Breastfeeding and Immunity in Ariaal Mothers and Infants

by

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In Memory of my Dad, Ronald A. Miller

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Chapter I

Anthropological Perspectives on Breastfeeding and Immunity

Introduction

For most of human history, infants were fed breastmilk or faced death from

malnutrition and disease. The introduction of infant formula in Western nations was

culturally popular and increasingly safe as sanitation measures improved throughout the 20th

century (Hrdy 1999; Small 1998). As birth rates in the developed world decreased, formula

companies began marketing in developing countries to expand their customer base. Their

marketing techniques focused on health care workers, asking them to promote formula as

more nutritious than breastmilk and urging new mothers to use free samples just long enough

for their milk supply to disappear, forcing their dependence on formula. This sparked outrage

among activists that lead to the late 1970s boycott against the Nestlé Corporation (Muller

1974). There were three main factors contributing to this public health disaster: 1)

contaminated water and unsanitary containers were used to mix the formula; 2) poor mothers

used diluted mixtures to save formula; and 3) the immune factors in breastmilk that protect

against disease are not present in formula. It is unknown how many infants were affected by

this advertising campaign, but researchers estimate that non-breastfed infants in the

developing world are seven times more likely to die of diarrheal disease and six times more

likely to die of respiratory infections than breastfed children (Black et al. 2003). Because of

the boycott, the World Health Organization published standards for ethical marketing of

formula (WHO 1981) and increased the visibility of their recommendations of exclusive breastfeeding during the first 6 months of life (WHO 2001). To this day, Nestlé funds breastfeeding research in developing countries to help improve their image around the world (Nestlé Foundation 2008).

In part due to the visibility of the boycott, fields ranging from public health to biomedicine to biological anthropology have tackled various questions that arise from breastfeeding. Although biological anthropologists have explored how breastfeeding influences maternal and infant health, they have not yet directly assessed its immunological effects. The focus of this dissertation is to investigate the immunology of the breastfeeding system from an anthropological perspective. Specifically, I will be concerned with the following questions: 1) What reproductive and nutritional characteristics influence mothers' production of breastmilk immunity? and 2) How does breastmilk immunity influence infant growth and immune development? These questions will be explored in the cultural, ecological and environmental context of the Ariaal people of northern Kenya.

In this chapter, I will discuss the cultural and evolutionary aspects of breastfeeding and immunity, focusing on immunoglobulin A. In order to provide context for the function of IgA, I begin with a short description of the human immune system. I then provide an overview of the physiology of breastfeeding and the content of human breastmilk with special focus on IgA characteristics. Next, breastfeeding will be discussed in the context of mammalian and human evolution. From there, I describe the nutritional and immunological effects of breastfeeding on mothers and infants. This will be followed by a section describing cultural variation in breastfeeding practices in populations around the world. Finally, I conclude by introducing the chapters of this dissertation.

Overview of Human Immune Function

The human immune system consists of two components: the innate immune system and the adaptive immune system (Janeway 2005). The innate immune system, found in some form in all animals, provides non-specific defense against invading pathogens. It does not retain immunological memory of prior infections, but is the second line of defense against infection behind physical barriers such as skin. It acts quickly using a wide array of chemical and cellular mechanisms. The primary actors in the innate immune system are phagocytes, natural killer cells, and the complement system. Phagocytes are white blood cells that travel in the bloodstream and through tissues, patrolling for pathogens and devouring them. Natural killer cells force pathogens and infected cells to self-destruct. The complement system is a system of proteins that destroy the cell membranes of bacteria, killing the cell. Together, these complex interacting systems destroy most pathogens encountered by members of the animal kingdom.

The adaptive immune system, found only in gnastosomates, or vertebrates with jaws, is responsible for immunological memory. That is, it can adapt to each pathogen and retain memory of each infection. Immunological memory is used to prevent re-infection by previously encountered pathogens. The adaptive immune system is divided into two parts: cell-mediated immunity and humoral immunity. Although they interact with each other, each part plays a different role in combating infectious diseases.

Cell-mediated immunity involves T-cell response to an antigen, particularly antigens that have infected the body's cells. Although it is most effective at removing viruses, it can also provide protection from unicellular parasites, bacteria, and fungi. The T-cells in cell-

mediated immunity work in several ways. They can activate white blood cells to destroy infected cells, stimulate other cells in both the innate and adaptive immune system, and destroy cells that show evidence of viral infection.

Humoral immunity is primarily concerned with antibody-secreting B-cells and is most effective against circulating antigens, such as bacteria, rather than antigens that have infected cells. B-cells that are fully mature retain a "memory" of the specific antibody that fought a particular infection. Antibodies (or immunoglobulins) fight infection in many ways, and each class of antibodies play different roles in the immune system. After an antibody has bound to an antigen, it can perform three functions: prevent toxic effects of pathogens by neutralizing them; enable white blood cells to kill the cell (called opsonization); or trigger the activation of the complement system. There are five classes of antibodies.

Immunoglobulin G (IgG) is the most common antibody in the bloodstream and has many different functions, including activating natural killer cells, enhancing the actions of phagocytic cells through opsonization, and activating parts of the complement system. It is the only antibody that can cross the placenta and plays a large role in protecting neonates from infection. IgM is the first antibody that is produced in response to infection and is the second most common serum antibody. Its main role is to activate some parts of the complement system. IgD accounts for only a fraction of the antibodies in the blood and is similar in structure to IgM. It is unclear if IgD performs any useful function in the bloodstream, but secretory IgD (sIgD) plays a role in activating immature B-cells. IgE is also very rarely found in the blood stream. It is primarily found attached to structures called mast cells, which are white blood cells that are found beneath the skin and mucosal surfaces. IgE antibodies are designed to fight parasites, particularly worms, by attaching to mast cells in

large numbers when a parasite invades. When enough IgE molecules attach to mast cells, they burst open, sending out a range of chemicals (notably histamines) to destroy the worm. IgA, the last antibody subclass, is concentrated in mucosal tissues and breastmilk. IgA is the biomarker of interest in this dissertation.

Immunoglobulin A

Immunoglobulin A Structure and Function. IgA has a similar structure to other immunoglobulins, a variable region that corresponds to each antigen and a constant region that calls upon innate immune mechanisms. In addition, the antibody is also composed of two heavy and two light chains that are bound together by flexible disulfide bonds. There are five classes of heavy chains corresponding to the five antibody types; IgA is defined by the presence of an α-chain (Janeway 2005). The constant region of the α-chain is consistent across all IgA molecules, while the variable region is specific to the B-cell that produced it.

There are two subclasses of IgA, IgA1 and IgA2. They are distinguished by structural differences within their α-chain. Although IgA1 is the more abundant of the two, the ratio of IgA1 to IgA2 varies depending on the location in the body (Mestecky and Russell 1986). Approximately 75% of the IgA in the blood is IgA1. Only 60% of the IgA in mucosal secretions is IgA1, partially due to the fact that IgA2 is resistant to digestion by enzymes produced by gut pathogens (Brown and Mestecky 1985). In addition, more IgA2-producing cells are found in the gut, demonstrating the importance of IgA2 for neutralizing the effects of toxic bacteria in the gut.

Immunoglobulin A is found as a single antibody in circulating plasma, although levels are considerably less than IgG, comprising around 12% of serum antibodies (Janeway

2005). Secretory IgA, on the other hand, is found in abundance in secretions from mucosal surfaces, such as the gastrointestinal, respiratory, and urogenital tracts, as well as in tears and breast milk. Secretory IgA is created by two IgA molecules connected by a secretory component and a compound called a J chain (Russell et al. 1999). Approximately 90-95% of the IgA present in mucosal secretions and breastmilk is in the dimeric secretory form (Mestecky and McGhee 1987). The secretory component draws IgA from the serum and lymphoid tissues found in the mucosa and passes them onto the mucosal surface (Russell et al. 1999). However, serum IgA contributes only a small amount to the total sIgA in the mucosal tissues, making mucosal and systemic immunity highly compartmentalized (Mestecky 1987).

Immunoglobulin A works primarily by coating epithelial cells in the mucosal respiratory, gastrointestinal, and urogenital tracts, preventing pathogens from passing through or adhering to the mucosal surfaces, and preventing colonization (Mestecky and McGhee 1987; Russell et al. 1999). It gathers pathogens into large "clumps" so that they can be passed out of the body. Specific IgA antibodies are generated by B-cells that are present in the mucosal tissues; after the initial infection, these antibodies recognize and bind to specific pathogens to prevent re-infection. These mechanisms work for bacteria, viruses, enzymes and toxins (Brown and Mestecky 1985; Russell et al. 1985). Interestingly, the mucosal immune system in the gut tolerates a wide variety of non-toxic, commensal bacteria through a system of antibody activation that differs slightly from the pattern found in serum (Mestecky and Russell 1986). In addition, IgA helps suppress inflammatory responses and inhibits complement activation by IgG (Russell et al. 1999).

Although infections in the mucosal tissues may be restricted to one local area, specific antibodies that form against those pathogens are distributed throughout the mucosal tissues in the body (Mestecky 1987). This demonstrates that mucosal immunity is an integrated system, with the mucosal tissues communicating with one another. This allows immunological memory of an infection in one mucosal tissue to be "remembered" in another to prevent later re-infection. Although IgA levels in secretions can vary widely depending on the fluid (Jackson et al. 1999), salivary IgA is a good indicator of the general functioning of the mucosal immune system (Mestecky 1993).

The mammary glands, though they are not mucosal tissue, are part of the common mucosal immune system. IgA is the main antibody passed from mother to infant via breastfeeding, a process known as passive immunity. During breastfeeding, immunoglobulin A from the mother's mucosal gut tissue migrates to her breasts and is passed to her infant (Brandtzaeg 2003). The IgA coats the infant's digestive tract, facilitating the development of healthy gut bacteria that can out-compete harmful bacteria and offer protection against pathogens that the mother has encountered in her environment (Cripps et al. 1991; Walker 2004; Weaver 1992). A wide variety of specific sIgA against diarrheal and respiratory diseases is found in breast milk, protecting the infant's respiratory and gastrointestinal systems from infection (Butte et al. 1984; Goldman 1993). The IgA found in breastmilk may also play a role in priming future immune function, with breastfed babies having lower incidences of allergy, asthma, and autoimmune disease compared to formula-fed infants (Hanson 1998; Hanson and Telemo 1999). Therefore, the mother and infant share a common mucosal immune system that has an effect long after the infant develops adequate endogenous defenses.

Immunoglobulin A and the Life Course. Infants are not capable of producing adult

levels of IgA in their mucosa. This competency increases with age. The IgA transferred to

from the mother helps protect the infant from diarrhea and can elevate infant IgA levels.

Some studies indicate that this maternal contribution decreases over time, leaving the infant

less protected against gut infection (Fitzsimmons et al. 1994), while others show no evidence

of this decline (Hennart et al. 1991; Weaver et al. 1998). Seasonality, smoking, mood,

infection, and socioeconomic status are all thought to influence breastmilk IgA production

(Groer et al. 2004; Groer et al. 1994). These confounders will be addressed in future

chapters. In general, however, the source of variation in breastmilk IgA is not well

understood.

Infants reach adult levels of sIgA in the mucosa during childhood and maintain these

levels until old age. The age at which children reach these levels is associated with

breastfeeding duration and community disease burden (Johansson et al. 1994; Weemaes et al.

2003). Secretory IgA levels also vary based on nutritional status, socioeconomic status,

gender and other factors (Nuesslein et al. 1995; Weber-Mzell et al. 2004); these potential

confounding factors will be addressed in later chapters. Children reach adult levels of serum

IgA more slowly than mucosal IgA (Weemaes et al. 2003).

There is considerable evidence that mucosal immune function decreases as people

age, due to both fewer immunoglobulins as well as drying and thinning of mucosal tissues

(Vissink et al. 1996). However, this may vary among individuals, in part due to the amount

of stress encountered in day-to-day life (Evans et al. 2000; Flinn and England 1997). The

relationship between stress and IgA levels is unclear; there does not appear to be an

association between cortisol and IgA in saliva (Kugler et al. 1992) but there is an inverse relationship between the two in breastmilk (Groer et al. 1994).

Energetics of IgA Production. About 66 mg/kg body weight of IgA is produced every day, an amount that far exceeds the amount of other antibodies produced (Conley and Delacroix 1987). Despite the large volume of IgA produced every day the energetic cost of IgA production is unknown. However, secretory IgA is known to fluctuate with nutritional status (McDade 2005), providing evidence that the production of IgA is an energetically costly component of immune function.

Breastfeeding Physiology and Breastmilk Composition

Breastmilk Secretion and Maintenance. Breast tissue is sensitive to reproductive hormones that fluctuate during the menstrual cycle and pregnancy. Increased estrogen and progesterone levels during pregnancy stimulate secretion of prolactin from the pituitary gland and increased placental lactogen decreases prolactin-inhibiting factor. These hormones prepare the mammary glands for lactation during pregnancy. After birth, the mother-infant interaction and neuroendocrinology dictate the course of lactation. Stimulation from nipple sucking releases prolactin and oxytocin, which regulate milk production and milk ejection ("let-down" reflex). The behavioral-hormonal feedback loop is complex, and the mother-child relationship plays a large role in mediating mutually positive lactation. Breastfeeding must be started relatively soon after birth and infants must suckle frequently to maintain maternal lactation. Lactation will cease quickly when suckling stops (Lunn 1992; McNeilly et al. 1994; Ogra et al. 2006; Stallings et al. 1996).

Breastmilk itself is produced in the mammary glands. Protein, fat and carbohydrates are synthesized in mammary secretory cells. Other milk components leak between the mammary epithelial cells and the interstitial fluid between milk and blood (Ogra et al. 2006). Breastmilk is produced "on-demand" so daily amounts vary based on the size, age, and appetite of the infant. However, the average woman produces 19-30 ounces per day depending on infant age (Dewey et al. 1984; Dewey and Lonnerdal 1983).

Breastmilk Composition. Although human milk is 86-87.5% water, there are many nutritional and immunological compounds that compose human milk. Approximately 7% of breast milk is carbohydrate in the form of lactose, 3-4.5% is fat, and 1% is protein. These percentages vary depending on what type of milk the mother is secreting: colostrum, transitional milk, or mature milk produced about two weeks after birth. Fats are primarily triglycerides and long-chain fatty acids with trace amounts of cholesterol. The nutritional proteins are classified as whey or casein proteins; however there are a significant proportion of non-nutritive proteins as well, such as immunoglobulins, carrier proteins, growth factors, hormones, enzymes, and cytokines (Ogra et al. 2006). The immunological effects of some of these proteins are discussed in more depth below.

Both vitamins and minerals are passed through breast milk as well. Breastmilk contains A, B-complex, C, D, E, and K vitamins, although D, E, and K vitamins are found at low levels. Some breastfed infants require supplements and may develop rickets if not exposed to sunlight for vitamin D synthesis. Minerals in breast milk are at much lower concentrations than in commercial formula and include sodium, potassium, calcium, phosphorus, chloride, magnesium, iron, zinc, copper, iodine, manganese, fluoride, selenium, and boron. Mineral levels tend to decline over the course of lactation (Ogra et al. 2006).

Harmful substances can be passed to infants through breastmilk as well. They can generally be categorized into two types: unsafe chemicals and infectious agents. Mothers can secrete a variety of prescription drugs and non-prescription drugs in breastmilk. A list of those known to cause health problems in infants can be found in Table 5-9 of Ogra et al. (2006). Environmental contaminants that do not cause much harm to the mother can also be passed, sometimes in high quantities. They include caffeine, alcohol, nicotine, DDT, lead, mercury, and radioactive isotopes. Infectious agents can also pass through breast milk. This includes bacteria such as *Salmonella*, *Escherichia coli*, *Mycobacterium*, *Streptococcus*, and *Staphylococcus* as well as viruses such as rubella, varicella-zoster, Hepatitis B and C, West Nile, herpes simplex, and human immunodeficiency virus (HIV). Approximately 16% of exclusively breastfed infants with an HIV positive mother become infected, making postnatal transmission a serious public health concern (World Health Organization 2004).

On the other hand, many immunological components pass through breast milk. They are considered part of the infant's protein intake; however most immunological substances are not broken down for energy in the gastrointestinal tract. Both adaptive and innate substances are passed through breastmilk. Innate compounds include a variety of complement and anti-pathogen proteins, lactoferrin, lysozyme, macrophages, and neutrophils. Adaptive compounds include all classes of immunoglobulins, histocompatibility antigens, cytokines, T lymphocytes and B lymphocytes. Aside from IgA, lactoferrin and lysozyme play the largest role in preventing infection. Lactoferrin, an iron-binding protein, has anti-bacterial functions that perform in conjunction with IgA. Lysozyme helps inhibit gut and skin bacteria (Ogra et al. 2006). Lactoferrin and IgA levels tend to decline over time, while lysozyme concentration increases over the course of lactation (Goldman et al. 1982). A

further discussion of the immunological effects of various substances is reviewed in Ogra et al. (2006).

Breastfeeding and Human Evolution

Breastfeeding is one of the defining characteristics of the class Mammalia – so named because all female members develop mammary glands that feed their offspring. The evolutionary origins of the mammary gland is lost in time, but it may have evolved from a sweat gland that was used to keep egg shells moist (Oftedal 2002). The length of time mammals breastfeed their infants depends on each species' life history. The life history of an organism is the collection of phenotypes that impact the survival and reproduction of an organism (Stearns 1992). Life history characteristics are driven by variation in extrinsic mortality and tend to correlate highly with one another (Charnov 1993). Life history characteristics include adult body and brain size, age at weaning, puberty, and first birth, age-specific mortality and fertility, interbirth interval, litter size, and duration of gestation. In general, small-bodied mammals have shorter lifespans and therefore earlier occurrence of life stages. Members of the order Primates have longer lifespans relative to their body size compared to other orders of placental mammals, which extends many life stages, including breastfeeding duration.

Within the order Primates, every segment of the lifespan increases with increasing body size. Large-bodied primates such as the great apes generally tend to wean their offspring later than do smaller primates such as strepsirrhines. Chimpanzees wean around 5 years of age (Watts and Pusey 1993), gorillas between 3 to 4 years (Watts and Pusey 1993), and orangutans between 5-7 years (van Noordwijk et al. 2009). Dettwyler (2004) used

several lines of evidence from primate life history characteristics to predict the expected age at complete weaning for humans. She used five primate traits that have been proposed to predict age at weaning: 1) tripling or quadrupling birth weight, 2) reaching one-third of adult body weight, 3) adult female body weight, 4) gestation length, and 5) age at 1st molar eruption. Based on the allometric relationships between these characteristics and age at weaning, she predicted that the "natural" age at weaning for human infants is between 2.5 and 7 years of age. Most values appear to center around 6 years of age (Dettwyler 1995).

However, cross-cultural evidence indicates that most non-industrial societies have an average age of complete weaning of less than 2.5 years (Sellen and Smay 2001). There are few, difficult-to-test hypotheses that explain this shorter breastfeeding period. Kennedy (2005) proposed that when early members of the genus *Homo* shifted to consumption of energy-dense meat, infants were given this food at younger ages to facilitate brain growth. However, this hypothesis does not consider that a long weaning process, in which infants are breastfed for long periods of time while eating high-calorie food, would provide more calories while offering protection from pathogens that may be introduced through food. It is also possible that human evolution selected for shorter interbirth intervals, requiring earlier weaning times (Wells and Stock 2007). Human infants have a higher percentage of fat than other mammals, helping buffer infants through malnutrition and poor health associated with early weaning and helping alleviate the heavy energetic demands of brain growth (Kuzawa 1998). In contrast to adaptive hypotheses, Dettwyler posits that shorter breastfeeding duration is a maladaptive artifact of cultural expectations (Dettwyler 1995). The cultural ecologies that contribute to individual and population variation will be discussed further below.

Although the evolution of a shorter human breastfeeding period is unclear, patterns of breastfeeding in living human populations can also be examined from an evolutionary perspective. Life history theory generates predictions that can also be used to examine physiological and reproductive phenotypes at the population and individual level. Variations in life history characteristics are based on the allocation of energy to different bodily functions. Adaptive life history strategies seek to allocate limited energy to growth, maintenance and reproductive efforts in a way that maximizes individual fitness (Stearns 1992). Immune function, along with cellular repair and organ function, is generally considered as part of the maintenance effort of an individual. Immune function, particularly adaptive immune function, is thought to compete with growth and reproductive efforts for available energy. There is evidence in the animal kingdom that this is the case (reviews in Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000). It is reasonable to assume that humans allocate energy in similar ways. In one of the few examples of trade-offs between growth and immunity, McDade et al. (McDade et al. 2008) found that acute inflammation predicted smaller gains in height after three months. In addition, I found that pregnant women have different immune profiles than non-pregnant women, down-regulating more energetically costly adaptive immunity while up-regulating less costly innate immunity (Miller 2009). Both results hint at energetic or immune life history trade-offs in humans.

Because breastfeeding is so energetically costly, it is an ideal system to investigate life history trade-offs in humans. Parent-offspring conflict, a hypothesis driven by competing reproductive, growth, and maintenance strategies, has been used to explain patterns of behavior in breastfeeding mammals. Trivers (1974) made several general predictions about the evolutionary patterning of transfer of resources between parents and children. Parent-

offspring conflict theory predicts that mothers will decrease their investment in offspring

relative to trade-offs they make between current offspring and future reproduction. This

resource patterning is time-sensitive relative to the reproductive interests of the mother and

the age-specific mortality probabilities of the offspring (Hill and Kaplan 1999). Often the

maternal decrease in investment occurs before it is in the infant's best reproductive interests,

leading to conflict between the two. This conflict can be evident in behavioral observations

(Fouts et al. 2005) or allocation of resources during fetal growth (Haig 1993).

Immunoglobulin A may be a proximate indicator of available somatic resources in both

mothers and infants. If it is provided at some cost to the mother for the benefit of the infant, it

may demonstrate patterns consistent with parent-offspring conflict. Specifically, IgA in

breastmilk would decline with increasing infant age, reflecting decreasing maternal

investment in current offspring with time. Conversely, higher levels of IgA would be

associated with better growth and health outcomes, demonstrating a benefit to the infant.

Breastfeeding has other evolutionary implications as well. The nutritional and

immunological molecules passed through breastmilk affect the phenotype of offspring,

making it a possible mode of inheritance. Growing evidence shows that maternal effects are

an adaptation that allows quick offspring phenotypic response to environmental variation

based on mothers' lifetime experience (Mousseau and Fox 1998). One good example is the

breastfeeding system in cows. IgA in cow milk passes through the gut of the calf and into the

bloodstream, facilitating serum immune development that persists throughout the calf's life

(MacDonald 1990). Human intestines do not operate the same way – IgA from breast milk

cannot be passed through the mucosal cells into the bloodstream – but the IgA can affect the

growth of bacteria in the gut, contributing to improved digestion and health (Koutras and

Vigorita 1989). These bacteria can, in turn, be passed to breastfed girls' future infants through two pathways: 1) during vaginal birth in which infants are colonized by bacteria from the mother's vaginal canal and rectum (Orrhage and Nord 1999) and 2) via bacteria in the mothers' mouth shared through kissing (Fessler and Abrams 2004). There is some evidence that IgA from the mother can prime infant B-cells as well (Hanson 1998). Clinical evidence has shown that breastfeeding can influence the long-term immunological phenotype of offspring in humans (Hanson and Telemo 1999), with non-breastfed individuals more likely to suffer autoimmune, allergy, and asthmatic disease. The non-clinical long-term immune effects of breastfeeding are much less well known and remain open questions for population biologists.

If the effects of the immunity gained from breastfeeding produce long-term effects on offspring, there is a possibility that these effects may be passed to the next generation of breastfed individuals. In the early 1990s, Barker advanced the hypothesis that low birth weight was associated with adult cardiovascular disease (Barker 1990). Since then, there is considerable evidence that prenatal nutrition influences adult phenotype by providing cues to the nutritional environment that the fetus can expect during adulthood (Gluckman et al. 2007). These cues can cause disease when they are incorrect; that is, an individual "expecting" sparse nutrition but encountering overnutrition is likely to experience adult disease. Kuzawa (2005) furthered these findings by suggesting that these cues last over several generations, providing information about somewhat short-term environmental fluctuations. These maternal effects may apply to immunity via two routes: transfer of maternal IgG through the placenta to the infants' blood stream and 2) transfer of maternal IgA via breastfeeding to the infants' mucosal surfaces. The immunological cues from the

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specific antibodies delivered by the mother may provide information about the disease ecology, possibly for several generations. There is evidence from animal studies that maternal transfer of immunity can influence immunological phenotype over two generations (Grindstaff et al. 2003). The potential for multigenerational effects of immune function transfer in humans exists, but data to examine such effects is currently unavailable. There is huge potential for long-term and multigenerational population studies of immune function in humans which have implications for mechanisms of human evolution.

The Effects of Breastfeeding on Maternal Reproduction

Breastfeeding is extremely energetically costly for mothers. Lactation requires about 600-700 kcal per day of energetic expense beyond maintenance costs (Hrdy 1999). The cost of producing IgA is likely a relatively small percentage of the energy required to synthesize all of the nutritional and immunological components of breastmilk. Even a small energetic cost, however, may play a role in shaping the total fertility of the mother. Therefore, IgA production may be a costly form of maternal investment. Reproductive energetics are subject to life history trade-offs; this allows humans to space births in a way that can maximize their fertility.

Breastfeeding can suppress ovulation through both nutritional and hormonal pathways. This phenomenon is known as lactational amenorrhea. Several factors influence the duration of lactational amenorrhea. There is evidence that levels of prolactin, released during breastfeeding, are positively associated with frequency of breastfeeding (Stallings et al. 1998). Since prolactin levels are also associated with ovarian suppression, some hypothesized that frequent feeding induces amenorrhea. In addition to prolactin, nutritional

factors also play a role. Decreased body mass index (BMI) and decreased intake of dietary fat are associated with increased duration of postpartum amenorrhea (Popkin et al. 1993). Better nourished Au women of Papua New Guinea return to menstruation faster than poorly nourished women (Tracer 1996). In a review of available studies, Ellison (Ellison 2001) found an interactive relationship between frequency of breastfeeding and nutritional status, with women who breastfeed frequently and who have low energy availability having the longest duration of lactational amenorrhea. Women who have high nutrition stores and breastfeed less often, such as many Western women, return to their menstrual cycle much more quickly. Women who have high nutritional stores and high breastfeeding frequency or low nutritional stores and low breastfeeding frequency have intermediate durations of amenorrhea. He concludes that prolactin levels must be higher to tip the scales towards milk production in well-nourished women while the threshold is much lower for poorly nourished ones. He also notes that milk production is relatively buffered against maternal energetic constraints as long as prolactin levels are high enough while conversely ovulation is particularly sensitive to energy during this time (Ellison 2003). This indicates a life history trade-off between investing in current offspring versus future offspring, allowing mothers with fewer nutritional stores to space births more widely in hopes of increasing fertility. However, women who are severely deficient in energy may experience decreased fertility, maintenance effort or both.

In order to buffer against energetic fluctuations that may occur during reproduction, women have considerably higher percent body fat, mostly located on the lower body, compared to men (Eveleth and Tanner 1990). Sexual dimorphism in fat deposition occurs during puberty, allowing women to store extra energy for reproduction. Women in

nutritionally stressed environments have fewer fat stores and may find it difficult to recover from the energetic demands of pregnancy and breastfeeding. This phenomenon is known as maternal depletion; it is defined as parity-related decline in fat reserves due to negative energy balance during pregnancy and lactation (Jelliffe and Maddocks 1964). Maternal depletion has been documented in many marginal nutritional environments. For example, Au women of Papua New Guinea demonstrate depletion of fat with parity independent of their age (Tracer 1991). Northern Kenyan women of low socioeconomic status experience parity-related decreases in BMI. This decrease is found in conjunction with alarmingly low caloric intakes and high workloads (Shell-Duncan and Yung 2004). Short-term weight decreases due to lactation that contribute to long-term depletion have been documented in Filipino women (Adair and Popkin 1992). Maternal depletion can occur in other ways as well; for example breastfeeding for longer than 6 months causes bone loss even when adequate dietary calcium is available (Sowers et al. 1993). Anemia, goiter, and edema may also be signs of maternal depletion of micronutrients (Winkvist et al. 1992). Despite an overall increase in total body fat, lower body fat percent is lower and central body fat is higher with increasing parity in US women, demonstrating a subtle form of maternal depletion (Lassek and Gaulin 2006).

The Effects of Breastfeeding and Weaning on Infant Growth and Health

Due to the proliferation of formula, some Westerners are unaware breastfeeding provides vital benefits for infants' growth and development. As described above, breastmilk provides complete nutrition as well as immunological benefits to infants. At some point in development infants must begin eating solid foods, a process known as weaning. Weaning can be very gradual, beginning with supplementation of foods and ending with the cessation

of breastfeeding (Bateson 1994). There are several nutritional and health factors that must be considered when weaning infants.

When to wean is a complicated matter that varies considerably across populations. Eating solid food increases infants' chance of pathogen exposure and reduced levels of protective breastmilk make weaning a potentially dangerous period for young infants. However, consuming breastmilk alone does not provide enough energy for the huge energetic demands of the developing brain (Kuzawa 1998). This balance between risk of malnutrition and risk of disease is known as the weanling's dilemma (McDade and Worthman 1998). To balance the cost of inadequate nutrition and higher pathogen load with the immunological benefits of breastfeeding, the World Health Organization recommends that infants be exclusively breastfed until the age of 6 months (Kramer and Kakuma 2004). However, some research indicates that it may be more advantageous for infants to begin supplemental feeding earlier to avoid mortality due to malnutrition (Wilson et al. 2006). Duration of exclusive breastfeeding and weaning must depend on the local nutritional and disease ecology (Gray 1996; McDade and Worthman 1998).

Humans' extremely high levels of infant body fat provide energy to buffer against energy fluctuations due to disease and growth during weaning (Kuzawa 1998). Despite large fat stores, weaning may be associated with poorer health and growth outcomes in marginal populations. For example, Ecuadorian infants experience severe growth retardation before 12 months, in part due to lower-quality supplemental foods (Leonard et al. 2000). Among the Amele of Papua New Guinea, infant growth begins to falter by 4 months of age and does not stabilize until 2 years of age (Jenkins et al. 1984). Kenyan infants are born with z-scores for height and weight close to international standards, but begin to lag behind infants in well-

nourished populations by the age of 6 months (National Council for Population and Development et al. 2003). Adding supplementary foods can increase infant morbidity as well. Infants in the Philippines who were supplemented with only liquids such as tea or water were two or three times more likely to suffer from diarrhea than exclusively breastfeed infants. Adding additional foods increased risk of morbidity due to diarrhea ten to thirteen times compared that suffered by exclusively breastfed infants (Popkin et al. 1990). Turkana infants experience high levels of infant mortality due in part to poor nutrition and early supplementation (Gray 1996). Cultural behaviors and competing interests due to parent-offspring conflict may also contribute to poor infant outcomes in nutritionally stressed groups (McDade 2001).

Culture and Breastfeeding Patterns

Along with physiological, nutritional, and disease factors, culture plays a large role in dictating the course of breastfeeding. Cultural attitudes regarding the roles of women and mothers, child development and care, diet, sexuality, medicine and breastmilk itself shape infant nutrition in interesting ways. Food itself offers an insight into cultural factors influencing the weaning process. Despite wide variation in adult diets worldwide, there are certain broad patterns associated with the feeding of supplementary foods. Liquids are often the first non-breastmilk food given to infants, followed by soft carbohydrates (Sellen and Smay 2001). Usually by 2 years, infants are eating the same foods as adults. An analysis of the Human Relations Area Files (HRAF) revealed differences in types and timing of supplementation between agriculturalists, pastoralists, and hunter-gatherers (Sellen and Smay 2001). Hunter-gatherers introduce liquids very early and completely wean their infants later

than other societies. Liquid supplementation in agricultural groups and solid food supplementation in pastoralists were relatively delayed compared to other subsistence groups. All societies have appropriate weaning foods and subsistence practice plays little role in the cessation of breastfeeding. Instead, breastfeeding patterns are more likely to be influenced by resource availability (McDade 2001) and mother's work schedule (Fouts et al. 2005) regardless of the subsistence base of the population.

Cultural attitudes towards breastfeeding can influence breastfeeding behaviors and weaning. Women living in the US face many choices when it comes to infant feeding: choosing to breastfeed or formula feed or pumping breastmilk to use in a bottle later. Feeding schedules become very important to mothers, influenced by beliefs about precise measurement of feeding and scientific approaches to childrearing (Small 1998). These choices are influenced by a variety of factors, including work schedule, family and personal decisions, the advice of experts, and cultural mores. For example, in the mid-twentieth century United States, formula was seen as a better source of nutrition and was recommended by childcare experts. Breastfeeding is only now making a comeback in this country. Public breastfeeding also continues to be stigmatized in the US as inappropriate and lewd, stemming from the strong association between breasts and sexuality (Hrdy 1999; Small 1998). In addition, women in the US are discouraged by medical professionals from sleeping with their infants during the night, reducing the ease of night feedings, making milk supply more difficult to establish and increasing the use of bottles (Small 1998). Co-sleeping improves ease of breastfeeding, regulates infant respiration, and may reduce the incidence of sudden infant death syndrome (McKenna et al. 2007). It is a prevalent practice among non-Western

and traditional societies. Some cultures, such as Maya women in Guatemala, express shock that US infants are made to sleep alone (Morelli et al. 1992).

More traditional societies tend to breastfeed more often than Western societies, sometimes whenever the infant demands it. For example, the !Kung San, a group of hunter-gatherers in Botswana, carry infants and feed on demand (Konner and Worthman 1980). The pastoralist Turkana breastfeed during the day and night as often as their work schedule allows (Gray 1995). Even in these societies, women's ability to breastfeed is constrained by their work (Fouts et al. 2005). Fetching water, searching for firewood, and paid labor can all influence an infant's feeding schedule. The need to work forces mothers to seek childcare. Infants at any age may be watched by kin or non-relatives. The need for mothers to have child care is the basis for the grandmother hypothesis, which suggests that a long postmenopausal period evolved so that grandmothers can help take care of their grandchildren (O'Connell et al. 1999). Often older sisters watch their young siblings. Friends and other adults sometimes have reciprocal childcare arrangements. These adult caretakers occasionally breastfeed their charges themselves.

A wet nurse is a woman who breastfeeds an infant who is not her own. Wet nursing is a well-known practice in current and historical societies although rare in developed countries due to the availability of formula or milk banks. The physiological ramifications of wet nursing and caring for unrelated children are mostly unknown, although drawbacks include diseases that may be passed through breastmilk and providing milk whose composition inappropriate for the age of the infant (La Leche League International 2008). Reasons for using a wet nurse include inability or unwillingness to breastfeed a child, insufficient milk, death of the mother, or desire to have another child quickly after birth (Hrdy 1999). For

example, high status women in medieval Europe, who were expected to have as many infants as possible during their reproductive lifetime, gave infants to wet nurses in order to resume ovulation. The combination of high infant survival from wet nursing and numerous pregnancies led to an extremely high fertility rate among elite women (Hrdy 1999). By the French Revolution, nearly all infants born were nursed by someone other than their mother, although infants from poor families still had lower survival rates (Hrdy 1999). Wet nurses are usually compensated for their milk and infant care, either monetarily, through reciprocal wet nursing, or through the status and security boost gained from nursing a high status child. In some cultures, infants who share the same wet nurse share a special type of kinship, known as milk kinship. Milk kinship was a widespread practice in Islam societies during the middle ages. There is a legal relationship between infants who nurse at the same breast that are governed by complex laws. Milk kinship was used to create alliances between families who did not want to be tied by marriage, as marriage between those related through milk is forbidden (Khatib-Chahidi 1995).

While these studies show an interesting picture of the variation found in breastfeeding in cultures around the world, very few connect mother and infant physiology with cultural practices. In order to fully explore evolutionary trade-offs in immunity within a cultural framework a population that experiences marginal nutrition, high disease load, and practices natural contraception is required. The Ariaal of northern Kenya fit all of these requirements, making them an ideal population to explore breastfeeding and immunity in depth. The culture and breastfeeding ecology of the Ariaal will be discussed in depth in Chapter 2.

Conclusion

The breastfeeding system generates several questions that are of interest to anthropology. This dissertation will attempt to answer biocultural and evolutionary questions relating to the transfer of IgA from mother to infant through breastfeeding. Chapter 2 will discuss the culture and ecology of the Ariaal and present the results of a cultural consensus designed to test mothers' knowledge of infant health and care. Mothers' knowledge of traditional medicine will then be correlated with infant immune function, illness and growth outcomes to investigate the relationship between cultural competency and health outcomes. It will attempt to replicate the findings of McDade et al. (2007), who found that ethnobotanical knowledge was positively associated with child growth and health, in an African population. Chapter 3 will review the field and laboratory methodology used, focusing on the development of an ELISA assay for IgA and its use in testing breastmilk and saliva samples stored on filter paper. Chapter 4 will use evolutionary hypotheses to address the variation found in Ariaal mothers' breastmilk IgA. Specifically, I propose that breastmilk IgA is a costly form of maternal investment that may be influenced by reproductive variables, such as time since birth, parity, and available energy. Chapter 5 will explore how breastmilk influences infant growth and immune development in the face of disease-exposing behaviors. Finally, Chapter 6 will synthesize the results of all chapters and suggest further work that can address unanswered questions.

Chapter II

Culture, Health and Breastfeeding Behaviors of the Ariaal

Introduction

Culture can play a major role in shaping human biological variation. Culture has allowed humans to adapt to a wide variety of ecological niches and can influence beliefs about, availability of, and use of various resources, all of which can have a profound effect on the human phenotype. Often, research in biological anthropology takes place in groups that exist outside mainstream Western research in order to understand the complete range of human variation. The research for this dissertation took place among the Ariaal, a group often cited by human biology researchers as an ideal population for studying life history questions, largely due to their adherence to a traditional way of life, high levels of malnutrition, and use of only natural forms of contraception. This chapter will begin by discussing the ecology, subsistence, and culture of the Ariaal people, present interview-based pilot work on infant health care and breastfeeding beliefs, and use the pilot data to test how mothers' knowledge of ethnomedicine affects infant health, growth, and immune function.

Overview of Ariaal Culture

The Ariaal are a group of about 10,000 semi-settled and settled pastoralists living in the deserts and mountains of Marsabit District, Kenya (Fratkin 1998). Figure 2.1

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shows a map of Marsabit District and Ariaal lands (Fratkin et al. 2004). The research

undertaken for this dissertation took place in Karare and surrounding areas, about 17

kilometers south of Marsabit Town, the district capital. Karare, one of the larger Ariaal

villages on Marsabit Mountain, has schools, small shops, churches, and a dispensary run

by the Catholic Church. However, there are no paved roads, electricity, running water, or

communication infrastructure available in Karare. Though the arid ecology takes a toll on

Ariaal, nutritional status and growth, they manage to adapt through a variety of cultural

and subsistence practices.

Ecology. Pastoralists occupy the vast majority of Kenyan lands, mostly areas

unsuitable for farming, even though they make up only a small percentage of the national

population (Fratkin 1998). The Ariaal live in semi-arid to arid land and raise a variety of

livestock as their main mode of subsistence. Pastoralists use a variety of strategies to

support their livelihood during both rainy and dry seasons. They maintain several

different livestock species that flourish in different ecologies. Camels thrive at lower

elevations, where rainfall is scarcer and water is saltier. Cattle are preferable in higher

elevations where there is more water and vegetation. Sheep and goats, while considered

less prestigious than larger livestock, help create a more diverse food base for the Ariaal

and can be raised at any elevation. A few families own donkeys to transport milk and

water and dogs to warn against predators. Livestock are fed by vegetation such as

acacias, scrub bush, and grasses. Generally, vegetation becomes sparser with decreasing

elevation. Despite this, the environment can support a wide variety of both domestic and

wild animals (Fratkin 1998).

Figure 2.1. Map of Kenya with Ariaal, Rendille, and Samburu geographic distribution and location of the study site.
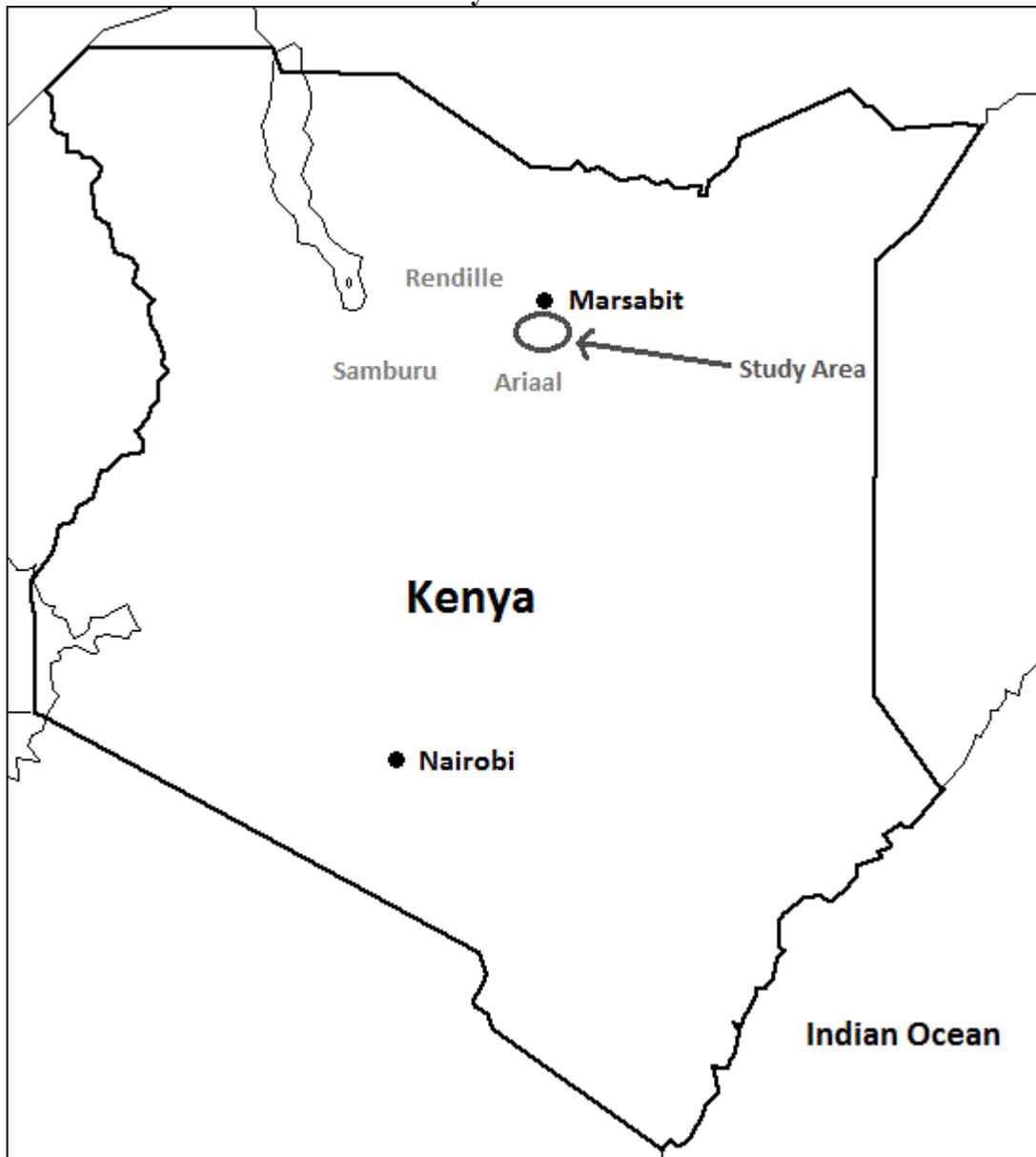
Marsabit District is the most arid region of Kenya. There are two wet seasons and two dry seasons per year, with the long rains occurring between March and May, and short rains occurring in October and November. The two dry seasons occur between November and March, and June through September. The rains allow vegetation to grow

and provide water for both livestock and people. During the dry season, the Ariaal must dig wells to find sufficient water. Many NGOs have responded to repeated droughts by constructing rainwater collection systems near many settlements. However, scarce water remains one of the most critical issues for Ariaal pastoralists (Fratkin 1998).

Many climactic and social developments have threatened the Ariaal ecology and way of life (see Fratkin 1998 for more detail). Global climate change has increased the length and severity of droughts in the area, in some cases causing the death of entire herds. Many other herds fall below replacement levels and cannot easily be replaced before drought strikes again. Several informants mentioned that 2006-2007 droughts were particularly devastating to cattle herds in the area. These droughts come on the heels of loss of communal lands to commercial ranches and population growth that restrict pastoralists to more marginal land. In response, various NGOs and religious groups have encouraged pastoral groups to settle into villages and towns by offering incentives such as schools, churches, health care, and water sources (Fratkin 1998). These groups have encouraged subsistence farming over pastoralism, a way of life that Ariaal consider less prestigious. Finally, Marsabit pastoralists are nearly constantly engaged in intergroup conflict. Cattle raids are intensified by the availability of guns from nearby Ethiopia. The Ariaal are relatively peaceful and often find themselves on the losing end of these disputes. The Gabra, Borana, and Turkana, who often cross the border into Ethiopia after attacks, are frequent antagonists (Galaty 2004). One of the few interventions from the Kenyan government has been to build airstrips in remote locations so that the military can quash some of these disputes.

Subsistence. The Ariaal in this study, including those that have settled, depend heavily on livestock for subsistence. They consume as much as possible from their animals: milk, blood, and meat are all used by the Ariaal. Milk and blood are renewable resources that provide calories, protein and micronutrients, particularly in the wet season when water is available to animals. Milk is available to all, while blood is primarily consumed by men (Fratkin 1998). Each food-producing species has a different productivity level and life history that allow the Ariaal to utilize resources at all times of the year. Camels need the least amount of water of any area livestock and can produce the most milk, blood and meat. However, their reproductive cycle is long, mortality is high, and the growth rate of herds is very low. For Ariaal in a camel ecology, it can be difficult to build and maintain herds; therefore, wealth is often inherited rather than built, and drought can make a rich man a poor one very easily (Spencer 1973). Camel herding is exclusively the domain of men and boys (Fratkin 1998). Ariaal (and Rendille) have a very complex system of camel sharing among unrelated men that signifies prestige to the stock owner. Poorer men, such as younger brothers that do not inherit camels, rely on patronage from wealthier men. In the worst case scenario, these men must rely on small stock (Spencer 1973).

Ariaal in the highlands keep drought-resistant cattle as their large stock. Cattle need to drink at least once every three days, making them unsuited to the arid lowlands (Fratkin 1998). They produce less milk than camels, but have a higher reproductive rate and higher population growth rate, making them more abundant and easier to replace. Cattle are also used more freely in the economy, with cattle forming the basis for bridewealth. This involves payment to a man in exchange for taking his daughter as a

wife. Cattle can be more easily sold for cash than camels. Cattle also play a large role in Ariaal ritual, slaughtered readily during weddings and age-set ceremonies (Spencer 1973). Young men in the warrior age-set are the primary cattle herders among the Ariaal, although sometimes young boys and girls will herd them. The Ariaal that participated in this research project are from the highlands and are primarily cattle owners, although some may have camels kept by relatives in the lowlands.

Although they are not considered prestigious, small livestock are a vital part of Ariaal subsistence and economy. Households generally have large herds of goats and/or sheep compared to cattle or camels. They can be easily sold for cash, given as a gift, or slaughtered for meat without drastically reducing the size of the herd (Fratkin 1998). Small livestock species have high reproductive rates and population growth rates and can rebound quickly in the face of drought. They are poor milk producers and are not used for blood; rather, sheep and goats are used mainly for their meat. They can be grazed close to settlements compared to cattle and camels, which must be taken across a large range. Sales of small stock can contribute significant cash to the household. Small livestock are generally herded by children (Fratkin 1998).

Encouraged by well-meaning religious groups and NGOs, some Ariaal have incorporated subsistence agriculture into their lifestyle. In drier areas such as Karare, only maize, beans and *sukuma wiki* (collard greens) can be grown; at higher elevations with more rainfall such as the community of Songa, fruits and vegetables can also be grown (Fratkin 1998). These crops can be eaten, sold in Marsabit Town for cash, or traded to more mobile pastoralists for herding labor. Agriculture has changed the structure of the community for settled Ariaal, providing more opportunities for the poor and granting

more autonomy to women. Farms are seen as less risky than livestock, particularly in the face of increasing drought due to climate change. According to Adano and Witsenberg (2004), agricultural residents on Marsabit mountain are happy with their way of life and do not wish to return to pastoralism. Most, however, maintain a mixed pastoralism/agricultural subsistence base.

Because of settlement, the Ariaal can participate in a wider regional economy than before. Livestock are sold to regional markets in Marsabit Town or south in Isiolo, contributing a significant amount to household income (Fratkin 1998). Cattle can command more money than small stock and are easier to replace than camels, making them an important contribution to household cash income. Women also benefit from the market economy. Women walk hours to Marsabit Town to sell milk or agricultural produce; however, this does not contribute much to household income. Wage labor from relatives who have moved away from the area, usually to Nairobi, also supplements household income (Fratkin and Smith 2004). Cash is used to buy food, including maize meal, cooking fat, tea, and sugar. Participation in the market economy varies based on geographic location and primary subsistence pattern, with settled agriculturalists participating the most and mobile pastoralists participating the least (Adano and Witsenburg 2004).

Despite their self-reported happiness, settlement has had, in general, a negative effect on Ariaal nutrition. Men in both settled and pastoral communities have body mass indexes that indicate acute undernutrition (Campbell et al. 2003). Women who reside in settlements, particularly agriculture-based settlements, have poor dietary intake and lower anthropometric outcomes compared to mobile pastoralists. This can be attributed to three

causes: lack of protein from milk, sale of maize and beans rather than consumption, and higher workloads. Poor and breastfeeding women are particularly hard-hit from lack of milk protein (Fujita et al. 2004a; Fujita et al. 2004b). Children have poorer anthropometric outcomes and growth rates in sedentary communities compared to pastoral communities as well (Nathan et al. 1996; Nathan et al. 2004). This finding is also tied to level of milk consumption with high protein levels in milk protecting children from protein-energy malnutrition during drought. Micronutrient deficiency is widespread, particularly vitamin A deficiency in reproductive-aged mothers (M. Fujita, personal communication). Despite the advantages to women's autonomy, lower risk of subsistence failure, and greater dietary diversity, poor diet is a critical problem in settled Ariaal women and children.

The health status of the Ariaal has been explored to a much lesser degree by anthropologists compared to nutritional status. The poor nutritional status among women and children suggest that health is a major concern to both settled and nomadic pastoralists. Children in settled communities experience significantly higher levels of anemia (Nathan et al. 1996). Child morbidity did not differ between settled and mobile populations; instead, incidence of fever and respiratory disease were tied to rainfall amounts. Further work on iron status indicated that over 30% of Rendille/Ariaal children are clinically anemic, with a higher anemia risk among girls than boys. In addition, this study found that 15.6% of children had elevated C-reactive protein levels, indicating inflammation due to recent infection (Shell-Duncan and McDade 2004; Shell-Duncan and McDade 2005). Pilot work undertaken in August 2006 indicated that up to 70% of infants experienced at least one symptom of illness during the previous 10 days as

reported by their mothers. Mothers reported diarrhea in 14% of the infants; the average

duration of diarrheal symptoms was six days. Forty-five percent of these mothers

reported at least one symptom of illness as well. NGOs attempt to vaccinate children

against a variety of diseases, although their coverage can be spotty in mobile

communities (Nathan et al. 1996). There are indications that poor health is a significant

issue for Ariaal women and children in addition to poor nutrition.

Culture. The Ariaal are a unique ethnic group not often listed in the Kenya tourist

brochures. Instead, they are the result of a cooperative relationship between the camel-

keeping Rendille of the arid lowlands west of Marsabit Mountain and the cattle-herding

Samburu of the western highlands (Fratkin 1998; Spencer 1973). The Ariaal are fluent in

both Rendille, a Cushitic language that is similar to Somali, and Samburu, a dialect of

Maa that comes from the Nilotic language group. The Ariaal in Karare prefer to speak

Samburu; therefore the foreign vocabulary used in this dissertation is Samburu unless

otherwise noted. The Ariaal, like the Samburu, are polygynous, although often men are

considerably older before they can afford to have a second wife. Both the Rendille and

the Samburu are organized into segmentary descent groups with moities, clans, subclans,

and families, and they are characterized by an age-set system in which male Ariaal follow

distinct life stages – boys, warriors, and elders – with particular behaviors prescribed for

each stage (Fratkin 1998; Spencer 1973). Women are characterized informally into girls,

adolescent unmarried girls, and married women. An exception is the *sapade* (Rendille),

who are women in the Rendille system whose marriage has been delayed until they are

much older (Spencer 1973). Although they are similarly organized, the Samburu and

Rendille use different clan names, exogamy systems, and perform age-set rituals during

different years (Fratkin 1998; Spencer 1973). In general, the Ariaal follow the age sets, clan organization, and rituals of the Samburu, while retaining camel-blessing rituals and prayers of the Rendille (Fratkin 1998).

The Ariaal, also referred to as Southern Rendille or *Masagera*, emerged from a complex interrelationship between the Rendille and Samburu due to their differing cultures, ecologies and animal husbandry systems (Spencer 1973). The size of the Rendille population in constrained by the arid conditions, the slow population growth rate of camels, and inheritance rules in which only the oldest son gains animals. The Rendille keep their population small in several ways: by keeping women as *sapade* thereby constraining their reproductive lifespans (Roth 1999; Spencer 1973), by younger sons joining the Samburu cattle economy, and by marrying women as second or third wives to Samburu husbands (Spencer 1973). This spillover from the Rendille to the Samburu helped form the Ariaal identity. Population rarely flows from Samburu into the Ariaal (Spencer 1973). Ariaal tend to own more cattle than camels, reflecting the relative ease of obtaining cattle over camels. However, most Ariaal own both cattle and camels, although camels may be kept by other kin or as part of camel sharing. Separate ethnographic accounts of Ariaal society by Spencer (1973) and Fratkin (1998) indicate that Ariaal ethnic identity may have shifted over time, to more Samburu-like traditions from more Rendille-like ones. However, oral histories that include the Ariaal date back to 1866 (Fratkin 1998), indicating a long association between the two groups. It is important to note that Ariaal identity is fluid and may change depending on context.

Kinship in Ariaal society is organized by segmental descent. Ariaal settlements follow Samburu clan rules. The Samburu population is divided into two moieties that

consist of several clans each, which are then divided into more subclans. These subclans

contain several lineages that can trace themselves back to a common ancestor.

Settlements are patrilineal and usually contain lineages from the same subclan (Fratkin

1998; Spencer 1973). Clan affiliation is vital in maintaining proper exogamy rules for

marriages – men marry women from outside their own clan. In addition, the Ariaal follow

the Samburu tradition of polygyny with bride wealth, usually reserved for wealthy, older

men. This practice often leaves many young widows in the Ariaal community, who

cannot remarry. Instead, they continue to bear children by their deceased husband's

relatives (Fratkin 1998).

Besides clan affiliation, the age-set system is one of the most important social

categories among the Ariaal and is a vital method for retaining knowledge through oral

histories. These categories govern how individuals may behave and relate to one another.

For men, age-sets follow a strict system punctuated by ritual circumcision (Fratkin 1998).

When it is time for a new age-set of young men (about every fourteen years), the young

men who are three age-sets below their fathers are gathered and ritually circumcised in

large groups. These ceremonies take place in large temporary camps and involve young

men aged 10-25 (Fratkin 1998; Spencer 1973). After circumcision, boys become morani

(which means literally circumcised guys, Cronk 2004) or warriors, and are responsible

for herding large livestock and protecting herds and camps from raids from other

pastoralists. A moran has considerable freedom to have girlfriends, sing late into the

night with other age-mates, carry weapons, and wear brightly-colored clothing. Two

years before the next age-set, morani are released from their warrior duties and are

allowed to marry and become elders (Fratkin 1998). Elder men are responsible for politics, managing wealth, resolving disputes, and arranging marriages.

Aside from the age-set system described above, there are many other rituals in Ariaal culture. Few of these rituals are linked to any religious belief, although the Ariaal who have not converted to Christianity or Islam maintain a belief in a supreme being, called *N'gai* (Samburu) or *Wahk* (Rendille, Fratkin 1998). They also believe in ghosts and spirits but have no tradition of ancestor worship. The Ariaal have a wide range of rituals, including those that involve rites of passage (of which circumcision is the most well-known), those that celebrate changes in season that the whole community participates in (such as traditional camel blessings that stem from Rendille traditions), and rituals that are designed to alleviate suffering from supernatural forces such as curses or sorcery (Fratkin 1998; Spencer 1973). These rituals can only be performed by specialized healers known as *laibon*. The *laibon* is a traditional role found in many Maa-speaking pastoralist groups, including the Samburu. *Laibon* are always male and possess considerable abilities to divine meaning from objects or acts (Fratkin 1991). They are well-versed in medicine and healing rituals, and their expertise generally makes them wealthy men with many wives. Further explanation of Ariaal, Samburu, and Rendille rituals are beyond the scope of this dissertation but instead can be explored in Spencer (1973).

Ariaal women have a more fluid age system than men although they do experience age-specific milestones (Fratkin 1998). Adolescent girls who have not yet married are free to dress colorfully and have boyfriends, provided they avoid pregnancy. The major transition in women's life is from unmarried to married, an occasion that is

also marked by circumcision ritual. Unlike male Ariaal circumcision, a woman's circumcision is done on an individual basis before her wedding. The procedure is a clitoridectomy, classified as a Type II circumcision according to the WHO (Shell-Duncan 2001). A more detailed account of the associated rituals can be found in Shell-Duncan (Shell-Duncan et al. 2004). Women who leave the area to pursue high school education, a privilege available to the wealthy, are generally circumcised before they leave for school. It is vital that women are circumcised before they bear children, and many Ariaal fear that high school girls will run off and marry before they are circumcised. Ariaal women have no choice in their marriage and are usually married to men that are at least one age-set older than their warrior boyfriends (Fratkin 1998). Marriage represents the loss of some freedoms but a gain in the ability to bear children, the most important and prestigious job of an Ariaal woman's life.

As in most other societies, women are responsible for more than child birth and care. They maintain the household, work in the gardens, and milk cattle. They are responsible for finding firewood and fetching water, activities that can take them far from home. Co-wives often share domestic tasks, reducing their labor (Fratkin 1998). Despite their hard work, Ariaal women cannot own animals, participate in politics, earn cash that they may keep, or control their children's futures (Fratkin and Smith 2004). Very few women have the means to support themselves away from the control of their male relatives or husbands, and many old widows lack basic security. As mentioned above, Ariaal women in agricultural settlements have more autonomy than pastoralist women at the cost of their nutrition and health.

Ariaal culture, particularly many of the Samburu aspects, corresponds fairly well to Kaplan et al.'s (2009) evolutionary predictions of how resource base influences society structure. For example, they predict that pastoralist societies have large resource inequalities, specific inheritance rules, high need for leadership roles, and practice polygyny and bride wealth. Interestingly, the Rendille do not conform as well as the Samburu to these predictions. For example, rates of polygyny are relatively low among the Rendille. This may be due to differences between cattle and camel ecologies. Both societies, however, have a high degree of status inequalities between men, entirely dependent on the size of their herds.

Village Differences. Three settled Ariaal villages were sampled as part of this dissertation. While they share many cultural, social, and subsistence values, the communities differ in terms of resource availability. The following is a brief description of each village.

Karare is one of the largest settled Ariaal communities on Marsabit Mountain. It consists of a village center with small shops, classrooms, religious buildings including a mosque and a Catholic church, and a Catholic-run dispensary. Karare is located on the main road between Marsabit Town and southern Kenya. Because of this, shop owners will often engage in trade with passing trucks, reselling the goods to community members. Smaller communities (manyattas) surround Karare including a moderately-sized agricultural area (Scheme); they are considered part of the general Karare area. Karare mixes agriculture with livestock ownership. Many families grow a household garden and maintain moderate- to small-sized flocks of livestock.

By contrast, Parkishon is drier and more heavily dependent on livestock ownership than gardening. Parkishon is a community that has developed within the last 5-10 years in a location near the road but slightly farther down the mountain away from water sources. Because of this, there is less infrastructure including no classrooms, no medical resources, no shops, no religious institutions, and few latrines. Women in Parkishon sell milk for money, which they will either sell by the side of the road to trucks passing through, or send to Marsabit Town to sell in the market there. Many individuals in Parkishon spend time in remote pastoral camps, making participant sampling a difficult endeavor.

Finally, Kituruni is located far from the road, but closer to available water sources on Marsabit Mountain. Because of this, they are more reliant on agriculture than pastoralism, although most families do own animals as well. Kituruni was settled early in the settlement process and has been relatively successful due to a slightly wetter climate. To this end, Kituruni has churches, a government-run dispensary, latrines, and classrooms. Despite this development, individuals in Kituruni are at some disadvantage; the distance from the road prevents some trade and can hinder transfer to Marsabit hospital in the case of a medical emergency. On the other hand, the government dispensary provides the cheapest care, including free visits to children under the age of 5.

This overview provides a general picture of the organization of Ariaal society. However, little research so far has explored areas of Ariaal life that are most relevant to this dissertation, particularly breastfeeding beliefs and behaviors and health status and care. A pilot study undertaken in Fall 2007 used both qualitative and quantitative

interviewing techniques to interview married women about both issues, providing more detailed insight into the lives of mothers.

Cultural Consensus Methodology

Data Collection. Cultural consensus is a mathematical model for determining information about cultural domains and the knowledge of local informants within a culture (Romney 1999; Romney et al. 1986). This analysis is based on the belief that cultural knowledge is shared by members of a culture and therefore can be reliably and validly measured. It assumes that the researcher does not know the answers to the questions about each cultural domain and that cultural knowledge may be unequally distributed between individuals. There are two separate cultural domains in this study: knowledge of infant health and treatment of illness and breastfeeding beliefs and behaviors. While I defined these two topics, Ariaal women defined the parameters of these topics and fleshed out culturally important issues related to the selected topics. This study defined factors that relate to each cultural domain, assessed the knowledge of Ariaal informants, and attempts to determine "culturally correct" answers to each factor.

The cultural consensus data collection was conducted in two phases. The first phase was a free listing phase that required participants to list everything they could think of about infant care and breastfeeding practices. Every issue mentioned by women in the free list phase was used to define the factors associated with infant feeding and health in the Ariaal community. The second phase, the cultural consensus phase, required participants to answer a yes-no question for each factor listed in response to the two cultural domains. This helped assess the "culturally correct" answer to each question and

to understand the factors in this community related to each cultural domain. This analysis required no prior knowledge of the cultural domains by the investigator and allowed the Ariaal to define and confirm aspects of their culture without interpretation from researchers.

In order to determine all possible factors associated with the cultural domains of infant health and breastfeeding, a small subsample of Ariaal women located in close proximity to Karare (n = 11) were asked to free list everything related to those cultural domains. Questions were posed in a semi-structured interview format (Bernard 1994) and included these types of questions as examples: "How long are babies usually breastfed in your community?" and "What foods are fed to babies when they first begin to eat and how are the foods prepared?". These questions were followed up with probing questions when necessary.

The women's answers were compiled into a list that included the frequency of each response. Every factor mentioned by women about breastfeeding was included in the second phase. All infant illnesses that women mentioned were included in the second phase. The three highest-frequency symptoms were included for each illness. Western medical treatments for each illness with a list frequency greater than 1 were included, as were all traditional medicine treatments that were mentioned. In addition, since some women mentioned the importance of using different treatments depending on illness severity, a question regarding treatment decision was added for each illness (e.g. "Is it better to treat malaria at home before going to the hospital?").

During the second phase of the cultural consensus analysis, a master list of true-false questions was compiled based on the responses from the free listing phase. This list

of 145 of questions included nine questions that asked about the individual characteristics of women being interviewed, including number of children, socioeconomic status, latrine use, and location of home. The remaining 136 questions were based on the responses from phase one. Questions were organized into twelve sections: individual characteristics, breastfeeding behaviors, and a separate section for each illness listed by the first set of women. The illnesses listed included: pneumonia, common cold, diarrhea, malaria, measles, ntingadu (joint pain, possibly brucellosis), eye infections, ear infections, worms, and an unspecified illness that is caused by a tick. Each illness category included questions about symptoms, treatment decisions, and Western and local medicine used to treat each illness. Thirty women (n = 30) took part in the second phase of the cultural consensus interviews. Fifteen of the women were located in or around Karare and the dispensary, and fifteen were located in the village of Parkishon, a 10 km walk from Karare and the dispensary. Parkishon is more oriented toward pastoralism, residents tend to be more mobile, and has fewer markers of settlement, including no schools, religious establishments, Western health care, or latrines.

Statistical Methods. Quantitative data from the cultural consensus interviews were compiled into a matrix with participant on one axis and each question on the other axis, and the true-false answer coded as either 0 or 1. The master matrix was reorganized into five submatrices based on the relevant domains: breastfeeding knowledge, illness knowledge, care decision-making, Western medicine knowledge, and traditional medicine knowledge. Submatrices were analyzed using the cultural consensus capabilities in ANTHROPAC v. 4.98 (Borgatti 2006). This method uses factor analysis to assess the "culturally correct" answer to each true-false question as well as the cultural

knowledge of each individual interviewee. It can obtain significant results with very small sample size (Romney et al. 1986). A list of consensus questions with their culturally correct answer can be found in Appendix I.

Cultural consensus analyses begin by assuming individuals in the same group share one cultural model. To determine the best fit of this cultural model, it uses a least squares factor analysis with the minimum residual method. This procedure estimates and compares the cultural knowledge of each woman as well as the relative correctness of each answer. This process generates several factors, or eigenvalues, that can account for the variation found in the consensus matrix. The first eigenvalue represents the variance in the matrix due to sharing one cultural model, while the second eigenvalue represents variance due to other factors (Smith et al. 2004). In order for a matrix to be considered a likely cultural domain, it should have a ratio of the first eigenvalue to the second eigenvalue greater than 3:1, with a ratio of 10:1 providing strong support (Borgatti 1996). This indicates that the greatest amount of variance in the data is due to shared cultural knowledge rather than some other effect, supporting the assumption of one cultural model. The matrices in this study had ratios that ranged from 6.2:1 to 30.2:1, indicating that there is a high degree of consistency of responses in each domain that is indicative of Ariaal cultural beliefs.

Consensus analysis yields three useful results: 1) it determines the "correct" answer to each question, 2) it assesses the level of knowledge of each individual, and 3) it provides information about how well each question fits with other questions in the domain through comparison of eigenvalues. Since not all women are equally knowledgeable in the domains of infant feeding and health, individual knowledge levels

of a given cultural domain are the variables of interest for this study. Therefore, maternal knowledge in each subdomain is the main dependent variables of interest in phase two of this pilot study. Knowledge of a given domain is determined on a scale from 0 to 1, with a higher number indicating higher levels of knowledge.

In order to show what factors might influence respondent knowledge, each woman's knowledge of the cultural domains were associated with their individual characteristics. Number of children, geographic location, latrine use, attendance at a non-governmental organization maternal/infant health seminar, and self-described socioeconomic status were associated with women's knowledge level of each domain using either a simple correlation or an independent two-sample t-test for populations with unequal variance. Significance level was set at $\alpha = 0.05$. Results are discussed in the sections below.

Means and frequencies of maternal characteristics and knowledge level can be found in the table below. Knowledge of each cultural domain, on average, tended to be high, with all women having a knowledge score of at least 0.65 (65% knowledge). Women were the least knowledgeable about Western medicine and were the most knowledgeable about infant illnesses.

Table 2.1. Knowledge and Characteristics of Ariaal Women in Phase II of Pilot Study (*n* = 30).

Variable	Mean (S.D.) or Freq (%)
Knowledge of breastfeeding	0.82 (0.06)
Knowledge of illness	0.91 (0.04)
Knowledge of local medicine	0.85 (0.05)
Knowledge of Western medicine	0.74 (0.15)
Knowledge of care decision-making	0.74 (0.06)
Parity	4.09 (2.03)
Lives near Karare (vs 'far', in Parkishon)	15 (50.0%)
Considers self poor (vs. not poor)	17 (56.7%)
Attended an non-governmental organization seminar	19 (63.3%)
Boils water for infants	15 (50.0%)
Uses a latrine	13 (43.3%)
Uses dispensary if infant is sick	29 (96.7%)

Infant Feeding among the Ariaal

During the free listing phase of data collection, women painted a fairly consistent picture of infant feeding in their community. Women indicated that in general, infants are breastfed between two to three years. Women tended to be very emphatic about not feeding infants longer than three years, while breastfeeding less than two years was not unheard of. For the most part, mothers indicated that they or their husbands, made the decision to stop breastfeeding; it was less common that infants would decide to stop breastfeeding. One reason cited for the decision to stop breastfeeding was that the infant was "big enough". Some women indicated that husbands told their wives to stop breastfeeding because they decided it was time to have another child. Women said that they only breastfeed one child at a time and that older infants were weaned so that they could become pregnant again. However, they claimed that becoming pregnant was not a good reason to stop breastfeeding, even though one woman mentioned that it was

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possible to become pregnant even while breastfeeding. Therefore, the extent of ovulation suppression due to breastfeeding in this population remains an open question.

Infants are breastfed on demand at night and during the day provided the mother is not working elsewhere. Women reported that for three months after giving birth, mothers must stay at home and not perform heavy work outside of the home. Three months was the general consensus, although some women mentioned that if a mother did not have anyone to help them perform these chores then she would return to work much sooner. Women in this community are responsible for collecting firewood and fetching water, tasks that require hours of walking with heavy loads. Ideally, women have a relative, older daughter, or friend that can watch the infant while she performs these chores. If she does not, she puts the baby to sleep in the house and works as quickly as she can.

Women indicated that the first supplemental food for infants is cow milk. It was unclear when they began supplementing children with cow milk; women stated that they did not begin feeding supplemental food until 6 months, but there were indications that women did not consider cow milk to be "food," possibly indicating that liquids are not on par with solid foods in this population. Supplemental foods were most likely to be cooked, mashed potatoes, mashed beans, tea with milk, or other soft foods. Women indicated that they did not feed full meals like the rest of the family until the child was 2 or 3 years old, about the same time full weaning occurs. There was not a wide variety of weaning foods fed, reflecting the low dietary variety available to the Ariaal in Karare.

Some women during the free listing phase, including one woman who identified herself as a community health care worker associated with the non-governmental

organization Food for the Hungry, International (FHI), reported that FHI sponsored seminars within the community that encouraged exclusive breast feeding for 6 months before supplementing food. A variety of other maternal/infant health care topics were included during this seminar. However, it was unclear how many women in the community attended these seminars and how well they influenced women's knowledge. A test of how well these seminars affected knowledge is reported below.

There was no significant association between parity and level of breastfeeding knowledge (r = -0.19, $p > 0.05$). Further analysis indicated no difference in breastfeeding knowledge between nulli- and primiparous women versus multiparous women ($p > 0.05$), although the small sample size in the former category renders these results unreliable. Independent t-tests indicate that living near the dispensary (t = -1.87, $p = 0.072$), regularly using a latrine (t = 1.52, $p = 0.14$), attending an FHI-sponsored seminar (t = 0.00, $p = 1.00$), regularly boiling water for infants (t = 1.31, $p = 0.20$), and considering self poor (t = -0.66, $p = 0.51$) were not associated with breastfeeding knowledge.

The Ariaal can be compared to research on infant feeding among the Turkana, west of Lake Turkana in northern Kenya. Gray (1996) used interview and behavioral observation to understand actual and ideal breastfeeding strategies in Turkana mothers. She found that infants nursed on demand during both the day and the night. Mothers were rarely separated from their infants during the first twelve months, even when the mother was working. The first food fed to Turkana infants is butterfat beginning around a few weeks after birth. Around three to four months whole milk was added to infants' diets; higher-fat cow milk was given first, with cow or goat milk added later. Around eight months, infants began to eat milky tea, animal fat, and maize porridge. By the time

infants are about two and a half years old, they begin to eat nearly everything Turkana adults eat, including blood and meat. Weaning is timed to coincide with the introduction of these foods. The two main reasons cited for weaning included new pregnancy and the developmental stage of the child. Grey mentions that these weaning behaviors are strategies to maximize reproductive success, particularly the early addition of butterfat. This extremely early supplementation adds energetic benefits to infants and possibly outweighs any protection from infection that exclusive breastfeeding provides. Also noted were the differing weaning patterns during the wet and dry seasons, indicating that Turkana mothers tie breastfeeding decision-making with food availability and odds of infection.

There are many similarities and a few differences between the Ariaal and the Turkana. Although both Ariaal and Turkana women feed on demand, Ariaal women tend to report leaving their infant with a relative to perform chores. Ariaal women reported a much later age for adding supplementary food, and do not mention butterfat or blood as weaning foods. In Karare, camels are rare; therefore cow milk is the preferred livestock milk supplement. These differences may be due to the differing ecologies of the Ariaal and Turkana – the Ariaal in this study were settled and the Turkana in Grey (1996) are mobile pastoralists. The Ariaal at higher elevation rarely use camels and women and children do not often drink blood. Most interestingly, Ariaal women mention that they do not supplement until six months of age while Turkana women add food much earlier. Ariaal mothers may perceive different needs for their infants, de-emphasizing infant energetic reserves from supplementation. The reasons for this difference cannot be comprehensively answered by the data in this study, although it is possible that influence

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from Western-sponsored health initiatives have changed Ariaal mothers' knowledge of breastfeeding behaviors.

Knowledge of ideal breastfeeding behaviors in this community were not associated with any of the characteristics reported in this study. It is possible that women learn breastfeeding behaviors well before they give birth (through observing their mothers or some other way) and that knowledge is not associated with experience due to greater parity. Breastfeeding behaviors also appear to be independent of other health-promoting behaviors such as latrine use or water boiling. This is in line with the belief in this community that breastfeeding does not promote infant health. Furthermore, these results indicate that FHI seminars have no impact on women's knowledge whatsoever, possibly because ideal breastfeeding behaviors within the Ariaal community are already similar to WHO recommendations (WHO 2006). Finally, interviews with women indicate that Ariaal patterns of breastfeeding and supplementation are somewhat similar to the Turkana, and may be representative of other pastoralist groups in the region.

Health among the Ariaal

Women tended to define infant illness along the same lines as Western definitions of disease, including diarrhea, common cold and pneumonia, measles, malaria, worms, and eye and ear infections. There were a few exceptions, including ntingadu (a description of symptoms that may correspond to brucellosis) and 'illness caused by a tick', which is characterized by common cold symptoms and different areas of hot and cold on the body. It is unknown whether this disease corresponds to an actual tick-borne

disease. Women indicated there were two different ways for treating each illness: traditional medicine based on local plants that could be mixed at home, and treatment at a local dispensary by a nurse who administers Western medicine. For severe disease, infants would be referred to the hospital in Marsabit Town, although traveling there presents a significant hardship. Informants did not present a clear picture of how these care decisions were made, indicating sometimes that one went to the dispensary first, others indicating that illness was treated with traditional medicine before going to the dispensary.

Women in this community were very clear about which local medicines treated each disease or symptom, and they were clear that there were others that were either too harsh to be used on infants or treated diseases that infants could not possibly have (such as an STD). A knowledgeable elder helped identify each plant used as medicine and pictures were taken to aid identification. When possible, plant species were identified using Beentje et al. (1994). A list of Ariaal medicines using their traditional names, the diseases they treat, and tentative species and genus of each plant can be found in Appendix II. Botanical identifications were taken from (Heine et al. 1988) which relied heavily on Fratkin (1975; 1980) for Samburu names and medicinal uses.

Four submatrices characterized cultural domains of 'health' in this study. The factors that predict knowledge of these cultural domains and their significance can be found in Table 2.2. Significance was assessed at $\alpha = 0.05$; this was not corrected for multiple comparisons due to the small sample size, the exploratory nature of the pilot study, and the risk of making a Type II error (Perneger 1998).

Table 2.2. Factors predicting knowledge of four health-related cultural domains.

Maternal Characteristics	Cultural Domains			
			Western	Care
	Illness	Local Medicine	Medicine	Decisions
Parity	$r = -0.27$	$r = 0.069$	$r = 0.0037$	$r = 0.32$
Lives far from Karare	$t = -2.07*$	$t = -0.76$	$t = -2.94*$	$t = -0.80$
Considers self poor	$t = -0.87$	$t = -0.41$	$t = 0.07$	$t = 0.05$
Attended an FHI seminar	$t = -0.08$	$t = 0.85$	$t = 0.72$	$t = -0.34$
Boils water for infants	$t = 0.33$	$t = 1.18$	$t = 0.56$	$t = -0.22$
Uses a latrine	$t = 1.29$	$t = 2.21*$	$t = 2.14*$	$t = 2.13*$

* indicates $p < 0.05$

Only two factors significantly predicted knowledge of the four cultural domains: geographic location and latrine use. Women who lived far away from Karare, the location of the dispensary, had less knowledge of illness and Western medicine than women who lived near Karare. Women who used a latrine had higher knowledge of local medicine, Western medicine, and care decision-making than those women who did not use a latrine. Latrine use and distance from Karare are related variables, because two-thirds of the women living near Karare used latrines ($n = 10$), while only 20% of the women living far from Karare used latrines ($n = 3$). The area around Karare has more infrastructure including nearby water supplies, pit latrines, and greater visibility of NGOs. Therefore, it is difficult to separate the influence of each of these factors on knowledge of health. However, it is likely that there is some effect of Western ideas of health on women's cultural knowledge.

Maternal Knowledge of Local Medicine and Infant Health and Growth Outcomes

Introduction. Culture is an important mediator of human health behavior. Knowledge of traditional medicine derived from local plant resources is culturally

mediated and may play a role in health outcomes in populations with restricted access to Western medicine. Previous research has found that mothers' ethnobotanical knowledge is associated with better child health and nutrition in the Bolivian Amazon (McDade, 2007). These results have not been replicated in other communities. Using data collected for this dissertation, I will test the idea that mothers' knowledge of the use of local plants as medicine is associated with better infant health and nutritional outcomes in the Ariaal.

Methods. The questions in the local medicine subdomain (46 total) were administered to a larger sample of mother-infant pairs in November-December 2008 ($n = 251$) and analyzed using the methods described above. In this study, the ratio of first to second eigenvalue for the consensus analysis is 1:9.2, indicating that the set of 46 questions does indeed belong to the same cultural domain. Individual knowledge levels ranged from 0.70 to 0.95 (on a scale from 0 to 1; mean = 0.87 *s.d.* = 0.05), demonstrating that the women on the whole were fairly knowledgeable about traditional medicine.

Three dependent variables were infant illness within the past month (coded as 0 or 1), infant upper arm fat percentage, infant height for age z-score (HAZ), and infant salivary IgA. Infant illness was analyzed using PROC LOGISTIC and infant upper arm fat and infant HAZ were analyzed using PROC REG in SAS 9.2. Mothers' knowledge of traditional medicine was the main independent variable; mothers' BMI, mothers' parity, infant sex, infant age, total livestock units, monthly per capita food budget, presence of a garden, and village were included as covariates. In addition, each model contained the other two dependent variables as covariates. Significance was assessed at $\alpha = 0.05$. Details of data collection and analysis can be found in Chapter III.

Table 2.3. Odds ratios and estimates of infant health indicators for model predictors.

Independent Variables	Dependent Variables			
	Infant Illness (OR)	Upper Arm Fat Area (β)	HAZ (β)	Log IgA (β)
Model R^2	0.11	0.068	0.17	0.041
Mothers' Knowledge (unit = 0.01)	0.90*	0.029	0.012	0.0054
Mothers' BMI	1.15*	0.10	0.13*	0.014
Mothers' Age-Adjusted Parity	0.96	-0.078	-0.020	0.053
Log Total Livestock Units	0.97	-0.10	-0.12	0.011
Per capita monthly household food expenditure	1.00	1.6×10^{-5}	-4.8×10^{-5}	2.8×10^{-5}
Presence of Household Garden	1.07	0.31	-0.16	0.089
Uses latrine	0.58	-0.23	0.32	0.0034
Living in Parkishon	1.67	0.64*	-0.32	-0.40*
Living in Kituruni	1.28	0.88*	-0.15	-0.53*
Infant Sex	0.99	0.17	-0.29	0.20
Infant Age (Months)	1.00	0.062*	-0.070*	0.012

* $p < 0.05$

Results. Descriptive analyses are introduced in later chapters and are thus not reported here. Table 2.3 details the results of multivariate analyses. Maternal knowledge was significantly associated with reported infant illness ($p < 0.05$) but not infant upper arm fat, HAZ, or infant salivary IgA levels. Most covariates were not significant; however, maternal BMI was significantly associated with reported infant illness and HAZ, village was significantly associated with infant IgA and infant fat, and infant age was significantly associated with infant fat and HAZ (all $p < 0.05$). These relationships will be explored further in later chapters.

Discussion. As mothers' knowledge of traditional medicine increases, their infants are significantly less likely to have been ill in the past month. Specifically, for every 0.01 increase in knowledge (on a 0 to 1 scale), infants are 9% less likely to have been ill in the past month. However, mothers' knowledge is not significantly associated with infant upper arm fat area, infant height-for-age z-scores, or infant salivary IgA. These results differ somewhat from those found by McDade et al. (2007), who found that height-for-age z-scores, skinfold thickness, and C-reactive protein levels were positively associated with Tsimané mothers' ethnobotanical knowledge. It appears that maternal knowledge among the Tsimané improves both child health and child nutritional status while knowledge among the Ariaal improves only child health. This may be explained by the substantially different ecologies in which the Tsimané and the Ariaal reside. The Tsimané live in Bolivian lowland forests which may be exploited fairly readily for fruits and other foods. The Ariaal live in arid and semi-arid lands; very few plants produce edible food and the caloric content of these foods is fairly low. Another possible reason is that the children in the current study are breastfeeding infants while the Tsimané children

are older. The breastfeeding Ariaal children may be buffered against the nutritional disadvantages that may exist due to having less knowledgeable mothers. Finally, the current study tested knowledge of traditional ethnobotanical medicine, while the Tsimané study assessed knowledge of plants that can have both nutritional and medicinal value. It may be that Ariaal women's knowledge of traditional medicine is separate from their knowledge of botanical food sources, making nutritional status unconnected to traditional medicine knowledge.

McDade et al (2007) were unable to distinguish between three causes for their results: 1.) more knowledgeable adults can better exploit the natural resources in their environment to feed their children better-quality diets, 2.) local plants may have beneficial pharmacological properties that influence children's well-being, or 3.) children who have more knowledgeable parents learn more about their environment and thus are better able to forage on their own. The current study among the Ariaal can distinguish better between these three causes. Because the Ariaal children are very young infants they cannot forage for themselves, eliminating cause three. Since the current study did not ask mothers about ethnobotanical food sources and because the northern Kenyan ecology does not support sustainable foraging, the possibility that number one causes the study results is small. This leaves cause 2, that local plants have beneficial pharmacological properties that improve children's health. There is evidence that some of the Kenyan medicinal plants named by Ariaal women in this study have anti-malarial properties (Kirira et al., 2006); future work may find more beneficial effects in these plants. Further research should go beyond ethnobotanical knowledge to address how the

use of traditional medicines may reduce the duration and frequency of illness in the Ariaal community, confirming the pharmacological benefits of traditional herbs.

Conclusion

The purpose of this chapter was to present the ethnography of the Ariaal, study the infant feeding and health care in both a qualitative and quantitative way, and to use this information to test how well mothers' cultural knowledge predicted infants' health, immune function, and growth. This study found that Ariaal women tend to be very knowledgeable about infant health, treatment, and care. Proximity to a medical clinic and latrine use predicts knowledge in many subdomains, particularly knowledge of Western medicine. In addition, a woman's cultural knowledge may have an effect on the health and well-being of their infant, highlighting the importance of a biocultural approach to human health.

Culture is a set of symbols and beliefs that are shared by a group of people that can have profound effects on health behavior. Because culture is a collective property rather than an individual one, it can be difficult to measure the effect of culture on immune function, health and growth outcomes. Using the cultural consensus method allows culture to be collectively defined while assessing an individual's competence within their culture. This research shows that effective human biology research should account for cultural and ecological factors that contribute to well-being.

The culture of the Ariaal plays a vital role in how they adapt to the stressors in their environment. For example, the long duration of breastfeeding culturally valued by the Ariaal help protect infants against the diseases in their environment and may improve

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maternal fitness through increased interbirth intervals. There appears to be an effect of mothers' knowledge of ethnomedicine the frequency of infant illness, which may represent knowledge of a true medicinal effect of plants found in the environment. Although the results of this study did not find a relationship between cultural knowledge and immunity in Ariaal infants, there may be other significant intersections between culture, immune function and the environment that remain to be found.

Chapter III

Methodology

Introduction

This chapter discusses the methods used in this dissertation research. First, I will detail the methods used at the field location in northern Kenya. Second, I will describe the development and quality of an enzyme-linked immunosorbent assay (ELISA) for immunoglobulin A (IgA). Next, I will evaluate whether that breastmilk and saliva collected on filter paper and stored at ambient temperature can provide samples that can be used to accurately quantify IgA levels. Finally, I will outline statistical methods used the analyses throughout the dissertation.

Field Methods

Field Location. This research took place in Marsabit District in Kenya among the Ariaal. Data were collected in the communities of Karare, Kituruni, and Parkishon, located approximately 17 kilometers from the district capital of Marsabit Town. The Ariaal are a population of approximately 10,000 settled pastoralists residing on Marsabit Mountain. Due to its location on Marsabit Mountain, the climate is relatively cool and somewhat humid compared to the surrounding arid desert making subsistence agriculture a somewhat sustainable alternate mode of subsistence. A more complete description of the cultural and social ecology and history of the Ariaal can be found in Chapter 2.

Sampling. Before the start of the study, research assistants made a door-to-door survey in the communities of Karare (and surrounding manyattas), Parkishon, Kituruni, and Hulahula to find women and infants that met the following criteria: 1) currently breastfeeding and 2) infant age between 0 and 18 months. They compiled lists of eligible participants and their location to aid sampling and recruitment. There were 181 eligible women in Karare, 43 in Parkishon, 60 in Kituruni, and 96 in Hulahula. In addition, women who gave birth after the creation of sampling lists and who met the above criteria were considered for the study.

In the interest of geographical proximity and area security, I decided to recruit all interested women in Karare, followed by Parkishon, Kituruni and finally Hulahula. Women were selected from the lists and asked to attend the study the next day. They were asked to stop breastfeeding an hour before the start of the study and to bring health cards that contained the vaccination records and birth date of their infant. One hundred and thirty mother-infant pairs were recruited from Karare, sixty-eight from Parkishon, and fifty-three from Kituruni. At that point, the target sample size was reached and the study was concluded. No women were recruited from Hulahula.

An analysis of women who participated versus those who were on sampling lists but did not participate indicated the infants of participating women were the same age as infants of non-participants ($t = 0.16$, $p = 0.88$). An analysis of Karare, Parkishon, and Kituruni versus Hulahula indicate no systematic difference in infant age between included communities and Hulahula ($t = 0.77$, $p = 0.44$). There may be other significant differences between the communities that make this study's sample not representative of the settled Ariaal community. However, it does significantly cover the population of

breastfeeding women and infants in Karare, Parkishon and Kituruni, communities that represent a wide spectrum of Ariaal life, particularly in regards to the availability of health, education, religious, and market resources.

The protocol for this research was approved by the University of Michigan's Institutional Review Board, project number HUM00017927. In addition, this protocol was approved by the Kenyatta National Hospital's Ethics Review Committee and the Ministry of Science and Technology of the Republic of Kenya.

Structure of Data Collection. Women and their infants arrived at the study site between 8 and 10 am. Between 3 and 20 women arrived each day, averaging 10.9 mother-infant pairs per day. Women were read, in translation, the informed consent form and agreed to participate. Mother and child's names were recorded with their participant ID number in a notebook kept separate from other study records to safeguard the privacy of women's responses.

After the consent process and check-in, women and infants went through the study in the following order: 1. breastmilk collection (approximately 10 minutes), 2. infant saliva collection (approximately 30 minutes to 1 hour), 3. maternal saliva collection (approximately 10 minutes), 4. maternal capillary blood sampling (approximately 1 hour), 5. questionnaire (approximately 20 minutes per woman), and 6. anthropometry (approximately 1 hour total). The study was generally completed between 2 and 5 pm. Women were compensated 300 Kenyan shillings (approximately 4.00 USD). The details of data collection are discussed below. Total maternal and infant *n* for each subset of data collection is noted in Table 3.1.

Biomarker Collection. Biomarkers were collected from women and infants in the morning so that infants could be fed as soon as possible. Capillary blood, breastmilk, and saliva were collected from mothers; saliva was collected from infants. Whole samples of saliva and breastmilk and dried capillary blood spots were frozen in liquid nitrogen at -212°C. Dried saliva and breastmilk were stored at ambient temperature (average temperature at 1:00 pm: 26.7°C).

1. <u>Capillary blood</u>. Dried blood spots (DBS) were collected from 251 women and hemoglobin readings were collected from 200 women. Capillary blood was collected using standard techniques. The tip of the middle or fourth finger of the right hand was cleaned with a rubbing alcohol swab and allowed to dry. While applying pressure to the last joint of the middle finger, a single-use spring-loaded lancet (2.0 x 1.0 mm) was used to create a puncture on the medial side of the tip of the finger. The first blood drop formed was wiped away with a lint-free tissue. The second drop of blood was used to fill a HemoCue microcuvette. Between two and four drops were collected on Whatman 903 filter paper by touching the edge of one blood drop to the center of each pre-printed circle and allowing the drop to spread to the edge. Blood flow was then stopped by applying the tissue to the puncture with gentle pressure. The site was covered with a sterile bandage (McDade et al. 2007).

The time of blood collection was noted on the questionnaire. The HemoCue microcuvette was inserted in a HemoCue 201+ reader within 5 minutes of collection and the hemoglobin level recorded on the questionnaire. The filter paper was dried between 3-5 hours before being placed in a triple-layer plastic bag with a desiccant and sealed. The bag was generally deposited in the liquid nitrogen container when it was opened the

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next day; however, later samples were compiled in one bag over several days before being placed in liquid nitrogen due to space shortages in the container. Average time to deposit was 61.3 hours (*s.d.* = 43.9 hours).

2. <u>Breastmilk</u>. Women were asked to stop breastfeeding their infants about 1 hour before the start of the study so that the expressed milk would be foremilk (as opposed to hindmilk, which has a higher fat content). Women expressed milk by hand from their right breast into a clean paper cup which was then poured into a 2 mL polypropylene centrifuge tube that had been coated with 40 μL of a 0.4% sodium azide (NaN_3) solution and allowed to dry. When filled two-thirds to three-fourths of the way full of sample, the NaN_3 concentration was approximately 0.001%. Sodium azide is a bacteriostatic preservative that inhibits the breakdown of proteins, including IgA, by bacteria.

After collection, each breastmilk sample was stored on filter paper. One hundred μL of sample was applied to the center of each pre-printed circle of a Whatman 903 filter paper card (400 μL total). After drying 2-3 hours, samples were stored in a triple-layer plastic bag with desiccant and left at ambient temperature between 56-19 days before freezing (mean = 37.2 days). Whole samples were frozen in liquid nitrogen within 5 hours of collection (mean = 160 minutes).

3. <u>Saliva</u>. Infants refrained from consuming breastmilk or food at least 1 hour before saliva collection so that samples would not be contaminated with milk IgA. Whole saliva was collected either by inserting disposable pipettes into their mouths and extracting saliva or asking older infants to spit directly into the collection tube. Occasionally sugar or bottled water was given to infants to induce saliva formation. The collection tubes were the sodium-azide coated, 2 mL centrifuge tubes described above.

Due to the much smaller volume of infant saliva collected, however (generally less than 1mL), the NaN_3 concentrations are higher than 0.001%. Three infants had no saliva sample; one had insufficient sample for analysis.

Adult women rinsed their mouths with bottled water prior to spitting in the collection tubes described above. They were asked to fill the tube; final sample volumes fell between 1.0 and 2.0 mL. Samples that showed evidence of tobacco chewing were discarded and women were asked to re-rinse their mouths and provide another sample.

Whole saliva samples of both mothers and infants were applied to filter paper. As with breastmilk, 100 µL of saliva was applied to the pre-printed circles of Whatman 903 filter paper using a Finnpipette and disposable tips. Due to the small volume of infant saliva, samples were only applied to 1-2 circles or in some cases were not applied to filter paper at all when the sample volume was lower than 300-400 µL. There were 189 infant saliva samples on filter paper. Filter paper samples were stored in triple-layered plastic bags with desiccant after drying 2-3 hours. Filter paper was stored at ambient temperature between 56-19 days (mean = 37.2 days) before being frozen. Whole samples were frozen in liquid nitrogen within five hours of collection (mean = 125 minutes).

Questionnaire. After biomarker collection, women participated in a questionnaire to assess maternal, infant, household, and socioeconomic characteristics. Questionnaires were printed in English and were translated orally into Samburu by two female research assistants. The questionnaire contained the following sections: 1) maternal individual characteristics, 2) infant individual characteristics, 3) socioeconomic status, 4) household composition and kinship, 5) health status and health care practices for both mothers and infants, 6) hygiene practices, 7) weaning and breastfeeding behaviors and beliefs and 8)

traditional medicine consensus questionnaire developed in Chapter 2. A copy of the complete questionnaire is presented in Appendix III.

1) Maternal characteristics. The first section of the questionnaire related to the mother's age, reproductive history, family and kinship, marital status, and education. Variables in this section of the questionnaire included age, father's age set, list of children born, their birthdates, and their dates of death if applicable, number of miscarriages, number of siblings and relative birth order, education, marital status and rank, husband's education and husband's age set if applicable. Women were asked if their menstrual periods had resumed since the birth of their infant, and if so, when they started and how many she has had. It was assumed that the mother's husband is the infant's father; if she did not have a husband she was not asked further details because such questions are sensitive in Ariaal society.

2) Infant characteristics. The second section of the questionnaire assessed characteristics of the infant, including age, date of birth, sex, vaccination history, use of deworming drugs, tooth eruption, crying patterns, and developmental landmarks. Mothers were asked to bring vaccination cards to the study site to help determine the age of the infants and the schedule of immunizations they had received. A small number of children were too young to have received a vaccination, so mothers were asked to recall their infant's date of birth instead.

3) Socioeconomic status. Women were asked several questions about their economic and social standing within Ariaal society. Questions included amount of cash income per month, numbers of large and small livestock owned, use of famine relief food, amount of cash spent on food per month, growing a household garden, woman or

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husband holding a wage-earning profession, type of housing, visits to Marsabit Town, religion, and ethnic identity.

4) <u>Household composition</u>. Mothers were asked to list members of their household and their relation to the mothers. Mothers were also asked to list household members who do not live at home, such as a spouse that works in another area.

5) <u>Health status and health care practices</u>. Mothers were asked if either they or their infants had any of the following illnesses within the last month: high fever or malaria, severe upper respiratory infection or pneumonia, and diarrhea. They were asked where they treated the illness (home, dispensary, hospital, etc.) and what kind of medicine (traditional or Western) were used. They were also asked to list other illnesses or symptoms they or their infants may have had within the last month, where it was treated, and what kind of medicine was used.

6) <u>Hygiene practices</u>. This section of the questionnaire asked about water sanitation practices, shoe use, use of latrines, use of mosquito netting, flooring, infant crawling and playing outside, and infant bathing practices.

7) <u>Weaning, breastfeeding and infant care behaviors</u>. Women were asked to list how many times and how long their infants latch on to breastfeed during the day and night, what non-breastmilk foods they have given to their infant within the past 24 hours, amount of time spent fetching firewood and water, who typically watches the infant while the mother performs these chores, and how many months women stopped working after the birth of their infant.

8) <u>Consensus questionnaire for knowledge of traditional medicine</u>. Women were administered the knowledge of traditional medicine questionnaire as developed on a prior

pilot trip and described in Chapter 2. The questionnaire consisted of 46 true-or-false

questions relating to use of traditional medicine for treating infant illness. An example

question is "Lasaramai is used to treat babies who have malaria".

Anthropometry. Women's height was measured with a stadiometer and recorded

in centimeters. Weight was measured on a digital scale and recorded to the nearest 0.2

pounds. In order to adjust for the heavy and difficult-to-remove jewelry worn by some

women in the study, five beaded necklaces were weighed and the mean weight subtracted

from women observed to be wearing necklaces. Mid-upper arm circumference was

measured in centimeters and triceps skinfold measurements were taken in triplicate using

Lange calipers and recorded in millimeters (Bones and Behavior Protocol 2009).

Infant recumbent length was measured using measuring boards and recorded in

centimeters. Infant weight was calculated by weighing the mother holding the infant on a

digital scale, and subtracting the mothers' weight. Infants' mid-upper arm circumference

and triceps skinfold were measured as described above.

From raw anthropometric measurements, several indices were calculated,

including: 1) BMI, 2) Upper arm muscle area (UAMA) and 3) Upper arm fat area

(UAFA). Maternal and infant BMIs were calculated as:

$$BMI = weight(kg)/height(m)^2 \text{ (Frisancho 2008)}.$$

Both UAMA and UAFA were calculated for mothers and infants from their triceps

skinfold (TRI; mm) and mid-upper arm circumference (MUAC; cm) measurements

using:

$$UAMA \ (cm^2) = MUAC - ((TRI*0.1*\pi)^2/4\pi)$$

$$UAFA \ (cm^2) = (MUAC^2/4\pi) - UAMA \text{ (Frisancho 2008)}.$$

For infants, z-scores of height-for-age, weight-for-age, and weight-for-height were calculated against the WHO Child Growth Standards (2006) reference population using the WHO Anthro program version 3.

Infant triceps skinfold measurements were converted into triceps skinfold-for-age z-scores (TSFZ) based on Tanner-Whitehouse standards. Paul et al. (1998) and Davies et al. (1993). Davies et al. (1993) generated LMS values based on Tanner-Whitehouse curves for infants between 0-12 months at 0.1 year intervals and between 1-3 years at 0.5 year intervals. Ariaal infants were grouped into appropriate age categories and z-scores generated in SAS based on LMS equations discussed in Frisancho (2008). Unfortunately, while the Tanner-Whitehouse standards may be higher than other populations of well-nourished infants (Paul et al. 1998), they are the only triceps skinfold standards that contain reference values between birth and 3 months, a time of significant fat development in infants. The WHO Child Growth Standards represent a larger and more variable population but do not have triceps skinfold references for infants under the age of 3 months.

Sample Shipping. Samples were removed from the liquid nitrogen tank, packed on dry ice, and shipped via refrigerated freight from Nairobi, Kenya, to Detroit, Michigan. Upon arrival in the United States, whole and filter paper samples were placed in -80°C freezers. The time between the decanting of samples from the liquid nitrogen container to their placement in the freezer was approximately nine days.

Table 3.1. Number of participants within each data subset by mothers and infants.

Data Subset	Mother *n*	Infant *n*
Questionnaire	251	251
Anthropometry	251	251
Whole Saliva	251	248
Dried Saliva	251	189
Whole Breastmilk	248	-
Dried Breastmilk	248	-
Dried Blood Spot	251	-
Hemoglobin	200	-

Laboratory Methods

Assay Protocol. An enzyme-linked immunosorbent assay (ELISA) for

immunoglobulin A (IgA) was developed by EM Miller and DS McConnell at the Clinical

Ligand Assay Satellite Service (CLASS) Laboratory, affiliated with the department of

Epidemiology at the University of Michigan. All buffer solutions were formulated and

mixed at CLASS. Whole and filter paper samples of breastmilk and saliva can be

analyzed in this IgA ELISA.

The day before running samples, 96-well microwell plates (Fisher) were coated

with a solution of sodium carbonate buffer (pH = 9.6) and anti-IgA antibodies. The anti-

IgA antibodies were two monoclonal antibodies specific for the two IgA subtypes: mouse

anti-human IgA1 and mouse antihuman IgA2 (Southern Biotech). Coating solution

contained a 1:500 concentration of anti-IgA1 antibody and a 1:1000 concentration of

anti-IgA2 antibody. Forty seven μL of solution was added to each well, sealed, and

incubated at room temperature overnight.

Samples were prepared on the day of the assay. Samples were diluted in a

phosphate-buffered solution (PBS) containing 0.05% bovine serum albumin (BSA) and

0.05% Tween-20. Whole breastmilk was inverted to mix the whey and lipid layer and prepared in PBS for a dilution of 1:4000. Whole adult saliva was centrifuged and the supernatant diluted in PBS for a dilution of 1:1500. Whole infant saliva was centrifuged and diluted in PBS for a dilution between 1:60 and 1:700. Filter paper breastmilk sample "punches" were punched out of the sample card using a 1/8 inch hole punch and eluted in 2000 µL PBS for a dilution of 1:2000. Adult filter paper saliva sample "punches" were eluted overnight in 750 µL PBS for a dilution of 1:750. Samples that fell outside of the range of the standard curve were re-assayed at a different dilution.

During sample preparation, the coating solution was decanted from the microwell plate and a blocking buffer containing sodium carbonate buffer with 5% BSA was added to each well. Plates were incubated, shaken, at room temperature for one hour. After incubation, the plate was washed three times in an automatic plate washer with a PBS solution containing 0.2% Tween-20.

Standards, controls, and samples were added to the microplate and incubated for three hours. Secretory immunoglobulin A (sIgA) purified from human colostrum (Accurate Chemical) was used as the standard. The standard curve contained five values at concentrations of 600, 200, 60, 20 and 0 ng/mL. High, medium, and low controls were added in duplicate and unknown samples were run in triplicate. Twenty-six unknown samples were run per plate. There were two blank wells per plate to assess background color levels. After a three hour incubation plates were washed as described above.

A 1:333 solution of PBS and polyclonal goat anti-human IgA antibody conjugated with horseradish peroxidase (Accurate Chemical) was prepared and added to the plate. The plate was incubated for one and a half hour and was washed as described above.

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A 3,3',5,5'-tetramethylbenzidine (TMB) solution (Pierce) was added to each well and allowed to react for 20 minutes before being stopped by a 2M sulfuric acid solution. Plates were read in a SpectraMAX 340PC at 450 nm and 620 nm. Values were adjusted for absorbance at 620 and for background in blank wells. The SpectraMAX generated a standard curve and calculated unknown values. All standard curves had an $R^2 > 0.98$.

Assay Performance. Inter assay percent coefficient of variation (%CV) is 8.05% ($n = 10$ each saliva and breastmilk on 3 plates). Intra assay %CV is 10.68% ($n = 10$ each breastmilk and saliva across three plates). The minimum detectable amount (defined as the 2 standard deviations above the zero standard) is 10.1 ng/mL. To investigate linearity of dilution, a saliva and breastmilk sample were assayed serially at 1:500, 1:1000, 1:2000, 1:4000, and 1:8000 dilutions. The correlation coefficients for breastmilk and saliva linearity were $R^2 = 0.992$ and $R^2 = 0.999$, respectively.

IgA standards and samples were run in a commercial sIgA assay (ALPCO) to compare their values with the commercial kit. The sIgA standard was assayed at four dilutions ranging from 20-600 ng/mL. The correlation coefficient between standard concentration and commercial assay results was 1.0; however the commercial assay results indicated that the sIgA standards were 1.85 more concentrated than the value stated by the standard manufacturer ($\beta = 0.53$). To adjust for differences between the stated sIgA concentration and kit results, the sIgA standard was considered twice as concentrated as its stated concentration and standard values were adjusted accordingly. To account for the remaining difference in concentration of unknown sample between the commercial kit and in house assay, adjusted standard was run at several known concentrations as "unknown" sample. Known values were regressed against "unknown"

assay value ($R^2 = 0.99$), giving an estimate of $\beta = 0.50$. Therefore, in order for the "unknown" value of the standard to equal the known concentration the "unknown" values must be multiplied by two, an adjustment that was made on all unknown breastmilk and saliva samples in the Ariaal population. Overall, these multipliers increased the sample values fourfold, approximating the commercial kit values and putting saliva and breastmilk concentrations within published ranges.

High, medium, and low saliva controls taken from one US female were added to each microwell plate for all analyses. Mean O.D. values for controls were 1.22, 0.77, and 0.13. The %CVs for the high, medium, and low controls for all analyses in this study were 9.1%, 9.4%, and 24.8%, respectively. These values corresponded well to standard %CVs, indicating that variation was likely not due to differences between standard and sample matrix.

Filter Paper Storage. Whatman 903 filter paper is a high-quality, medical grade filter paper used to collect small quantities of dried blood for biomarker analysis. Originally used to collect blood from newborns for neonatal health screening, blood spots dried on filter paper have been increasingly used by anthropologists for minimally-invasive, easily stored biomarker collection. This study investigated the storage capacity of Whatman 903 filter paper to store breastmilk and saliva for IgA analysis in a fieldwork setting. Previous research by Brown et al. (1982) found that anti-rotavirus IgA titer and anti-enterotoxin titers could be recovered from breastmilk stored on filter paper. In addition, a recent study found that filter paper that had been placed in infants' mouths and allowed to dry could be stored at room temperature for up to six months for cortisol assay (Neu et al. 2007). Although neither study used Whatman 903 filter paper, it does indicate

that substances other than blood can be dried, extracted, and reliably assayed in an ELISA. IgA is an ideal biomarker for this analysis because it is abundant, easy to detect in bodily fluids and remains relatively stable in a wide variety of environmental conditions.

Matched whole and dried filter paper breastmilk and saliva samples were collected and stored with the methods and storage conditions described above. There was a total n of 248 matched breastmilk samples and 251 matched adult saliva samples.

Table 3.2 contains descriptive statistics of IgA values in breastmilk and saliva. Three breastmilk samples had unusually high values (either dried or whole IgA values greater than 20,000 µg/mL); these high values were found in samples that visually appeared not to be foremilk. Filter paper analyses were run with and without these high values. The median breastmilk IgA, a better indicator of central tendency due to outliers, were within the published normal range of 470-1632 µg/mL (Jackson et al. 1999). The whole saliva IgA mean was within commercial assay range of 102-471 µg/mL (ALPCO Diagnostics 2008). A paired t-test of whole versus dried samples yield no significant difference between breastmilk samples ($t(247) = -0.46$, $p = 0.65$) but did reveal a significant difference between saliva samples ($t(248) = 8.18$, $p < 0.0001$).

Table 3.2. Descriptive statistics of whole and dried breastmilk ($n = 248$) and whole and dried saliva ($n = 251$) IgA concentration. Units are in µg/mL for whole samples and paper "punch"/mL for dried samples.

	Whole Breastmilk (µg/mL)	Dried Breastmilk (punch/mL)	Whole Saliva (µg/mL)	Dried Saliva (punch/mL)
mean	1004.1	1118.8	325.3	218.9
s.d.	1530.0	4424.7	189.4	196.7
median	808.6	551.5	296.2	174.7
maximum	21155.2	56150.0	1425.8	1571.2
minimum	245.9	116.6	39.5	6.1

Dried breastmilk was regressed against whole breastmilk with amount of time at ambient temperature (in days) as a covariate. Next, the regression was repeated after excluding the three outlying breastmilk samples. Finally, both dried and whole breastmilk values were log transformed and the regression was repeated, which can be seen in Figure 3.1. Results and regression equations are reported in Table 3.3. While there was a significant relationship between whole and dried samples, there was no effect of time until storage on dried sample values.

Table 3.3. Estimates, R^2, and equations for whole breastmilk IgA and time until storage at -80°C regressed against dried breastmilk IgA.

Model	Breastmilk IgA β (p)	Time to Storage β (p)	R^2
Not Transformed	0.16 (<0.0001)	-8.7 (0.28)	0.21
Not Transformed, No Outliers	0.31 (<0.0001)	-2.0 (0.31)	0.26
Log-Transformed	0.42 (<0.0001)	-0.0012 (0.59)	0.41
Regression Equation			
Not Transformed	WBM = 0.16*DBM – 8.7*TIME + 1142.9		
Not Transformed, No Outliers	WBM = 0.31*DBM – 2.0*TIME + 717.7		
Log-Transformed	$WBM = e^{0.42*Ln(DBM) - 0.0012*TIME + 4.0}$		

WBM = Whole Breastmilk
DBM = Dried Breastmilk
TIME = Time to Storage in Days

Figure 3.1. Scatterplot and regression line of log-transformed whole breastmilk IgA and dried breastmilk IgA.
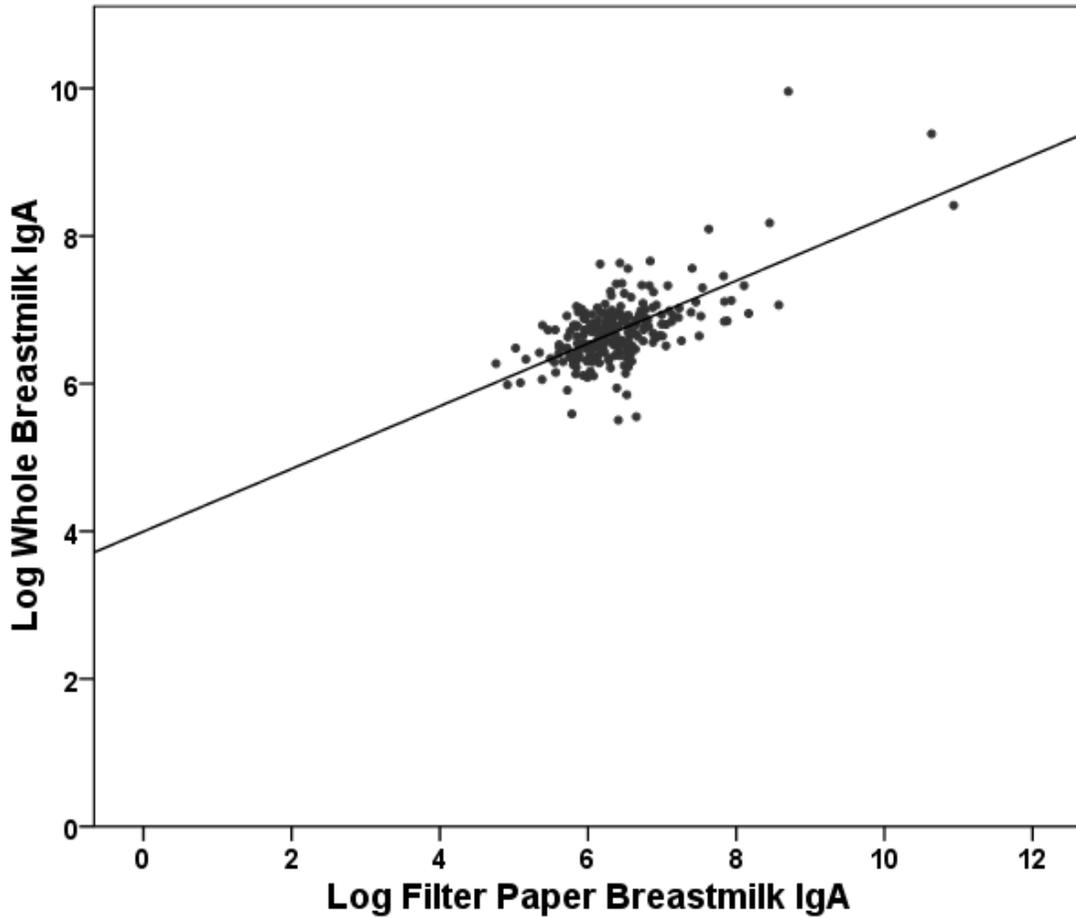
Dried saliva IgA values were regressed against whole saliva IgA levels with length of time at ambient temperature (in days) as a covariate. Both dried and whole salivary IgA values were log transformed and the regression repeated. Results and regression equations are given in Table 3.4. There was a significant relationship between whole and dried samples, while there was no significant effect of time until storage in any equation.

Table 3.4. Estimates, R^2, and equations for whole saliva IgA and time until storage at -80°C regressed against dried saliva IgA

| Model	Saliva IgA β (*p*)	Time to Storage β (*p*)	R^2
Not Transformed	0.43 (<0.0001)	-1.2 (0.26)	0.18
Log-Transformed	0.38 (<0.0001)	-0.0049 (0.09)	0.34
Regression Equations			
Not Transformed	WSAL = 0.43*DSAL – 1.2*TIME + 274.3		
Log-Transformed	WSAL = $e^{0.38*Ln(DSAL) - 0.0049*TIME + 3.9}$		

WSAL = Whole Saliva
DSAL = Dried Saliva
Time = Time to Storage in Days

Figure 3.2. Scatterplot and regression line of log-transformed whole saliva IgA and dried saliva IgA.
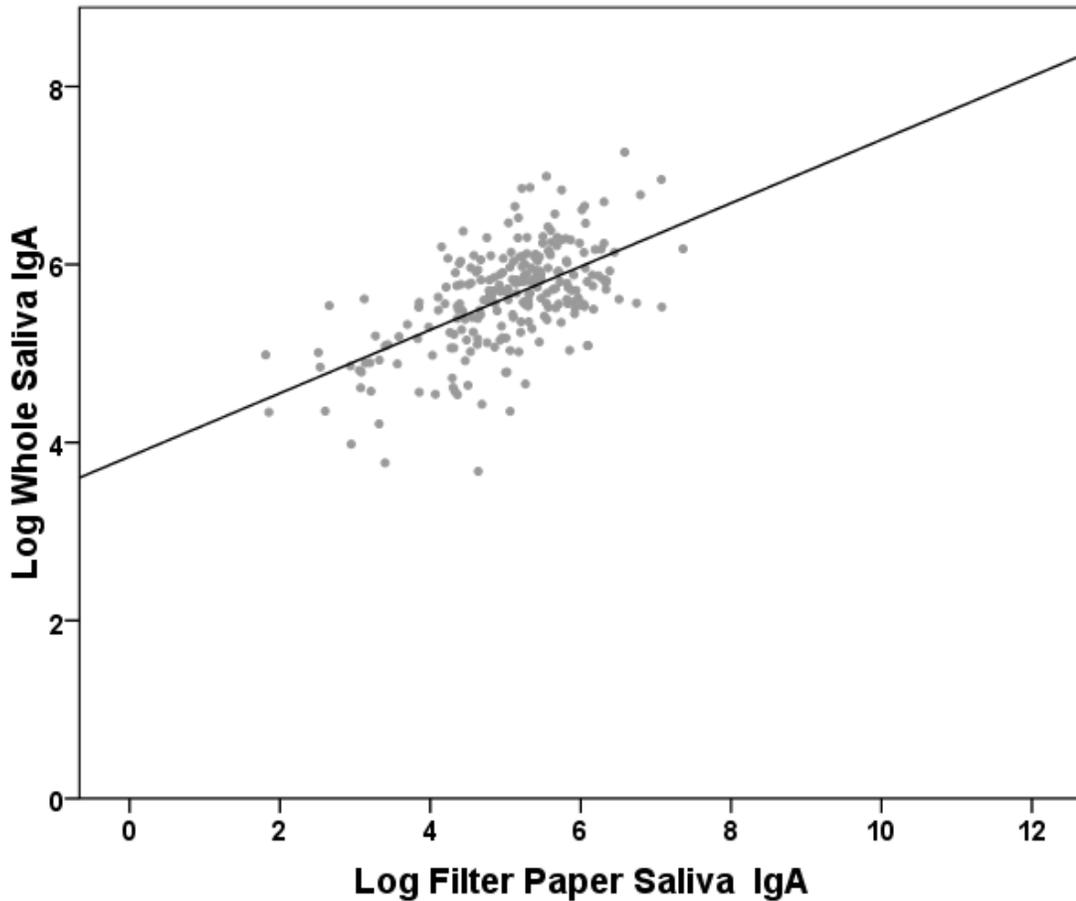
Data from both saliva and breastmilk were combined into one regression equation

for a total *n* of 499 matched whole and filter paper samples. Regressions were run on

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non-transformed and transformed IgA concentrations. Results can be found in Table 3.5; a scatterplot with both log-transformed breastmilk and saliva samples combined can be found in Figure 3.3. For the combined regression equation, whole and dried samples remained significantly related; in this case, there was a significant effect of time to storage as well in the log-transformed equations, which will be discussed below.

Table 3.5. Estimates, R^2, and equations for whole sample IgA and time until storage at -80°C regressed against dried sample IgA.

Model	Sample IgA β (p)	Time to Storage β (p)	R^2
Not Transformed, no outliers	0.49 (<0.0001)	-0.57 (0.38)	0.40
Log-Transformed	0.55 (<0.0001)	-0.0055 (0.0052)	0.62
Regression Equation			
Not Transformed	WHOLE = 0.49*FILTER − 0.57*TIME + 203.1		
Log-Transformed	WHOLE = $e^{0.55*\text{Ln(FILTER)} - 0.0055*\text{TIME} + 3.2}$		

WHOLE = Whole Sample
FILTER = Dried Filter Paper Sample
TIME = Time to Storage in Days

Recovery of IgA sample from one 3.2 mm "punch" compared to 1 µL whole sample appears, based on the regression estimates, to be fairly low ($\beta = 0.42$ for breastmilk and $\beta = 0.38$ for saliva, corresponding to a 42% and 38% recovery, respectively). However, because the y-intercepts of the regression equations are relatively high (exponentiating the intercepts in the log models gives values of 55.0 µg/mL for breastmilk and 49.4 µg/mL for saliva), the overall amount of IgA recovered from filter paper is higher. Analyzing both saliva and breastmilk together yields a regression estimate of $\beta = 0.55$ (55% recovery) and a correspondingly lower y-intercept of 3.2 (24.5 µg/mL). The ratio of median filter paper concentration to median whole concentrations of breastmilk is 0.682, or 68.2% recovery. The ratio of mean filter paper to whole concentrations of saliva is 0.673, or 67.3% recovery. These recovery rates are high

enough to successfully analyze breastmilk and adult saliva IgA without loss of signal.

Two possible reasons for the high equation intercept are: 1.) high concentrations in

breastmilk and saliva guarantee that a certain threshold of IgA will be present in the filter

paper diluent; including mucosal fluids with lower IgA concentrations may give a

regression line with an intercept closer to zero and 2.) Whatman 903 filter paper,

designed for blood, may interact differently with fluids of different compositions such as

breastmilk and saliva.

Figure 3.3. Scatterplot and regression line of log-transformed whole sample IgA and dried sample IgA by sample type.
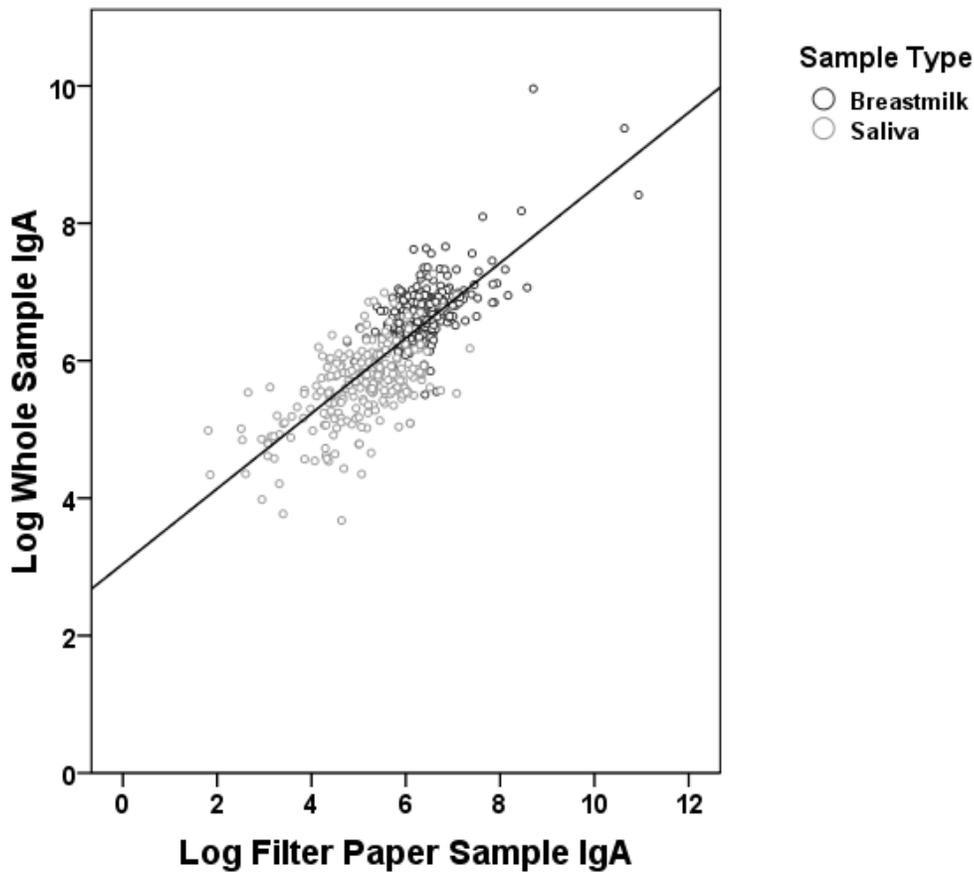
The regression coefficients (R^2) of the models indicate how well filter paper samples approximate their whole counterparts. The higher the regression coefficient, the greater the ability of dried filter paper samples to give an accurate representation of IgA concentration in whole samples. While there is no gold-standard rule for concordance between dried blood samples and serum samples in biomarker assays, published papers have regression coefficients ranging between 0.39 (Fujita et al. 2007) and 0.96 (McDade et al. 2004). The regression coefficients for breastmilk and saliva in this study, when regressed separately, fall at the very low end of this range. Combining breastmilk and saliva improved the regression coefficient to acceptable levels, making the combined equation WHOLE = $e^{0.55*\text{Ln(FILTER)} - 0.0055*\text{TIME} + 3.2}$ useful for extrapolating whole IgA values from filter paper IgA values. It is important to note that many studies of matched serum-filter paper assay results are not log-transformed; however the majority of these studies are of biomarkers that lack the 100x range that is observed for IgA in this sample. In this case, data transformation is necessary because it reduces the impact of large values and creates a normal distribution of IgA values.

Regression analysis of breastmilk and saliva separately found no significant effect of time stored at ambient temperature on dried filter paper IgA concentration. Combining breastmilk and saliva does, however, indicate a small but significant storage effect, equal to a loss of about 1 µg/mL of sample per day. Repeating the regression analysis without time at ambient temperature as a covariate gives an $R^2 = 0.61$, nearly equal to the regression coefficient in the model that includes time. Because storage time, although significant, does not appear to affect the model variation, the storage effects can be adjusted for mathematically using the regression equation above. This indicates that

storing dried mucosal fluid on filter paper at ambient temperature is a valid fieldwork

strategy. It is worth noting that in this natural experiment all filter paper samples were

stored at ambient temperature for at least 19 days before freezing. Some degradation may

have occurred between application of sample to filter paper and the date frozen.

An alternate method for assessing differences between two measurements is the

Bland-Altman method (Bland and Altman, 1986; Leonard et al. 1997). In this method,

the difference between two measurements is compared to their mean in order to assess

systematic biases in a measurement system along different levels of the measurement. To

apply the Bland-Altman method to filter paper IgA analysis, the log mean of and

difference between each whole-dried sample pair were calculated and plotted against

each other, excluding samples whose means were greater than 3000 μg/mL (n = 494).

The results can be seen in Figure 3.4. If there is no measurement bias, there should be no

relationship between the two measures. However, regression analysis shows a significant

relationship between mean and difference for dried and whole IgA samples (β = -0.55, p

= 0.018). Exponentiating this result yields the following interpretation: For every 1

μg/mL change in mean IgA, the difference between whole and dried samples increases by

0.20 μg/mL This indicates a significant bias between whole and dried samples in samples

with high levels of IgA; that is, dried filter paper results will increasingly underestimate

IgA levels as sample concentration increases. However, a visual inspection of the Bland-

Altman plot suggests that this result may be due to an extreme outlier, and that most

samples under e^7 or about 1100 μg/mL do not appear to show measurement bias. The

mean difference between whole and dried samples indicates that dried samples

underestimate whole samples by 70.7 μg/mL. The limits of agreement, that is, the ± 2SD

of the difference, are 52.5 µg/mL and 95.0 µg/mL. Ninety-five percent of the difference between whole and dried samples falls within this range. While dried samples do underestimate whole samples by an average of 70.7 µg/mL, there is no systematic bias between the two measures when the samples have a concentration of 1100 µg/mL or lower. While this range encompasses all saliva and most breastmilk IgA levels, caution should be used when interpreting dried filter paper results for highly concentrated breastmilk samples.

Recovery of IgA in breastmilk and saliva stored on filter paper is acceptable and concordance to whole samples is moderate to low. There is a small but correctable effect of time at ambient temperature up to 56 days. There is no systematic measurement bias between whole and dried samples when the sample concentration is below 1100 µg/mL, although there may be inconsistent results in high-concentration breastmilk samples above that value. Individual researchers should determine if the fieldwork advantages of filter paper storage outweigh the moderate laboratory results.

Figure 3.4. Bland-Altman plot showing the log mean of whole and dried IgA samples versus the log differences of whole and dried IgA samples.
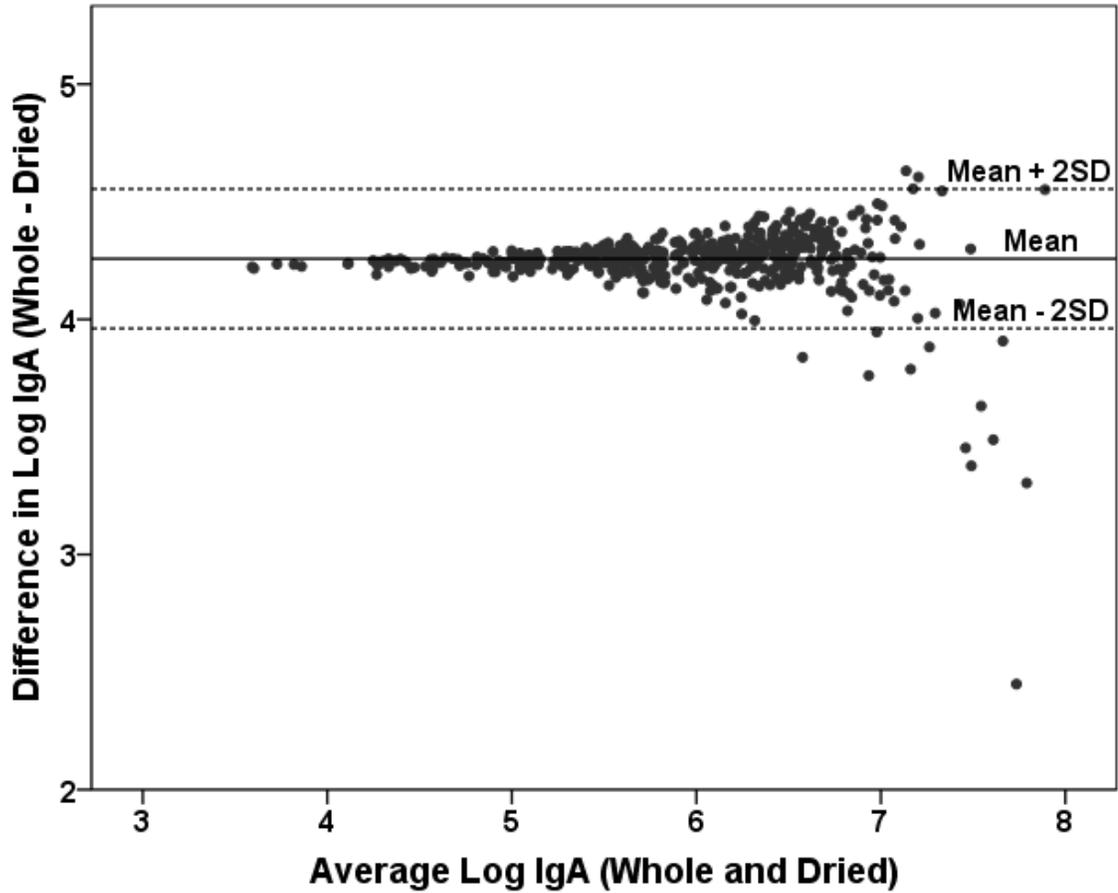
Statistical Methods

Continuous variables were checked for normality and natural log transformed if

necessary. IgA concentrations in particular were log-transformed before analysis.

Descriptive, bivariate, and multivariate statistics were performed in SAS 9.1. Figures and

charts were generated in PASW (formerly SPSS) 16.0.2. This study used a significance level of $\alpha = 0.05$.

Conclusion

This chapter described the field, laboratory, and statistical methods that were used in this dissertation. In addition, it tested the possibility of drying saliva and breastmilk samples on filter paper in field conditions for later immunoglobulin A analysis. Results indicate that immunoglobulin A can be extracted from 100% of dried samples, can be stored at ambient temperature for up to two months, and are moderately accurate compared to matched whole samples. This method will be useful for researchers who want to study saliva and particularly breastmilk in remote locations where freezing and shipping whole samples is not feasible or cost-effective.

Chapter IV

Sources of Variation in Breastmilk IgA Production in a Natural Fertility Population

Introduction

The immunoglobulin A (IgA) in breastmilk is thought to play a significant role in preventing infant disease and promoting infant immune development. Information on the variation in breastmilk IgA throughout the course of lactation conflicts and is found mainly in Western populations. This chapter will use predictions from evolutionary theory to explore variation in IgA levels in the breastmilk of Ariaal women.

Breastmilk. Breastmilk is a highly nutritious infant food. Approximately 7% of breastmilk is lactose, 3-4.5% is fat, and around 1% is protein. The remaining 86-87.5% is water. Both fat and water soluble vitamins are found in breastmilk as well as most nutritive minerals, albeit at low levels (Ogra et al. 2006)). Breastmilk is synthesized in the mammary glands and is produced "on demand"; that is, a complex hormonal-behavioral feedback loop helps produce and maintain enough breastmilk to keep up with infant appetite, usually about 19-30 oz per day (Dewey et al. 1984; Dewey and Lonnerdal 1983). Given the high nutrient value of breastmilk, it is unsurprising that breastmilk synthesis is energetically costly to the mother, adding approximately 600-700 kcal per day to her normal metabolic needs (Hrdy 1999).

Breastmilk has a large advantage over synthesized infant formulas: it contains immunological compounds that protect the immature infant from disease. Substances

from both the adaptive and non-specific innate immune are passed from the mother to the infant through breastmilk, including all classes of white blood cells, cytokines, immunoglobulins, and anti-pathogen proteins such as lactoferrin and lysozyme. Immunoglobulin A (IgA), found predominantly in its secretory form (sIgA), is found in large quantities in human breastmilk. IgA is part of the adaptive immune system and therefore provides pathogen-specific protection to infants based on the diseases their mothers have been exposed to in their environments (Brandtzaeg 2003; Walker 2004; Weaver 1992). Mothers and infants are said to be sharing a common mucosal immune system during breastfeeding, particularly before infants develop greater levels of IgA production. IgA attaches itself to pathogens, preventing them from adhering to, colonizing, or passing through mucosal surfaces into the body (Mestecky and McGhee 1987; Russell et al. 1999). It also inhibits inflammatory responses and complement activation (Russell et al. 1999). Interestingly, IgA assists in tolerance mechanisms for non-pathogenic commensal bacteria in the gut (Mestecky and Russell 1986) and aids the development and maintenance of beneficial bacterial communities (Bollinger et al. 2003). IgA protects infants from diarrheal and respiratory infections and may provide helpful immune activation long after the infant develops its own mucosal immune system.

There is considerable variation in breastmilk IgA. There is some evidence that IgA levels decrease through the postnatal period (Fitzsimmons et al. 1994) while other studies have found a consistent concentration of IgA in breastmilk throughout the lactational period (Hennart et al. 1991; Weaver et al. 1998). Breastmilk IgA is elevated in smokers, low-income women, women who report more positive life events, and women who had recently experienced an infection, while breastmilk IgA is lower in

stressed women and older women (Groer et al. 2004). Seasonality may also play a role, with lactating Gambian woman experiencing lower IgA levels in the wet season, which is a period of low food, compared to the dry season (Weaver et al. 1998). However, the effect of maternal characteristics on IgA levels has not been studied extensively, and the source of variation in breastmilk IgA remains unclear.

Theoretical Orientation. Although there are no direct measures of the metabolic cost of IgA production in kcal, several lines of evidence suggest that IgA production is energetically costly to individuals. First, undernutrition is associated with lower levels of IgA in the mucosal immune response (Chandra 1992; Chandra 1997; Chandra and Wadhwa 1989). Second, breastmilk IgA levels have been tied to food availability, with woman having higher levels during seasons with higher food availability (Weaver et al. 1998). Finally, lactating women who exercise to exhaustion have much lower levels of breastmilk IgA, indicating that IgA production may be reduced in the face of severe energetic stress (Gregory et al. 1997). This mucosal immune response during exercise is seen in non-lactating athletes' salivary IgA levels as well (Gleeson 2000). Since IgA production is likely costly, the transfer of IgA in breastmilk can be considered a form of maternal investment in offspring. Therefore, it can be tested using insights from life history theory.

If IgA is conceptualized as a costly form of maternal investment, evolutionary theory can predict the pattern of resource transfer from mother to infant through the postnatal period. Adaptive life history strategies attempt to allocate energy to growth, reproduction, and maintenance in a way that maximizes reproductive fitness. Mothers will maximize their fitness by investing in their current infant until it becomes

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advantageous to invest energy in their future offspring. Their investment in their current infant is time-sensitive based on the age specific mortality of the infant, which generally decreases with age. Therefore, maternal investment in the current offspring will decrease throughout the postnatal period. The more infants a woman has invested in, the fewer resources she will have for future infants, with women with higher parity having lower IgA levels in their breastmilk. Finally, women with greater overall resources, whether through higher nutrition, greater socioeconomic status, or better health, will be able to invest more IgA in their offspring compared to women with fewer resources.

Research Questions. Several hypotheses relating to IgA concentration can be derived from evolutionary theory. These will be tested in Ariaal women, a group of settled pastoralists in northern Kenya who experience considerable disease and nutritional stress:

1. IgA levels in breastmilk will decline over the postpartum period as mothers prepare to invest in future offspring.

2. IgA levels in breastmilk will correlate negatively with higher parity.

3. Finally, if IgA is costly to produce, its concentration in breastmilk will be positively associated with better indicators of maternal health, nutritional status, current reproductive status and socioeconomic status.

Methods

Field Site, Field Methods and IgA Assay. A detailed description of the study site can be found in Chapter 2. Field methods and IgA ELISA methods are found in Chapter

3. In addition to IgA, breastmilk was analyzed for total protein and creamatocrit; detailed methods are described in the following.

Protein Assay. Total breastmilk protein was assayed with a bicinchoninic acid (BCA) assay kit (Pierce), a colorimetric assay performed in a microplate and read with a spectrophotometer at 562 nm. Some modifications were made to the kit protocol. Breastmilk samples were assayed at a 1:10 dilution to fit within the standard curve. Due to the high concentration of protein in breastmilk, samples were incubated at room temperature for 15 minutes rather than 30 minutes at 37°C to avoid over-reacting both standards and samples. Acceptable duplicates were within 15% coefficient of variation with most values falling within 10%. Samples that fell outside the range of the standard curve were re-assayed at 1:20. One control value was included on each plate with a CV of 2.38%. Previous research indicated that the Pierce BCA assay kit has the greatest precision and sensitivity for measuring breastmilk protein compared to the Kjeldahl method for determining total nitrogen (R^2 =0.99), although it overestimates the total amount of protein by 13% (Keller and Neville 1986). Therefore, all samples were divided by 1.13 for a more accurate estimate of total breastmilk protein.

Creamatocrit. Percent fat in breastmilk was assessed by creamatocrit following the methods outlined in Lucus et al. (1978). Whole breastmilk samples were drawn into capillary tubes and sealed on one end with tube sealant. Tubes were spun in a microhematocrit centrifuge at 12,000 rpm for 15 minutes. Total milk column length and cream and fat layer length were measured with vernier calipers to the nearest 0.05 mm. Creamatocrit % is expressed as the length of the fat layer divided by the total milk column times 100. Creamatocrit % was converted to g/dL using the regression equation

in Wang (Wang et al. 1999). This method was tested for precision and accuracy by the operator (E. Miller). Twenty-four 2% cow milk capillary tubes were centrifuged and measured using the method above. The CV of the creamatocrit results was 5.43% and the mean creamatocrit percent was 2.3%.

Statistical Methods. Data were analyzed with SAS 9.1. First, univariate (descriptive) statistics were analyzed to characterize each variable, bivariate statistics were examined to assess the relationship between dependent variables and independent variables and covariates, and finally multivariate statistics combined these factors into one model. Descriptive statistics were compiled using PROC MEANS and PROC FREQ. Bivariate relationships were analyzed with PROC CORR or PROC ANOVA. Multivariate relationships with breastmilk IgA as a dependent variable were assessed with PROC REG for all three hypotheses. Women with breastmilk IgA values greater than 3000 µg/mL (>3 times normal IgA values) and/or reported abnormal milk samples were dropped from analysis, giving a final sample of 243 individuals. Variables were checked for normality by assessing their skew and kurtosis; variables with non-normal distributions (breastmilk and salivary IgA levels and Total Livestock Units) were log-transformed. Creamatocrit % and total protein, although significantly related to each other, had variance inflations < 10 in regression models and could be used together as covariates without violating the collinearity assumptions of the models. Results were considered significant at $\alpha < 0.05$.

Results

Maternal Characteristics. Means and percents of selected characteristics of Ariaal women are listed in Table 4.1 below for the total sample and by home village. One-way ANOVA indicate there are significant differences between villages for maternal age ($F = 5.43$, $p = 0.0050$), reported illness, ($F = 5.40$, $p = 0.0051$), BMI ($F = 4.19$, $p = 0.016$), triceps skinfold ($F = 6.59$, $p = 0.0016$), mid-upper arm circumference ($F = 3.15$, $p = 0.045$), education ($F = 3.10$, $p = 0.047$), wage-earning ($F = 34.4$, $p < 0.0001$), total livestock units ($F = 9.25$, $p = 0.0001$), household garden ($F = 14.31$, $p < 0.0001$), and per capita food budget per month ($F = 10.11$, $p < 0.0001$).

Tukey pairwise comparisons on these results indicate that Karare women are significantly older than women in Kituruni and Karare and reported significantly more illness than women in Parkishon. Women in Kituruni had significantly higher BMI and triceps skinfold than women in Karare. Women in Kituruni were significantly more educated than women in Parkishon. Women in Kituruni and Parkishon were more likely to have a source of income than women in Karare. Parkishon women had greater total livestock units than women in Karare and Kituruni, while all three communities differed from each other in frequency of growing a garden. Women in Karare had a lower per capita food budget compared to women in Parkishon and Kituruni. Finally, there was no significant pairwise difference between community and mid-upper arm circumference.

Breastmilk Characteristics. The breastmilk composition of Ariaal women and comparative values from the literature are shown in Table 4.2. Ariaal women have slightly lower IgA values and fat grams per deciliter. Their total protein is comparable to reference values. IgA comprises 9.1% of total breastmilk protein, similar to but slightly

lower than published amounts. Bivariate relationship between breastmilk components can

be found in Table 4.3. Immunoglobulin A, total protein, and creamatocrit % are all

significantly positively associated with each other. Percent IgA of total protein is

significantly negatively associated with total protein and creamatocrit.

Table 4.1. Maternal characteristics, total population and by village.

Mean ± S.D. or %	Total ($n = 243$)	Karare ($n = 124$)	Parkishon ($n = 66$)	Kituruni ($n = 53$)
Maternal Characteristics				
Age*	27.4 ± 6.1	28.6 ± 6.4	26.2 ± 5.9	26.0 ± 5.2
Months Postpartum	10.4 ± 6.1	10.2 ± 6.0	11.4 ± 6.7	9.8 ± 5.4
Salivary IgA (µg/mL)	323.7 ± 190.7	341.3 ± 193.3	301.8 ± 177.3	310.0 ± 199.8
Number of Live Births	3.5 ± 2.2	3.3 ± 2.0	3.5 ± 2.4	3.7 ± 2.2
Total Pregnancies	3.8 ± 2.3	3.7 ± 2.1	4.0 ± 2.6	3.9 ± 2.2
% Reported Illness*	38.3%	47.6%	24.2%	34.0%
% Returned to Menstruation	38.0%	33.8%	39.4%	45.3%
% Married	96.7%	94.4%	100%	98.1%
% Polygynous	40.9%	40.2%	45.5%	36.5%
Nutritional Status				
BMI (kg/m^2)*	18.5 ± 2.2	18.1 ± 2.2	18.8 ± 2.4	19.1 ± 2.0
Triceps Skinfold (mm)*	16.5 ± 6.5	15.2 ± 5.7	16.8 ± 7.7	19.0 ± 6.3
MUAC (cm)*	24.4 ± 2.5	24.0 ± 2.4	24.6 ± 2.6	24.9 ± 2.2
Socioeconomic Status				
% With Any Education*	12.8%	14.5%	4.5%	18.9%
% Earning Wage*	65.3%	43.5%	93.9%	79.2%
% "poor"	18.1%	23.4%	13.6%	11.3%
Livestock Units*	4.3 ± 4.5	3.4 ± 3.4	6.2 ± 6.3	4.2 ± 3.1
% Growing Garden*	49.6%	52.8%	25.8%	71.7%
Household Size	3.3 ± 1.3	3.2 ± 1.3	3.4 ± 1.2	3.6 ± 1.4
Food budget per month per capita (Kenyan shillings)*	889 ± 1242	1220 ± 1618	452 ± 359	658 ± 506

* $p < 0.05$

Table 4.2. Means of breastmilk components of Ariaal women and published sources from industrialized countries.

Mean ± S.D.	Ariaal Women	Published Values
IgA Concentration (µg/mL)	842.1 ± 311.1	1000[1]
Total Protein (g/dL)	0.97 ± 0.3	0.9-1.03[2]
Creamatocrit (%)	4.4 ± 2.8	5.1 ± 1.9[3]
IgA/Total Protein (%)	9.1 ± 3.6	10-15[2]
Total Fat (g/dL)	2.8 ± 1.5	3-4.4[2]

[1] (Prentice 1996)
[2] (Ogra et al. 2006)
[3] (Wang et al. 1999)

Table 4.3. Correlation coefficients and p-values of breastmilk components in Ariaal women.

	IgA	Protein	Creamatocrit %	IgA/Protein %
IgA	___			
Protein	$r = 0.27$ $p < 0.0001$	___		
Creamatocrit %	$r = 0.14$ $p = 0.034$	$r = 0.66$ $p < 0.0001$	___	
IgA/Protein %	$r = 0.73$ $p < 0.0001$	$r = -0.29$ $p < 0.0001$	$r = -0.29$ $p < 0.0001$	___

Hypothesis 1. This hypothesis predicts that IgA concentration in breastmilk will decrease as time since birth increases. Breastmilk IgA concentration was significantly positively associated with infant age ($r = 0.14$, $p = 0.028$). However, visual inspection of breastmilk IgA and months postpartum indicated that the relationship between the two was not linear, but actually 2nd degree polynomial (Figure 4.1), so time since birth squared was included in the multivariate analysis. Creamatocrit percent, total protein (g/dL), log maternal salivary IgA levels, maternal BMI, reported number of night feedings, and home village were included in the analysis as covariates.

Figure 4.1. Relationship between breastmilk IgA and months postpartum.
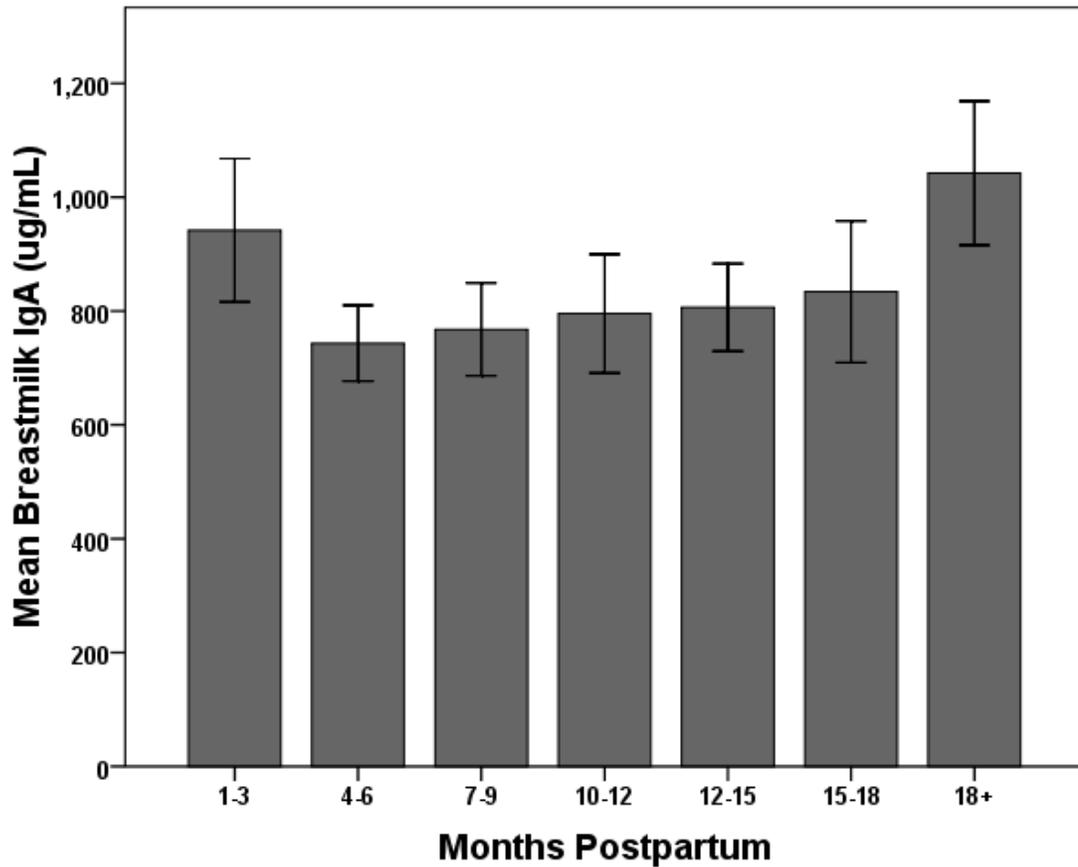
Results of the regression are listed in Table 4.4. They indicate that IgA is significantly associated with months postpartum in a polynomial U-shaped pattern, with IgA concentrations initially high, declining, then rising again after 18 months postpartum. Total protein was significantly positively associated with IgA concentrations while creamatocrit %, salivary IgA, maternal BMI, number of night feedings, and village were not significant.

Table 4.4. Estimates and p-values for months postpartum and covariates regressed against breastmilk IgA.

	Log Breastmilk IgA		Log Breastmilk IgA/Fat	
	β	p-value	β	p-value
Model R^2	0.097		0.30	
Months Postpartum	-0.031	0.023	-0.10	<0.0001
Months Postpartum^2	0.0017	0.0047	0.0042	<0.0001
Creamatocrit %	-0.0063	0.56	----	---
Total Protein (g/dL)	0.31	0.0074	-1.41	<0.0001
Maternal Age	0.0028	0.44	0.0026	0.69
Log Salivary IgA	-0.0035	0.93	-0.064	0.33
Maternal BMI	0.012	0.23	0.031	0.074
Number of Night Feedings	0.018	0.38	-0.0065	0.85
Living in Parkishon*	0.020	0.71	-0.13	0.16
Living in Kituruni*	0.074	0.21	-0.22	0.032

* "Living in Karare" was the reference category for these variables

Fat is a known indicator of amount time breastmilk has spent in the breast (Daly et al. 1993). Older infants tend to breastfeed less often (Prentice 1996), and as a result breastmilk and its components accumulate in the breast for longer periods of time. In this sample, fat appears to increase over the course of the postpartum period compared to other breastmilk components (see Figure 4.2). Therefore, to adjust for the amount of time breastmilk has spent in the breast, the regression was rerun using the ratio of IgA to creamatocrit, minus creamatocrit % as a covariate. The results can be found in Table 4.4. Breastmilk IgA remains in a significant polynomial relationship (it is not linearly significant), although the shape of the curve has changed, with higher levels seen during the first 6 months postpartum and a much smaller upward curve at greater than 18 months. Total protein is still significant, but the sign has changed compared to the model with IgA concentration alone. In addition, IgA/fat is lower in Kituruni. Finally, maternal BMI is marginally significantly positively associated with IgA to fat ratio.

Figure 4.2. Fat (g/dL), protein (g/dL), IgA concentration (g/L), and IgA/fat (g/L) over the course of the postpartum period.
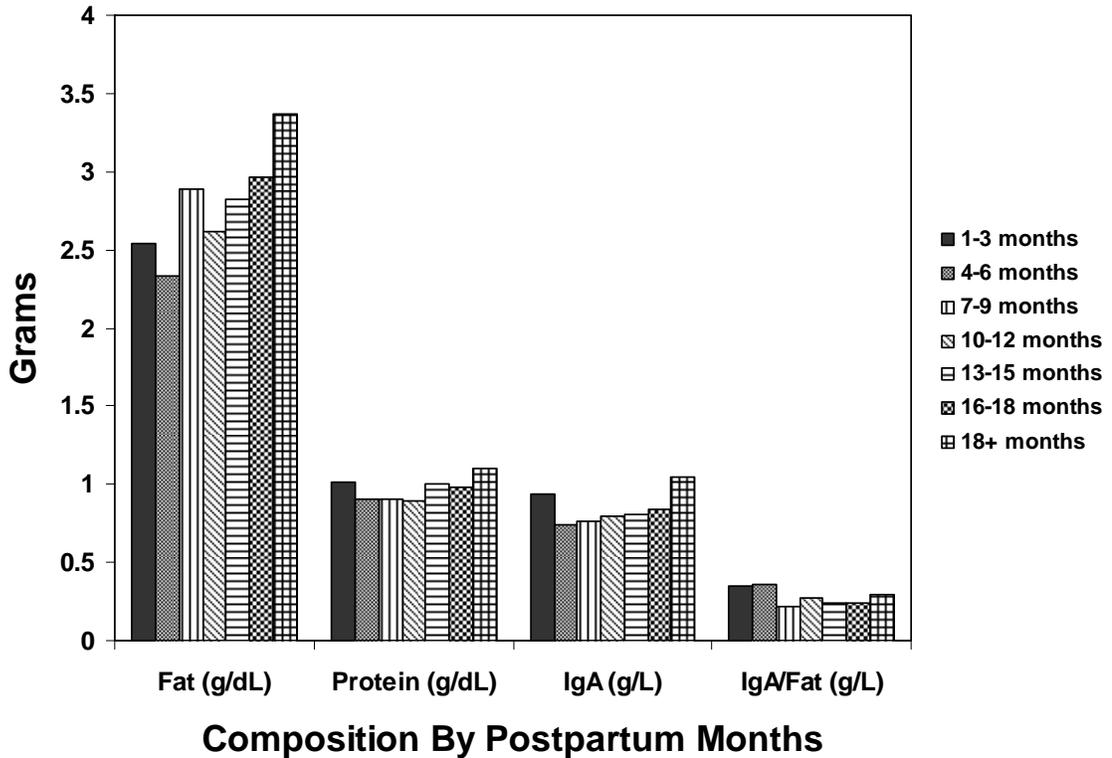
Hypothesis 2. This hypothesis predicts that breastmilk IgA levels will be lower in women with higher parity. Bivariate correlation indicates no relationship between parity and breastmilk IgA ($r = 0.055$, $p = 0.39$), however, again the relationship between the two is non-linear. Figure 4.3 shows that the relationship between parity is non-linear, with breastmilk IgA concentrations increasing to 4 live births and declining in females who have given birth seven or more times. Therefore, a squared parity term was included in the multiple regression model. Covariates included creamatocrit %, total protein, log salivary IgA, BMI, number of night feedings, and village. Another regression model was run with IgA/fat ratio as a dependent variable. Results are presented in Table 4.5. Parity has a significant 2nd degree polynomial relationship with breastmilk IgA. Total protein is also significantly associated with breastmilk IgA, while the other covariates are not. In

the model with IgA/fat ratio as the dependent variable the polynomial relationship with parity remained, as did the significant association with total protein. In addition, IgA/fat ratio was significantly positively associated with maternal BMI and negatively associated with living in Kituruni.

**Figure 4.3. Breastmilk IgA concentration by number of children.**
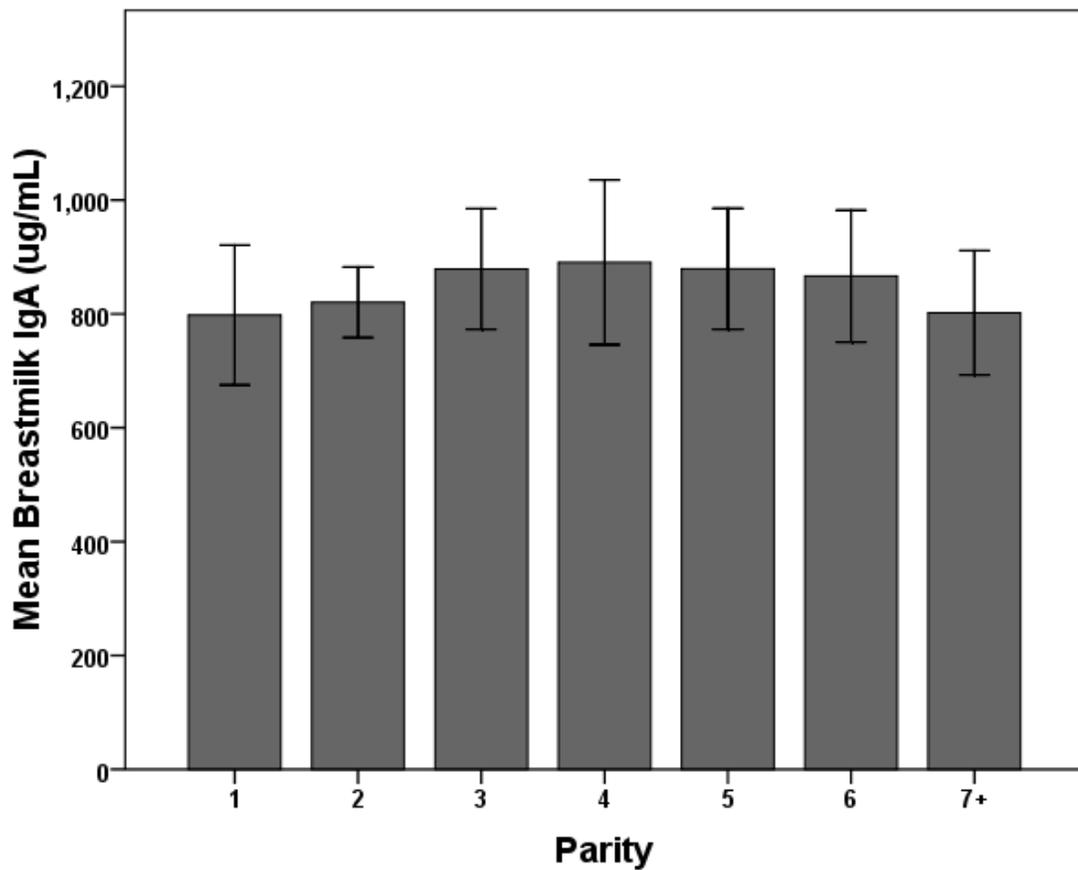
Table 4.5. Estimates and p-values for parity and covariates regressed against breastmilk IgA.

	Log Breastmilk IgA		Log Breastmilk IgA/Fat	
	ß	*p-value*	*ß*	*p-value*
R^2	0.075		0.26	
Parity	0.089	0.032	0.17	0.017
Parity^2	-0.0010	0.017	-0.016	0.025
Creamatocrit %	-0.0059	0.58	----	----
Total Protein (g/dL)	0.39	0.0009	-1.30	<0.0001
Maternal Age	0.0018	0.76	-0.0079	0.43
Log Salivary IgA	-0.0084	0.83	-0.068	0.32
Maternal BMI	0.015	0.16	0.037	0.040
Number of Night Feedings	0.011	0.57	-0.0024	0.94
Living in Parkishon*	0.041	0.46	-0.13	0.19
Living in Kituruni*	0.052	0.40	-0.30	0.0053

* "Living in Karare" was the reference category for these variables

Hypothesis 3. The final hypothesis tested the association between breastmilk IgA and current reproductive status, nutritional status, health, and socioeconomic status. Resumption of menstrual period (coded 0/1) was used as an indicator of reproductive status. Frequency of women reporting a menstrual period increased as postpartum months increased, as shown in Figure 4.4. Nutritional status was indicated by triceps skinfold. Women were considered "sick" (coded 0/1) if they had reported a fever, respiratory infection, or diarrheal episode within the past month. Several variables assessed socioeconomic status in this model: total livestock units, growing a garden, earning a wage, education level, food budget per capita, and having a polygynous marriage. Finally, the following variables were added as covariates in the model: total breastmilk protein, creamatocrit %, maternal age, salivary IgA, number of night feedings, and village.

Figure 4.4. Proportion of women who have resumed menses versus months postpartum.
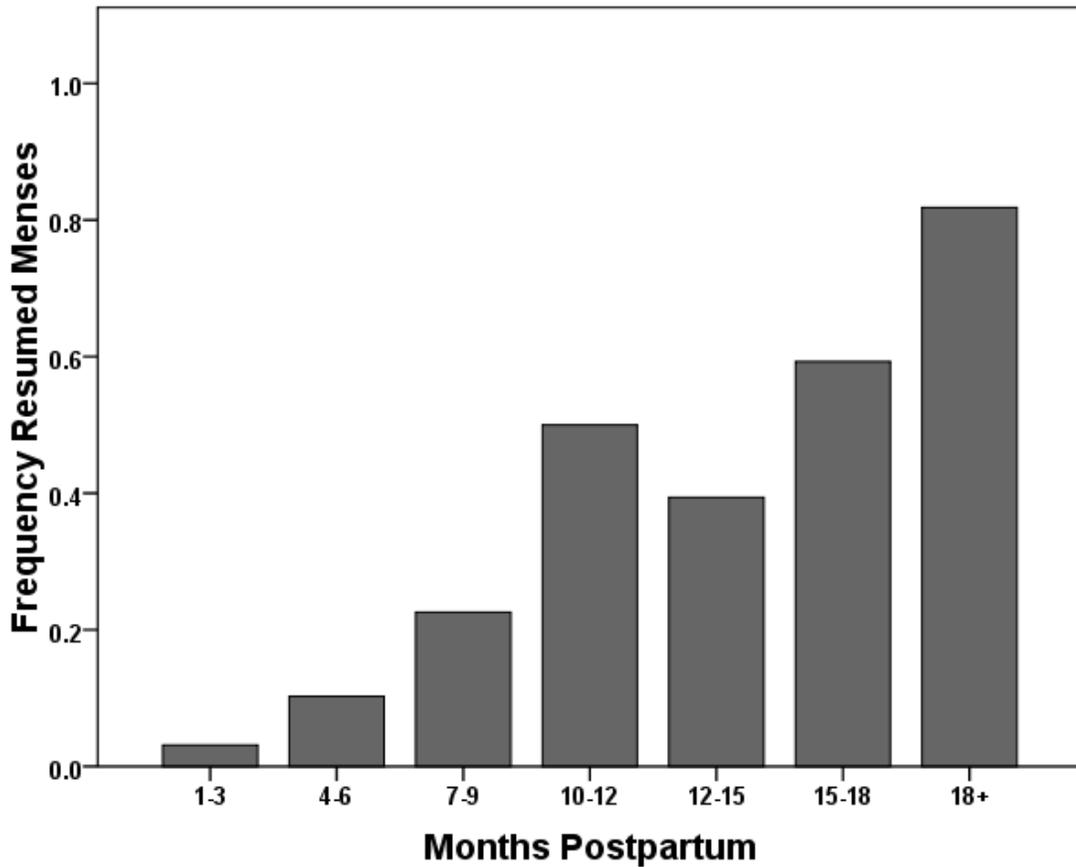
Results are listed in Table 4.6. No reproductive, nutritional, health, or socioeconomic variable is significantly related to breastmilk IgA. For the IgA-only model, total protein remained highly significantly associated with IgA concentration while other covariates were not significant. In the IgA/fat model, total protein is also highly significant. Living in Kituruni, having an illness within the past month, and being in a polygynous marriage are significantly associated with a lower IgA/fat ratio. Log total livestock unit is marginally positively associated with IgA/fat ratio. By contrast, the per capita household food budget is significantly negatively associated with IgA/fat ratio, although the size of this effect is small.

Table 4.6. Estimates and p-values of reproductive, nutritional, health, and socioeconomic variables regressed against breastmilk IgA.

	Log Breastmilk IgA		Log Breastmilk IgA/Fat	
	β	p-value	β	p-value
R^2	0.048		0.26	
Resumed Menses	0.0080	0.87	-0.078	0.34
Reported Illness	-0.043	0.38	-0.17	0.049
Mean Triceps Skinfold (mm)	0.0010	0.77	0.0032	0.59
Log Total Livestock Units	0.039	0.31	0.12	0.062
Earn Wage	0.029	0.63	-0.088	0.40
Grows Garden	-0.039	0.43	-0.081	0.34
Any Formal Education	0.051	0.51	0.062	0.64
Food Budget Per Capita	-7.9×10^{-6}	0.69	-8.3×10^{-5}	0.013
Polygynous Marriage	-0.070	0.13	-0.16	0.041
Creamatocrit %	-0.0075	0.51	----	----
Total Protein (g/dL)	0.40	0.0010	-1.34	<0.0001
Maternal Age	0.0027	0.50	0.0064	0.34
Log Salivary IgA	-0.012	0.76	-0.048	0.49
Number of Night Feedings	0.0075	0.71	-0.0017	0.96
Living in Parkishon*	-0.025	0.70	-0.19	0.094
Living in Kituruni*	0.052	0.43	-0.24	0.033

* "Living in Karare" was the reference category for these variables

Discussion

Immunoglobulin A is significantly associated with number of months postpartum; however the effect was not linear as predicted. Breastmilk IgA concentration does decrease through the first three months before leveling off until 18 months, when it rises again. Previous reports of Western women who lactated for longer than 12 months indicate that the fat and energy content of the milk increases with increasing months postpartum (Larnkjaer et al. 2006; Mandel et al. 2005). However, the higher IgA concentration persists in Ariaal women even after correcting for the protein and fat content of the breastmilk. There may be several explanations for this finding. First, even within the Ariaal population where women breastfeed for extended periods of time, the women who lactate beyond 18 months may do so because they have higher quality breastmilk and higher IgA concentrations than women who stop breastfeeding. This

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scenario seems somewhat unlikely, as there are no outward signs that would signal to these women that they are high IgA producers. Second, since the majority of women who have been lactating for longer than 18 months have resumed menstrual periods (> 80%), they may have greater total energy reserves and thus more IgA-intense breastmilk than mothers lactating for shorter periods. Finally, because older infants are likely breastfeeding much less often than younger infants (the data in this study are unclear), it may be that breastmilk contents accumulate in the breast, becoming more concentrated with greater time (Daly et al. 1993). The ratio of IgA concentration to creamatocrit % revealed a different shaped polynomial curve that favored a higher slope during postpartum months 0-6 and a still-raised, but lower increase at 18+ months. This seems to favor the last explanation for the rise in IgA in women who are 18+ months postpartum. Other studies have found an increase in creamatocrit and energy content in women who prolong lactation greater than 18 months (Larnkjaer et al. 2006; Mandel et al. 2005); however, they have no explanation for why these higher concentrations exist.

There was a significant 2nd degree polynomial relationship between parity and IgA levels. IgA levels peak at around 4 live births and drop dramatically in women with 8 or more live births (not shown). These women were not necessarily at the end of their reproductive careers, with a mean age of 35 years old. The hypothesis was partially predicted, in that IgA levels did decrease in high parity women; however women with fewer than four children had slightly lower breastmilk IgA concentrations. This relationship is difficult to interpret. It is unknown why IgA levels are lower in low parity women and high parity women and highest in mid-parity women, especially when the model has been adjusted for maternal age. However, there is not a statistically significant

difference between low parity women and mid-parity women. It may be that women who have experienced a certain number of pregnancies have higher IgA due to greater exposure to pregnancy hormones or the immunological tolerance effects of pregnancy, an effect that creates a "peak" in immune function followed by a decline. Other studies have indicated either no association between breastmilk IgA and parity (Hennart et al. 1991; Weaver et al. 1998), or reduced milk "quality" in highly parous mothers (> 9 children; (Prentice et al. 1989)); however it is likely that investigators were not looking for a polynomial relationship between the two. Further research should investigate this interesting association.

Breastmilk IgA is not associated with some of health, nutritional and socioeconomic markers than indicated higher resources levels in Ariaal women. For example, being in a polygynous marriage, being ill within the past month, and having fewer livestock were associated with lower IgA/fat ratio. On the other hand, spending more money on food per month was associated with lower breastmilk IgA/fat, a result that contrasts with the other findings. Some of the models suggest a slight positive association between maternal BMI and IgA/fat ratio, indicating a relationship between energy balance and IgA production. However, further analysis showed that IgA concentration is not directly associated with fat reserves. IgA concentration is also not significantly associated with the resumption of menstrual periods. On the other hand, the frequency of resuming menstrual periods increases with increasing postpartum months, indicating that changes in energy balance may still be involved but acting below a threshold that would create changes in body composition.

A comparison of Ariaal women to US reference values indicates that Ariaal IgA levels may be on average lower than better-nourished Western women (although still within the normal range of 400-1600 μg/mL; (Ogra et al. 2006)) despite their normal protein composition. It is unclear if this effect is due to differences in resource availability, although it is somewhat surprising that Ariaal women who live in a high disease environment would have lower IgA concentrations than relatively hygienic Western women. Exploring the IgA levels in Western women using the same IgA assay can shed light on the possibility of population differences in breastmilk immunoglobulin A.

To some degree the predicted associations between breastmilk IgA and months postpartum and parity were supported by the data presented here; however, there was mixed evidence for a role for resource availability in determining breastmilk IgA concentration. Therefore, it may be that this system does not involve an energetic trade-off, but rather a trade-off with time. Time is a very important resource in an individual's live history, guiding when and how much energy will be allocated particular reproductive events. In this case, time rather than energy may be interacting with breastmilk physiology to influence the life history of the mother and the infant. Measuring time vs. energy trade-offs is fraught with difficulty (Stearns 1992), but with careful study design these questions could potentially be answered in a human population.

There remain several questions about the role of women's reproductive status on her immune function and particularly her breastmilk IgA. Longitudinal studies of lactating women would help indicate if the relationship between breastmilk IgA and postpartum months were a typical pattern among women or an artifact of some other

characteristic of this particular sample of Ariaal women. A comparative study between Kenyan and US women can further elucidate the differences in breastmilk IgA concentration and nutritional and energy status using comparable methodology. Finally, more sensitive measures of energy balance such as insulin and C-peptide levels (Valeggia and Ellison 2009) and ovarian function can provide more accurate estimations of maternal energy and reproductive status than those used in this study.

Conclusion

There is considerable variation in breastmilk IgA in Ariaal women. IgA levels are high then decline after 3 months postpartum, before rising again in women who lactate for 18 months or more. IgA levels are highest in women who have 4 children and are considerably lower in women with 8 or more children. This result can be linked to life history theory, which predicts that maternal IgA investment will decrease with increasing months postpartum and preparation for the next reproductive event. Women who have "spent" energy by having high numbers of children have fewer overall somatic resources for their infant, explaining the decrease in IgA in high parity women. This study, however, did not find an association between available resource, nutritional and reproductive status and breastmilk IgA concentrations. Future research will follow women longitudinally, compare high and low resource populations, and use more sensitive measures of energy balance to further study the relationship between IgA and women's reproduction.

Chapter V

IgA and Infant Nutritional Status

Introduction

Overwhelming evidence suggests that breastfeeding protects infants in developing countries from diarrheal and respiratory disease (Arifeen et al. 2001; Brown et al. 1989; Clemens et al. 1997; Jason et al. 1984; Kovar et al. 1984; Morrow and Rangel 2004). Breastmilk is nutritionally complete, does not require purchasing and relatively much more sterile than local water sources in many countries. It is therefore a safer and more cost-effective method of feeding infants compared to using formula. Mothers also transfer considerable immune protection to their infants through breastfeeding (Cripps et al. 1991; Walker 2004; Weaver 1992).

Breastmilk contains many immunological elements that protect against diarrheal disease. Lactoferrin, lysozyme, anti-pathogen proteins, macrophages, neutrophils, T and B lymphocytes, immunoglobulins and cytokines are all transmitted through breastmilk (Ogra et al. 2006). The most abundant immunological factor in milk is immunoglobulin A (IgA). IgA works primarily by coating the epithelial cells on mucosal surfaces to prevent pathogens from passing though cell walls into the body (Mestecky and McGhee 1987; Russell et al. 1999). As part of the adaptive immune system, pathogen-specific IgA is secreted by maternal B-cells and transferred to the infant, forming a protective barrier that is specific to the diseases the mother has experienced in her environment. Specific

IgA molecules also gather in large "clumps" around pathogens to prevent re-infection and help them pass out of the body. In addition, IgA inhibits local inflammatory responses and complement activation (Russell et al. 1999). Interestingly, IgA molecules tolerate a wide variety of commensal, non-pathogenic bacteria in the gut which also help prevent infection (Cole et al. 1999; Mestecky and Russell 1986). Approximately 90% of the IgA secreted in mucosal tissues and breastmilk is in the dimeric form, secretory IgA (sIgA).

Weaning, the process of introducing solid foods to an infant, opens a potential vector of pathogen invasion to the developing infant. After around 6 months of age, breastmilk alone cannot meet the nutritional needs of the growing infant but the introduction of solid food can increase the risk of malnutrition and disease. This is a predicament known as the "weanling's dilemma". The timing of the beginning and the end of the weaning process can vary depending on the reproductive needs of the mother and infant disease risk and nutritional status. For example, the World Health Organization recommends infants be exclusively breastfed until 6 months of age (Kramer and Kakuma 2004). However, in some populations nutritional supplementation is recommended before the age of 6 months due to insufficient energy from breastmilk alone (Wilson et al. 2006). The end of the weaning process is marked by the cessation of breastfeeding and may occur much earlier in developed countries than in developing countries due to lower disease risk. For infants in high disease ecologies, breastmilk can provide immunological protection against food-borne pathogens introduced by weaning. There is likely a protective nutritional effect of fat stores as well. Kuzawa (1998) notes that fat deposition in infants is accelerated postnatally but before weaning begins. During weaning, fat stores decline to childhood levels. It is likely that this fat exists to protect infant growth during

periods of nutritional upsets due to infection or food shortage during weaning. Both IgA-rich breastmilk and infant fat stores protect the weaning infant.

Weaning is not the only behavior that introduces pathogens to the naïve immune system. Fessler and Abrams (2004) hypothesize that infant mouthing behavior, commonly thought to help infants explore the environment or aid in teething, actually serves the purpose of exposing infants to small, non-lethal amounts of pathogens present in the environment. They note that mouthing behaviors peak between 6 and 12 months of age, the same time as the beginning of the weaning process. Both weaning and mouthing, behaviors that can "calibrate" the immune system, are sheltered by the protective immune effects of breastfeeding to buffer the infant against deadly consequences of dangerous pathogens. These behaviors, in conjunction with other hygienic and/or disease exposing behaviors, such as crawling, bathing, and latrine use, can adjust the infant's mucosal immune system to local diseases.

While it is well-known that infection during development can take a severe toll on infant nutritional status, few studies have assessed the energetic costs of immune maintenance and activation in human populations. The vertebrate literature provides slightly more evidence for the costs of activating and maintaining an immune response. Previous research in rodents has found that infection by an antigen, even one that does not produce active symptoms such as fever, increases the amount of oxygen consumed by an animal between 20-30% (Demas et al. 1997). Lochmiller and Deerenberg (2000) review literature that notes that severe illness can significantly raise metabolic rates and promote wasting by the mobilization of protein sources for energy. They suggest that while short-term immune activation is beneficial to the individual by protecting the body

from death by infection, long-term immune up-regulation can produce significant costs to individuals' growth, reproduction, and ultimately their fitness. They further propose that adaptive immune responses are more energetically costly than innate immunity in animals.

Other research has indicated that fat stores are a more likely source of energy than carbohydrates for immune responses and that the hormone leptin may modulate the mobilization of fat stores during immune responses in Siberian hamsters (Demas and Sakaria 2005). These observations support the empirical data that underdeveloped infant immune responses can lead to malnutrition and death without the protective umbrella of breastmilk and body fat. It is not known, however, how the development and activation of the infant immune system may impact the growth and nutritional status of infants with high exposure to disease and limited energetic reserves.

The development and the adaptation of the mucosal immune system to the local disease ecology is a critical process during infancy. The IgA present in breastmilk likely protects infants from some of the lethal and non-lethal costs of disease exposure. On the other hand, immune development likely imposes some cost on growth, particularly in high pathogen environments. To date, researchers have not addressed the effects of breastmilk IgA on infant growth and immune system development. Likewise, there are few investigations of the links between mucosal immune function and nutritional status. In this chapter, I will explore the benefits of passive immunity from breastmilk on growth and mucosal immune development in infants in the Ariaal population, a settled pastoral group in northern Kenya that experiences considerable nutritional and disease stress.

Research Questions. Infant immune function and nutritional status are complex phenotypes that are interconnected with breastmilk, disease-exposing behaviors, and the environment. Specifically, I will investigate three questions that address the interplay between infant IgA, nutritional outcomes, breastmilk IgA, behavior, and the environment.

1. Is the development of IgA in infants energetically costly and associated with poorer nutritional outcomes?

2. Are higher levels of breastmilk IgA associated with better infant health and nutritional outcomes?

3. Does breastmilk IgA protect against the health and nutritional effects of disease-exposing behaviors such as the consumption and preparation of supplemental foods, vaccination status, crawling behavior, family toilet use, frequency of water boiling for infant consumption, frequency of bathing, and mosquito net use?

Methods

Field and Laboratory Methods. Refer to Chapters II and III for a detailed description of the study site, field methods, and laboratory methods.

Statistical Methods. Infant height and weight measurements were converted into z-scores with the computer program WHO Anthro, which uses the WHO Child Growth Standard reference population values (WHO 2006). The WHO Child Growth Standard is a multiregional standard based on optimal growth of breastfed infants. It better represents breastfed infants compared to formula fed infants in other child reference standards, who tend to be shorter and heavier (de Onis et al. 2007). Height-for-age (HAZ), weight-for-

age (WAZ) and weight-for-height (WHZ) z-scores were generated for each infant using this program. HAZ is a measure of long-term nutritional stress; infants with HAZ at -2 or below are said to be stunted. WAZ (underweight) and WHZ (wasting) z-scores, when they are less than -2, are measures of more immediate or short-term nutritional stress. Appendix IV contains charts of sex-specific height-for-age and weight-for-age growth curves with Ariaal infants plotted against WHO reference percentiles.

An infant was considered to have a reported illness if mothers stated that their infant suffered a fever, respiratory infection, or diarrhea within the past month. Maternal recall of health events generally tends to be very high for up to a year, with mothers of younger children having better recall (Pless and Pless 1995). Breastmilk and infant salivary IgA concentrations had non-normal distributions and were therefore log-transformed before analysis. The amount of livestock owned by the family was converted to livestock units, a measurement developed by the Food and Agriculture Organization (FAO 2009). Using this method, individual cattle were multiplied by 0.5, small livestock by 0.1, and camels by 1.1 and the total added together. A dichotomous variable that indicated whether an infant was appropriately vaccinated for his or her age was generated based on the vaccine schedule for Kenya recommended by the WHO (WHO 2009). Among the Ariaal, older infants appear to be well-vaccinated while younger infants lag behind, indicating barriers to following an age-specific vaccine schedule in this population.

Infant age was a problematic covariate in models with HAZ, WAZ, WHZ, and TSFZ (triceps skinfold-for-age) because it is correlated with each of these variables. In addition, it is associated with many hygiene and supplemental food variables, possibly

obscuring the effects of these independent variables. When infant age is regressed against crawling, living in a traditional house, boiling water for the infant, using a mosquito net, using a family latrine, supplementing with milk, and supplementing with solid food, these variables account for 65.9% of the variance seen in infant age ($R^2 = 0.659$), while collinearity between these variables remains low (all variance inflation values are well below 10). Unsurprisingly, many of these variables are significantly associated with infant age. While I retained infant age in analyses, it is important to note that the effects of independent hygiene and food variables may not operate directly on infant nutritional outcome in these models but instead may have effects that may be subsumed by infant age.

Data were analyzed using SAS 9.1. First, univariate (descriptive) statistics were analyzed to characterize each variable, bivariate statistics were examined to assess the relationship between dependent variables and independent variables and covariates, and finally multivariate statistics combined these factors into one model. Bivariate continuous relationships were analyzed with PROC CORR, giving the Pearson product-moment correlation. Relationships between dichotomous independent variables and continuous outcomes were analyzed using PROC TTEST. The Welch-Satterthwaite method of computing degrees of freedom was used because it does not assume that the underlying population variances are equally distributed. The bivariate relationship between dichotomous hygiene and food variables and reported illness (coded yes/no) was assessed by Pearson's chi-square test using the "chisq" function of PROC FREQ. The "chisq" function assumes column and row frequencies are independent to calculate expected values. Multivariate relationships were analyzed using PROC REG for continuous

dependent variables and PROC LOGISTIC for dichotomous dependent variables.

Statistical significance was assessed at $\alpha = 0.05$. Although a Bonferroni correction could

be appropriate for some questions in which multiple models are reported, the decision

was made to keep the $\alpha = 0.05$ level because each growth indicator represented a

different aspect of nutritional status. Although this approach is less conservative, it allows

each nutritional pathway to be assessed at a greater power (lower Type II error rate).

Results

Descriptive Statistics and Community Differences. Means and standard deviations

or percentages of infant characteristics were assessed using PROC MEANS and PROC

FREQ. Total and by community values for these variables can be found in Table 5.1.

One-way ANOVA and chi-square analyses were used to test possibility of

significant differences in infant characteristics between the three communities. Infants

did not differ significantly in age ($F(2) = 0.74$, $p = 0.48$) but did differ significantly in

salivary IgA concentration ($F(2) = 5.77$, $p = 0.0036$). Observed frequencies of diarrhea,

respiratory infection, fever, and total illness by village did not differ from expected

values (all $p > 0.05$).

A chi-square analysis of BCG vaccination and location found no significant

differences between expected and observed values ($\chi^2(2) = 3.38$, $p = 0.18$). There were

significant differences between villages in frequencies of first polio vaccine ($\chi^2(2) = 9.7$,

$p = 0.0080$) but not first DPT vaccine ($\chi^2(2) = 3.0$, $p = 0.22$) in infants that were older

than 2 months. Significant differences between villages remain between expected and

observed frequencies of third polio vaccine dose in infants above the age of 4 months

$(\chi^2(2) = 17.8, p = 0.0001)$; differences are also found in expected and observed

frequencies of infants above 4 months old who have received the third dose of DPT

vaccine $(\chi^2(2) = 11.6, p = 0.0031)$. There are no significant differences between expected

and observed frequencies of measles vaccination by village in infants who are greater

than 9 months old $(\chi^2(2) = 5.2, p = 0.076)$. No infant had received a second,

recommended measles booster vaccine.

There were no significant differences between village and HAZ, WHZ, or WAZ

(all $p > 0.05$) or between expected and observed frequencies of stunting, and underweight

($p > 0.05$). There were significant mean differences between villages for TSFZ ($F(2) =$

8.64, $p = 0.0002$) and infant averaged triceps skinfold ($F(2) = 9.13, p = 0.0002$).

Most hygiene practices and behaviors did not significantly differ from expected

frequencies between villages. Always boiling water for the infant, crawling behavior, and

living in a traditional house did not differ significantly between communities from

expected frequencies ($p > 0.05$). Only household latrine use frequencies ($\chi^2(2) = 21.4, p <$

0.0001) and mosquito net use frequencies ($\chi^2(2) = 18.0, p = 0.0001$) were significantly

different from expected frequencies. In addition, one-way ANOVA of number of times

the infant was bathed per week per village was not significant ($F(2) = 0.48, p = 0.62$).

There were no differences between expected and observed frequencies of

excusive breastfeeding, drinking cow milk, or eating starch for infants in the three

villages ($p > 0.05$).

There were significant differences in socioeconomic status between villages.

There were significant differences in TLU between communities, as revealed by one-way

ANOVA ($F(2) = 8.89, p = 0.0002$). There were also differences in garden ownership

between villages compared to expected values ($\chi^2(2) = 26.3$, $p < 0.0001$). However, there were no significant differences in observed frequency of self-report as "poor" between villages compared to expected frequencies.

Tukey's pairwise comparisons on significant ANOVA relationships revealed community differences. Infants in Karare had higher salivary IgA, lower triceps skinfold, and a lower TRFZ than infants in Parkishon and Kituruni. Families in Parkishon had greater total livestock units than families in Karare and Kituruni. Finally, there were significant differences between all communities in growing a household garden.

Table 5.1. Infant characteristics by community.

Mean ± S.D. or %	Total (*n* = 239)	Karare (*n* = 122)	Parkishon (*n* = 66)	Kituruni (*n* = 51)
Infant Characteristics				
% Male	46.4%	47.5%	45.5%	45.1%
Age (months)	10.6 ± 6.0	10.3 ± 5.9	11.4 ± 6.7	10.1 ± 5.3
Salivary IgA (μg/mL)*	89.4 ± 116	113 ± 154	63.2 ± 42.4	64.6 ± 44.8
% Reported Diarrhea	10.5%	10.7%	10.6%	9.8%
% Reported Fever	9.2%	9.0%	4.5%	15.7%
% Reported Respiratory Illness	42.7%	38.5%	54.5%	37.3%
% Total Reported Illness	58.6%	54.9%	66.7%	56.9%
Vaccination Status				
% BCG (all ages)	95.0%	93.4%	93.9%	100%
% PV 1 (> 2 mo)*	84.1%	86.1%	73.0%	93.9%
% PV 3 (> 4 mo)*	81.8%	86.7%	60.8%	93.3%
% DPT 1 (> 2 mo)	89.4%	90.4%	84.1%	93.9%
% DPT 3 (> 4 mo)*	78.8%	79.6%	63.6%	93.3%
% Measles (> 9 mo)	68.3%	59.4%	75.6%	79.3%
Nutritional Status				
HAZ	-0.82 ± 1.4	-0.69 ± 1.5	-1.1 ± 1.4	-0.77 ± 1.2
% Stunted (< -2.0 HAZ)	17.6%	16.4%	21.2%	15.7%
WAZ	-0.97 ± 1.3	-0.95 ± 1.3	-1.2 ± 1.3	-0.73 ± 1.0
% Underweight (< -2.0 WAZ)	15.1%	16.4%	18.2%	7.8%
WHZ	-0.70 ± 1.3	-0.70 ± 1.2	-0.77 ± 1.2	-0.34 ± 1.3
% Wasted (< -2.0 WHZ)	19.2%	18.0%	27.3%	11.8%
Infant Average Triceps Skinfold*	9.4 ± 2.5	8.7 ± 2.3	9.9 ± 2.6	10.3 ± 2.4
Infant TSFZ*	-0.78 ± 0.99	-1.0 ± 0.96	-0.58 ± 1.0	-0.45 ± 0.9
Hygiene Practices and Behaviors				
% Families Using Latrine*	24.3%	22.1%	10.6%	47.1%
% Always Boil Water for Infant	57.3%	53.3%	65.2%	56.9%
% Always Use Mosquito Nets*	33.1%	36.9%	13.6%	49.0%
% Infants Crawling	56.9%	56.6%	57.6%	56.9%
% Living in Traditional House	92.1%	90.2%	98.5%	88.2%
Baths Per Week	8.2 ± 3.9	8.3 ± 3.4	8.2 ± 4.5	7.7 ± 4.2
Supplemental Foods				
% Exclusive Breastfeeding	12.1%	15.6%	7.6%	9.8%
% Consuming Cow Milk	85.4%	82.0%	92.4%	84.3%
% Consuming Boiled Milk	66.6%	65.0%	62.3%	76.7%
% Consuming Fresh Milk	32.4%	35.0%	34.4%	23.3%
% Consuming Staple Food	32.2%	28.7%	34.8%	37.3%
Socioeconomic Status				
Livestock Units*	4.4 ± 4.5	3.4 ± 3.4	6.2 ± 6.3	4.2 ± 3.2
% "poor"	17.6%	22.1%	13.6%	11.8%
% grow a garden*	49.6%	52.9%	25.8%	72.5%

* Denotes significant community difference ($p < 0.05$)

Sex Differences. Sex differences in anthropometric measurements and salivary

IgA levels can be found in Table 5.2. One-way ANOVAs found significant differences in

salivary IgA ($F(1) = 5.97$, $p = 0.015$) and HAZ ($F(1) = 5.53$, $p = 0.020$). Chi-squared

analyses revealed a significant observed sex difference in stunting ($\chi^2(1) = 10.45$, $p =$

0.0012) compared to expected frequencies. There were no differences between male and

female infants on WAZ, WHZ and TRIZ or in the frequencies of children classified as

wasted or underweight.

**Table 5.2. Infant anthropometric indices and salivary IgA measurements by sex.**

Variable	Female (n = 128)	Male (n = 111)
Salivary IgA (μg/mL)*	74.2	106.8
Height-for-age*	-0.62	-1.05
Weight-for-age*	-0.83	-1.13
Weight-for-height	-0.64	-0.77
Triceps skinfold-for-age	-0.80	-0.76
% Stunted*	10.2%	26.0%
% Wasted	16.4%	22.5%
%Underweight	13.3%	17.1%

* Denotes significant sex difference ($p < 0.05$)

Hypothesis 1. I hypothesized that there would be significant relationships between

infant IgA development and indices of nutritional status in Ariaal infants. Bivariate

correlations of log infant IgA with infant UAFA, TRFZ, WAZ, and WHZ indicate no

significant associations (all $p > 0.05$), although HAZ was significant ($r = -0.135$, $p =$

0.037). Independent t-tests of IgA in underweight vs. non-underweight and wasted vs.

non-wasted infants were not significant (all $p > 0.05$). However, salivary IgA was

significantly higher in stunted infants than in non-stunted infants ($t(64.4) = -3.36$, $p =$

0.0013). Figure 5.1 shows the difference in salivary IgA in stunted versus non-stunted infants.

Figure 5.1. Mean salivary IgA levels in stunted vs. non-stunted Ariaal infants.
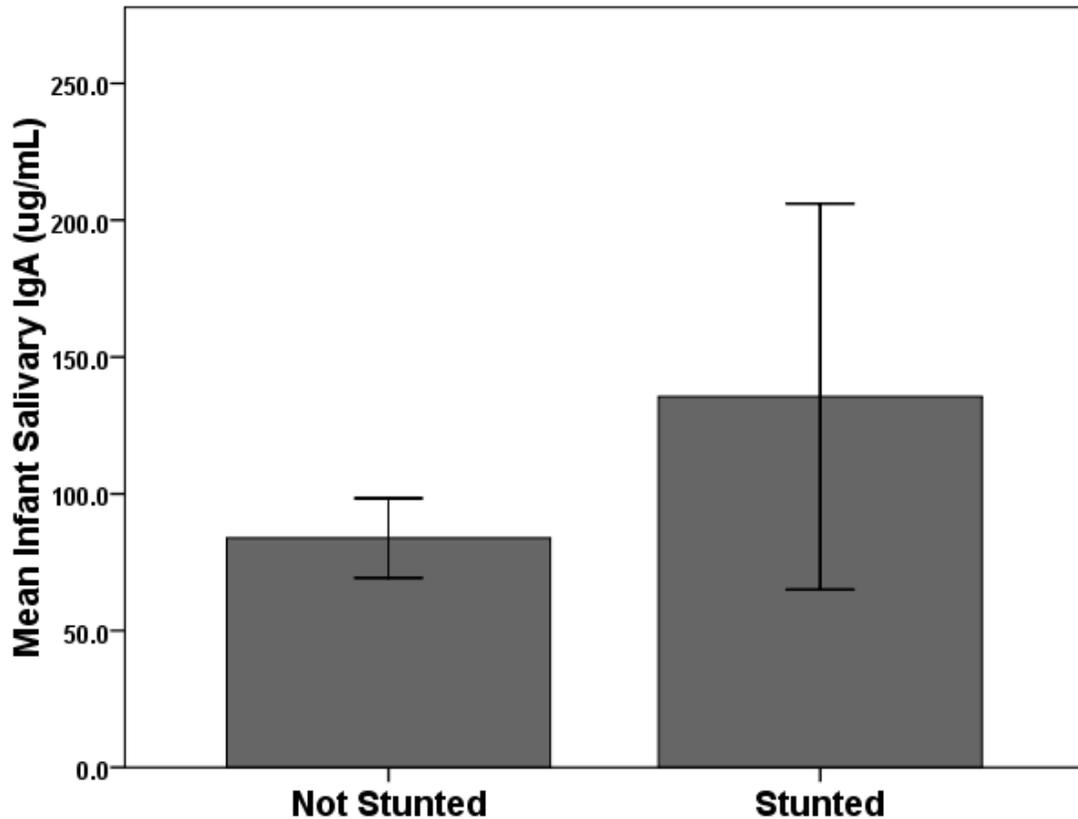
Multivariate analysis of infant IgA as the dependent variable with nutritional indices as the main independent variables (adjusted for infant age, infant sex, village, TLU, and breastmilk IgA) tended to show the same patterns as above. Infant age and TLU were not reported as they remained non-significant in all models. Models were repeated with the ratio of breastmilk IgA to fat in place of breastmilk IgA, but results were not significant, did not significantly alter the estimates of other variables, and therefore are not reported here. Living in Parkishon and Kituruni remained significantly

associated with infant IgA in all models as reported in the previous section. Models with HAZ, WAZ, WHZ, UAFA, and TSFZ as independent variables, adjusted for breastmilk IgA and infant sex, can be found in Table 5.3. Of the dependent nutritional variables, only HAZ was significantly associated with infant IgA. Infant IgA was also significantly negatively associated with being male and positively associated with breastmilk IgA levels in most models. One interesting finding is that the inclusion of HAZ in statistical models lowers the estimate of the effect of infant sex by 16%, making HAZ a considerable confounding effect on infant sex. Some of the effect of sex on IgA can therefore be attributed to long-term nutritional status, a finding that will be discussed below.

To summarize this hypothesis, it appears that several factors predict salivary IgA levels in infants. HAZ significantly predicts IgA development; however infant sex may play a mediating role between the two. Breastmilk IgA level also appears to be positively associated with infant IgA level. However, other indicators of infant nutritional status do not predict infant IgA level.

Table 5.3. Multivariate linear regression of nutritional status, breastmilk IgA, and infant sex against infant IgA, adjusted for infant age, village, and total livestock units.

	Salivary IgA β (p)
**HAZ **	-0.087 (0.03)
Log Breastmilk IgA **	0.30 (0.04)
Infant Sex **	0.20 (0.05)
R^2	0.091
WAZ	-0.0013 (0.98)
Log Breastmilk IgA *	0.30 (0.04)
Infant Sex **	0.24 (0.02)
R^2	0.073
WHZ	0.068 (0.1)
Log Breastmilk IgA *	0.30 (0.04)
Infant Sex **	0.25 (0.02)
R^2	0.083
UAFA	-0.020 (0.5)
Log Breastmilk IgA *	0.29 (0.06)
Infant Sex **	0.25 (0.02)
R^2	0.075
TSFZ	-0.0030 (0.96)
Log Breastmilk IgA **	0.30 (0.04)
Infant Sex **	0.24 (0.02)
R^2	0.073

* Marginally significant $0.05 < p < 0.1$ ** Significant $p < 0.05$

Hypothesis 2. This hypothesis explored the link between the IgA in breastmilk with infant health and nutritional outcomes.

Bivariate Pearson correlations indicate that breastmilk IgA is not significantly associated with HAZ, WAZ, WHZ, UAFA, or TSFZ. Independent t-tests of children who were stunted, underweight, wasted, experienced diarrhea, respiratory infection, or fever compared to children who were not had mothers with insignificant differences in breastmilk IgA ($p > 0.05$).

Multivariate linear regression models with either HAZ, WAZ, WHZ, UAFA or TSFZ as the dependent variables, breastmilk IgA as the main independent variable, and

infant IgA, infant age, infant sex, village, and livestock units as covariates. Results are presented Table 5.4. Breastmilk IgA was only significant when regressed against infant UAFA ($p = 0.037$) but not TSFZ, indicating an association between breastmilk IgA and absolute infant fat deposition in comparison to reference norms. A graph of the relationship can be found in Figure 5.2. Salivary IgA was significantly associated with HAZ but no other nutritional indices. Infant sex was significantly associated with both HAZ and WAZ, with female infants having better nutritional status than male infants. UAFA was significantly higher in Parkishon and Kituruni compared to Karare. Total livestock units were not significantly associated with nutritional markers. As discussed above, the inclusion of infant age is a highly significant predictor of nutritional status, possibly obscuring the effects of other factors on nutrition.

The regression models above were repeated but with breastmilk IgA/creamatocrit % (fat-adjusted breastmilk IgA) as the main independent variable. This was done to separate the effects of the IgA from the nutritive content of the breastmilk. The results can be found in Table 5.5. In these models, breastmilk IgA/fat is not significantly associated with UAFA, but it is significantly positively associated with HAZ and WAZ. That is, better nourished infants have mothers who feed them higher levels of IgA per unit of fat. This effect is separate from the effect of infant age.

Table 5.4. Estimates and significance levels for linear regression of breastmilk IgA against dependent infant nutritional status indicators, adjusting for infant IgA, age, sex, village, and total livestock units.

Independent Variables	HAZ $\beta(p)$	WAZ $\beta(p)$	WHZ $\beta(p)$	UAFA $\beta(p)$	TSZ $\beta(p)$
R^2	0.13	0.17	0.14	0.079	0.043
Log Breastmilk IgA	0.024 (0.92)	-0.053 (0.81)	-0.011 (0.96)	-0.73 (0.034)	-0.11 (0.54)
Log Infant IgA	-0.24 (0.033)	-0.0011 (0.99)	0.16 (0.11)	-0.11 (0.49)	-0.0036 (0.97)
Infant Age	-0.072 (<0.0001)	-0.083 (<0.0001)	-0.079 (<0.0001)	0.069 (0.0006)	0.0037 (0.73)
Infant Sex	-0.39 (0.024)	-0.33 (0.032)	-0.19 (0.25)	0.21 (0.37)	0.060 (0.64)
Living in Parkishon	-0.38 (0.043)	-0.18 (0.33)	0.090 (0.65)	0.59 (0.046)	0.45 (0.0050)
Living in Kituruni	-0.20 (0.38)	0.18 (0.35)	0.47 (0.025)	0.96 (0.0022)	0.59 (0.0005)
Livestock Units	-0.033 (0.79)	0.040 (0.72)	0.057 (0.62)	-0.0015 (0.99)	0.014 (0.88)

Table 5.5. Estimates and significance levels for linear regression of breastmilk IgA/Fat against dependent infant nutritional status indicators, adjusting for infant IgA, age, sex, village, and total livestock units.

Independent Variables	HAZ $\beta(p)$	WAZ $\beta(p)$	WHZ $\beta(p)$	UAFA $\beta(p)$	TSZ $\beta(p)$
R^2	0.15	0.18	0.14	0.061	0.042
Log (Breastmilk IgA/Fat)	0.28 (0.024)	0.23 (0.040)	0.12 (0.29)	-0.011 (0.95)	-0.040 (0.66)
Log Infant IgA	-0.24 (0.027)	-0.0094 (0.92)	0.16 (0.12)	-0.15 (0.33)	-0.0096 (0.91)
Infant Age	-0.068 (<0.0001)	-0.080 (<0.0001)	-0.078 (<0.0001)	0.063 (0.0017)	0.0023 (0.83)
Infant Sex	-0.38 (0.025)	-0.32 (0.034)	-0.18 (0.25)	0.20 (0.41)	0.056 (0.66)
Living in Parkishon	-0.36 (0.093)	-0.13 (0.50)	0.12 (0.54)	0.54 (0.074)	0.43 (0.0076)
Living in Kituruni	-0.11 (0.61)	0.24 (0.22)	0.50 (0.017)	0.88 (0.0055)	0.57 (0.0008)
Livestock Units	-0.042 (0.73)	0.028 (0.80)	0.052 (0.65)	-0.047 (0.78)	0.0086 (0.93)

Figure 5.2. Relationship between breastmilk IgA concentration and infant upper arm fat area.
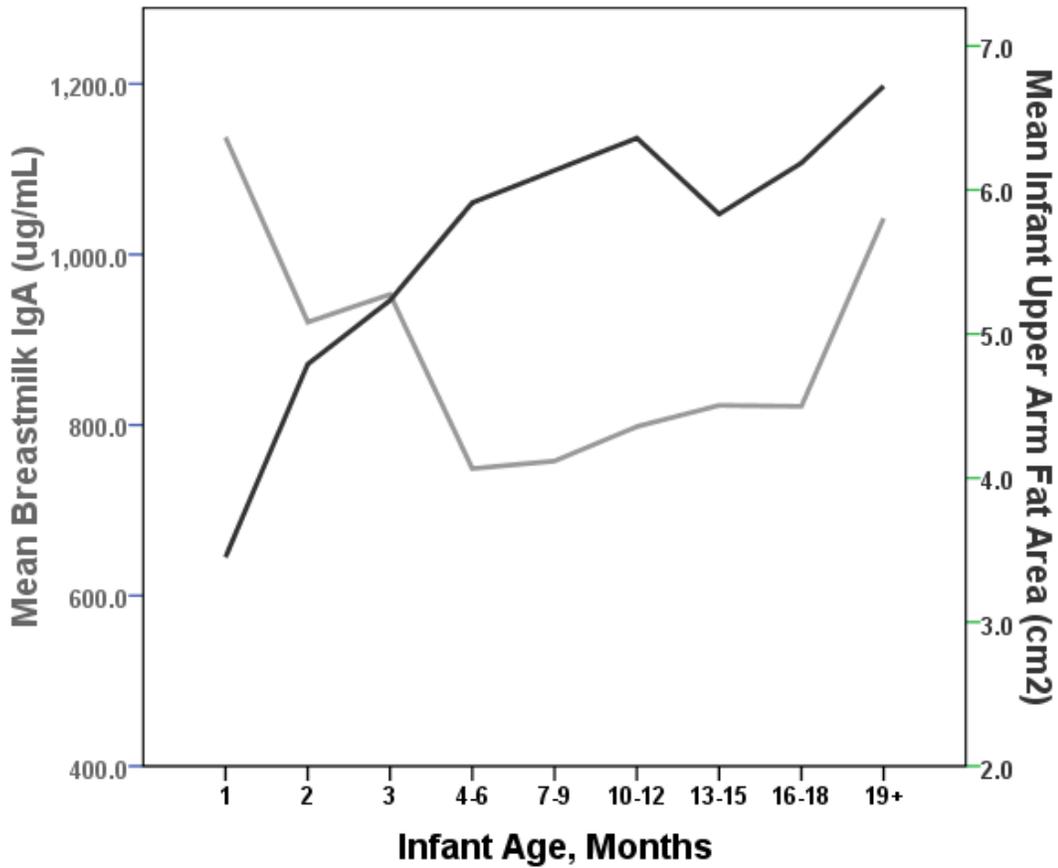
Maternal reports of infant health were analyzed by multivariate logistic regression; results are tabulated in Table 5.6. Breastmilk IgA did not significantly change the likelihood of the infant experiencing diarrhea, respiratory infection, fever, or all illnesses within the past month. Older infants were marginally more likely to experience diarrhea, while infants living in Parkishon were 1.77 times more likely to have experienced a respiratory infection, although this relationship was only marginally significant. No other relationship approached significance. These models were re-run

with fat-adjusted IgA as the main independent variable. No significant results were found; they are not reported here.

Table 5.6. Odds ratios and significance for logistic regression of breastmilk IgA against dependent infant illness indicators, adjusting for infant IgA, age, sex, village, and total livestock units.

	Diarrhea	Respiratory Illness	Fever	All Illness
	OR (p)	OR (p)	OR (p)	OR (p)
R^2	0.027	0.046	0.055	0.018
Log Breastmilk IgA	1.12 (0.86)	1.26 (0.56)	1.15 (0.83)	1.11 (0.79)
Log Infant IgA	1.04 (0.89)	0.84 (0.34)	1.20 (0.54)	0.93 (0.68)
Infant Age	1.06 (0.10)	0.97 (0.16)	1.01 (0.72)	0.99 (0.53)
Infant Sex	0.89 (0.79)	1.08 (0.78)	0.60 (0.28)	0.95 (0.84)
Living in Parkishon	0.94 (0.91)	1.77 (0.085)	0.46 (0.27)	1.57 (0.18)
Living in Kituruni	0.94 (0.92)	0.85 (0.66)	1.91 (0.22)	1.03 (0.94)
Livestock Units	0.98 (0.95)	1.08 (0.70)	1.17 (0.65)	1.04 (0.83)

In summary, there is no association between breastmilk IgA levels and health and nutritional variables as predicted by hypothesis two.

Hypothesis 3. This research question addressed the effects of hygiene and supplemental food variables on nutritional and health status and investigated the effects of breastmilk IgA on mitigating the possible effects of these variables. First, bivariate *t, r* or χ^2 associations between independent hygiene and supplemental food variables versus dependent nutritional and health measurements were investigated. Next, multivariate linear or logistic regression models investigated the multiple effects of the hygiene and food variables on each dependent nutritional and health variable, adjusted for infant age, sex, breastmilk IgA, and village. Finally, the degree to which the inclusion of breast milk IgA changes the estimates of the independent variables on the dependent variables, known as confounding, was checked to see if the effects of breastmilk IgA reduced the impact of the food and hygiene variables on HAZ, WHZ, and WAZ.

1.) Bathing: Frequency of infant bathing was not associated with nutritional or health measurements ($p > 0.05$).

2.) Toilet use: Infants whose families used a latrine were had significantly higher HAZ ($t(121) = -2.61$, $p = 0.010$) and WAZ ($t(113) = -2.88$, $p = 0.0047$) than infants whose families did not. WHZ, TSFZ, and infant UAFA were not significantly associated with latrine use ($p > 0.05$). Toilet use was not significantly associated with reported illness ($p > 0.05$).

3.) Water boiling: Women who reported that they always boiled water for their infants had infants with significantly higher HAZ ($t(219) = -2.37$, $p = 0.018$) and WAZ ($t(226) = -2.58$, $p = 0.011$). Water boiling was not associated with WHZ, UAFA, TSFZ, or illness ($p > 0.05$).

4.) Mosquito net use: Women who always used a mosquito net on their infant at night had infants what had significantly higher WHZ than those that did not ($t(145) = -2.56$, $p = 0.011$). Infants whose mothers always used a mosquito net had lower than expected frequency of reported illness ($\chi^2(1) = 5.34$, $p = 0.021$). Mosquito net use had no effect on HAZ, WAZ, TSFZ, or UAFA ($p > 0.05$).

5.) Crawling Behavior: Crawling is associated with significantly lower HAZ ($t(196) = 3.03$, $p = 0.0028$), WAZ ($t(173) = 5.45$, $p < 0.0001$) and WHZ ($t(170) = 6.01$, $p < 0.0001$) Crawling is not significantly associated with reported illness, UAFA, or TSFZ ($p > 0.05$).

6.) Traditional House: Living in a traditional house versus a cement house was not significantly associated with HAZ, WHZ, or TSFZ, UAFA, or reported illness ($p > 0.05$).

Infants who live in traditional houses had significantly higher WAZ $t(23.2) = 2.14$, $p = 0.043$)

7.) Vaccine-for-age: There was no significant effect of vaccination status-for-age on HAZ, WAZ, and WHZ, UAFA, TSFZ or reported illness ($p > 0.05$).

8.) Milk: Drinking cow milk is not significantly associated with reported illness, TSFZ or HAZ ($p > 0.05$). Drinking milk is associated with significantly higher UAFA ($t(43.3) = -2.15$, $p = 0.037$) but significantly lower WAZ ($t(41.5) = 2.57$, $p = 0.014$) and WHZ ($t(43.7) = 2.45$, $p = 0.018$).

9.) Milk Preparation: Among infants who drink cow milk, there is no association between drinking boiled or fresh milk and nutritional status z-scores. However, infants who drank fresh milk had higher than predicted frequencies of reported illness ($\chi^2(1) = 5.28$, $p = 0.022$).

10.) Solid Starch: Infants who consumed solid starch staples had significantly lower HAZ ($t(168) = 2.60$, $p = 0.010$), WAZ ($t(183) = 4.28$, $p < 0.0001$), and WHZ ($t(183) = 4.32$, $p < 0.0001$). There was no significant effect of consuming solid food on UAFA, TSFZ, or reported illness.

Five multivariate linear regression models were run with HAZ, WAZ, WHZ, TSFZ or UAFA as the dependent variable. The independent variables were latrine use, frequency of infant bathing, water boiling for the infant, mosquito net use, crawling, living in a traditional house, drinking milk, and eating starch weaning food, with breastmilk IgA, infant age, infant sex, and village as covariates. Results can be found in Table 5.7. A multivariate logistic model was run with the same independent variables and covariates as above, but with reported illness as the dependent variable. No independent

variables were significant in the logistic model and are not reported. Infant age is significantly negatively associated with most nutritional dependent variables ($p < 0.05$) except triceps WHZ and TSFZ. The effect of infant age largely removed the significant relationships between hygiene variables and nutritional status. Crawling remained significantly associated with reduced WHZ ($p = 0.015$). Use of a mosquito net is significantly associated with higher WHZ ($p = 0.044$). The effect of sex on HAZ remains significant ($p = 0.0049$), with male infants demonstrating lower HAZ scores than female infants. In this model WAZ was also significant, with male infants having lower WAZ as well ($p = 0.034$). Infant fat stores, measured by TSFZ and UAFA, are significantly higher in the communities of Parkishon and Kituruni compared to Karare ($p < 0.05$). Finally, breastmilk IgA levels remain significantly negatively associated with infant UAFA ($p = 0.022$) but not TSFZ.

Table 5.7. Multivariate analysis of hygiene variables regressed against five dependent nutritional status variables, adjusted for breastmilk IgA, infant age, infant sex, and village.

	HAZ $\beta\,(p)$	WAZ $\beta\,(p)$	WHZ $\beta\,(p)$	TSZ $\beta\,(p)$	UAFA $\beta\,(p)$
R^2	0.14	0.18	0.16	0.032	0.073
Log Breastmilk IgA	-0.038 (0.88)	-0.082 (0.70)	-0.020 (0.93)	-0.12 (0.50)	-0.80 (0.020)
Infant Age	-0.094 (0.0002)	-0.063 (0.0042)	-0.033 (0.15)	0.018 (0.35)	0.10 (0.0036)
Infant Sex	-0.45 (0.0087)	-0.30 (0.045)	0.11 (0.48)	0.062 (0.63)	0.18 (0.44)
Living in Parkishon	-0.38 (0.073)	-0.094 (0.61)	0.17 (0.38)	0.45 (0.0049)	0.54 (0.073)
Living in Kituruni	-0.27 (0.27)	0.070 (0.73)	0.39 (0.070)	0.63 (0.0004)	1.11 (0.0007)
Latrine Use	-0.37 (0.086)	0.26 (0.18)	-0.036 (0.86)	-0.072 (0.66)	-0.20 (0.51)
Baths/week	-0.035 (0.12)	-0.027 (0.16)	-0.0091 (0.66)	-0.0013 (0.45)	-0.0096 (0.76)
Boil Water for Infant	0.25 (0.18)	0.098 (0.55)	-0.080 (0.65)	-0.090 (0.52)	-0.063 (0.81)
Use Mosquito Net	-0.34 (0.084)	0.019 (0.91)	0.38 (0.041)	-0.070 (0.64)	-0.18 (0.53)
Crawling	0.21 (0.44)	-0.30 (0.22)	-0.62 (0.016)	-0.20 (0.34)	-0.53 (0.17)
Traditional House	-0.31 (0.36)	-0.40 (0.18)	-0.33 (0.30)	-0.22 (0.40)	-0.16 (0.74)
Drinking Milk	0.15 (0.57)	-0.11 (0.63)	-0.18 (0.46)	0.031 (0.88)	0.42 (0.25)
Eating Solid Starch	0.13 (0.55)	-0.059 (0.75)	-0.23 (0.25)	-0.15 (0.35)	-0.28 (0.35)
Vaccine for Age	0.17 (0.37)	0.19 (0.25)	0.11 (0.54)	0.0049 (0.97)	-0.033 (0.90)

Table 5.8. Multivariate analysis of hygiene variables regressed against five dependent nutritional status variables, adjusted for breastmilk IgA/Fat, infant age, infant sex, and village.

	HAZ $\beta\,(p)$	WAZ $\beta\,(p)$	WHZ $\beta\,(p)$	TSZ $\beta\,(p)$	UAFA $\beta\,(p)$
R^2	0.15	0.19	0.17	0.031	0.050
Log (Breastmilk IgA/Fat)	0.27 (0.033)	0.20 (0.065)	0.10 (0.37)	-0.049 (0.60)	-0.017 (0.93)
Infant Age	-0.090 (0.0003)	-0.061 (0.0049)	-0.032 (0.17)	0.015 (0.42)	0.091 (0.011)
Infant Sex	-0.45 (0.0086)	-0.30 (0.044)	-0.11 (0.48)	0.056 (0.66)	0.16 (0.52)
Living in Parkishon	-0.33 (0.12)	-0.057 (0.76)	0.19 (0.33)	0.44 (0.0072)	0.48 (0.12)
Living in Kituruni	-0.17 (0.46)	0.13 (0.52)	0.42 (0.052)	0.60 (0.0007)	1.04 (0.0020)
Latrine Use	0.36 (0.10)	0.24 (0.20)	-0.043 (0.83)	-0.075 (0.64)	-0.24 (0.44)
Baths/week	-0.035 (0.12)	-0.027 (0.16)	-0.0091 (0.66)	-0.013 (0.43)	-0.012 (0.70)
Boil Water for Infant	0.28 (0.13)	0.12 (0.47)	-0.069 (0.70)	-0.096 (0.50)	-0.065 (0.81)
Use Mosquito Net	-0.35 (0.076)	0.014 (0.94)	0.37 (0.042)	-0.071 (0.63)	-0.19 (0.51)
Crawling	0.25 (0.37)	-0.26 (0.27)	-0.60 (0.018)	-0.19 (0.37)	-0.43 (0.28)
Traditional House	-0.24 (0.34)	-0.35 (0.25)	-0.30 (0.35)	-0.23 (0.38)	-0.13 (0.80)
Drinking Milk	0.14 (0.59)	-0.12 (0.59)	-0.18 (0.45)	0.026 (0.89)	0.38 (0.30)
Eating Solid Starch	0.10 (0.63)	-0.081 (0.66)	-0.24 (0.23)	-0.15 (0.35)	-0.31 (0.30)
Vaccine for Age	0.15 (0.43)	0.18 (0.29)	0.099 (0.57)	0.011 (0.94)	-0.019 (0.94)

The five multivariate models were repeated with fat-adjusted breastmilk IgA as an independent variable rather than breastmilk IgA. The results are listed in Table 5.8. Fat-adjusted breastmilk IgA is significantly positively associated with HAZ ($p = 0.043$) and marginally positively associated with WAZ ($p = 0.069$), but is not longer significant with UAFA. Infant age remains significantly negatively associated with HAZ, WAZ, and UAFA ($p < 0.05$). Significance levels for hygiene and food variables remain similar to the models with breastmilk IgA only.

Statistical confounding is the ability of one independent variable or covariate to influence the effect of another independent variable on the dependent variable. The covariate does not necessarily need to have a direct significant effect on the dependent variable; rather, it can exert its effect through its influence on another independent variable. Generally, confounding can be difficult to detect in statistical models. There are no direct statistical tests for confounding, but a rule of thumb that can be used is if the addition of a covariate changes the estimate of an independent variable by 10% or more, it is said to be a confounding variable (Maldonado and Greenland 1993). While the data have shown that breastmilk IgA is not significantly associated with nutritional z-scores, it may exert influence on these dependent variables through confounding the effects of hygiene and weaning variables. Table 5.9 shows the percent change in estimate for each independent variable for the five dependent variables. There appears to be some confounding effect of breastmilk IgA on infant upper arm fat through starch consumption, family latrine use, and living in a traditional house. Breastmilk IgA enhances the positive effect of starch consumption and using a latrine on infant fat. However, it makes the positive effect of living in a non-traditional become a negative

effect; on the other hand, the size of this effect is extremely small. Otherwise, there is no

confounding effect of breastmilk IgA on the independent variables in this model.

Table 5.9. Percent confounding effects of breastmilk IgA on supplemental foods and hygiene behaviors for five dependent nutritional variables.

Independent Variable	Percent Change in Effect Size				
	HAZ	WAZ	WHZ	UAFA	TSFZ
Consuming Milk	5.6	2.7	0.8	6.1	1.3
Consuming Starch	3.8	1.9	0.2	19.4	0.4
Latrine Use	2.9	2.6	2.1	16.2	2.0
Boiling Water	1.4	1.3	0.7	3.3	0.3
Mosquito Net Use	6.7	2.1	0.5	4.7	0.1
Crawling	1.3	0.6	0.2	3.0	0.4
Baths Per Week	3.2	3.3	3.1	2.5	0
Traditional House	6.7	3.7	1.8	847.4	0.7
Vaccine-for-Age	3.7	2.4	0.6	2.3	0.3

Statistical confounding of fat-adjusted breastmilk IgA on food and hygiene

variables were completed as above, and results can be found in Table 5.10. There is some

confounding of fat-adjusted IgA on mosquito net use, crawling, and living in a traditional

house in models with HAZ as the dependent variable. Fat-adjusted IgA appeared to alter

the effect of latrine use, mosquito net use, crawling, and living in a traditional house. On

the whole, fat-adjusted breastmilk IgA appears to exert a greater effect on measures of

chronic nutritional status and fat-for-age measures rather than immediate measures of

nutritional status such as WAZ, WHZ, or UAFA.

Table 5.10. Percent confounding effects of breastmilk IgA/fat on supplemental foods and hygiene behaviors for five dependent nutritional variables.

	Percent Change in Effect Size				
Independent Variable	**HAZ**	**WAZ**	**WHZ**	**UAFA**	**TSFZ**
Consuming Milk	8.1	3.9	2.3	1.9	7.8
Consuming Starch	5.0	2.7	1.4	6.1	9.7
Latrine Use	5.3	4.0	4.4	10.5	55.8
Boiling Water	3.8	3.2	2.5	2.5	7.2
Mosquito Net Use	30.4	8.1	2.2	6.5	11.5
Crawling	10.5	4.4	1.5	5.9	63.8
Baths Per Week	9.3	8.0	18.8	2.3	2.5
Traditional House	31.7	14.6	8.3	1354.8	32.8
Vaccine-for-Age	4.6	2.7	1.0	0.8	5.4

In conclusion, there was no consistent effect of disease-exposing behaviors on infant growth and health, and breastmilk IgA level did not modify the effect of these behaviors on growth indicators as predicted by hypothesis three.

Discussion

The results of this study indicate that infant IgA development may have complex costs that are associated with poor growth outcomes, which may be modulated by breastmilk IgA and hygienic behaviors. Particularly, the intersection between infant fat and breastmilk IgA warrants further explanation.

The test of hypothesis 1 shows that stunted infants have much higher concentration of IgA in their saliva, indicating greater mucosal immune activation in infants that are chronically malnourished. While male infants have significantly lower levels of IgA than female infants in most models, closer investigation revealed that the sex difference in IgA is largely due to the sex difference in stunting, where a greater proportion of male infants are stunted compared to female infants. More immediate

measures of undernutrition and adiposity do not significantly affect infant IgA levels. The causal relationship between chronic undernutrition and IgA levels is unclear: do high levels of immune activation (and possibly, underlying infection) contribute to poor long-term growth, or do stunted infants increase their immune function to compensate for their poor nutrition to protect against possible infection? This question bears investigation into the complex relationships between energetics, nutritional status, and immune function.

Hypothesis 2 showed that most growth outcomes are unaffected by breastmilk IgA concentration. The lack of relationship between IgA and nutritional outcome may be obscured by the fact that all of the breastfeeding infants were exposed to at least some level of IgA in breastmilk. A more conclusive examination of the differing growth outcomes of breastfed and formula-fed infants may shed more light on the impact of IgA on growth. The relationship between infant fat deposition and IgA will be discussed in more depth below.

On the other hand, there was a positive association between fat-adjusted breastmilk IgA and height-for age and weight-for-age z-scores. When adjusted for the amount of fat in breastmilk, a known correlate of breast fullness (Daly et al. 1993), it appears that there is some effect of breastmilk immunity on growth. Fat-adjusted breastmilk IgA does not have a significant relationship with infant fat deposition, in contrast to total breastmilk concentration.

Bivariate results from hypothesis 3 indicate that several hygiene and supplemental food variables are significantly associated with negative non-adiposity growth outcomes, including not boiling water, no family toilet, not using a mosquito net, crawling, vaccine status for age, and consuming supplemental starch foods. Drinking cow milk increased

upper arm fat area but was associated with negative non-adiposity related growth outcomes. Multivariate analyses that include infant age as a covariate negate most significant effects of these variables. Since hygiene and age variables predict infant age to a high degree, it is possible that the negative effects of disease-introducing behaviors remain significant but are subsumed in the global variable of "infant age" and all of the developmental milestones that appear with age. Unfortunately, these effects are impossible to separate in the cross-sectional design of this study. In addition, breastmilk appeared not to confound the effect of hygiene and supplemental food variables on nutritional status in most cases, indicating that the concentration of IgA in breastmilk may not have an influence on the negative effects of environmental exposure to disease. Fat-adjusted breastmilk IgA exerts slightly more confounding effects but the results are not consistent within and across models. It is possible that any amount of IgA is protective against disease exposure or that the main protective effects of breastmilk IgA occur at a different point in infant development.

Although overall breastmilk IgA concentration was not associated with height or weight indicators, fat-adjusted IgA was significantly associated with height-for-age and weight-for-age, independent of the effect of infant age. Mothers with higher levels of IgA in their breastmilk, adjusting for fat, had infants with greater height-for-age and weight-for-age. This may represent a true benefit of breastmilk IgA on infant growth. Higher concentrations of IgA relative to breastmilk composition may decrease the infant's need for immune defenses, freeing more energy for growth. It is unclear if this relationship is directly associated with infection-induced growth disruptions.

Overall, infant fat levels and breastmilk IgA levels generally appeared to operate separately from disease-exposing behaviors, calling into question the function of infant fat and breastmilk IgA in buffering the weaning process. The beneficial effects may be most prominent during the pre-weaning phase, where IgA and infant adiposity appear to be inversely related to each other. Although much of infant weight and adiposity gain can be explained by appetite (Drewett and Amatayakul 1999), the regulation of gut bacterial communities by IgA in breastmilk may contribute to energy regulation, growth, and fat deposits. Recent research has shown that gut bacteria contribute to energy intake, metabolism, and storage (Cani and Delzenne 2009; DiBaise et al. 2008; Kleerebezem and Vaughan 2009; Neish 2009). Gut bacteria populations develop in infancy and are influenced by a wide range of factors, including natural versus c-section delivery, breast versus bottle feeding, weaning diet, and hygiene conditions (Fanaro et al. 2003; Kohler et al. 2002; Orrhage and Nord 1999). *Bifidobacterium* and *Lactobacillus* species tend to dominate the guts of breastfed infants, particularly during the first three months before the introduction of weaning foods . The IgA in breastmilk promotes the stability of these bacterial colonies, even in developing countries with poor hygiene conditions and high bacterial exposure (Fanaro et al. 2003). However, by 1-2 years of age formula and breastfed infants have more similar gut bacteria profiles due to the introduction of weaning foods. In addition to its protective effect against disease, immunoglobulin A may be involved in aiding adiposity development in young infants, at least indirectly through the promotion of certain gut bacteria. Individuals with lower numbers of *Bifidobacterium* and *Lactobacillus* species tend to have higher levels of obesity, indicating that these bacteria actually lower rates of adipose tissue accumulation.

Unfortunately, many of the mechanisms underlying IgA, gut bacteria proliferation, and energetic modulation are unclear, especially in undernourished individuals, making the association, if any, between IgA and infant fat deposition unknown.

In contrast to the normal pattern of fat accumulation in early infancy, the infants in this study do not appear to show a decline in triceps skinfold past the 6 month period as described by Kuzawa (1998), but instead remain fairly constant throughout the weaning period. Infants in this population may preserve their fat stores over other growth indicators such as height and weight, which decline relative to age. This fat retention may be aided by the energy infants derive from early supplementation with cow milk, a source of protein that has been associated with better growth outcomes in children in this population (Fratkin et al. 2004). In fact, infants supplemented with formula or cow's milk tend to be fatter than only breast- or bottle-fed infants in Western societies (Dewey 2009). The breastfeeding environment that supports high levels of *Lactobacillus* species also aids in the extraction of energy from the lactose in cow milk. The combination of human and cow milk consumption in Ariaal infants may promote fat retention during the weaning period.

This study has several limitations. First, the variable of infant illness in this study is extremely limiting for investigating the impact of infection on growth and immune function. Reporting an illness within the past month can lead to faulty or biased recollection from the mother, little indication of the severity of the illness, and what impact, if any, this illness has on current outcomes. In addition, there is no age-comparable non-breastfeeding group within this society, since nearly every infant is breastfed for almost two years (see Chapter 2). An investigation of nutritional outcomes,

gut flora, and IgA in a population that uses both breast- and formula-feeding would help expand on the differences in adiposity development between the two. Finally, the cross-sectional design heightens the importance of age as an explanatory variable in developing Ariaal infants. Longitudinal investigation of pathogen exposure, IgA and breastfeeding in infants would reduce the variation due to age-related development in statistical models and would more clearly show the effect of disease-exposing behaviors.

Conclusion

The immunological benefits of breastmilk have been hypothesized to play a large role in infant growth and the development of the infant immune system. Breastmilk IgA, along with the development of large amounts of infant fat, is predicted to buffer the infant from the nutritional and pathogen stress introduced to the infant during the weaning process. In this study, infant immune development does appear to be influenced by chronic nutritional stress. When breastmilk IgA is adjusted for the percent fat in breastmilk, it appears to have some beneficial effect on infant growth. However, breastmilk IgA concentration is not directly associated with improved growth indicators and does not significantly buffer against the negative effects of disease-introducing behaviors. This infant population may have alternative mechanisms for survival during the weaning period, such as maintaining fat stores but allowing slower growth rates for length and weight. Instead, the protective effects of breastmilk IgA may occur earlier in infancy, during the development of fat stores. Future research in the mechanisms of gut bacterial development, immunity, and growth may clarify the mechanisms involved in early infant immune development.

Chapter VI

Summary and Conclusions

The main objective of this dissertation was to explore the immune components of the breastfeeding system from an evolutionary perspective within the disease, nutritional, and cultural ecology of the Ariaal people of Kenya. Chapter II introduces the culture and ecology of the Ariaal and tests how maternal knowledge of local infant health care influences the nutritional, health, and immune status of Ariaal infants. Chapter III describes the methodology of dissertation and tests a method for storing breastmilk and saliva samples in fieldwork conditions for later IgA analysis. Chapter IV tests the hypothesis that IgA in breastmilk is an investment in offspring that is subject to evolutionary trade-offs. Chapter V tests the effect of breastmilk IgA on infant growth and development and studies the effect of the nutritional and disease ecology on infant immune function. The current chapter will summarize and synthesize the findings of this dissertation into a larger context of immune ecologies.

Summary of Research Findings

Chapter II began by reviewing the literature on the unique culture and ecology of the Ariaal, demonstrating how some cultural beliefs and practices help improve survival in the harsh deserts of northern Kenya. Next, it described the results of structured and semi-structured ethnographic data on breastfeeding practices, infant care, and local and

Western medicine and how this information was translated into a quantitative

questionnaire using the cultural consensus method. Finally, a cultural consensus

questionnaire based on how local medicine is used to treat infants was administered to the

full sample of 251 women, and their resultant knowledge of local medicine regressed

against infant health, growth, and salivary IgA. Mothers' knowledge of local medicine

was significantly associated with infant health status, with more knowledgeable mothers

less likely to report that their infant has been ill within the past month. Growth status and

infant IgA level was not significant. While there is no direct causal information available

to explain this association, it may be possible that medicinal plants have true

pharmacological properties and that more knowledgeable mothers can use them in a way

that benefits their infant's health.

The results of Chapter III, while not directly addressing immune function among

the Ariaal, showcased the development of an IgA ELISA and tested the possibility of

using Whatman 903 filter paper to dry and store breastmilk and saliva spots under field

conditions for later IgA analysis. The ELISA for immunoglobulin A performs

adequately, with acceptable inter- and intraassay coefficients of variation and high

linearity of dilution. The minimum detection limit is well below the necessary amount

needed to detect IgA in even low-level infant saliva samples; 100% of the samples in this

study could be assayed within range. After mathematical adjustment, the unknown

sample results are very similar to a commercial kit for secretory IgA. Quality control of

many runs suggests a high degree of consistent performance, making this assay a cost-

effective and accurate alternative to commercial assay kits. In addition to assay

development, a natural, field-based study of the capacity of filter paper to store

breastmilk and saliva was undertaken. It showed that saliva and breastmilk stored on

filter paper were reasonably concordant with their whole sample counterparts ($R^2 = 0.62$)

and an adequate amount of sample can be recovered from filter paper in order to

undertake the analysis (between 55.4-68.2%). There was a significant negative effect of

time stored at ambient temperature; that amount equaled a loss of approximately 1 µg/mL

IgA per day. Despite this loss, there remained more than enough IgA to analyze in this

ELISA, and because time stored at ambient temperature did not affect the variation

present in the model, the loss of IgA at ambient temperature can be controlled statistically

for up to 8 weeks.

Chapter IV discussed the sources of variation in IgA levels in breastmilk from an

evolutionary perspective using data from 245 Ariaal women. A review of the literature

suggests that IgA production is energetically expensive for mothers, indicating that it is a

costly maternal resource that may be subject to the trade-offs analysis characteristic of

life history theory. This idea produces three testable hypotheses. Hypothesis 1 predicts

that IgA levels in breastmilk will decline as infant age increases, indicating mothers' slow

switch from investment in their current infant to investment in future offspring.

Hypothesis 2 predicts that IgA levels will be lower as Ariaal women's parity increases,

since multiparous women have a diminishing store of resources to devote to their

offspring. Hypothesis 3 predicts that women with more resources will have higher IgA

levels than do women with fewer resources; in this case resources include

somatic/nutritional, health and socioeconomic indicators. Results indicated that the IgA

in the breastmilk of Ariaal women does decline over the first 3 months of life, stabilizes,

and then rises, somewhat conforming to the prediction in hypothesis 1. The rise post 18-

months is reduced somewhat by adjusting for breastmilk fat percent. Hypothesis 2 was also somewhat confirmed; IgA in breastmilk did indeed decrease after about 4 live births, declining precipitously in women who had given birth to 8 or more offspring. However, breastmilk IgA levels increased up to four months, creating a polynomial relationship between parity and breastmilk IgA. There were mixed results for hypothesis 3; results indicated that decreased number of livestock but increased household food budget were associated with lower breastmilk IgA. Polygynous marriage was also associated with lower breastmilk IgA. In some models, higher breastmilk IgA was associated with higher maternal BMI. In addition, the IgA levels in Ariaal breastmilk appears to be slightly lower than the breastmilk of Western women, indicating there may be some support for the idea that IgA is depressed in low-resource environments. This study provided some lines of evidence that breastmilk IgA is a costly resource that conforms to some of the predictions of evolutionary theory; however, a direct association between overall maternal resources and breastmilk IgA concentration could not be found. Changes in energy balance that are not reflected in women's nutritional status may be responsible for the regulation of maternal reproductive cost-benefit trade-offs.

Chapter V investigated the relationship between infant IgA, breastmilk IgA, infant disease exposure risk, and infant growth and health outcomes. The first hypothesis acknowledged that the energetic requirements of immune function and growth may interact in infants, and hypothesized that higher levels of IgA may be associated with poorer growth outcomes. The second hypothesis predicted that breastmilk IgA levels would be positively associated with infant growth and health outcomes. The final hypothesis predicts that breastmilk IgA would mitigate the negative effects of infant

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disease-exposing behaviors, such as the consumption of supplemental food, crawling, vaccination, and family hygiene behaviors, on infant nutritional and health indicators. The first hypothesis was confirmed for chronic but not acute undernutrition. Stunted infants had significantly higher salivary IgA levels than infants that were not stunted. However, underweight and wasted conditions were not associated with infant IgA levels. For the second hypothesis breastmilk IgA was only significantly associated with infant upper arm fat area and not other infant growth or health variables. For hypothesis 3, breastmilk IgA levels did not appear to adjust the effect of disease-exposing behaviors on infant nutritional status and health. While there appears to be an interaction between immune function and long-term nutritional outcomes in infants, it appears that the variation in IgA in breastmilk does not play a direct role in protecting infants from disease during the weaning period. The protective effects of IgA may occur earlier in postpartum development, during the first 3-6 months when infant fat stores increase dramatically. From there, infant fat stores may play a more important role in buffering infants against the nutritional and disease stresses of the weaning period. Interestingly, in this population infants do not experience a post-weaning fat decline and in fact experience an increase of fat in infants greater than 18 months. This is especially curious in light of the increasing rates of malnutrition as infants age through the postpartum period. Ariaal infants may prioritize fat storage over growth, an effect that may be in part due to the prolonged consumption of breastmilk.

What Can We Learn from IgA as a Biomarker?

As reviewed in Chapter I, immunoglobulin A plays an important role in the protection of mucosal surfaces from infectious threats. IgA prevents pathogens from adhering to mucosal surfaces and helps pass them out of the gastrointestinal tract, promotes the colonization of commensal bacteria within the gut, and reduces inflammation (Mestecky and McGhee 1987). In addition, breastmilk contains large quantities of IgA to protect infants' mucosal surfaces while their immune system matures (Mestecky 2001). Based on these interesting properties, the variation in the IgA system is a topic of potential interest to human population biologists.

Despite the well-known proximate activities of IgA within the human body, the beneficial or detrimental effects of variation in mucosal immunity in a population are less clear. There is only one clinical diagnosis associated with IgA: selective IgA deficiency. This disease is marked by extremely low or absent levels of serum IgA. Most patients are asymptomatic but some experience a higher rate of certain infections and have a higher risk of autoimmune disease (Azar and Ballas 2007). It is hereditary and found in the highest frequency in people with European ancestry (Azar and Ballas 2007). Because a lack of IgA may not result in poor health or mortality outcomes, this may limit the usefulness of studying IgA variation within a population.

In addition, much of the variation in IgA levels in the Ariaal population of women and infants could not be explained by the hypotheses and variables in this study even though several variables were significant. There is some interesting but inconclusive evidence that IgA is a costly resource, particularly the decline of IgA in breastmilk at the beginning of the postpartum period and the higher IgA levels in stunted infants.

However, IgA could only be connected with self-reports of health status in one case, indicating that if IgA changes in response to an infection, the effect is short-lived. And finally, the widely touted immunological benefits of breastmilk could not be determined within the Ariaal infant population. In light of this information, should IgA be considered a useful biomarker for population studies of immunity?

Despite the limited results in Ariaal women and infants, IgA likely remains one of the more sensitive immune markers to changes in health and energy status and is the easiest to collect in field settings. First, IgA is a front-line defense and elevated levels can indicate an infection in mucosal tissues. However, this elevation should be assessed in the context of individual variation, meaning that IgA should be evaluated longitudinally or paired with another marker of infection, such as C-reactive protein. Next, because it is produced in such large quantities, it is more susceptible to changes in nutritional status and energy balance (McDade 2005). Therefore, it remains an important factor when considering the energetic costs of immune function and how they affect population life history. Finally, it is likely that some variation in IgA can be attributed to other biomarkers. Knowing what these biomarkers are would help researchers control for the effects of one upon the other, leading to more accurate predictions. Ultimately, with more understanding of IgA physiology it can be a useful complement to the study of immune function within human populations.

Evolutionary Perspectives

Reproducing organisms face trade-offs between investing in their current offspring and investing in their future offspring. Life history theory predicts that mothers

will invest optimally in current and future offspring to maximize their fitness. As risk of

infant mortality decreases with increasing infant age, mothers will decrease their

investment in their current offspring (represented by breastmilk IgA in this study) in

order to invest somatically to prepare for their next pregnancy. This prediction was

partially supported in lactating Ariaal women: breastmilk IgA does decline over the first

3 months of lactation to a low between months 4-6. This low level remains consistent

until about the 18th month of lactation, then rises again. This result is less pronounced

when IgA is divided by breastmilk fat percent. There may be alternate, non-evolutionary

explanations for this decline. For example, breastmilk IgA may decline because its

protective value is of decreasing value to the infant, not to the mother. Or, the decline

may be a proximate side-effect of postpartum changes in maternal hormones and immune

function. In addition, the increase in breastmilk IgA greater than 18 months postpartum is

puzzling. Dividing IgA level by the amount of fat in the breast helps adjust for the

amount of time milk has spent in the breast reduces but does not eliminate the increase in

IgA. This finding detracts from the evolutionary prediction that IgA concentration will

decrease over postpartum months. Non-evolutionary explanations for this phenomenon

include an increase in energy balance that accompanies the return of menses may boost

breastmilk production or that the 18+ month sample of women is biased toward good IgA

producers, with women who produce less opting to stop breastfeeding earlier. Even if

breastmilk IgA concentration does not follow an evolutionary pattern, the maternal

effects of immune function transfer from mother to infant still plays a role in infant

phenotype, and may be evolutionarily adaptive as well.

Maternal effects are phenotypic characteristics of the mother that directly contribute to phenotypic characteristics in their offspring. Rather than representing environmental "noise", these effects may be adaptive strategies that manipulate offspring phenotype relatively rapidly in the face of maternal experience to environmental conditions (Kuzawa 2005; Mousseau and Fox 1998). The transfer of immunity is a major area of maternal effects in birds and mammals. Immunological transfer between mothers and infants provide specific, up-to-date information about the disease environment and potentially provide phenotypic adjustments to infant growth and immune development (Boulinier and Staszewski 2008; Grindstaff et al. 2003). There is some possibility of maternal effects of IgA transfer in Ariaal women; for example women with higher IgA in their breastmilk had infants with higher salivary IgA, although the relationship was not significant. However, it is unclear if the IgA in breastmilk actually represents the maternal phenotype of mucosal immunity because maternal salivary IgA and breastmilk IgA were not statistically associated with each other. It may be that IgA concentration itself is not a good indicator of maternal immune phenotype; specific IgA antibodies may be more important than concentration when considering maternal effects. In addition, variation in breastmilk IgA did not influence infant growth, but there may be longer-term growth and immune effects that are not yet known. Unfortunately, other factors such as infant feeding patterns, infection load, and available resources confound the potential maternal effects in the Ariaal population.

It can be difficult to evaluate the true evolutionary impact of evolutionary forces without including indicators of fertility and mortality. Researchers have convincingly documented the greater infection and mortality rate in non-breastfed infants in

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developing countries (Arifeen et al. 2001; Brown et al. 1989; Clemens et al. 1997; Popkin et al. 1990; Prentice et al. 1984), indicating that the immune factors within breastmilk are adaptive. It is still unclear, however, if IgA concentration itself is tied to mortality risk. Follow-up studies within this population can indicate if infants whose mothers have higher breastmilk IgA levels have a lower risk of mortality.

Dual Protection of Breastmilk IgA and Infant Fat During Weaning

Ariaal infants do not follow a typical pattern of fat depletion during weaning. Generally, infants gain fat during the first 3-6 months of life and begin to lose fat during month 6, slowly declining to childhood levels. Ariaal infants, on the other hand, gain fat during the first 6 months but maintain this level over the weaning period, even increasing fat levels past 18 months postpartum. This happens even as infant growth indicators (height-for-age, weight-for-age and weight-for-height z-scores) decline significantly over the postpartum period. It appears that Ariaal infants conserve fat over growth during the weaning period, in opposition to predictions that infant adiposity buffers growing infants against disease risk of weaning (Kuzawa 1998). This pattern may be in part due to breastmilk immunity, the other major factor protecting infants from disease during weaning.

There is an interesting co-relationship between adiposity and breastmilk IgA in the Ariaal which may be related to protection from disease and fat conservation. Fat growth appears to be accelerated in Ariaal infants when breastmilk IgA concentrations are the highest, at both ages 0-3 months and beyond 18 months. This may indicate a different role for breastmilk than protection over the weaning period. One possible role of

breastmilk IgA in the gut is to help train the infant immune system to neither overreact nor underreact to pathogens. It does this by selectively tolerating certain commensal bacteria colonies and attacking pathogenic bacteria (Bollinger et al. 2003). Studies indicate the most sensitive developmental period for the establishment of gut bacteria populations is between the ages of 0 and 2 years of age, and that the effects of these populations on later allergy and asthma risk are profound (Bjorksten 2008). Immunoglobulin A, in particular, appears to favor *Lactobacillus* bacteria that aid in the digestion of milk (Fanaro et al. 2003), a possible benefit to the Ariaal population in which the first weaning food is cow milk. There may be other, unknown, interactions between IgA and the priming of future digestive functions in the gut, possibly helping explain the relationship seen between adiposity and breastmilk IgA in the Ariaal. Future work should consider more carefully the proximate mechanisms between breastmilk IgA, gut bacteria, and infant developmental outcomes.

Future Directions

Many of the results in this dissertation are limited by the cross-sectional design. Addressing infant development longitudinally would reduce or eliminate many of the confounding effects of developmental stage and behavior on infant age and would more accurately determine when growth begins to lag in Ariaal infants. It could also more accurately assess maternal reproductive status, particularly return to menstruation and possible pregnancy. A longitudinal study would also eliminate the self-selecting bias of prolonged lactators and determine more accurately the mean duration of lactation within the population. It can also form the basis to compare the benefits of lactation length.

Another weakness of this study is that the Ariaal population is relatively homogenous in terms of available nutritional resources. A supplemental comparative study of lactating US women and their infants using the same IgA assay would address population differences in breastmilk composition and IgA production.

As understanding of physiology increases, research in human biology, biomedicine, and public health is trending toward including more biomarkers in studies. Multiple biomarkers help researchers adjust for interactions between hormonal and immunological systems. In this study, for example, it is known that IgA interacts with cortisol in some way (Groer et al. 2004), and including cortisol in statistical models would adjust for the effects of cortisol on IgA. Further, more biomarkers would also allow research on the interaction between different aspects of the adaptive and innate immune system, a growing area of interest in human biology (e.g. Miller 2009). Besides IgA, lysozyme and lactoferrin are also significant immunological compounds present in breastmilk, each with their own unique pattern over the course of lactation. Unfortunately, increasing the number of biomarkers in a study also increases the cost and can be a major barrier to research in programs with limited funding.

This dissertation research has highlighted the need for more research into the direct cost of maintaining and mounting immune responses in human populations. Reviews of the literature suggest the cost of immunity is fairly high, particularly during direct infectious threats (Lochmiller and Deerenberg 2000). In humans, fevers are a high energetic burden associated with high metabolic rate and weight loss (Chiolero et al. 1997; Long 1977). More detailed work into the costs of immunity should be possible. For example, administering a vaccine and using indirect calorimetry to assess the changes in

metabolic rate during immune activation could be one area of research. Another possibility is to investigate more closely the changes in immune function that occur with intense exercise. Understanding the true costs of immunity would help link the proximate research done by immunologists to ultimate hypotheses that are of interest to biological anthropologists.

Finally, more research should investigate the interaction between breastmilk IgA and bacterial flora with a focus on growth and immune outcomes. Research into the effects of the amount and types of specific IgA antibodies on gut bacterial communities would set the stage for longitudinal studies of infant growth, fat deposition, and long-term immune development.

Final Remarks

This dissertation represents an attempt to integrate proximate mechanisms and descriptive understanding of immunoglobulin A biology with ultimate questions that are the purview of biological anthropologists. The findings in this project, namely, 1) that the IgA concentration in breastmilk follows a U-shaped pattern over the postpartum period (Chapter 4), 2) that breastmilk IgA exerts some influence on infant IgA profiles (Chapter 5), 3) that chronic malnutrition is accompanied by elevated salivary IgA levels in infants (Chapter 5), and 4) that IgA and infant fat deposits may work in concert to protect infants from negative outcomes associated with disease (Chapter 5), highlight the need for more research in the area of evolutionary and population immunology. These results suggest that the system of IgA transfer between mothers and infants is of considerable interest to biological anthropologists and worthy of more careful study. Evolutionarily, these results

hint that 1) the transfer of resources from mothers to infants is based on the maximization of maternal fitness and that 2) the maternal effect of IgA may have an impact on infant immune phenotype in a way that may ultimately increase fitness.

Appendix I

Cultural Consensus Phase II Questionnaire

Question	**Answer**
1. How many children do you have?	
2. Are you poor or not poor?	
3. What village are you from?	
4. Where do you get water for your family?	
5. Do you boil water for your family to drink?	
6. Does your family use a toilet?	
7. Have you attended a seminar sponsored by FHI or other NGO?	
8. Do you use the hospital in Karare if someone in your family is sick?	
9. Have your children received all vaccinations?	
10. It is best to breastfeed a child for less than one year.	no
11. It is best to breastfeed a child for two years.	yes
12. It is best to breastfeed a child between 2-3 years.	yes
13. It is best to breastfeed a child for three years.	yes
14. It is best to breastfeed a child for more than three years.	no
15. Becoming pregnant is a good reason to stop breastfeeding.	no
16. Women stop breastfeeding when the child wishes to stop.	no
17. Women can breastfeed a young baby and an older baby at the same time.	no
18. Women stop breastfeeding when they (women) want to stop.	yes
19. Mothers can start introducing foods other than breast milk before the child is 6 months old.	no
20. Mothers can start introducing foods other than breast milk when the child is 6 months old.	yes
21. Mothers can start introducing foods other than breast milk only when the child is older than 6 months.	yes
22. When you first start introducing food, cow milk is the first food other than breast milk given to babies.	yes
23. By 6 months, it is best to give babies the same food as the rest of the family.	no
24. By 1 year, it is best to give babies the same food as the rest of the family.	no
25. By 2 years, it is best to give babies the same food as the rest of the family.	yes
26. At 6 months old, it is best to give babies soft foods.	yes
27. After giving birth, mothers do not work for 3 months.	yes
28. When a mother works, the baby goes with her.	yes
29. When a mother works, the baby stays at home with a relative.	yes
30. When a mother works, the baby stays home alone and sleeps.	yes
31. When a mother is home, the baby nurses often.	yes
32. Mothers and fathers prefer boy and girl babies equally.	yes
33. Mothers and fathers prefer boy babies only.	no
34. Mothers and fathers prefer girl babies only.	no
35. If a baby is sick, it is best to treat at home before going to the hospital.	yes
36. If a baby is sick, it is best to treat at the hospital before treating at home.	no
37. If a baby is sick, it is best not to treat the illness.	no
38. People in my area follow all instructions for medicines given at the hospital.	yes
39. If a baby has a high fever, does not eat, and feels weak, the baby probably has malaria.	yes
40. Malaria is caused by mosquito bites.	yes

41.	If a child has malaria, it is best to treat at home before going to the hospital.	no
42.	At the hospital, a yellow medicine is used to treat malaria.	yes
43.	At the hospital, a pink medicine is used to treat malaria.	yes
44.	At the hospital, amodiaquine is given to treat malaria.	no
45.	At the hospital, panadol is given to treat malaria.	yes
46.	At the hospital, a powder is given to treat malaria.	yes
47.	At the hospital, an antibiotic is given to treat malaria.	no
48.	Silalei is used to treat babies who have malaria.	no
49.	Lmisingiyoi is used to treat babies who have malaria.	no
50.	Lasaramai is used to treat babies who have malaria.	no
51.	Lokirdingai is used to treat babies who have malaria.	no
52.	Lmaimim is used to treat babies who have malaria.	no
53.	Miti arbaini is used to treat babies who have malaria.	yes
54.	Ltungomi is used to treat babies who have malaria.	no
55.	If a baby has frequent stools, the baby probably has diarrhea.	yes
56.	Diarrhea is caused by dirt.	yes
57.	Diarrhea is caused by dirty water.	yes
58.	Women in my area boil water for babies to drink.	yes
59.	If a child has diarrhea, it is best to treat at home before going to the hospital.	yes
60.	At the hospital, ORS is given to treat diarrhea.	yes
61.	At the hospital, syrup is given to treat diarrhea.	yes
62.	At the hospital, powder is given to treat diarrhea.	yes
63.	Women in my area mix salt and sugar in water to give to babies with diarrhea.	yes
64.	Breastfeeding protects babies from getting diarrhea.	no
65.	Ltudupei is used to treat diarrhea in babies.	yes
66.	Ldule is used to treat diarrhea in babies.	yes
67.	Lerai is used to treat diarrhea in babies.	yes
68.	If a baby has spots, red eyes, and a fever, the baby probably has measles.	yes
69.	Babies in my area get one injection to vaccinate against measles.	yes
70.	Babies in my area get two injections to vaccinate against measles.	no
71.	It is better to treat babies at home for measles before going to the hospital.	yes
72.	At the hospital, an injection is given to treat measles.	yes
73.	Lmasaduku is used to treat babies who have measles.	yes
74.	Sheep oil is used to treat babies who have measles.	yes
75.	Sheep soup is given to babies who have measles.	yes
76.	If a baby has a cough and a runny nose, the baby probably has a common cold.	yes
77.	Common colds are caused by dirt.	yes
78.	Common colds are caused by other people who are sick.	yes
79.	Common colds are caused by cold air.	yes
80.	If someone has a common cold with a fever, it is cause by a tick.	yes
81.	It is best to treat babies at home for a common cold before going to the hospital.	yes
82.	At the hospital, cough syrup is given to treat a common cold.	yes
83.	At the hospital, a powder is given to treat a common cold.	yes
84.	At the hospital, antibiotics are given to treat a common cold.	no
85.	Lmisingiyoi is used to treat common cold in babies.	yes
86.	Soup is used to treat common cold in babies.	yes
87.	Silapani is used to treat common cold in babies.	yes
88.	Malmal is used to treat common cold in babies.	no
89.	Ltungomi is used to treat common cold in babies.	no
90.	Lakirdingai is used to treat common cold in babies.	yes

91.	Loyapasei is used to treat common cold in babies.	yes
92.	Silalei is used to treat common cold in babies.	yes
93.	If a baby has difficulty breathing, a cough, and a fever, the baby probably has pneumonia.	yes
94.	Pneumonia is caused by a common cold.	yes
95.	Pneumonia is caused by not wearing enough clothing.	yes
96.	Pneumonia is caused by rain.	yes
97.	It is best to treat pneumonia at home before going to the hospital.	no
98.	At the hospital an injection is given to treat pneumonia.	yes
99.	At the hospital a powder is given to treat pneumonia.	yes
100.	At the hospital antibiotics are given to treat pneumonia.	yes
101.	At the hospital panadol is given to treat pneumonia.	yes
102.	Loyapasei is used to treat pneumonia in babies.	no
103.	Ldepe is used to treat pneumonia in babies.	no
104.	Lmisingiyoi is used to treat pneumonia in babies.	no
105.	Silalei is used to treat pneumonia in babies.	no
106.	Losung is used to treat pneumonia in babies.	no
107.	Sokoni is used to treat pneumonia in babies.	yes
108.	If a baby has pain in the legs and back, cannot stand, and has swollen joints, the baby has ntingadu.	yes
109.	Ntingadu is also known as brucellosis.	no
110.	Ntingadu is caused by unboiled milk.	yes
111.	Ntingadu is caused by uncooked meat.	no
112.	Ntingadu is caused by following animals.	yes
113.	It is best to treat ntingadu at home before going to the hospital.	yes
114.	It is best to avoid the hospital when a baby has ntingadu.	no
115.	At the hospital, ntingadu is treated with injections.	yes
116.	At the hospital, ntingadu is treated with panadol.	yes
117.	A baby with ntingadu can be treated with miraa.	yes
118.	A baby with ntingadu can be treated with lamurei.	yes
119.	A baby with ntingadu can be treated with ldepe.	yes
120.	A baby with ntingadu can be treated with lemishiria.	yes
121.	A baby with ntingadu can be treated with lmakutukuti.	yes
122.	If a baby has red, watery eyes with discharge, the baby probably has an eye infection.	yes
123.	It is best to treat an eye infection at home before going to the hospital.	no
124.	The hospital does not treat young babies who have eye infections.	yes
125.	Infected eyes in babies should be washed with cow milk.	yes
126.	Infected eyes in babies should be washed with breast milk.	yes
127.	Infected eyes in babies should be washed with water.	yes
128.	Infected eyes in babies should be washed with strong tea.	yes
129.	If a baby has sores on his mouth, it is best to treat at home before going to the hospital.	yes
130.	At the hospital, a purple medicine is given for mouth sores.	yes
131.	Lmerapare/Lmerepari can be used to treat mouth sores in babies.	yes
132.	If a baby has a big stomach, loses weight, and has visible worms in the stool the baby is probably infected with worms.	yes
133.	It is best to treat a baby for worms at home before going to the hospital.	no
134.	People in my community receive medicine from FHI to treat worms in babies.	yes
135.	Seketet can be used to treat worms in babies.	yes
136.	Lmunguten can be used to treat worms in babies.	yes

137. If a baby has a rash starting in the fingers, the baby probably has scabies.   yes
138. It is best to treat a baby at home for scabies before going to the hospital.   no
139. At the hospital, scabies is treated with lotion.   yes
140. In babies, scabies should be washed with Omo.   yes
141. In babies, scabies should be washed with cow urine.   yes
142. If a baby has a common cold with eyes that "stand up", it is caused by a tick.   yes
143. Malmal is used to treat illness caused by a tick.   yes
144. Ltungomi is used to treat illness caused by a tick.   yes
145. If a baby is sick from a tick, you can find the tick on the baby's body.   no

Appendix II

Ariaal Traditional Medicine and Diseases They Treat

Medicine	Identification	Disease Treated[1]	Disease(s) Treated[2]
Lmaimim	*Commiphora* sp.	n/a	polio, gonorrhea, arthritis
Ldepe	*Acacia nubica*	ntingadu	stomach pains, hepatitis, arthritis, rheumatism, polio, colds, cough, fever, malaria, headache, gonorrhea, liver and spleen
Sokoni	*Warbugia ugandensis*	pneumonia	fever, malaria, colds, childbirth, abortions, upset stomach, chest pains
Lmasaduku[3]		measles	
Ldule	*Senecio petitianus*	diarrhea	heart, liver, mental illness
Lemishiria (leminciria)	*Sesethamnus rivae* *S. busseanus*	ntingadu	upset stomach, back pain, gonorrhea, rheumatism, arthritis
Silalei	*Boswellia hildebrandtii*	common cold	chest pain, diarrhea, strength
Ltungomi (Ltigomi)	*Cardiospermum corindum* *C. halicacabum*	'tick illness'	malaria, polio
Lmakutukuti	*Clerodendrum myricoides*	ntingadu	rheumatism, arthritis, malaria, colds, polio, congestion, headache, abortions, clear head
Lmerepari	*Cadaba farinosa*	mouth sores	n/a
Lakirdingai	*Croton dichogamus* Pax	common cold	chest congestion, stomach upset, malaria, fever
Loyapasei	*Aspolia mossambicensis*	common cold	n/a
Lmisingiyoi	*Rhus vulgaris* Meike	common cold	young children's pains
Ltudupei (Ldupai)	*Sanseveria robusta*	diarrhea	gonorrhea, arthritis
Silapani	*Cordia sinensis*	common cold	chest pains
Lmtungtan	*Albizia anthelmintica* Brong	worms	tapeworms, worms, malaria, upset stomach, arthritis, malaria, gonorrhea

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Lerai	*Acacia hockii* *A. seyal* *A. xantholoea*	diarrhea	stomach pains
Lamurei	*Carissa edulis*	ntingadu	polio, gonorrhea, malaria
Lasaramai	*Harrisonia abyssinica* *Pistacia aethiopica*	n/a	chest congestion, malaria, fever gonorrhea, strength
Miti Arbaini[3]		malaria	
Loisungi	*Zanthoxylum chalybeum* *Fagara chalybea* *F. usambarensis*	n/a	sore throat, chest congestion, tonsillitis
Seketet	*Myrsine africana* *Kyllinga flava* *Lantana trifolia*	worms	strength, TB, tapeworms, malaria, fever, wounds, diarrhea
Malmal[3]		'tick illness'	
Miraa	*Catha edulis*	ntingadu	malaria, stimulant

[1]Medicinal uses of plants for infants as reported by consensus analysis.
[2]Medicinal uses of plants as reported by (Heine et al. 1988).
[3]Plant could not be identified.

Appendix III

Questionnaire

Participant Number:	Interviewer:		Date:
Village:	Manyatta:		

1. Maternal Characteristics
001. What is your age (in years)?   _____
002. Do you have a health card or other documentation? *Interviewer, please record the information here:*
003. *Interviewer, please estimate the age of the participant*: _____
004. What is your father's age set? _____
005. I know that this may be difficult for you, but I would like to ask you about the children you have had, including those who have died. Please start with your firstborn child and list them, in order, ending with the child you are currently breastfeeding

Child's First Name	**Child's Sex (M/F)**	**Date of Birth (dd/mm/yyyy)**	**Age of Child (years and months)**	**Date of Death, if applicable (dd/mm/yyyy)**
i.				

ii.				
iii.				
iv.				
v.				
vi.				
vii.				
viii.				
ix.				
x.				
xi.				
xii.				

006. Now I would like to ask you about your siblings. Please start with your **mother's** oldest child and list them in order, *including yourself*. To the best of your knowledge, include any siblings that may be deceased. Please include sex of sibling and age set of any brothers you have.

Sibling's First Name	Sibling's Sex (M/F)	Age Set of Brother

i.		
ii.		
iii.		
iv.		
v.		
vi.		
vii.		
viii.		
ix.		
x.		

007a. How many wives does your father have? _____

    b. Is your mother his 1st, 2nd, 3rd, etc. or only wife? _____   [_] *Check this box if the woman's parents are unmarried.*

    c. How many children does each wife have (aside from your mother)?

_____

008. Is your…
    a. Mother alive? [_] yes [_] no   b. Husband's mother alive? [_] yes [_] no
    c. Father alive?   [_] yes [_] no   d. Husband's father alive? [_] yes [_] no

009. Have you had a menstrual period since the baby has been born? [_] yes [_] no   If yes, please state when they began and how many you have had since they started

_____

010. What is your highest level of education (Indicate highest Standard, Form, or Post-High School level)?
_____

011. Are you married? [_] yes   [_] no

012. What is your husband's age set? _____

013. What is your husband's highest level of education (Indicate highest Standard, Form, or Post-High School level)?
_____

014. Have you attended a seminar on breast feeding or infant care sponsored by Food for the Hungry International or other Non-Government Organization (NGO)? [_] yes   [_] no

2. Infant Characteristics

015. What is the sex of your baby? [_] male   [_] female

016. What is your baby's age, in months? _____

017. What season was your baby born in? [_] cold dry season   [_] short rains   [_] hot dry season   [_] long rains

018. Did you bring a health or vaccination card for the baby?   [_] yes   [_] no   *Interviewer: if yes, use card for Question 017*

019. Please indicate which vaccines your baby has received and at what age.

Immunization	Has Baby Received this Vaccine?	How many doses of this vaccine have been given?	Age(s) Given In Months
BCG (tuberculosis)	[_] yes [_] no [_] don't know		i.
DPT (diphtheria, pertussis, tetanus)	[_] yes [_] no [_] don't know		i. ii. iii.
PV (polio)	[_] yes [_] no [_] don't know		i. ii. iii.
Measles	[_] yes [_] no [_] don't know		i. ii.
Hepatitis B	[_] yes [_] no [_] don't		i.

	know		ii. iii.
Yellow Fever	[_] yes [_] no [_] don't know		i.
Haemophilus influenzae (HIB Meningitis)	[_] yes [_] no [_] don't know		i. ii. iii.

020. Has your baby received any drugs for worms from Food for the Hungry International (FHI) or other NGO? [_] yes   [_] no

021. Can your baby:
[_] Grab your finger?   [_] Reach with his/her hands?   [_] Put things in mouth?   [_] Walk alone?
[_] Hold up his/her head?   [_] Roll over?   [_]Sit up alone?   [_] Say any words?
 [_] Crawl?   [_] Pull self up to stand?   [_] Walk with help?

022. a. How many times does your baby cry during a typical day? _____

       b. When your baby cries during the day, how long does he/she typically cry before stopping? _____

       c. What time of day does your baby cry the most? _____

023. a. How many times does your baby cry during a typical night? _____

       b. When your baby cries at night, how long does he/she typically cry before stopping? _____
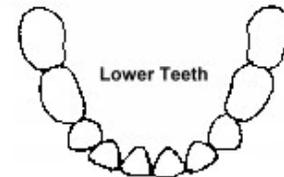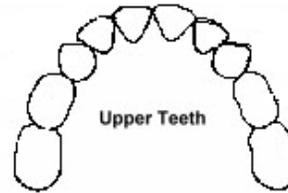
024. Please record infant tooth eruption:

Upper Teeth   Lower Teeth

3. Socioeconomic Status

025. What is your household's cash income for one month in Kenyan shillings? _____

026. How many of the following animals does your household own?
   Cattle _____   Goats and Sheep _____   Camels _____   Donkeys _____

027. Does your household keep a garden?   [_] yes   [_] no

   What do you grow in the garden?

   _____

   How big is the garden in footsteps? _____ footsteps in length   _____ footsteps in width

   Does your household eat or sell most of the food grown in the garden?   [_] Eat [_] Sell

028. Have you used famine relief foods in the past month? [_] yes   [_] no

029. How much money, in Kenyan shillings, did you spend on food in the past month? _____

030. Do you consider yourself poor or not poor? [_] poor   [_] not poor

031. What is your religion?

[_] Catholic	[_] African Inland Church	[_] Full Gospel Church	[_] Islam	[_] Traditional	[_] none	[_] other

_____

032. Do you live in a traditional house? [_] yes [_] no

033. How many times a month do you visit Marsabit Town? _____

034. Do you have a wage-earning profession or own a business (excluding selling milk, livestock, or vegetables)? [_] yes [_] no   What is it?   _____

035. Does your husband have a wage-earning profession or own a business (excluding selling milk, livestock, or vegetables)? [_] yes [_] no   What is it?   _____

036. Which ethnic group best describes you? [_] Ariaal [_] Rendille [_] Samburu [_] Other (please list)_____

4. Household Composition

037. Please list all people that are members of your household. This includes people that spend most nights in your house as well as people who would usually live in your household but for some reason do not (examples would be a husband that works in another area or a child living away at school). Include their relationship to you (the mother) and whether they spend most nights in the house.

First Name	Is Person a Child or Adult?	Relationship to Woman	Currently Living in Household?
	[_] child [_] adult		[_] yes [_] no

	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no
	[_] child [_] adult		[_] yes [_] no

5. Health and Treatment

038. Have you had any of the following diseases in the past month? Please indicate the number of days the disease lasted, where the disease was treated, and which medicines (both local and from the hospital) you have used to treat these symptoms.

Disease	Number of Days	Where Treated?	Medicines Used
Malaria or High Fever [_] yes		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:

[_] no			
Diarrhea [_] yes [_] no	No. Stools/Day: _____	[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:
Pneumonia/Severe Cold [_] yes [_] no		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:

039. Please list any other symptoms you have had in the past month, the number of days you have had each symptom, where you have gone for treatment, and which medicines (both local and at the hospital) you have used to treat these symptoms.

Symptom	Days	Where Treated?	Medicines Used
		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:
		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:
		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:

040. Has your baby had any of the following diseases in the past month? Please indicate the number of days the disease lasted, where the disease was treated, and which medicines (both local and from the hospital) you have used to treat these symptoms.

Disease	Number of Days	Where Treated?	Medicines Used
Malaria or High Fever [_] yes [_] no		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:
Diarrhea [_] yes [_] no	No. Stools/Day: _____	[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:
Pneumonia/Severe Cold [_] yes [_] no		[_] home [_] other [_] Karare dispensary [_] none [_] Marsabit hospital	Traditional: Hospital:

041. Please list any other symptoms your baby has had in the past month, the number of days he/she have had each symptom, and which medicines (both local and from the hospital) you have used to treat these symptoms.

Symptom	Number of Days	Where Treated?	Medicines Used
		[_] home [_] other	Traditional:

		[_] Karare dispensary [_] none [_] Marsabit hospital	Hospital:
		[_] home [_] other	Traditional:
		[_] Karare dispensary [_] none [_] Marsabit hospital	Hospital:
		[_] home [_] other	Traditional:
		[_] Karare dispensary [_] none [_] Marsabit hospital	Hospital:

6. Hygiene Practices

042. What shoes do you usually wear when walking outside? [_] sandals [_] closed-toe shoes   [_] no shoes [_] other _____

043. Does your family use a toilet?   [_] yes   [_] no

044. How often do you boil water or use water purification tablets for your baby? [_] always   [_] sometimes [_] never

045. How often do you boil water or use water purification tablets for yourself? [_] always   [_] sometimes [_] never

046. How often do you boil water or use water purification tablets for the rest of your family? [_] always   [_] sometimes [_] never

047. Where do you get water from your family? _____

048. How often does your baby use a mosquito net? [_] always   [_] sometimes [_] never

049. How often do you or the rest of your family use a mosquito net? [_] always   [_] sometimes [_] never

050. Does your baby crawl or walk on the ground outside? [_] yes   [_] no

051. What type of floor do you have in your house? [_] dirt/skins   [_] concrete [_] wood [_] other: _____

052. How often do you bathe your baby? _____ times per [_] day [_] week [_] month

7. Breastfeeding and Weaning

053. In a normal day this past week, how many times does your baby latch on to breast feed?
_____
    How many minutes do they typically breast feed each time they latch on? _____

054. In a normal night this past week, How many times are you woken up for breastfeeding? _____
    How many minutes are you typically awake each time you are woken up? _____ minutes

055. What foods have been given to baby in the past 24 hours, and how have they been prepared (including cow milk)?

056. In a normal day, how long does it take you to fetch water for your family? _____ [_] does not fetch water
    Does your baby go with you when you fetch water for your family? [_] yes [_] no

057. In a normal day, how long does it take you to fetch firewood for your family? _____ [_] does not fetch firewood

Does your baby go with you when you fetch firewood for your family? [_] yes [_] no

058. When you fetch water or firewood, who usually watches the baby?
    [_] mother   [_] husband's mother   [_] daughter   [_] friend   [_] husband   [_] no one   [_]
other_____

059. How long did you stop working after giving birth to this baby? _____ months   [_] have not returned to work

8. Anthropometry

Mother	Height (cm):	Weight (kg): Wearing beads? [_] yes [_] no	MUAC (cm):	Triceps Skinfold (mm): 1. _____ 2. _____ 3. _____
Infant	Recumbent Length (cm):	Mother + Infant Weight (kg):	MUAC (cm):	Triceps Skinfold (mm): 1. _____ 2. _____ 3. _____

9. Biomarker Collection

Please write the time each biomarker was collected in the space provided. Write the result of the HemoCue hemoglobin test in the box labeled Hb.

Mother	_____ breast milk _____ saliva _____ blood spot _____ hemoglobin	Hb:
Infant	_____ saliva	

10. Notes

11. Knowledge of Local Medicine		
1. Silalei is used to treat babies who have malaria.	[_] yes	[_] no
2. Lmisingiyoi is used to treat babies who have malaria.	[_] yes	[_] no
3. Lasaramai is used to treat babies who have malaria.	[_] yes	[_] no
4. Lokirdingai is used to treat babies who have malaria.	[_] yes	[_] no
5. Lmaimim is used to treat babies who have malaria.	[_] yes	[_] no
6. Miti arbaini is used to treat babies who have malaria.	[_] yes	[_] no
7. Ltungomi is used to treat babies who have malaria.	[_] yes	[_] no
8. If a child has diarrhea, it is best to treat at home before going to the hospital.	[_] yes	[_] no
9. Women in my area mix salt and sugar in water to give to babies with diarrhea.	[_] yes	[_] no
10. Breastfeeding protects babies from getting diarrhea.	[_] yes	[_] no
11. Ltudupei is used to treat diarrhea in babies.	[_] yes	[_] no
12. Ldule is used to treat diarrhea in babies.	[_] yes	[_] no
13. Lerai is used to treat diarrhea in babies.	[_] yes	[_] no
14. Lmasaduku is used to treat babies who have measles.	[_] yes	[_] no
15. Sheep oil is used to treat babies who have measles.	[_] yes	[_] no
16. Sheep soup is given to babies who have measles.	[_] yes	[_] no
17. Lmisingiyoi is used to treat common cold in babies.	[_] yes	[_] no
18. Soup is used to treat common cold in babies.	[_] yes	[_] no
19. Silapani is used to treat common cold in babies.	[_] yes	[_] no
20. Malmal is used to treat common cold in babies.	[_] yes	[_] no
21. Ltungomi is used to treat common cold in babies.	[_] yes	[_] no
22. Lakirdingai is used to treat common cold in babies.	[_] yes	[_] no
23. Loyapasei is used to treat common cold in babies.	[_] yes	[_] no
24. Silalei is used to treat common cold in babies.	[_] yes	[_] no
25. Loyapasei is used to treat pneumonia in babies.	[_] yes	[_] no
26. Ldepe is used to treat pneumonia in babies.	[_] yes	[_] no
27. Lmisingiyoi is used to treat pneumonia in babies.	[_] yes	[_] no
28. Silalei is used to treat pneumonia in babies.	[_] yes	[_] no
29. Losung is used to treat pneumonia in babies.	[_] yes	[_] no
30. Sokoni is used to treat pneumonia in babies.	[_] yes	[_] no
31. A baby with ntingadu can be treated with miraa.	[_] yes	[_] no
32. A baby with ntingadu can be treated with lamurei.	[_] yes	[_] no
33. A baby with ntingadu can be treated with ldepe.	[_] yes	[_] no
34. A baby with ntingadu can be treated with lemishiria.	[_] yes	[_] no
35. A baby with ntingadu can be treated with lmakutukuti.	[_] yes	[_] no
36. Infected eyes in babies should be washed with cow milk.	[_] yes	[_] no
37. Infected eyes in babies should be washed with breast milk.	[_] yes	[_] no
38. Infected eyes in babies should be washed with water.	[_] yes	[_] no
39. Infected eyes in babies should be washed with strong tea.	[_] yes	[_] no
40. Lmerapare/Lmerepari can be used to treat mouth sores in babies.	[_] yes	[_] no
41. Seketet can be used to treat worms in babies.	[_] yes	[_] no

42. Lmunguten can be used to treat worms in babies.	[_] yes	[_] no
43. In babies, scabies should be washed with Omo.	[_] yes	[_] no
44. In babies, scabies should be washed with cow urine.	[_] yes	[_] no
45. Malmal is used to treat illness caused by a tick.	[_] yes	[_] no
46. Ltungomi is used to treat illness caused by a tick.	[_] yes	[_] no

**Ariaal Infant Growth Compared to World Health Organization Reference
Standards**

**Figure 1. Female Ariaal infant mean weight-for-age versus WHO (2006) reference
population.**
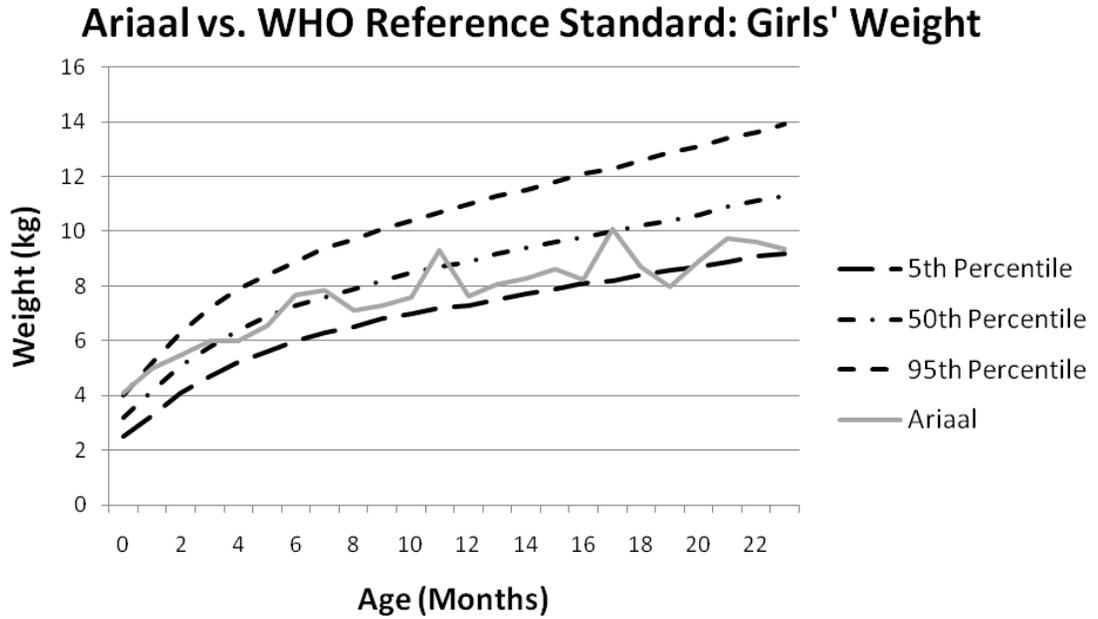
Figure 2. Female Ariaal infant mean height-for-age versus WHO (2006) reference population.
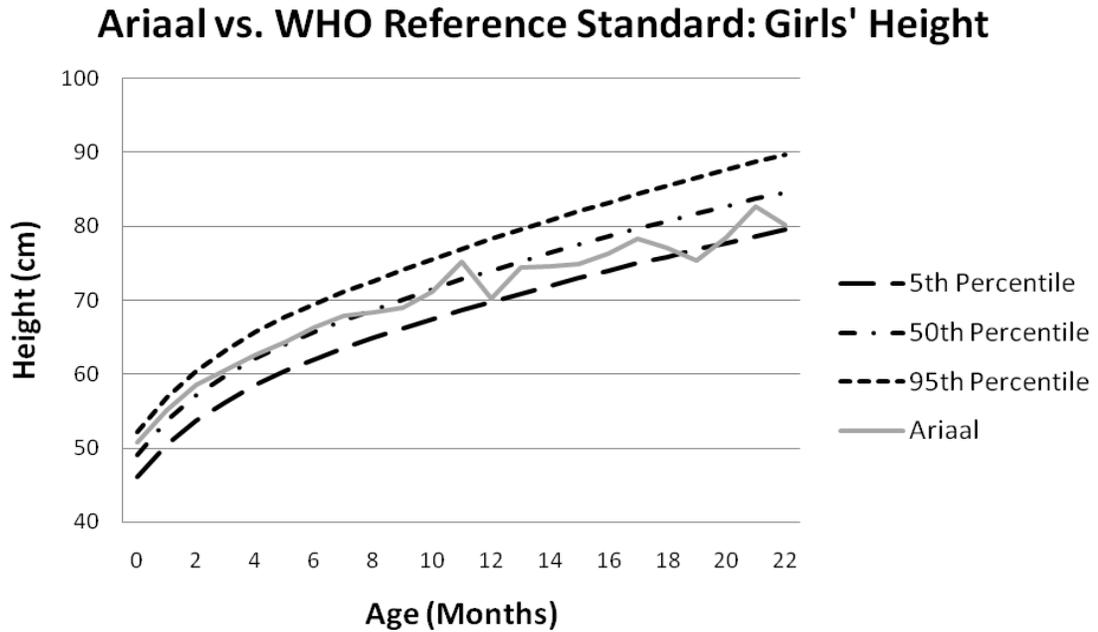
Figure 3. Male Ariaal infant mean weight-for-age versus WHO (2006) reference population.

Figure 3. Male Ariaal infant mean height-for-age versus WHO (2006) reference population.

Ariaal vs. WHO Reference Standard: Boys' Height
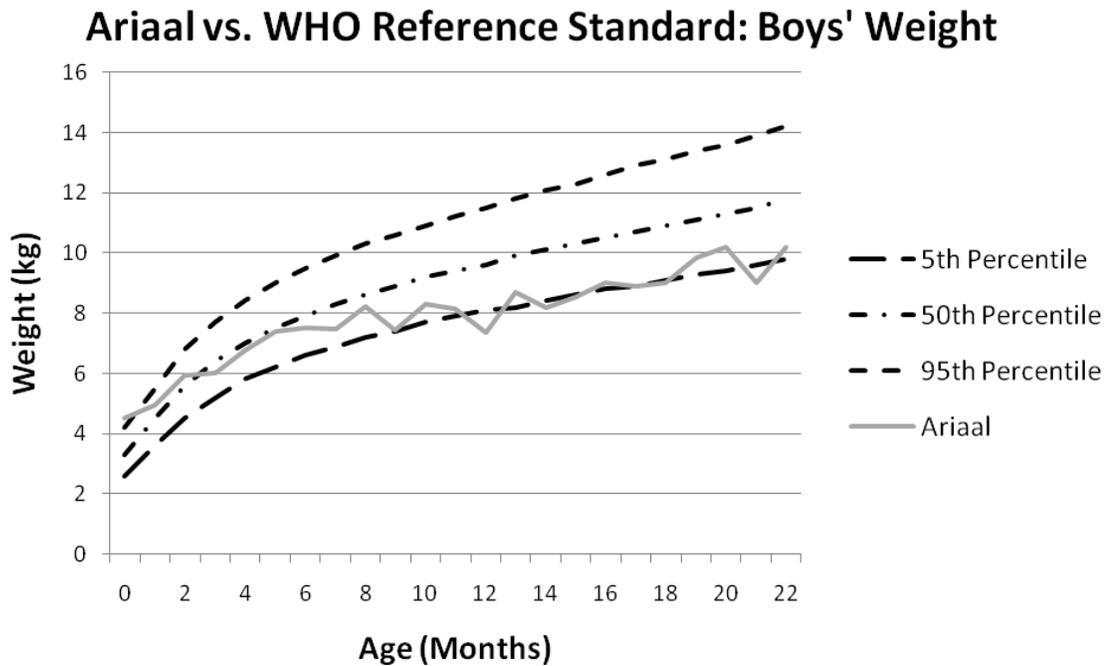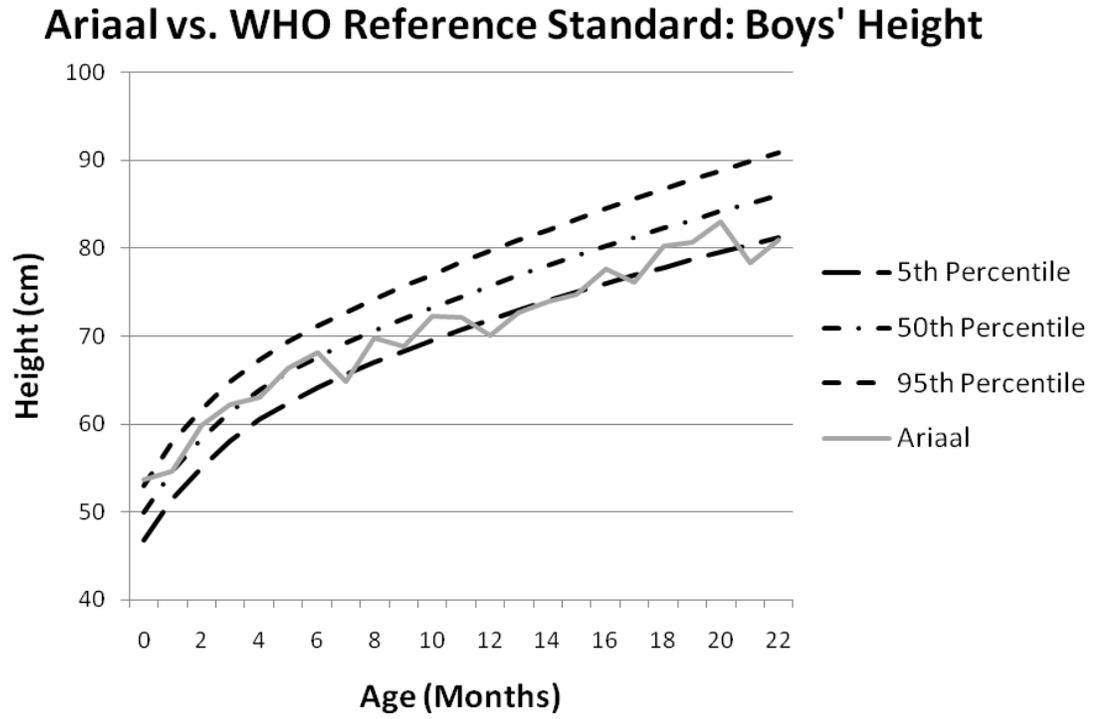
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