects reported no breast changes during pregnancy or engorgement post partum. One subject had close family members with histories of lactational failure and another had patchy palpable areas of glandular tissue in her larger breast. Breast diaphanography was used on two of the subjects. Light transmission was observed to a much greater degree than in normal lactating breasts suggesting a lack of normal glandular tissue in both subjects. Neifert et al. (1985) pointed out that early recognition of under-development of glandular tissue was beneficial to both mother and infant; physical explanation of failure to breastfeed adequately had two main advantages in that it dispelled guilt feelings on the part of the mother and failure to thrive in the infant could be prevented.

Hytten & Leitch (1964) stated that milk production was dependent on growth in the size of the breast and suggested that primiparous women under 30 years old produced more milk than older primiparae. Hytten and Leitch speculated on changes that may occur in breasts which do not function for some time after maturity and put forward the hypothesis that in older primiparae, mature breasts, unused for several years, could suffer from 'disuse atrophy' and be less efficient than the breasts of younger women. They further suggested that individual differences in breast enlargement may owe more to genetic difference than to age. In humans pre-pregnant breast volume is dependent upon the amount of adipose tissue the breast contains, and even then there are individual as well as racial differences in average breast volume, but as there has been no recorded selective breeding for milk production in humans this hypothesis of Hytten and Leitch (1964) cannot be confirmed. Permanent enlargement of the mammary glands is unique in humans among the primates.

Hytten and Leitch (1964) reported that many primiparae under 25 years, presented at antenatal clinics with enlarged, tender breasts in early pregnancy, whereas older primiparae showed
little change in early pregnancy. Hytten and Leitch ascribed this effect to vascular engorgement, which is prevalent in young primiparae, and subsides during the first trimester of pregnancy. From weeks 12-14 of pregnancy Hytten and Leitch (1964) reported increasing growth in breast volume up to 8 months of pregnancy and that breast size in primiparae tended to decline with age. Multiparae, they reported, presented a different picture as glandular enlargement occurred into the loose strome left by involution from previous lactation. Breast enlargement was therefore not noticeable in early pregnancy. They also reported that increase in breast size in primiparae tended to decline with age, and that in multiparae, breast enlargement was not noticeable in very early pregnancy.

Mammals have discrete adipose tissue deposits associated with various organs, viscera and skeletal muscles (Pond 1990) and they lay down reserves of fat during pregnancy (Low et al. 1987, Pond and Mattacks 1987). Humans are among the few species whose bodies contain significant amounts of white adipose tissue at birth. Gallup (1982) showed that a girl must have accumulated about 17% of her body weight as fat for menarche to begin. This finding verified the work done by Frisch and McArthur (1974) who also stated that well nourished girls must have deposited a certain quantity of fat before menstrual cycles could begin and that the required minimum increased with increasing age. A loss of 10-15% of body weight being sufficient to stop menstrual cycles which do not resume until the body weight for age is regained. However after reaching maturity body fat needs to fall below 22% of body weight for menstruation to cease (Gallup 1982). The subcutaneous thoracic depot is of special interest in this study. In both sexes it covers the dorsal, lateral and ventral surfaces of the chest and extends over the posterior and medial sides of the upper arms. In human males adipose tissue forms a usually, thin layer of uniform thickness on the ventral side of the thorax, but in females, the pectoral portion enlarges under the mammae and becomes the adipose tissue constituent of the forward protruding breasts. Because the thoracic and upper arm depots are part of the same adipose tissue sheet any increase in the anthropometric measurement of the upper arm during pregnancy could, by inference, suggest increase in breast volume due to fat deposition.
All higher primates including humans develop a single pair of pectoral nipples but only in girls does the pectoral adipose tissue depot become so enlarged at puberty. It continues in this state throughout the years of reproductive life, only regressing some time after menopause (Pond 1990). There are marked inter-species differences in the siting of adipose tissue depots and humans are among the very few in which there are sex differences; but in human females of reproductive age, breast and femoral depots are most pronounced. The general deposition of adipose tissue in the mammalian body evolved as a nutrient store for conversion to energy supporting life during periods of food shortage, and particularly by some mammals, e.g. bears and some seals, during pregnancy and lactation. The human breast itself provides a very small adipose tissue depot (Campagne et al. 1979), constituting about 4% of the total adipose tissue content of the body in well nourished females (Pond 1990). The adipocytes of the breast are relatively small and resemble those of the abdominal region rather than the larger cells of the inguinal region.

The thickness of adipose tissue in a specific region is dependent both on the number of adipocytes and on their lipid content. In humans adipose tissue has different metabolic properties in different regions of the body (Rebuffé-Scribe et al. 1986). In women during pregnancy increase in adipose tissue occurs in the thoracic depot, which encompasses breasts and upper arms and in the femoral region, preparatory to breast feeding. Lipoprotein lipase activity (LPL) has been shown to be higher in the adipose tissue of the femoral region than in that of the abdominal area of fertile women (Rebuffé-Scribe 1987). This difference is more pronounced in the first 10 weeks of gestation. Femoral LPL activity decreases in late pregnancy to a level approximating abdominal LPL activity by the end of the first month of breast feeding. Rebuffé-Scribe (1987) suggests that these metabolic events, occurring as they do in the adipose tissue of the femoral area, have the specialised function of accumulating energy to support lactation. The suggestion is supported by the fact that this femoral adipose tissue deposition occurs mainly in women. Rebuffé-Scribe (1987) demonstrated that in late pregnancy and during lactation triglycerides are mobilised as easily from femoral as from abdominal sources.
1.3 Measurement of breast volume

Accurate measurement of breast volume is difficult, partly due to the shape of the breast and partly due to its fluidity. Methods of breast measurement need the co-operation of the subjects most are messy and may cause subjects some discomfort. Methods fall into three categories, X-rays, casting and water or air displacement. Reiman and Seabold (1933) used X-ray shadows of breasts in profile in an attempt to quantify breast volume. This method is unsatisfactory in two respects: the measurements are 2-dimensional, and non-therapeutic exposure to X-rays especially during pregnancy is unacceptable. Wax impression of plaster casts of breasts was pioneered by Ingleby (1949) and casting later developed by Campagne et al. (1979) as a method of measuring breast volume.

Hytten (1954) used Archimedes' principle of water displacement to measure breast volumes of lactating women. His apparatus consisted of a perspex half-hemisphere shaped to fit closely to the chest wall with an inflatable rubber ring encasing the perimeter to make a leak-proof seal. Water was introduced through a tube at the top of the vessel and a small adjacent hole allowed air to escape. The vessel was drained by a tapped tube on the underside. The subject was seated in an upright position and the vessel held securely against the chest wall whilst being filled and emptied. The breast volume was calculated by subtracting the volume of water needed to fill the vessel covering the breast from the volume of water needed to fill the vessel. Hytten (1954) measured the left breast of 86 primiparae and 23 multiparae on day 7 post-partum, following complete emptying of the breast with a breast pump (humalactor). All measurements were done by one operator in an attempt at standardisation, but two people were needed to manage the apparatus. Hytten (1954) also ran a pilot experiment measuring nine primiparae and two multiparae at routine anti-natal clinic visits throughout their pregnancies, beginning at the third month. He reported that five of these subjects showed no increase in breast volume after the twenty-fourth week and that the increase in breast volume slowed down during the second half of pregnancy in the remaining six subjects.
A simple method based on the Archimedes principle was used by Milligan *et al.* (1975) when studying changes in breast volume during the menstrual cycle. This nulliparous group of women were asked to measure their breast volume on three consecutive occasions at the same time of day during a three month period. A simplified technique of water displacement was used. A 17.5 cm diameter glass mixing bowl was placed inside a larger container on the floor and filled to the brim with water. A plastic sheet, spread on the floor was marked for the position of hands, elbows, knees and the container. The subject knelt on the floor using the marker guides and lowered her breast into the bowl. The displaced water flowed into the outer container and was subsequently measured in a graduated cylinder. The volume of the breast was calculated by subtracting the overflow from the volume of the inner container.

In recent years, plastic surgeons have made use of Archimedes’ principle when measuring breast volume for breast reconstruction following mastectomy, so that volumetric symmetry between the remaining and prosthetic breast is assured. The apparatus developed by Wilkie and Ship (1977) consisted of a water filled, membrane-lined cup from which projected a calibrated cylinder. When the membrane was applied to the breast the membrane bulged upwards and the water was forced back into the cylinder providing a record of the breast volume. A variation of this method was developed by Morris (1978) and by Grossman and Rounder (1980) in which the cup was attached to a giant syringe. The plunger descended until the cup was firmly attached to the breast surface. Grossman and Rounder cited a limit in breast volume of 400 ml for this method. Ward and Harrison (1986) went a stage further and used air displacement measurements for rebuilding the breast mound after mastectomy. A breast cup, lined with a flaccid silicone membrane, was connected to an airtight, closed, calibrated 2 litre cylinder with a piston via a three-way tap and polythene tube. The cup was fitted over the breast and the volume measured by the position of the piston when resistance was felt as the membrane enclosed the breast. Ward and Harrison (1986) stated that the position of the membrane fitted over the breast depended upon the position of the piston within the cylinder. It was therefore possible to measure the differences in volume between the two sides of the chest wall by noting the position.
of the piston when the membrane embraced the normal breast on one side and the irregular contours of the mastectomy site on the other. Ward and Harrison (1986) reported that a definite point of resistance was reached by the piston when the membrane encased the object and that the compression of air within the cylinder was not a significant source of error. Fifteen external prostheses of various volumes were taped to a volunteer subject’s chest wall and measured using this apparatus. Each prosthesis (ranging in volume from 150-950 ml) was first measured using Archimedes principle before the 2 sets of results were compared. A significant correlation resulted, the value of r being 0.995. From a subject’s point of view the air displacement methods are more convenient than those involving water.

1.4 Preparations for breast feeding

Throughout pregnancy the body makes preparations for the nutrition of the foetus post partum. Prentice (1988) pointed out that mammals adopt various energy storage strategies to support lactation. At one end of the scale are the fasting seals and bears, with huge fat stores laid down during pregnancy, and at the other extreme are rats and cattle who lay down little extra fat during pregnancy, indeed may be simultaneously pregnant and lactating, and where food intake is immediately converted to milk. A female rat needs to increase her food intake by 300% whilst lactating. Most large mammals increase their body fat content during pregnancy and continue to take in extra food whilst lactating. Because the energy stress of lactation is very low in humans, Prentice (1988) estimated that the energy needed for lactation could be accommodated by the fat stored during pregnancy. When food is plentiful this energy source remains largely untapped but even so nutrients, in the form of fat, are stored in the mother’s body to supplement her energy budget during lactation. Hytten and Leitch (1964) estimated that an average increase of 4 kg in body fat during pregnancy, in Scottish women, represented an energy store of 35,000 Kcal, enough to supplement lactation for 4 months at a rate of 300 Kcal/day. Calcium is accumulated in the skeleton by females during pregnancy (Hytten and Leitch 1964) partly for the development of the foetal skeleton and partly to reinforce that of the mother as she carries the extra weight of
the developing infant. Hytten and Leitch (1964) thought it unlikely that protein was stored by human mothers as human milk contains only about 1% protein which is easily found in an adequate maternal diet. The glandular tissue of the breast begins synthesis of colostrum during the third trimester of pregnancy in humans and the nipples become larger and more mobile (Hytten and Leitch 1964).

1.5 Initiating breast feeding

The proportion of mothers who initiate breast feeding varies widely in different cultures. The timing of the first suckling can vary from a few minutes to several hours after birth. In a DHSS survey of England and Wales carried out by Martin and White (1985) the researchers found that the time elapsing after birth before an infant was suckled, strongly influenced the success or failure of breast feeding in the first two weeks. Their survey showed that in 1985 nearly 80% of women who subsequently breast fed had held their baby immediately after birth compared with 63% five years earlier. Martin and White also reported in the 1985 survey that 59% of women had put their baby to the breast within the first hour compared with 16% in 1980. The proportion of mothers who had experienced delay in starting breast feeding for more than four hours after birth had fallen from 35% in 1980 to 24% in 1985.

This improvement in initiation of breast feeding did not result in fewer mothers discontinuing breast feeding in the first 2 weeks post partum. In 1985, 14% of mothers who started breast feeding within the first hour stopped breast feeding within the first 2 weeks (13% in 1980), whereas 24% of those who first breast fed between 4-12 hours post partum gave up within 2 weeks as had 21% in 1980. A delay of more than 12 hours significantly increased the likelihood of terminating breast feeding within the first 2 weeks; 31% of such mothers gave up. Martin and White (1985) also noted that mothers who had their babies with them were more likely to initiate breast feeding than those whose infants were removed to a nursery.

According to Martin and White (1985) the number of mothers initiating breast feeding had declined in England and Wales from about 50% in 1930 to only 20% in 1970. A
considerable increase in the incidence of breast feeding took place in the following 10 years to 1980. In their survey, Martin and White (1985) reported an increase from 51% to 67% in mothers initiating breast feeding in England and Wales in the years 1975 - 1980, which levelled to 65% in 1985. This is comparable to an incidence of 64% by 1985 in the whole of Great Britain.

In some developing countries, the incidence of breast feeding new-born infants is very high; 100% initiation was reported by Prentice et al. (1980) in the Gambia, and Covington et al. (1985) reported 98% in Bangladesh and 93% in Egypt. Patterns in the developing countries are beginning to change, particularly among the educated and affluent classes, and are following very much the pattern of western societies in the past. Nursing patterns in developing countries, as in the west are dependent upon tradition and cultural background. Some cultures regard the colostrum as ‘bad medicine’ and fear that its consumption damages the baby. On the Indian subcontinent, initiation of breast feeding may be delayed for 2, 3 or 4 days when the milk supply proper is established (Huffman et al. 1980). During the intervening period, neonates may be given supplements of honey, mustard oil, diluted cow’s milk or sugar syrup, any of which could carry life threatening infections to the newborn. Curiously, Allen et al. (1986) noted that women in cultures with such practices rarely experience subsequent difficulty in establishing and maintaining lactation.

1.6 Suckling

Lactation is stimulated in the mammary glands by hormonal changes occurring at about the time of parturition. In many mammals, including humans, the mammary glands are sufficiently developed before the birth of the fetus to enable milk secretion to begin. This is inhibited by the presence of progesterone, synthesised in the placenta, which acts on the alveolar cells of the mammary glands blocking secretion (Robinson 1992).
Following the birth and delivery of the intact placenta, levels of human placental lactogen and progesterone decline rapidly and it is this rapid fall in placental hormones in the mother's bloodstream that provides the trigger for milk secretion to begin. It appears that once the source of progesterone is removed the mammary glands respond to prolactin.

Labbock (1985) noted that some women reported increased nipple sensitivity very soon after parturition and attributed this to the decline in placental hormones coupled with the suckling stimulus of the neonate.

The hormones, prolactin and oxytocin are both required to establish and maintain an adequate milk supply. Short (1984) recognised the importance of the sucking stimulus in the production of prolactin as did Labbock (1985) in the release of oxytocin. Suckling appears to be a major factor in the release of prolactin, growth hormone and adrenocorticotropic hormone (ACTH) in several mammal species. In humans, suckling causes the release of prolactin from the anterior pituitary by removing the prolactin inhibitory factor (PHI), dopamine, which is produced by a group of neurones in the hypothalamus.

In response to suckling, the afferent nerve pathway for prolactin release proceeds from the nipple to the hypothalamus via the spinal cord and brain stem. Release of PHI from the hypothalamus is modulated as a result of afferent nerve input and prolactin secretion from the anterior pituitary is increased.

In humans the level of prolactin rises during pregnancy reaching its peak just before parturition. *Post partum*, the level of prolactin falls slowly to the level it maintained during pregnancy, showing only transient rises during suckling.

Milk is secreted at a fairly constant rate into the alveolar luminae and drains into the large lactiferous ducts and sinuses. Milk removal from the mammary gland is intermittent and depends upon the frequency of suckling which can range from the young being continuously attached, as in marsupials, to the Arctic seal which may only suckle once a week. Milk can only be removed from the mammary gland by active expulsion through the milk ejection reflex triggered by suckling.
The hormone oxytocin is the crucial factor in causing milk ejection. Suckling causes stimulation of the sensory receptors in the nipple activating nerve impulses which pass via the spinal cord and brain stem to the hypothalamus and is secreted from the posterior pituitary.

Its main effect is to cause contraction of the myoepithelial cells surrounding the nipple resulting in the expulsion of milk into the lactiferous ducts causing milk to spurt from the nipple or teat. In women, oxytocin levels rise steadily to peak 10 minutes after the start of suckling although milk ejection occurs about 80 seconds after suckling begins. Howie et al. (1979) stated that successful lactation was dependant on the establishment of satisfactory milk flow during the first week post partum.

Carvalho et al. (1983) researching into the effect of breast feeding on early milk production and weight gain showed that milk production and early weight gain of the infant could be significantly increased by increased nursing and noted that work with animals showed early stimulus by suckling increased the number of receptors for prolactin in the mammary glands.

Suckling is a contributory factor in inhibiting conception during lactation although the precise mechanism is not understood. In his experiments with sheep, Short (1984) found that when sheep teats were denervated the contraceptive effect ceased whilst suckling carried on normally. The contraceptive effect has been demonstrated in dairy cows whose calves suckle at will, whereas those whose calves suckle for only 30 min/day return to estrus almost immediately post partum. Short (1984), observing a group of women in Edinburgh, reported that ovulation was impeded when women were breast feeding 6 times or for 60 min/day, continuing night feeds and not giving supplements to their infants. Research by Gray et al. (1990), Kennedy and Visness (1992) and Perez et al. (1992), following the guidelines of the Bellagio Conference, have shown low lactational amenorrhea can be used as a reliable contraceptive in the first few months post partum (Chapter 1.2).
Suckling patterns

In humans, true demand feeding was reported by Berman et al. (1972) studying the Alaskan Eskimos, who suckled their infants for about 5 minutes from each breast every 2 - 3 hours or when they were restless or seemed hungry. Prentice et al. (1988) studied women in the Gambia where the frequency of feeding was 14 - 18 feeds/24 hours in early lactation. Konner and Worthman (1980) studied the !Kung people whom they reported suckling their infants several times an hour during the day. In western societies, where it is not customary for mothers to carry their infants all the time, ad libitum nursing is difficult to assess. However in Boston, USA, Barr and Elias (1988) studied two groups of mother/baby pairs matched for social background but with differing ideas of child rearing. The first group were La Leche League followers who habitually carried their infants and fed them ad libitum. The second group were designated 'Standard Carers' and fed their infants 'on demand'. The frequency and duration of the 'La Leche' group was the same at 2 and 4 months, 11 episodes/day totalling 165 minutes, whereas both frequency and duration dropped in the other group to 7 and 5.5 episodes/day totalling 77 and 55 minutes respectively.

Nutritive sucking was described by Wolff (1968a) as "any repeated mouthing on the nursing nipple, associated with negative intra-oral pressure, sufficient to deliver a palatable liquid from that nipple." This statement was applied to bottle fed babies where there are differences in temporal organisation between nutritive and non-nutritive sucking (Wolff 1968a). Breast fed babies alter their sucking pattern on the breast as the milk flow changes during a feed (Wolff 1968b, Drewett and Woolridge 1979). Wolff studied sucking patterns over a wide range of mammals and concluded that sucking times are specific to species: human neonates suck at the rate of about 1 Hz. Drewett and Woolridge (1979) showed that when milk flowed freely the rate of sucking was about 1 Hz, increasing to 2 Hz as the milk flow slowed down towards the end of a feed. Drewett and Woolridge concluded that the highest and lowest sucking rates on the breast corresponded to nutritive and non-nutritive rates seen during bottle feeding. Bowen-Jones et
al. (1982) studying milk flow and sucking rates verified that nutritive and non-nutritive suckling rates were continuously variable in breast-fed babies.

Self-regulation

The fear of insufficient milk continues to be a preoccupation of many nursing mothers. Although demand feeding is recommended by clinicians, in western societies frequent demand is often interpreted as indicating a poor milk supply (Carvalho et al. 1983). Studies into the milk consumption of breast milk have shown infants to be self-regulating in their intake (Drewett and Woolridge 1980, Dewey et al. 1981, Hofvander et al. 1982.) Research done by Drewett and Woolridge (1980) showed that breast fed babies are self-regulatory in their intake. Drewett and Woolridge (1980) studied a group of 5 - 7 day old babies and showed that infants, offered both breasts at a feed, often refused or took little milk from the second breast. Drewett and Woolridge (1980) concluded that infants regulated their own intake by this refusal even though milk was still available. Hofvander et al. (1982) showed that infants are self-regulatory and noted wide individual variation in the amount of milk consumed. In their study of milk volumes at the onset and continuation of lactation Neville et al. (1988) reported that many mothers produce more milk than their infants can take and that it was infant demand rather than maternal capacity which controlled milk intake after the third day post partum. Dewey et al. (1991) in their research with two groups of infants in a longitudinal, breast feeding study described one group as average and the other as low breast feeders. Infants in both groups were allowed to breast feed normally and residual milk was stripped mechanically. Dewey et al. (1991) reported that there was no significant difference in residual milk between the groups, pointing again to the fact that breast fed infants control their own intake. However Hofvander et al. (1982) showed that heavier infants generally consumed more milk than lighter ones; but when consumption was calculated as intake/kg body weight, energy intakes were very similar.
1.7 Milk composition

Milk composition differs from species to species in protein, fat and carbohydrate content. Ben Shaul (1962) analysed the milk content of a wide range of animals both in the wild and in captivity and classified them into 5 groups depending on their nursing pattern. She found that the protein, carbohydrate and fat content of milk was dependent upon whether the mother was available at all times or visited infrequently. Ben Shaul's first group included mammals whose milk is fairly dilute such as marsupials where the mother is always available. A similar low fat concentration is seen in the second group whose young are born fairly mature and which follow or are carried by the mother and nurse frequently e.g horse and chimpanzee. Although humans are immature at birth and mature slowly, they belong to this group with dilute milk of low fat but relatively high carbohydrate content i.e. milk typical of a readily available mother. The third group have very concentrated milk of high fat content and are mammals which leave their young concealed for long intervals e.g. cows, lions, red deer. Group 4 include nesting or burrowing animals where young are born in a relatively immature state e.g. rabbits and hedgehogs and where nursing is more likely to be 'on schedule' than on 'demand'. Polar bears become dormant during the nursing period and have milk which is 50% fat, more as a result of the diet of the mother rather than nursing frequency. Ben Shaul (1962) includes polar bears in Group 4 because of the milk's high fat content. The fifth group consists of animals that live in cold water such as whales or whose young are frequently wetted by the mother e.g. otters and beavers. This group have concentrated milk with an extremely high fat content. Fat is a food of high energy content present in species living in cold conditions and where growth to maturity is rapid.

The nutrient quality of milk within species remains remarkably constant even though the mother's energy balance may be compromised. Female mammals can only reproduce if their nutritional intake is sufficient to cope with both their normal physiological needs and the demanding needs of lactation (Sadleir 1984). Using allometric analysis, Prentice (1988) demonstrated a correlation between milk energy yield at peak lactation and maternal metabolic size. Within this relationship Oftedal, (1984) had defined three groups which showed special
adaptations; species with many young e.g. rats, ungulates with single young e.g. cows, and primates. Primates have a much lower rate of milk production: Prentice (1988) estimated it as being four to fifteen-fold less than the other species listed. As the stress of human lactation is spread over a relatively long period, the woman only increases her food intake by about 25% during lactation.

The natural length of human lactation is not known but is considerably longer than in most other species, so that the overall energy costs may be similar to other species with higher milk yields.

1.8 Milk intake

The most widely used method of measuring milk intake in babies is test weighing. The method is simple and the one against which other methods are evaluated. It entails weighing the baby before and after every feed over a period of at least 24 hours, preferably 48 hours or longer. Modern electronic balances are extremely accurate and integrate weighings over a pre-set period of time to compensate for movements of the baby being weighed. A digital weight-display eliminates operator bias (Drewett et al. 1984). Portability and their need for an electricity supply has hindered the use of sophisticated balances beyond the confines of laboratory or clinic but lighter, battery operated models have been introduced by some manufacturers. Sampling average milk intake over a period poses problems. Interaction between mother and baby must be disturbed as little as possible, and all the milk taken, within a specified time, must be recorded. Such sampling presents few problems in western societies but can be difficult in cultures where mother and baby are in close proximity day and night and *ad libitum* feeding is the norm. Biologists studying milk intake in wild mammals (Coward et al. 1982) developed a method using a non-radioactive isotope, deuterium oxide ($^{2}$H$_{2}$O) for estimating milk intake by water transfer; the method is also applicable to human subjects. Originally administered to the infant, Coward et al. (1984) found that administration of deuterium oxide to the mother as an oral dose of 0.1 mg/kg body weight was more acceptable. Samples of the mother's milk and infant's saliva are
collected for mass spectrometry, for 14 days after administration of the isotope. Coward et al. (1984) compared this method with traditional test weighing carried out under hospital conditions and produced comparable results. Another technique using ultrasound was developed by How et al. (1979) to measure milk intake and flow. Although detailed studies can be made during feeds and a sampling line incorporated the authors suggest that this apparatus is potentially for use in controlled hospital conditions and not in the field.

1.9 Milk Secretion

The volume of milk secreted at the beginning of lactation is very small and increases rapidly during the first few days to a peak level at about one month. Longitudinal studies of infant feeding undertaken by Houston et al. (1983) in Edinburgh and two by Neville et al. (1988) in the USA followed groups of breast feeding infants from day 1, and showed large increases in milk intake (g) in the first few days (Table 1.1)

Table 1.1

Three comparative studies, the first in Edinburgh (UK) and the others in Denver (USA), which show the large increase in milk production (g) from day 1 to 9 post-partum.

<table>
<thead>
<tr>
<th></th>
<th>day 1</th>
<th>day 2-4</th>
<th>day 5</th>
<th>day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Houston et al. (1983)</td>
<td>30</td>
<td>396</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neville et al. (1988)</td>
<td>50</td>
<td>550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neville et al. (1988)</td>
<td>94</td>
<td>449</td>
<td>529</td>
<td>959</td>
</tr>
</tbody>
</table>

Maternal secretory capacity and infant intake are two different things (Hytten 1954, Butte et al. 1985). Milk production can be measured by emptying the breast completely either manually by squeezing or mechanically with a breast pump. Butte et al. (1985) pointed out that even in
successful lactation, the divergence of the two is not fully understood. Inaccuracies in mechanical measurement may occur because the suckling stimulus of the infant is absent, and a truer record of lactation capacity might be obtained by test weighing the infant before and after a normal feed then mechanically depleting the breast. The two amounts added together are more representative of the breast's capacity, but not, of course, of the infants' natural intake. Carvalho et al. (1983), in their experiments with nursing mothers in Cleveland U.S.A, proved that early milk production and therefore infant weight increase could be significantly increased by unrestricted and frequent breast feeding. They also showed, in their experiments on animals, that early suckling increases the number of receptors to prolactin in the mammary gland and suggested that frequent nursing in humans could have the same effect though experimental verification of this is not possible.

1.10 Aim of study

The aim of this study was to examine the extent to which breast enlargement during pregnancy predicts breast milk intake in infants feeding naturally on the breast. Hytten and Leitch (1964) found a strong correlation between breast enlargement over pregnancy and subsequent milk production (at 7 days post partum). In Hytten's study, however, the breast was completely emptied by a 'humalactor' at every feed, so the study does not show that the functional capacity of the breast is a determinant of natural milk intake, or that it accounts for any of the natural variation in intake over mother/infant pairs. The aim of this study was to examine this question and the methodology was intended to replicate closely Hytten's (1954) method, except that natural milk intake was measured by test weighing. Two other relevant variables (birth weight and nursing patterns) were also taken into account.
CHAPTER 2

METHOD

The general plan of the study was to record breast enlargement and some related anthropometric measurements during pregnancy; birth weight; and suckling patterns at 1/2 days and 8/9 days of age. These variables were then to be related to milk intake by the baby over 48 hours, measured by test weighing at 8/9 days post partum (Fig 2.1). The time sequence of the study is illustrated in Fig 2.1.

2.1 Protocol

Permission was sought and granted by both Durham Area and North West Durham Area Ethical Committees, as all subjects were National Health Service maternity patients being delivered in local hospitals. The protocol of the study was sent to the senior pediatrician, obstetricians and chief maternity nursing officers at both Dryburn and Shotley Bridge hospitals. In the event it was never necessary to visit subjects in hospital as all mothers were home by day 5 post partum and, having been well briefed, were able to undertake the first recordings of suckling patterns at day 1/2 without supervision. Interested general practitioners and their midwives were also provided with the protocol.

2.2 Subjects

Twenty-two subjects were recruited at the rate of 1 or 2 a month over a period of 2 years. Women from within a 10 mile radius of Durham City who expressed a commitment to breast feeding were recruited from a variety of sources. Several general practitioners were approached to facilitate introduction to suitable recruits primarily through an interested general practitioner who had herself been a subject in a previous study. These general practitioners allowed me to visit their ante-natal clinics and to talk to midwives in the hope of recruiting women before they
Figure 2.1
Time sequence of study.
were 3 months pregnant. Personal contacts with other women of all ages was a more rewarding source of subjects, however, as many women are interested in breast feeding whether or not they have been successful breast feeders themselves. Women were recruited as near to 12 weeks pregnant as possible so that any prior breast enlargement was minimal. Both primiparae and multiparae were accepted into the study.

Each subject was visited in her home in the third and eighth month of pregnancy and at 7 days post partum. At each visit the subject was instructed in her role in the project. During the first visit the general procedure was explained and the subject was invited to read the protocol and sign the Informed Consent form. Her personal details were then recorded (Appendix 1). The first breast volume and other anthropometric measurements were made and recorded (Appendix 2). At 8 months the visit included measuring the subject as before and explaining the first of the 'diary' forms (Appendix 3) to record the suckling pattern of the new infant. At the visit on day 7 post partum, the mother was measured for the third time and instructed in the use of the electronic balance for test weighing her baby. A set of weighing instructions (Appendix 4) was left with the mother. The second of the diaries was introduced and it was emphasised that the baby must be weighed before and after every feed, day and night for 48 hours on days 8 and 9 post partum, and the weights recorded in the diary. This written record was a back-up precaution in the event of the failure of the balance printer. The mother was also reminded to record the suckling times of the infant over the 48 hour weighing period, as she had done immediately after the birth.

2.3 Data records

An 'Informed Consent' form (Appendix 1) was designed to explain the aims of the study simply to subjects and each was asked to read and sign it to indicate understanding the procedure in which they were consenting to take part. It was made clear to subjects that they were free to withdraw at any time, should they feel the need to do so. A 'Subject Data' record form
Appendix 1 was developed to record the relevant personal details of recruited subjects which was completed at the first visit to the subject at 12 weeks pregnant.

Other data

A group of three forms was designed to record other data at 12 weeks and 8 months into pregnancy and at 7 days post partum (Appendix 2). These data included the volume of the breast by water displacement, the bra size and anthropometric measurements of the subject’s limbs. A further group of forms was designed as diaries (Appendix 3) to be used by the mothers to record infant suckling patterns and milk intake. On the first each mother recorded every suckling bout in minutes for the day of birth up to midnight and the subsequent 48 hours. The second was used to record both suckling time and milk intake over 48 hours on days 8 and 9 post-partum. A detailed instruction sheet for the use of the Sartorius electronic balance and its accompanying printer was also prepared (Appendix 4). A group of forms for each subject was ring bound into a booklet together with the printed tape from the Sartorius balance’s accompanying printer. Several volunteers including a nurse, a psychologist, a nursery nurse and several young mothers, none of whom was taking part in the study, read and were asked to comment upon and criticise the forms as they were compiled to ensure clarity to study subjects.

2.4 Apparatus development

Hytten (1954) measured the breast volumes of women during pregnancy (Chapter 1.2). A local plastics firm was able to manufacture a dome to replicate that of Hytten (1954). In a small pilot study, in which one of the subjects was herself a graduate psychologist, it was soon evident that this dome had disadvantages. Firstly, the dome was very big and leaked consistently as it did not fit snugly against the chest wall. The second and more important feature of this dome was that it needed two people (4 hands) to use it successfully: one pair to hold the dome against the chest wall of the seated subject and the other to pour the water in through a funnel attached to the upper tube and drain the vessel into a measuring cylinder through the lower tube. Although
subjects were willing to help this proved impracticable as the movement of the subject to hold the vessel altered the contour of the chest wall.

Some modifications were made and the resulting vessel manufactured. The apparatus was constructed of 13 cm diameter plastic tube 12 cm in height stuck on to a base plate. Two of these cylinders were made having the same attachments as the original globe i.e. a tube for filling near the rim and one for emptying near the base. A rubber flange was added which was not inflatable but T-shaped in cross-section; the leg of the T had a groove which fitted over the edge of the vessel so that the flat top of the flange would lie against the chest wall. Although the flange prevented leakage, in practice, it restricted the mouth of the vessel as the breast was lowered into it; the flange was therefore discarded. It was subsequently found that the vessel was just as watertight when held firmly against the skin, without a rubber seal. One cylinder had the open end cut at right angles to the vertical wall and the open end of the other was cut at an oblique angle. The angled vessel was the better fit but limited measurement to one side because of the attachments and air vent. It still needed more than two hands to cope with it successfully and proved too wide for narrow chested subjects.

Experimenting with one subject, whose breasts were quite small at 12 weeks pregnant, a simple polythene measuring jug, with a diameter of 12 cm, full of tepid water was used. The subject leaned over from the waist and suspended her breast in the jug until the rim fitted comfortably against her chest wall, the displaced water overflowed and she withdrew. The remaining water was poured into a measuring cylinder. The advantage of this vessel and method was that it could be used by a single operator. The straight sided cylinder was therefore adapted for this method by cutting 2.4 cm from the open end below the inlet tube, smoothing off the rim and clamping the drainage tube. This vessel was used for subjects with larger breasts and after withdrawing the breast from the vessel the remaining water could be drained off into a measuring cylinder as before. Three vessels were then available for measuring subjects of varying physiques (Fig 2.2). The original vessel was only used for two subjects; the jug or smaller vessel were used for all the others. The pilot study showed that when the volume of a breast was measured three
Figure 2.2

Apparatus used in gathering data.
times, replication of measurement was acceptable. The fact that the same person was carrying out all the measurement was an advantage to consistency of the data. Before using any of the vessels each was filled to the brim with water at approximately body temperature and the volume of water that each contained was recorded.

2.5 Measuring procedures

Breast measurement

A standing position was adopted in my project as being more comfortable for subjects as pregnancy progressed rather than the kneeling position used in the study of Milligan et al (1975). Reliability in both projects was maintained by control of posture, duplication of measurements, and a single investigator carrying out all observations.

It was suggested that the subject’s bathroom was the most convenient place in which to do the displacement measurements and all subjects complied. Subjects were asked to remove their upper garments. The appropriate measuring vessel was selected and filled with water at body temperature. The subject was instructed to lean over from the waist suspending her breast in the vessel until the rim fitted against her chest wall. At the same time she was instructed to hold her arm at right angles to her body to keep the arm from obstructing the operator holding the vessel and to keep the curvature of the chest wall similar among subjects. Both right and left breasts were measured recording the amount of water remaining in the vessel when the breast was withdrawn.

The volume of the breast was calculated as the volume of the vessel minus the water remaining after withdrawal of the breast. The mean of the two breast volumes was used, because this study looked at total breast enlargement throughout pregnancy. No subject presented with unilateral breast development, nor did any subject show a preference for a particular breast to begin each feed. Present advice from midwives being to alternate the breast offered at each feed.
Breast measurements were made in triplicate when subjects were 3 and 8 months pregnant and at 7 days post partum.

Bra size

A record was kept of each subject’s bra size which included the size worn before pregnancy, at 3 and 8 months pregnant and at 7 days post partum. Broadly speaking, British manufacturers formulate increase in bra size in units of 2 inches. I found only one manufacturer measuring in centimetres, so all bra measurements are given in inches.

An under-bust measurement is taken by passing a tape around the subject from the front where the breasts leave the chest wall. Five inches is added to this measurement resulting in the familiar bra sizes of 32, 34 or 36 which increase in units of 2 inches to 40 or 42. To determine the cup size, a further measurement is taken around the subject over the fullness of the breasts. The cup size is calculated by subtracting the measurement around the rib cage +5 inches from the over breasts measurement. Should the result be a minus quantity the cup is designated AA. If the two measurements are the same the cup size is A, +1 inch is a B cup, +2 inches is C, +3 inches is D, +4 inches is E, +5 inches is EE or F. This calculation gives a wide range of fittings from 32AA to 42F although not all cup sizes are made in all fittings.

Anthropometric measurement

Anthropometric measurements were made to ascertain any increase in adipose tissue depots in selected areas of the body during pregnancy e.g. upper arm and thigh (Chapter 1.1). Girth measurements were made at 3 and 8 months pregnant and at 7 days post partum over the skin of the wrist, forearm, elbow, upper arm, ankle, calf, knee and thigh of subjects, using a centimetre tape. Both right and left sides were measured in triplicate at each stage and the mean values calculated.
Body mass index

Body mass index (BMI) is an index of adiposity in humans and is calculated by dividing body mass (kg) by standing height^2 (m^2); (Table 3.1). At 3 months pregnant subjects were asked to state their pre-pregnancy standing height and body mass. These measurements were given in avoirdupois and feet and inches and converted to metric. The data were recorded in order to give some indication of the total fatness of subjects relative to their breast volume. Indices of 22 - 25 are regarded as normal for women, 27 as moderately obese and over 30 as definitely obese, but under 20 excessively lean (Keys et al. 1972). It could therefore be assumed that the breasts of subjects with high indices would contain more adipose tissue than the breasts of subjects with lower indices.

2.6 Suckling patterns

Each subject was asked to record her baby’s suckling pattern from birth to the first midnight (day 0) and for the subsequent 48 hours (days 1 and 2). One feed was designated as feeding from one or both breasts within a 20 minute period. The suckling pattern was again recorded on days 8 and 9 post partum. Each bout of suckling was timed, to the nearest minute, from the time the infant latched on until it relinquished the nipple. The number of feeds during each 48 hour period was also recorded. The means of suckling times and the number of feeds over each 48 hour period were calculated and expressed as results day 1 and day 8/24 hours.

2.7 Milk intake

Natural milk intake was recorded by test weighing infants using a Sartorius 3865 MP portable Top Pan Balance with a maximum loading of 16,000 g accurate to 0.1 g, fitted with a baby weighing pan. It was programmed to integrate 20 weighings in 20 seconds, and the subject was instructed to repeat each weighing three times. The balance was coupled to a Sartorius Universal Printer, type 7080 with integrated data output printed onto paper tape (Fig 2.3). All test weighing was carried out by the mothers in their own homes. The tester explained the
Figure 2.3

Sartorius balance and printer.

Figure 2.4

Balance and baby Adam.
weighing procedure and demonstrated the use of the balance to each mother following the prepared instruction sheet (Appendix 4). It was suggested to each subject that the balance be set up in her bedroom so that weighings during the night caused the minimum of disturbance. It was pointed out that the baby must be weighed in the same clothing before and after a feed so a nappy must be changed before the first or after the second weighing. Each mother was advised to prepare the balance well before her baby was ready for a feed so that she could nurse in a relaxed manner leaving the balance switched on ready for weighing afterwards. Both balance and printer were plugged into the electricity supply and switched on. A 6-key programme was typed into the printer resulting in the figure 20 appearing on the display panel of the balance. At this point a blanket could be placed in the balance pan, for the infant’s comfort, and left there for the duration of the weighings. Pressing the ‘Tare’ bar of the balance resulted in a zero reading. Pressing the printer key activated the balance which showed a digital count up to 20 and then displayed a weight of +/-1 g which was printed on the paper tape. The balance was then ready for use. With practice, this preparation took about 90 seconds. The baby, clothed (Fig 2.4), was placed in the balance pan and the printer key pressed. The balance repeated the count to 20, displayed the weight on the balance and printed it onto the paper tape. The mother was instructed to repeat this procedure 3 times before and after every feed day and night for 48 hours on days 8 and 9 post partum. After being instructed in the use of the balance each mother was given a day in which to become familiar with it before doing the actual test weighing. She was also given the investigator’s telephone number, in case of emergency.

2.8 Statistical analysis

Data were transferred from record forms to text files on the Durham University SUN 4 Mainframe Computer and analysed using SPSSx and Minitab. The data are essentially concerned with patterns of individual differences in numerical variables (milk volume, time spent suckling, birth weight etc.). Individual variables are described using means, standard deviation and ranges, and the relationships between them using regression analysis.

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CHAPTER 3

RESULTS

3.1 Subjects

Twenty-two women were recruited to the study. Their ages ranged from twenty years to forty-one years, with a mean of thirty-one. Five were primiparae, and, of the seventeen multiparae, eleven were expecting their second baby, three their third baby and the remaining three their fourth baby. All the infants were singletons; fifteen were male and seven were female.

Full sets of data were collected on seventeen of the infants recruited, twelve males and five females. Two of the original group of women gave birth prematurely and were lost to the study before the eighth month of pregnancy. Two decided to bottle feed and one withdrew due to family problems.

All the women were asked to give their pre-pregnancy weight and height so that Body Mass Indices of subjects could be calculated as described in Chapter 2.5 and Table 3.1.

3.2 Breast volume and its changes during pregnancy

Change in breast volume was measured in two ways, volumetrically and by recording the increase in bra size. The histogram (Fig. 3.1a) shows the volumetric breast measurements of all twenty-two subjects at 3 months pregnant and (Fig. 3.1b) records the measurements of twenty subjects at 8 months pregnant. The results of measuring each subject's breasts volumetrically showed changes in volume, from 3 - 8 months of pregnancy, ranging from a minimum of -47 ml to a maximum of +344.5 ml; a mean increase of 114 ml with a Standard Deviation of 102 (Fig. 3.2).

Increase in breast volume was calculated by subtracting breast volume at 3 months of pregnancy from that at 8 months. Breast volume at 7 days post partum was collected as a back up should the 8 month measurements be unobtainable. In the event the 7 day data was not needed
Figure 3.1a
Breast volume (ml) of 22 subjects at 3 months pregnant.

Figure 3.1b
Breast volume (ml) of 20 subjects at 8 months pregnant.
Figure 3.2

Changes in breast volume (ml) of 20 subjects from 3 - 8 months pregnant.
as that at 8 months was complete (Table 3.2), but some interesting data emerged from it.

Negative values were recorded in two subjects 10.CB and 16.PG in the 3 - 8 months of pregnancy (Table 3.2). The former established and continued breast-feeding her infant successfully, but the progress of the other subject could not be followed as she withdrew from the study when her baby was born. All other subjects established and continued to breast feed successfully. It was noted, however that subject (9.FM), had an increased breast volume of only 17 ml during her pregnancy, her total breast volume was still < 400 ml at 7 days post partum and her baby weighed 4536 g at birth. She had no trouble in initiating breast feeding.

By plotting the changes in upper arm girth (Fig 3.3a) against changes in breast volume from 3 - 8 months of pregnancy it can be seen that there is no correlation between breast enlargement and changes in upper arm girth in this study. Nor is there any correlation between increase in breast volume and increase in either thigh or knee girth during the same period (Figs 3.3b and 3.3c).

Sixteen subjects remained in the study for the final breast measurements at 7 days post partum. In all but one there were further increases in breast volume from the eighth month of pregnancy. Changes ranged from a minimum of -18 to a maximum of 298 ml, with a mean increase of 129 ml i.e. about the same as between 3 to 8 months pregnant (Table 3.2). In three subjects 1.CS, 17.HG, 18.JR (Table 3.2) there was a substantial increase in breast enlargement from 8 months of pregnancy to 7 days post partum although increase from 3 - 8 months had been modest. Upper arm girth decreased or remained the same in all but three subjects during this period (Table 3.5).
Table 3.1

The pre-pregnancy body mass, height and body mass (kg/m²) index of each subject recorded together with age, parity and infant birth weight.

(The five subjects indicated withdrew from the study.)

<table>
<thead>
<tr>
<th>ID mother</th>
<th>Age:y:m</th>
<th>Parity</th>
<th>Body mass (kg)</th>
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Table 3.2  Mean Breast Volume (ml) throughout Study

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Figure 3.3a

Changes in upper arm girth (cm) plotted against changes in breast volume (ml) in 20 subjects from 3 - 8 months pregnant.
Figure 3.3b

Changes in thigh girth (cm) plotted against changes in breast volume (ml) in 20 subjects from 3 - 8 months pregnant.

Figure 3.3c

Changes in knee girth (cm) plotted against changes in breast volume (ml) in 20 subjects from 3 - 8 months pregnant.
Changes in bra size

All twenty-two subjects were able to state their pre-pregnancy bra sizes which were recorded together with those at 3 and 8 months pregnant and at 7 days post partum. At 3 months pregnant, sixteen subjects were still wearing the same size bra as they had before pregnancy, three subjects had increased the cup size and two the girth. One had decreased the cup size and another had decreased the cup size whilst increasing the girth. Of the twenty subjects in the study at 8 months pregnant, one reported not wearing a bra. Fifteen had increased the girth size and eleven the cup size, but four had not changed their bra size from that at 3 months pregnant. At 7 days post partum the remaining 17 subjects had established breast feeding and the pattern of bra sizes was still varied. One subject, feeding successfully, retained her pre-pregnancy bra size. Eleven retained the same girth as at 8 months of pregnancy, one had reduced the girth and four had increased it. Ten subjects had retained their 8 month cup size, three had changed to a larger cup and one to a size smaller. The subject who had not previously worn a bra was now doing so.

Relationship between bra and volumetric measurement

The reason for recording subjects’ bra measurements stemmed from the need to simplify the measurement of breast volume for future work. The water displacement method proved reliable and whilst there was some inconvenience to the subject, all who took part did so willingly. This method however, greatly delays the collection of data, since 6 months of pregnancy must elapse before milk intake can be measured. The possibility of using reported changes in bra measurement was therefore investigated as part of this study.

Two measurements are available - the girth and cup size. A predicted value for breast volume can be established using the multiple regression of (measured) breast volume of these two variables, giving an equation of the form

Predicted volume = a = b (girth) + c (cup size)

were a, a constant, and b and c, are coefficients estimated from the regression analysis.
This analysis was carried out separately for the 3 month and the 8 month data, using all the cases for which the required three measurements were available (22 at 3 months; 19 at 8 months, as one mother did not wear a bra.

At 3 months the estimate for a is -888.4, for b 36.0 and for c 33.9, giving

\[
\text{Predicted volume} = -888.4 + 36.0 \text{ (girth)} + 33.9 \text{ (cup size)}
\]

The analysis of variance is

<table>
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<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
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</tbody>
</table>

Multiple R, the correlation between the predicted and the measured values, is 0.6. A plot can be seen as Figure 3.3d.

At 8 months the estimate for a is -1287.3, for b 48.1 and for c 28.2, giving

\[
\text{Predicted volume} = -1287.3 + 48.1 \text{ (girth)} + 28.2 \text{ (cup size)}
\]

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean square</th>
<th>F</th>
<th>p</th>
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<td>499844.0</td>
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Multiple R, the correlation between the predicted and the measured values, is 0.8. A plot can be seen as Figure 3.3e.

Although these estimated volumes do not have the precision of measured volumes, it is possible that they could be of some value in situations where measured volumes are not available.
Figure 3.3d
Showing the correlation between the measured and predicted breast volumes of 22 subjects at 3 months pregnant.

Figure 3.3e
Showing the correlation between the measured and predicted breast volumes of 19 subjects at 8 months pregnant.
Precision of measurement of breast volume

Since each measurement of breast volume was made three times on each side, it is possible to obtain an indication of the precision of measurement of breast volume from this data.

The study depended on the measurement of the change in breast volume from 3 to 8 months of pregnancy, so we need to compare the between subject variability in this change with the within subject variability in the breast volume measurements. These values can be obtained from a random coefficient regression analysis (the regression analysis equivalent of a random effects analysis of variance).

A dummy variable taking the values 0 and 1 was used to code for the 3 and the 8 month measurement, and the breast volume regressed on a constant and on this dummy variable. The analysis was carried out using ML3 (Prosser et al, 1991).

Parameter estimates were as follows:

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<th>standard error</th>
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<table>
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<tr>
<th>RANDOM PARAMETERS</th>
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<th>estimate</th>
<th>standard error</th>
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<td></td>
</tr>
<tr>
<td>Month</td>
<td>9645</td>
<td>3147</td>
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</table>

|                      | within subjects   | Constant | 912.8 | 90.6 |
In the fixed parameter estimates the constant term is the average breast volume at 3
months, and the month term the average difference between 3 months and 8 months.

The within subject constant term is the within subject variance, i.e. the variability of
measurements with subjects around their means. The between subject constant term is the
between subject variability of breast volume at 3 months. The between subject month term is the
between subject variability of the change from 3 to 8 months.

The important comparison is between this term and the within subject constant term. This
shows that the variance of the change in breast volume across subjects (9645) is about 10 times
the within subject error variance (912.8).

3.3 Body mass index

Body mass indices were calculated for all subjects in the study (Chapter 2.5 and Table
3.1) The range of BMI can be seen by comparing three of the subjects in this study. Subject
(10.CB) had a pre-pregnancy body mass of 69.3 kg and a height of 1.6 m, her BMI was 27.1 an
above average body fat content. Subject (14.MB) had a body weight of 52.6 kg and a height of
1.5 m; her BMI was 23.4 indicating an average body fat content. Subject (9.FM) had a body
weight of 59.8 kg and a height of 1.7 m, her BMI was 20.7 indicating a less than average body
fat content.

3.4 Anthropometric measurements and their changes during pregnancy

The mean changes in anthropometric measurements of twenty subjects between 3 and 8
months pregnant are recorded in Table 3.3. Fourteen subjects had no increase in wrist girth and
twelve had no increase in ankle girth during pregnancy. The results are to be expected as these
areas are not normal adipose tissue depots. The changes in girth of the forearm ranged from -1.0
to +2.5 cm, and elbow from -1. to +3.0 cm. The changes in the girth of upper arm and thigh
were of particular significance in this study. An increase in upper arm girth was recorded in
twelve subjects and ranged from 0.5 cm to 2.5 cm which represents a substantial fat deposition
from 3 - 8 months pregnant (Table 3.4). However eight subjects showed no increase in upper arm girth, three of these changes being decreases in girth. The records of two others were incomplete.

An increase in thigh girth was recorded in nineteen subjects from 3 - 8 months pregnant varying from 0.5 to 6 cm. Eighteen subjects had an increase in knee girth varying from 0.0 to 4.5 cm. A decrease in thigh with an increase in knee girth was recorded in one subject and reduced knee girth in another in the 3 - 8 month period of pregnancy (Table 3.3). Similar increases in knee and thigh girth would be expected as the adipose tissue of these two depots tend to merge.
Table 3.3

Mean changes in the anthropometric measurements (cm) from 3 to 8 months pregnant in 20 subjects, (blank lines indicate subjects who withdrew owing to premature births.

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<td>1.5</td>
<td>1.0</td>
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<tr>
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<td>1.0</td>
<td>0.0</td>
<td>1.5</td>
<td>-1.0</td>
<td>2.5</td>
<td>0.0</td>
<td>4.5</td>
</tr>
<tr>
<td>18.JR</td>
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<td>0.0</td>
<td>1.0</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
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<td>0.5</td>
<td>0.0</td>
<td>1.5</td>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21.CC</td>
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<td>0.5</td>
<td>0.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>22.JM</td>
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<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>4.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Using a hypothetical subject with upper arm girths of 23 and 24 cm at 3 and 8 months pregnant and an upper arm length of 15 cm the increase in fat deposition can be estimated by treating the upper arm as though it is a cylinder whose circumference increases from 23 to 24 cm and its height is 15 cm. The estimated increase in volume can be calculated by subtracting the former from the latter (Table 3.4).

The volume of a cylinder is $\pi r^2 h$, and $\pi = 3.14$. The radius can be calculated by dividing the measured circumference by 2 $\pi$.

Using the formula $\pi (\text{girth}/2 \pi)^2 h$ the volumes of the upper arm at 3 and 8 months pregnant would be:

\[
\begin{align*}
\pi (3.7)^2 h & \quad \text{and} \quad \pi (3.8)^2 h \\
3.14 \times 13.7 \times 15 & \quad \text{and} \quad 3.14 \times 14.4 \times 15 \\
= 645 \text{ cm}^3 & \quad \text{and} \quad = 678 \text{ cm}^3
\end{align*}
\]

This calculation estimates the increase in the volume of adipose tissue as 33 cm$^3$ from 3 to 8 months pregnant.

Using the same formula and assuming the upper arm length to be 15 cm the amount of adipose tissue gained by subjects during pregnancy can be estimated and the range of differences observed. The changes in one subject is illustrated below, and all subject differences are recorded in Table 3.4.

Subject 10.CB had mean upper arm girths of 31 and 32.5 cm at 3 and 8 months pregnant (Table 3.4) and BMI of 27.1 (Table 3.1), an above average body fat content.

\[
\begin{align*}
\pi (31/2 \pi)^2 h & \quad \text{and} \quad \pi (32.5/2 \pi)^2 h \\
3.14 (31/6.28)^2 h & \quad \text{and} \quad 3.14 (32.5/6.28)^2 h \\
3.14 (4.9)^2 15 & \quad \text{and} \quad 3.14 (5.2)^2 15 \\
3.14 \times 24 \times 15 & \quad \text{and} \quad 3.14 \times 27 \times 15 \\
= 1130 \text{ cm}^3 & \quad \text{and} \quad = 1272 \text{ cm}^3
\end{align*}
\]

The difference in volume is 142 cm$^3$, which is an estimate of the amount of adipose tissue deposited in the upper arm from 3 - 8 months pregnant.
Subject 3.VC had mean upper arm girths of 36.5 and 36 cm at 3 and 8 months pregnant and BMI of 30.7, an above average body fat content. This subject had an estimated decrease in upper arm volume of 52 cm during her pregnancy.

Subject 9.FM whose BMI of 20.7 indicated a less than average body fat content, showed no change in upper arm volume during pregnancy (Table 3.4).
Table 3.4

Changes in upper arm girth measurements from 3 - 8 months pregnant in 20 subjects and estimated changes in volume.

<table>
<thead>
<tr>
<th>ID</th>
<th>3 month girth (cm)</th>
<th>8 month girth (cm)</th>
<th>Girth change 3 - 8 mths (cm)</th>
<th>Estimated change in volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.CS</td>
<td>24.0</td>
<td>24.0</td>
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<td>0.0</td>
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<td>2.JG</td>
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</tr>
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<td>36.0</td>
<td>-0.5</td>
<td>-52.0</td>
</tr>
<tr>
<td>4.JB</td>
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<td>25.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5.CD</td>
<td>23.0</td>
<td>24.0</td>
<td>1.0</td>
<td>33.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7.JC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.JP</td>
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<td>-0.5</td>
<td>-33.0</td>
</tr>
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<td>9.FM</td>
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<td>24.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10.CB</td>
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<td>1.5</td>
<td>142.0</td>
</tr>
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<td>24.5</td>
<td>0.5</td>
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</tr>
<tr>
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<td>22.5</td>
<td>1.0</td>
<td>66.0</td>
</tr>
<tr>
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<td>26.5</td>
<td>0.5</td>
<td>38.0</td>
</tr>
<tr>
<td>14.MB</td>
<td>24.5</td>
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<td>1.5</td>
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</tr>
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<td>15.PM</td>
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</tr>
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</tr>
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<td>18.JR</td>
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</tr>
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<td>26.0</td>
<td>1.0</td>
<td>37.0</td>
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<td>22.JM</td>
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<td>27.0</td>
<td>1.5</td>
<td>117.0</td>
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50
<table>
<thead>
<tr>
<th>ID</th>
<th>8 month girth (cm)</th>
<th>7 day <em>post partum</em> girth (cm)</th>
<th>Changes in girth 8 months to 7 days <em>p p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.C</td>
<td>24.0</td>
<td>23.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>2.JG</td>
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<td>23.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>3.VC</td>
<td>36.0</td>
<td>35.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>4.JB</td>
<td>25.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.CD</td>
<td>24.0</td>
<td>23.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>6.EC</td>
<td>26.5</td>
<td>27.0</td>
<td>0.5</td>
</tr>
<tr>
<td>7.JC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.JP</td>
<td>22.0</td>
<td>22.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9.FM</td>
<td>24.0</td>
<td>23.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>10.CB</td>
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<td>30.5</td>
<td>-2.0</td>
</tr>
<tr>
<td>11.CH</td>
<td>24.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.RC</td>
<td>22.5</td>
<td>22.0</td>
<td>-0.5</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.MB</td>
<td>26.0</td>
<td>25.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>15.PM</td>
<td>22.0</td>
<td>22.0</td>
<td>0.0</td>
</tr>
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<td>16.PG</td>
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<td>-</td>
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<td>17.HG</td>
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<td>21.CC</td>
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<td>25.0</td>
<td>-0.5</td>
</tr>
<tr>
<td>22.JM</td>
<td>27.0</td>
<td>27.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
3.5 Birth weights of babies in study

Mothers were asked to record the birth weights of their infants. These weights, originally given in avoirdupois, were those recorded by midwives immediately post partum (Table 3.1). The mothers of two premature babies were subsequently lost to the study as measurements were not available at 8 months pregnant or at 7 days post partum. None of the remaining infants had birth weights less than 2500 g: the lightest was 2637, and the heaviest 4536 g.

3.6 Suckling Patterns

Each subject was instructed to time her baby’s suckling bouts from the first attachment post partum and record them in the diary provided. Suckling times were recorded post partum to the first midnight (day 0) and for the subsequent 48 hours (days 1 and 2). None of the subjects reported any difficulty in establishing breast feeding during this time and all records were conscientiously kept. Results are given in Table 3.6.

Seventeen subjects recorded their infants’ suckling patterns and the mean of the two days was calculated (day 1). The number of times infants were put to the breast varied from 7.0 to 20.5 / 24 h, with a mean of 12.5 (SD 3.6). Suckling times varied from 7.1 to 20.0 minutes/feed with a mean of 12.8 (SD 4.2). The total time spent suckling (day 1) varied from 65.5 minutes to 351.5 minutes / 24h period with a mean time of 157 minutes (SD 74.6).

By day 8 the number of feeds was between 5 and 12 with a mean of 8.4 (SD 2.1). The time spent suckling varied between 9.2 and 34.9 minutes / feed with a mean of 20.0 (SD 6.7). The total time spent suckling ranged from 76.5 to 257 minutes, a mean of 158.5 minutes (SD 46.4).

For suckling time, the difference is not statistically significant (t is 0.075 with 16 df, p >0.1). For number of feeds, there is a statistically significant reduction (t is 5.34 with 16 df, p < .001).
Table 3.6

Mean suckling times (min) on days 1 and 8 post partum /24 hours together with the number of feeds on those days /24 hours.

The mean milk intake on day 8 g /24 hours is also shown together with the mean milk intake / feed.

<table>
<thead>
<tr>
<th>ID</th>
<th>Suckling time (min) day 1</th>
<th>No. of feeds day 1</th>
<th>Suckling time (min) day 8</th>
<th>No. of feeds day 8</th>
<th>Total milk intake (g) day 8</th>
<th>Mean milk intake/feed day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.CS</td>
<td>287</td>
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<td>111</td>
<td>12</td>
<td>685</td>
<td>57</td>
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<td>16</td>
<td>145</td>
<td>10</td>
<td>865</td>
<td>87</td>
</tr>
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<td>147</td>
<td>6</td>
<td>339</td>
<td>57</td>
</tr>
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<td>197</td>
<td>10</td>
<td>650</td>
<td>65</td>
</tr>
<tr>
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<td>16</td>
<td>102</td>
<td>7</td>
<td>463</td>
<td>66</td>
</tr>
<tr>
<td>8.JP</td>
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<td>7</td>
<td>150</td>
<td>7</td>
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<td>87</td>
</tr>
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<td>6</td>
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<td>28</td>
</tr>
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<td>11</td>
<td>201</td>
<td>10</td>
<td>588</td>
<td>59</td>
</tr>
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<td>39</td>
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<td>10</td>
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<td>64</td>
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<td>716</td>
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3.7 Relationship between milk intake and other variables

The aim of this study was to examine two variables that have a bearing on milk intake i.e. breast enlargement during pregnancy and suckling patterns in the first two days post-partum. The effect of birth weight and of anthropometric variables was also of interest, though subsidiary to the purpose of the main study.

Milk intake at day 8 (means for 2 days) ranged from 169 to 865 g/day, with a mean of 547 (SD 180) (Fig 3.4). When these values were plotted against the changes in breast volume from 3 to 8 months, no clear relationship was evident (Fig 3.5). The regression of milk intake/24 h on the number of feeds was statistically not significant.

\[
predicted \text{ milk intake} = 618 - 0.62 \times \text{breast volume increment}
\]
\[
t = .17 \text{ with 1,15 degrees of freedom, } r = -0.36, p > 0.1
\]

A clear relationship was, however, shown between milk intake and the number of feeds on day 8 (Fig 3.6):

\[
predicted \text{ milk intake} = 43 + 60.8 \times \text{number of feeds on day 8}
\]
\[
t = 3.67 \text{ with 1,15 degrees of freedom, } r = .69, p < 0.01.
\]

This regression shows that for every additional feed/24h the baby drank about 61 g more milk.

A definite relationship was also found to exist between milk intake on day 8 and the number of feeds on day 1 (Fig 3.7):

\[
predicted \text{ milk intake} = 227 + 26 \times \text{number of feeds on day 1}
\]
\[
t = 2.28 \text{ with 1,15 degrees of freedom, } r = .51, p < 0.05.
\]
Birth weight was found to have no significant effect on milk intake on day 8 (Fig 3.8):

\[ t = 0.403 \text{ with } 1.15 \text{ degrees of freedom, } r = 0.1, \ p = >0.5. \]

Nor did birth weight correlate with the number of feeds on day 8 (Fig 3.9). However, one subject (9.FM) is clearly atypical in Fig 3.7 - a very large baby with a very low intake. The data points were checked carefully, with no indication of error. When subject 9.FM was weighted out, the relationship was clear and statistically significant:

\[
\text{predicted milk intake} = -353 + 0.27 \times \text{birth weight}
\]

\[ t = 3.18 \text{ with } 1.14 \text{ degrees of freedom, } p = 0.001. \]

Subject 9.FM was 1.7 m tall weighing 59.8 kg and had a BMI of 20.7. At 3 months pregnant her mean breast volume was 323 ml and it increased by only 17 ml to 340 ml by 8 months pregnant and her upper arm girth had not increased at all during pregnancy. Her baby (male) had a birth weight of 4536 g and on day 8 took only 169 ml of milk/24 hours at 6 feeds. He did not receive supplements and at 8 days post partum did not appear distressed in any way. This subject’s first pregnancy and early post-natal period had followed an almost identical course and she had been obliged to bottle feed her previous infant by three weeks post partum as her milk supply was insufficient. This subject is indicated in all relevant graphs.
Figure 3.4

Milk intake (ml) of 17 infants on day 8 post partum.
Figure 3.5

Milk intake (g) of 17 infants on day 8 post partum/24 hours plotted against the increase in breast volume (ml) of their mothers from 3 - 8 months pregnant.

There is no statistically significant relationship.
Figure 3.6

Milk intake (g) of 17 infants on day 8 post partum/24 hours plotted against the number of feeds of each infant on day 8 post partum/24 hours.
Figure 3.7

Milk intake (g) of 17 infants on day 1 post partum/24 hours plotted against the number of feeds of each infant on day 1 post partum/24 hours.
Figure 3.8
Milk intake of 17 infants on day 8 post partum/24 hours plotted against birth weight.
Figure 3.9

Number of feeds on day 8 post partum/24 hours plotted against birth weight.
CHAPTER 4

DISCUSSION

The intake of milk is of paramount importance to the new-born of every mammalian species. In her essays on the evolution of lactation, Pond (1977) noted that although the mammalian offspring is mechanically separated from the mother at birth, it remains dependant upon her for nourishment for a period thereafter. Lactation enables the offspring to continue growth after birth in an 'embryonic' manner without compromising the mother's ability to follow her normal pattern of activity, encumbered by the increasing size and weight of a growing foetus. Milk provides a highly nutritious, completely digestible food source for the offspring. The colostrum may also have an effect in maturing the infant's intestinal brush border enzymes (Labbok 1985).

The non-pregnant female breast contains mainly adipose tissue. The problem is in defining how much of the enlargement during pregnancy is due to the deposition of fatty tissue and how much to the growth of alveolar tissue; there being no non-invasive way of distinguishing the two.

The main aims of this thesis have been first, to investigate an important anatomical predictor of milk production in lactating women, the increase in breast volume during pregnancy; and second, to investigate the part that suckling, in the first 48 hours post partum, plays when milk production is established by day 8 post partum. The method used to measure the changes in breast volume in this research followed Archimedes' principle as used by Hytten (1954), and had similarities to the method of Milligan et al. (1975). The method of water displacement was chosen as being most acceptable to pregnant women in this study and within the technical ability of the tester. Wax impressions of plaster casts (Ingleby 1949, Campagne 1979) are time consuming and specialised in their application and development. Air displacement was another option considered but information on this proved too fragmentary.
Hytten (1954) was principally interested in the milk production of the mother and stripped the breast mechanically, after each feed. The studies in this thesis were based on the naturally occurring milk intake of the infants. This distinction is very important and may explain the discrepancy between his conclusions and mine.

There were some other differences in approach. Hytten (1954) measured only the left breast whereas in this study both breasts were measured and the mean calculated (Chapter 1.2). The advantage of the method in this study over that of Hytten (1954) was that the displacement vessel could be used to measure both breasts and could be handled efficiently, by a single operator; an important factor in attaining standardisation of measurements. A standing position was chosen, rather than the kneeling position of Milligan et al. (1975) as being more comfortable for subjects as pregnancy progressed. Anthropometric limb measurements proved simple and errors in their accuracy were again minimised by being done by one tester. All measurements, linear and volumetric, were done in triplicate as an aid to accuracy and means calculated.

Arguing from comparative anatomy and biochemical and anatomical measurements, Pond (1990) described the adipose tissue of the thorax and upper arms as being derived from the same subcutaneous depot, the pectoral portion of which in girls becomes permanently enlarged at puberty forming the adipose tissue component of the breasts (Chapter 1.1). Gallup (1982) suggested that this enlargement signalled sexual maturity. Measuring the relative increase in girth of the upper arm during pregnancy provides a simple, if indirect indication of the relative increase in fat deposited in the breast during the same period. The small measure of fat deposition in the upper arms and by inference in the breasts, of subjects in this project agrees with the work of Campagne et al. (1979) who stated that the breast provides a relatively small adipose tissue depot, estimated by them as being only 4% of the total adipose tissue of a normal, healthy, young female (Chapter 1.1). Work carried out by Johnson et al. (1988) studying obese females also showed that arm circumference did not contribute to upper body fat loading but that the circumference of the thigh contributed to that of the lower body. Although one subject in the
current study was obese the results of upper arm and thigh measurements during pregnancy, followed this pattern of fat deposition. The theory also agrees with the research findings of Rebuffé-Scrive (1987) who reported that triglycerides were more easily mobilised from the femoral region during lactation than from the adipose tissue of the breast itself. Anthropometric measurement is a way of estimating the changes in adipose tissue deposits and was chosen as being more acceptable than skin-fold measurements, to this group of women.

Women taking part in this study were not a random sample of pregnant women but chosen because they were not more than 3 months pregnant when first recruited and were committed to breast feeding. The measurements of all subjects recruited to the project were recorded at 3 months pregnant, although 5 subjects withdrew at various stages thereafter. The data of 17 subjects was completed and early data from the five withdrawals was also used. The drop-out rate of 22.7%, by the end of the project, is unlikely to have biased the outcome of this study. This study provided the opportunity to find out whether breast volume could be assessed from bra size and if measuring increase in bra size could be a valid method of measuring increase in breast volume during pregnancy. In this country, bra design is not done volumetrically but by using sets of rather complicated linear measurements. However, knowing cup size and girth measurement and using a regression equation the volume of any bra could be estimated (Chapter 3.2). Such estimations, though less accurate than direct volumetric measurement of the breast could be of use when volumetric methods were unacceptable. Bra volumes could also prove useful in recruiting subjects at term, provided that women could give a history of their bra requirements throughout their pregnancy. The objection to these data is that, although the majority of subjects showed an increase in their breast size, by no means all found the need to change their bra size, possibly because the increase in breast volume incorporates the lateral spread of breast tissue as well as forward fullness.

Not all pregnant women experience a marked increase in breast volume as the present study has shown. One subject (9.FM) increased her bra size only once, from 34A to 34B, during the whole period from pre-pregnancy to full lactation at 8 days post-partum (Chapter 2.5), her
breasts were symmetrical but small. She had a mean breast volume of 323 ml at 3 months which had increased to 340 ml at 8 months pregnant, an overall increase of only 17 ml. This subject gave birth to a male infant weighing 4536 g. No problems were encountered in establishing lactation but by day 8 the infant was feeding six times and taking only 169 ml/24 hours. A previous pregnancy had followed a similar pattern and the subject began to bottle feed when her milk supply was inadequate at 3 weeks post partum, a history which was subsequently repeated with the infant in this study. It is also of interest to note that the upper arm girth of this subject did not increase during pregnancy suggesting that by inference, the increase in breast volume although very small, was due to the proliferation of glandular tissue (Chapter 1.1). The mean increase in thigh girth, in this subject, was 2 cm which was towards the lower end of the range of increases in thigh girths recorded (Table 3.3).

In the present study increase in breast volume from 3 - 8 months pregnant had no significant relationship with milk intake on Day 8 (Fig 3.5). It showed a clear negative, not an equivocal result. This conclusion apparently contradicts the results of Hytten (1954). The explanation is perhaps that in this study the natural intake of the infant was measured, and not the milk that could be stripped from the breast. Work published by Drewett and Woolridge (1980), Neville et al. (1988), Dewey et al. (1991) proved that infants control their own milk intake whilst not necessarily depleting the breasts.

In contrast a clear relationship between milk intake and the number of feeds/24 hours was seen. Those infants who had the greatest number of feeds took in the most milk (Fig 3.6). Milk intake on day 8 was also greatest in those infants who had spent the most time suckling on day 1 (Fig 3.7). These results are consistent with the findings of Dewey et al. (1991) who showed that the total time nursing was positively associated with milk intake in 3 month old infants. The amount of suckling from birth through the first few days post partum appears to stimulate the breast to produce milk at an increasing rate up to day 8. This finding agrees with those of Carvalho et al. (1983) who showed experimentally that early and frequent suckling resulted in greater milk output by day 15.
Birth weight in this study did not have any effect on either milk intake (Fig 3.8) or on the number of feeds on day 8 (Fig 3.9), but when the single grossly discrepant case, of a heavy birth-weight infant with a low milk intake, was weighted out, the statistical significance of the association with birth weight was clear. Neville et al. (1988) showed, in their studies of early lactation, that the milk volume transfer of each mother/infant pair was not significantly related to milk yield on day 6 or to birth weight, but to infant weight at 3 months. The results of the present study are however similar to the findings of Dewey et al. (1991) who showed that birth weight was positively associated with intake. In explanation Dewey et al. (1991) suggested that the association of birth weight with intake could be due to the greater energy demands of larger infants who suck more strongly and frequently to fulfil their needs, whereas smaller birth weight infants have smaller energy needs and are therefore less demanding in their intake of milk to support adequate growth.

In conclusion, the results of this study differ from those of Hytten (1954). Breast enlargement during pregnancy had no effect on the natural intake of milk by 8 day old infants, in contrast to Hytten's (1954) findings that breast enlargement affected the amount of milk produced by the mother on day 7 post partum. The amount of time spent suckling during the first 2 days post partum had a definite effect on infant milk intake on day 8. The number of feeds on day 8 also showed a clear relationship with milk intake on that day. Birth weight was found to have no significant effect on the number of feeds, but was associated with milk intake on day 8 post partum.

Infant milk intake is necessary for infant growth and whilst there has been much interest and research into energy intake from the first month of lactation, research during the first few days post partum is meagre. However, it is during this crucial few days that the suckling pattern of the infant is established, upon which may depend the success or failure of breast feeding.
APPENDIX
APPENDIX 1 : Informed Consent

RESEARCH ON BREAST FEEDING

As part of a programme of work on breastfeeding we are carrying out a study with breastfeeding mums from early pregnancy until the introduction of weaning foods.

The commonest single problem breastfeeding women report worldwide is concern for their milk supply, and the aim of the project is to throw some light on this. The measurements involved are very simple: they include measurement of changes in breast volume and of upper arm circumference during pregnancy and of nursing patterns and baby’s milk intake after birth.

Breastfeeding mums who have taken part in our studies in the past have generally found them interesting, and we would be very pleased if you would join the project by signing below.

Shirley Whiteley (Mrs)
Robert Drewett (Dr)

Department of Psychology,
University of Durham,
Science Laboratories,
South Road,
Durham.
DH1 3LE.

Tel: 091 374 2619

I am willing to take part in the breastfeeding project. I understand that I am free to withdraw from the project at any time if I wish to do so.

Signed ........................................

Name (please print) .........................

Date .........................................
APPENDIX 1: Subject Data

Mother's Name ........................................................................

Date of Birth (as 24: Jun: 58) ........................................

Address .................................................................................

Tel No. ................................................................................

Contact ...............................................................................

Mother's Height .....................................................................

   ... ft ... ins

Weight (at beginning of pregnancy) ........................................

   ... st ... lbs

Bra. size (before pregnancy - as 36B) ...............................  

L.M.P. ...................................................................................

Hospital/Home Delivery .....................................................

Baby Due ...............................................................................

Number of previous pregnancies ........................................

   (born alive) .....................................................................

Others (miscarriage, abortion, still birth: enter M,A or SB)  

No. of children breast fed ....................................................

(i.e. put to the breast at least once) ....................................

Handedness (right : left) .......................................................  

Name of G.P. .........................................................................

Name of Obstetrician ...........................................................

Hospital ................................................................................

Date of First Visit (12 wks) ................................................

Date of Second Visit (8 mths) .............................................

Date of Third Visit (7 days post-partum) .............................
### APPENDIX 2: Data Record at 12 weeks pregnant

<table>
<thead>
<tr>
<th>Metrical Parameter</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mother’s name</td>
<td>.......</td>
<td>.......</td>
</tr>
<tr>
<td>Breast Volume (ml)</td>
<td>.......</td>
<td>.......</td>
</tr>
<tr>
<td>Vessel used</td>
<td>.......</td>
<td>.......</td>
</tr>
<tr>
<td>Volume of vessel</td>
<td>: ml</td>
<td>: ml</td>
</tr>
<tr>
<td>Water remaining in vessel after breast measurement (ml)</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>Displacement (i.e. breast volume)</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>Anthropological measurement (cm)</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>Wrist</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>F’arm</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>Elbow</td>
<td>: .......</td>
<td>: .......</td>
</tr>
<tr>
<td>U’arm</td>
<td>: .......</td>
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<tr>
<td>Ankle</td>
<td>: .......</td>
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<tr>
<td>Calf</td>
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<tr>
<td>Knee</td>
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</tr>
<tr>
<td>Thigh</td>
<td>: .......</td>
<td>: .......</td>
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</tbody>
</table>
**APPENDIX 2 : Data Record at 8 months pregnant**

<table>
<thead>
<tr>
<th>Breast volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel used</td>
</tr>
<tr>
<td>Volume</td>
</tr>
<tr>
<td>Water remaining in vessel after breast measurement (ml)</td>
</tr>
</tbody>
</table>

<table>
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</table>

<table>
<thead>
<tr>
<th>Displacement (i.e. breast volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
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<td>Left</td>
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<tr>
<td>:</td>
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<tr>
<td>:</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Anthropological measurement (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
</tr>
<tr>
<td>Left</td>
</tr>
<tr>
<td>Wrist</td>
</tr>
<tr>
<td>F'arm</td>
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<tr>
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<td>Calf</td>
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<td>Knee</td>
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<tr>
<td>Thigh</td>
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| :     | :    |
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| :     | :    |
| :     | :    |
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| :     | :    |
| :     | :    |
| :     | :    |

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APPENDIX 2 : Data Record at 7 days post partum

Mother's name ...........................................

Breast Volume (ml)

Vessel used ...............

Volume of vessel : ml

Water remaining in vessel after breast measurement

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>Left</th>
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<tbody>
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</table>

Displacement (i.e. breast volume)

<table>
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<th>Right</th>
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<tbody>
<tr>
<td></td>
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</table>

Anthropological measurements (cm)

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Wrist</td>
<td>:</td>
<td>:</td>
</tr>
<tr>
<td>P'arm</td>
<td>:</td>
<td>:</td>
</tr>
<tr>
<td>Elbow</td>
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<td>:</td>
</tr>
<tr>
<td>U'arm</td>
<td>:</td>
<td>:</td>
</tr>
<tr>
<td>Ankle</td>
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</tr>
<tr>
<td>Calf</td>
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<tr>
<td>Knee</td>
<td>:</td>
<td>:</td>
</tr>
<tr>
<td>Thigh</td>
<td>:</td>
<td>:</td>
</tr>
</tbody>
</table>

Baby's Name ................. Sex M : F ....

D.O.B. : : Birth weight .......

Type of Delivery (SVD : Forceps : CS) ...........

Breast feeding problems:-
APPENDIX 3 : Suckling Diary

Suckling Record from Birth

Please keep this record from the birth of your baby for the remainder of the day of birth up to midnight, and for the following 48 hours.

Please record every occasion when your baby suckles, to the nearest minute. Even very short feeds, or comfort feeds should be recorded; but only breast feeds.

<table>
<thead>
<tr>
<th>Name</th>
<th>Time of birth</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Start time</th>
<th>End time</th>
<th>Which breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>eg. 3.5.89</td>
<td>4.20</td>
<td>4.25</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>4.27</td>
<td>4.40</td>
<td>/</td>
</tr>
</tbody>
</table>

"Start time" : the time when the baby takes the nipple into his/her mouth.
"End time" : the time when the nipple comes out of the baby's mouth.
APPENDIX 3 : Weighing and Suckling Diary

Please keep this diary from 10 a.m. on ....... until 10 a.m. on ....... (over 48 hours).

Please record *every feed* day and night over this period.

<table>
<thead>
<tr>
<th>Feed No.</th>
<th>Weight before feed</th>
<th>Time feed starts feed</th>
<th>Time feed ends feed</th>
<th>Weight after feed</th>
<th>Which breast</th>
</tr>
</thead>
</table>

Please add any comments that you think would be relevant.
APPENDIX 4 : Weighing Instructions

Please weigh your baby before and after every feed between:

10 a.m. on ....... and 10 a.m. on ......

SETTING UP BALANCE

Please use the following procedure:

BEFORE FEEDING

1) Make sure both Balance and Printer are switched on.
2) Press Printer Keys ....

\[ 2 \ 0 \ \text{STO} \ 5 \ \text{PRO} \ 2 \]

\text{(the balance will display \ 20)}

3) Place sheet or blanket in the balance pan and leave it there until you switch everything off.

4) Press RED bar marked "T" on balance

\text{(the balance will display \ 0)}

5) Press # key on printer and wait for the balance to count to 20.

\text{(it will display a number less than 1.0 which will also be printed on the paper tape.)}

WEIGHING THE BABY

1) PLACE THE BABY (CLOTHED) ON THE BLANKET IN THE PAN. Check that baby and blanket are not touching anything e.g. yourself or a wall.

2) PRESS KEY ON PRINTER # \text{wait for balance to count to 20 (it will then display the baby's weight and print it on the tape.)}

3) Please write this WEIGHT in the DIARY provided.

4) Press # a second time, wait and record.

5) Press # a third time, wait and record.

When you have finished all the weighings at a feed, switch off both the balance and printer.

THANK YOU.
REFERENCES


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Rebuffé-Scrive, M. 1987, Regional Adipose Tissue Metabolism in Women During and After Reproductive Life and in Men. *Recent Advances in Obesity Research* 5, 82-91.


