Maternal and Infant Essential Fatty Acids Status in Havana, Cuba

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Dedication

I would like to dedicate the effort put into the completion
of this thesis to all of the people
and things through which God so graciously shows us His face.

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Abbreviations

Fatty Acid Terminology:
Saturated Fatty Acids = SAFA
caprylic acid = C8:0
capric acid = C10:0
lauric acid = C12:0
myristic acid = C14:0
palmitic acid = C16:0
stearic acid = C18:0
intermediate and medium chain saturated fatty acids (C6:0 to C14:0) = IMCSAFA

Monounsaturated Fatty Acids = MUFA
oleic acid (C18:1 n9) = OA

Polyunsaturated Fatty Acids = PUFA
linoleic acid (C18:2 n-6) = LA
γ-linolenic acid (C18:3 n-6) = γ-LNA
α-linolenic acid (C18:3 n-3) = LNA
dihomo-γ-linolenic acid (C20:3 n-6) = DGLA
arachidonic acid (C20:4 n-6) = AA
eicosapentaenoic acid (C20:5 n-3) = EPA
docosatetraenoic acid (C22:4 n-6) = DTA
docosapentaenoic acid (C22:5 n-6) = DPA(n-6)
docosapentaenoic acid (C22:5 n-3) = DPA(n-3)
docosohexaenoic acid (C22:6 n-3) = DHA
essential fatty acid (any n-3 or n-6 PUFA) = EFA
long chain polyunsaturated fatty acids (C_{20} and C_{22} n-3 and n-6 PUFA) = LCP
sum of long chain (C_{20} and C_{22}) n-6 fatty acids = ΣLC n-6
sum of long chain (C_{20} and C_{22}) n-3 fatty acids = ΣLC n-3

Terminology Related to Infant Feeding Practices
standard formula = SF (refers to infant formula with no LCP but adequate LA and LNA)
breast fed, breast feed or breast feeding = BF
breast milk = BM

Anthropometrical Terminology:
Body Mass Index (Kg/m^2) = BMI
Mid Upper Arm Circumference = MUAC

Other Terminology
red blood cell = RBC
phosphatidylethanolamine = PE
phosphatidylcholine = PC

central nervous system = CNS
rod outer segment = ROS
electroretinogram = ERG
visual evoked potential = VEP
forced choice preferential looking acuity = FPL
Teller Acuity Card = TAC

Abstract

An adequate ingestion of essential fatty acids is required for optimal development of the central nervous system and visual acuity in infants. For breast feeding mothers, it is important that a diet containing an adequate balance of essential fatty acids of the n-6 and n-3 series be consumed as this is reflected in breast milk. The objective of this investigation was to determine the essential fatty acid status of breast feeding women and their infants in Havana, Cuba, with particular focus on the n-3 series. The group of 56 Cuban mothers and infants under investigation did not show biochemical or functional signs of poor essential fatty acid status. Mothers possessed a good profile of essential fatty acids in their milk, plasma and erythrocytes. The content of docosahexaenoic acid in breast milk samples was 0.43 ± 0.26% of total fatty acids, which is within typical value ranges. In combination with an adequate content of total fat (5.29 ± 1.81 g/100 ml), breast fed Cuban infants had a good source of this essential nutrient, which was reflected in their plasma and erythrocytes. The functional test assessing visual acuity development using the Teller Acuity Card system in the infants revealed an average of 2.00 ± 0.68 cycles/degree, which coincided with large sample age norms and the scores of all infants fell within the 99% prediction limits. As has been demonstrated previously in healthy infants, no relations were found between essential fatty acid status and visual acuity score. As 45% of the infants were weaned on different milks, some of which are known to be poor sources of n-6 fatty acids, further promotion of exclusive breast feeding and healthy diets for breast feeding mothers, as well as education regarding optimal weaning practices are required to ensure adequate development of infants in Cuba. Based on the biochemical and functional data collected, it is conceivable to conclude that n-3 fatty acid deficiencies must be exceedingly rare, if they exist at all, in the population of breast feeding women and their infants residing in Havana, Cuba.

Résumé

Une ingestion adéquate d'acides gras essentiels est nécessaire au développement optimal du système nerveux central et de l'acuité visuelle chez les nourrissons. Il est important pour les mères qui allaitent leur enfant de consommer une diète contenant un équilibre adéquat en acide gras essentiels des séries n-6 et n-3 puisque cela se reflètent dans la composition de leur lait. Cette étude avait pour objectif de vérifier si une carence des acides gras essentiels de série n-3 était présente chez les femmes allaitant et chez leurs nourrissons à la Havane, à Cuba. Aucun signe biochimique ou fonctionnel de carence en acide gras essentiel n'a été trouvé pour le groupe de 56 mères et nourrissons cubains faisant partie de la présente étude. Le profil en acides gras essentiels des mères au niveau du lait, du plasma et des érythrocytes était satisfaisant. La proportion de l'acide docosahexaénoïque dans les échantillons de lait était de 0.43 ± 0.26% du total des acides gras, ce qui correspond à une valeur typique. Le contenu en gras total du lait maternal était lui aussi adéquat (5.29 ± 1.81 g/100 ml), donc les nourrissons avaient une bonne source de ce nutriment essentiel et cela était reflété dans leur plasma et érthrocytes. L'évaluation de l'acuité visuelle des nourrissons avec le système Teller Acuity Card a révélé une fonction normale avec une moyenne de 2.00 ± 0.68 cycles/degré, ce qui coïncide aux normes d'un vaste échantillon, et les scores de tous les nourrissons étaient parmi les 99% des limites de prédiction. Tel qu'il a été démontré auparavant auprès de nourrissons en santé, cette recherche n'a pas mis à jour une corrélation entre acide gras essentiels et le score d'acuité visuelle. Puisque 45% des nourrissons ont été sevrés avec des laits différents, don't certains sont des sources faibles d'acides gras essentiels de série n-6, il faut continuer de promouvoir l'allaitement exclusif et une saine alimentation pour les mères qui allaitent, tout en assurant une éducation des pratiques de sevrages optimal afin d'assurer une croissance adéquate des nouveau-nés cubains.

1. INTRODUCTION:

1.1 Thesis Structure

The introduction commences with a brief overview of the economic situation in Cuba, which may have an impact on the nutritional status of the population. Some aspects of nutrition and disease patterns are also discussed, to give an idea of whether severe malnutrition or the possibility of n-3 fatty acid deficiency exists or existed. Rationale for the original protocol is then discussed, as well as reasons for changes in the project. This is followed by a critical review of the literature. It begins with a discussion about breast milk (BM) fatty acid composition, reporting investigations from various countries. This section considers reasons for the appearance of different levels of certain fatty acids in BM, particularly essential fatty acids (EFA), in different regions around the world.

The second section of the literature review examines investigations which assessed the EFA status of infants, and the functional implications of poorer EFA status often found among formula fed infants. For ease of discussion of later topics, the second section of the literature review begins with a synopsis of central nervous system (CNS) structure, including the retina, as related to fat composition. This is followed by a brief discussion of different methods of assessing visual acuity and retinal function for the purpose of clarification for the reader who may not be familiar with the terminology or methodology of this aspect of the project. The main discussion then follows, citing clinical evidence in mammalian infants which related dietary EFA to tissue fatty acid composition and retinal function as well as the limited number of studies relating diet to human infant CNS fatty acid composition. The effect of dietary EFA supply on blood levels of these fatty acids in human infants is then discussed, presenting a number of observational and clinical investigations. Research which further delved into the relation between fatty acid status and visual acuity development in human infants are presented lastly. A variety of studies which are retrospective cohorts, prospective cohorts or experimental in nature, are presented to illustrate the contradictory results obtained in this

field over the past decade, discussing some of the possible reasons for a lack of agreement amongst the studies.

The remainder of the thesis deals with an in depth discussion of objectives, procedural details, including statistical analyses, presentation and discussion of results, and recommendations for the maintenance of adequate EFA levels and adoption of healthy eating practices in the Cuban population.

1.2 Cuba: The Economy, Nutrition and Patterns of Disease

Due to the tightening of the US embargo and the loss of the Soviet Bloc as trade partners, the Cuban population may have suffered difficulties in obtaining an optimal diet, quantitatively and/or qualitatively, in the early to mid 1990's. The Soviet Bloc subsidized up to 85% of the Cuban economy up until the period following 1987-1990, (Sadun & Martone 1995). This economic pullout led to an economic crisis referred to as the "Special Period", which was further compounded by the introduction of the Cuban Democracy Act and the Helms Burton Act which precludes third countries from doing business with Cuba (Sandun & Martone 1995). Such sanctions have limited food and medicine delivery to this nation, resulting in the possibility of direct negative effects on the health status of vulnerable groups, which could include infants and women of child bearing age (Kirkpatrick 1996; American Association for World Health (AAWH) 1997).

One of the most serious public health problems occurring in Cuba, which was at least in part attributable to the embargo and ensuing economic problems, was an epidemic of optic and peripheral neuropathies starting late in 1991 and ending in 1995. Morphological characterization of the peripheral nerve lesions revealed that the lesions were compatible with nutritional, toxic or metabolic etiologies, and not of virus, genetic or inflammatory origin (Borrajero *et al.* 1994). This appears to be true as the epidemic was eradicated by ensuring that every citizen received adequate nutrition or vitamins which were produced locally and administered through the family doctors (Sandun & Martone 1995). It is estimated that the epidemic affected over 50,000 inhabitants in all, with the majority of cases occurring in adults between the ages of 25 and 64 years. This

is thought to be due to the fact that vulnerable groups such as children, pregnant women, and the elderly, received extra nourishment through the governments food distribution system, putting them at lower risk for malnutrition (AAWH 1997). Clinical manifestations associated with these neuropathies were blurred vision, photophobia, loss of visual acuity and loss of colour vision in the optic form and sensory ataxia, burning pain and dysesthesias in the extremities, decreased or absent ankle reflexes, increased urinary frequency, and weakness in legs in the peripheral form (Bern *et al.* 1995; Borrajero *et al.* 1995).

The exact etiology of the epidemic has to this point never been determined. However, neuropathies of nutritional origin usually show generalized signs of under nutrition such as loss of subcutaneous fat (Gay *et al.* 1995). The per person weight loss in Cuba between 1992 and 1993 was 4.5 kg to 9 kg, with larger weight losses in people affected by the neuropathies (Tucker & Hedges 1993). By observing the magnitude of the weight losses, one possible cause, which was never thoroughly investigated, could be loss of cerebral docosohexaenoic acid (DHA). This could occur with a prolonged energy and fat deficient diet leading to extremely low body weight and body fat stores. In general, it is difficult to produce the n-3 fatty acid deficiency syndrome, consisting of symptoms paralleling those mentioned for neuropathies in mammals, as n-3 fatty acids are highly conserved in neural tissues and are difficult to deplete (Nettleton 1995). However, the phenomena has been demonstrated in humans (Holman *et al.* 1982; Anderson & Connor 1989). The possibility of this cause was one reason the original protocol was to be implemented.

1.3 Rationale for Original Protocol and Changes

The investigation described in this thesis was planned to be a clinical trial assessing the impact of maternal n-3 fatty acid supplementation with an imported oil on the EFA status of breast feeding (BF) mothers and infants. Supplementation was to be given to mothers and not infants, as BF rates are high upon hospital discharge in Cuba (97.8%), possibly due to the implementation of The United Nations Children's Fund

(UNICEF) Friendly Baby Hospital Initiative (Aliño-Santiago *et al.* 1997). The national average of exclusive BF at 4 months is also high at 71.7%, and the lowest regional average reported was in City of Havana (58%), the study site (Aliño-Santiago *et al.* 1997).

It was argued that n-3 EFA intakes were low due to the fact that there were no existing sources of dietary n-3 fatty acids for the population, including no availability of fish (Cabrera - Personal Communication 1997). It was also argued that energy and fat intakes in pregnant and BF women may be low, which could have an impact on EFA status. As lack of dietary n-3 fatty acids was thought to have persisted for a number of years, low n-3 status in this group was considered a serious possibility. However, it became apparent that all citizens in the country do, and have long been receiving, 454 g of a high fat dark meat fish on a biweekly basis, as well as a fair number of eggs, known to be good sources of n-3 fatty acids. Upon closer assessment of food items available in the country, other possible sources of linolenic acid (LNA), an n-3 fatty acid, included soy based products such as yogurt, which are produced by the National Food Industry.

Fluid milk was also presumed to be a potential source of LNA. It was rumoured that fluid milk given to children in Cuba resembles infant formula from Western countries. It was mentioned that it was based on defatted cow milk with added fat, which was said to be soy oil by some. Unfortunately, it was not possible to acquire accurate information regarding the exact ingredients of the milk preparation. As such, a sample of the milk preparation was analyzed for its fatty acid profile (Appendix A), revealing that it is most likely supplemented with a blend of fats. This is a logical conclusion as the levels of both linoleic acid (LA) and oleic acid (OA) were substantially higher than which is typically found in cow milk (12.5% LA, and 45% OA), indicating that it is supplemented with fat from other sources. The LA/LNA ratio of the milk was found to be approximately 8:1 which is in accordance with the ratio found in infant formulas from Western nations. This milk preparation was found to contain a fair amount of LNA and an optimal LA/LNA ratio for the formation of longer chain n-3 fatty acids. It also became apparent that each infant receives an entire food ration starting at birth, which is intended

for consumption by the BF mother. This includes an equivalent of 1L of the milk preparation each day and the same amount of fish as every other citizen receives. Therefore, it is unlikely that a mother would suffer from poor n-3 fatty acid intakes during the period of pregnancy or BF, provided she consumes the n-3 rich food items available to her. Therefore, the logic for supplementation with an imported oil lost its significance. Also, if supplementation were necessary, it would be more logical and economical to employ a source available locally, such as soy yogurt or fish, rather than use an imported product.

Upon close assessment of available data, it became apparent that cerebral DHA loss was most likely not the underlying cause of the neuropathy epidemic, despite large weight losses in the country at the time. Though substantial weight losses occurred in the population as a whole, the percentile rankings for various measures of body composition were not indicative of severely low weight or fat status, indicating that a complex number of issues including various micronutrient deficiencies occurring at that point in time, may have been the most prominent cause (Bowman *et al.* 1996). In an investigation by Gay *et al.* (1995), 77.3% of people suffering from the neuropathies living on the Isle of Youth had anthropometric measures made on them, revealing the following percentile rankings for body mass indices (BMI) in cases; 25th percentile = 20.2 kg/m^2, 50th percentile = 21.5 kg/m^2, 75th percentile = 22.6 kg/m^2. Percentile rankings for BMI in controls were 25th percentile = 21.7 kg/m^2, 50th percentile = 24.9 kg/m^2, 75th percentile = 27.1 kg/m^2. Values of percent body fat were also parallel to BMI patterns in both groups. As weight losses in the country were substantial, and BMI's remained high in the control group, and relatively adequate in the cases, it appeared as though average weights in the country before the onset of the epidemic were on the higher end of normative values. This was evident through public health problems in the country related to obesity such as heart disease, which continues to be the leading cause of death (AAWH 1997; Cabrera-Hernandez *et al.* 1997), and diabetes (Collado-Mesa & Diaz-Diaz 1995). Also, it may be that cultural norms for appropriate weight may influence reference standards against which adequate status is assessed. The country's set of nutritional recommendations indicate that a

minimum weight for height be equal to a BMI of approximately 21 kg/m^2 for women and 21.2 kg/m^2 for men (Porrata *et al.* 1996), which is 2.5 points (approximately 6.4 kg) higher than the lower end of normal stipulated by international standards (World Health Organization (WHO) 1995; DeOnis & Habicht 1996).

It appears as though mortality due to obesity related diseases has decreased since the onset of the "Special Period". In 1988, an investigation of workers in Havana revealed that 26.3% of men and 35.4% of women were obese, which declined to 17.8% of men and 19.6% of women in a recent investigation assessing a similar group (Cabrera-Hernandez *et al.* 1997). Average BMI's in the more recent investigation were also indicative of adequate energy intake, being 24.3 kg/m^2 for women and 22.3 kg/m^2 for men. Another recent investigation regarding obesity as public health problem in Havana, found a similar proportion of obese people in the population (Garcia-Ferrera *et al.* 1996). It appears as if the decrease in obesity has led to decreased mortality due to cardiovascular disease (Cabrera-Hernandez *et al.* 1997), although, it remains the leading cause of death (AAWH 1997). As the economic situation in Cuba changes, attempts to educate the public about the negative effects of obesity and its causes may be warranted.

There was no apparent need for an intervention once all of this information became clear. However, it was still argued that an n-3 deficiency in pregnant and BF women may exist as our collaborators insisted that the population is not partial to the items containing n-3 fatty acids in their food supply, and therefore may not be consuming them. Therefore, the investigation became cross sectional in nature rather than experimental. Assessment of the EFA profile of mature BM, and maternal and infant plasma and erythrocytes at 2 months postpartum, as well as a visual acuity test in the infants to assess functional normality were to be carried out in the modified protocol. Therefore, the rationale for this investigation became to assess whether or not there was a deficiency of energy or EFA intake as measured by infant growth, visual acuity scores and EFA profiles.

2. LITERATURE REVIEW:

2.1 Fatty Acids in Human Breast Milk

The fatty acid composition of human BM clearly reflects maternal dietary patterns. This statement is of notable significance when assessing levels of EFA, as they cannot be synthesized within the body. Therefore, the degree of their presence in human BM is entirely dependant on dietary intake. With reference to EFA, mature human milk has typically been found to contain 8% to 17% of total fatty acids as LA, 0.5% to1% of total fatty acids as LNA (LA/LNA ratio = 8:1 to 34:1), while levels of long chain polyunsaturated fatty acids (LCP), including DHA and arachidonic acid (AA) vary in their concentrations (Anonymous 1998). For women consuming a typical North American diet, AA has been found to comprise 0.5% to 0.7% of total BM fatty acids while, DHA makes up 0.2% to 0.4% (Innis 1992). However, levels far exceeding this amount have been found in different regions (Anonymous 1998). Average levels of DHA ranging from 0.12% to 2.78% of total BM fatty acids have been found in countries such as Italy, Nigeria, Belize, Dominica, Germany, Spain and China (Serra *et al.* 1997; Koletzko *et al.* 1991; van Beusekon *et al.* 1990; Koletzko *et al.* 1988; and Pita *et al.* 1985; Chulei *et al.* 1995).

2.11 ASIAN COUNTRIES:

Chulei *et al.* (1995), related regional and dietary influences to human BM fatty acid composition. They obtained BM samples from women living in 5 distinct geographic regions in China. They found that EFA profiles of BM varied among groups, contending that the differences observed were due to variations in maternal diet across regions. A group living in a coastal area had an average DHA concentration in BM of 2.78 ±1.2%, which was 3 to 6 times greater than that of any of the other groups studied. The authors attributed this to the group's high intake of seafood, which is known to be high in DHA. A pastoral group had the lowest BM levels of DHA and LNA which was attributed to relatively low intakes of soybean oil, the main source of LNA for all groups,

and a lack of fish or shellfish consumption due to geographical location. However, the LNA content of milk in all regions was found to be greater than that reported in studies conducted in other countries. With respect to DHA, even the pastoral group was slightly above the normal range for North American women, having 0.44 ± 0.29% of total BM fatty acids in this form.

The fatty acid profiles of BM during various stages of lactation from two regions in China were investigated by Chen *et al.* (1997). The authors found similar levels of DHA from milk samples pooled over all stages of lactation in both groups, being approximately 0.55% of total fatty acids in each group. The authors argued that higher levels of this fatty acid were found in the Chinese samples than in BM from Western countries due to dietary habits. The authors stated, that due to cultural norms, the group from Chongqing will commonly eat more than 8 eggs and 200 g of chicken per day during the first month postpartum. This was reflected in significantly higher DHA levels after 2 weeks of lactation as compared to samples taken at 6 weeks in the same group. As eggs are known to contain a substantial quantity of n-3 fatty acids (Simopoulos & Salem 1992), this could have been their main source of DHA, and provided the bulk of it for incorporation into their BM. The authors also speculated that negligible quantities of trans fatty acids in the groups they studied may be attributable to better DHA status than in Westernized countries. This is possible as trans isomers may inhibit chain elongation and desaturation so that LCP are not formed to the same extent as in their absence (Koletzko 1992; Rosenthal & Doloresco 1984). This investigation also demonstrated that notable differences in AA intake were not greatly reflected in BM levels of this fatty acid. Despite the authors calculation of a high intake of AA for the group from Chongqing (700mg to 800mg per day), and a lower intake for the group from Hong Kong (120mg to 140mg per day), both groups had similar BM AA levels. In summary, both groups presented adequate DHA status which was related to food consumption patterns, while wide variations in AA ingestion were not found to significantly alter the level of this fatty acid in BM.

In a recent investigation, mature milk form Israeli mothers was found to be rich in

polyunsaturated fatty acids (PUFA) (Budowski *et al.* 1994). The authors contended that Israelites have a known background of high LA consumption, mainly through soy oil, which was reflected in their BM fatty acid profiles at 6 to 10 weeks postpartum. The LA content of BM averaged approximately 20% of total fatty acids, being slightly higher than what is typically found in human milk (Anonymous 1998). However, the BM AA content was not elevated as compared to normal values found in other countries, containing 0.6% of total fatty acids in this form. Despite the high levels of LA, DHA levels were within the normal range, comprising an average of 0.37% of total fatty acids. This may have been due to the fact that LNA levels were also above the normal range (Anonymous 1998), making up an average of 1.53% of total fatty acids. These phenomena may be due to frequent soy oil consumption which has an LA/LNA ratio of approximately 8:1, which should favour DHA formation. The dietary LA/LNA ratio was also reflected in the BM samples of this group of women, averaging 13.3:1.

Kneebone *et al.* (1985), assessed the fatty acid composition of mature milk from women of 3 different ethnic groups living in Malaysia. All groups had high levels of LCP, with DHA comprising 0.71% of total fatty acids in the Chinese group, and 0.9% in the groups of Malaysian and Indian descent. The authors attributed this to the consumption of local fish by all groups. Differences between the Chinese and the 2 other groups existed in terms of the saturated fatty acid (SAFA) and LA content of BM. The higher SAFA levels found in the Indian and Malay groups were attributed to frequent consumption of coconut and highly saturated oils, while the higher LA content of Chinese samples was attributed to frequent consumption of vegetable oil. Adequacy of LCP in the milk of all 3 ethnic groups was evident and existed, despite diets based on different types of fat between groups, due to the common consumption of fish.

2.12 AFRICAN COUNTRIES:

Van der Westhuyzen *et al.* (1988), studied the fatty acid composition of human milk in rural South African women consuming a traditional maize diet, and in urban South African women consuming a partially Westernized diet. Maize was the main

source of EFA in the rural women's diet having a high content of LA and practically devoid of LNA, increasing their risk for n-3 EFA insufficiency. The BM fatty acid profiles of this group reflected their dietary intake in that low levels of LNA and DHA were found relative to LA and AA respectively. Rural women had abnormally high ratios of LA/LNA and AA/DHA in their BM, being 157:1 and 10:1 respectively, while urban women had more typical values of 40:1 and 3:1 respectively. Total DHA content of rural mothers was low at 0.1 ± 0.2% of total BM fatty acids. In addition to low LNA intakes, the diets of the rural women were low in fat and energy, high in carbohydrate and practically devoid of DHA. Due to these dietary patterns, the amount of fatty acids synthesized de novo in the mammary gland, which are the intermediate and medium chain saturated fatty acids (IMCSAFA) C8:0 to C14:0, were higher in the rural women. This study demonstrated the fact that poor DHA BM levels are related to low maternal n-3 fatty acid intake and/or an extremely high n-6 fatty acid intake relative to n-3 intake, which may compromise the cognitive and visual development of BF infants.

Rocquelin *et al.* (1998), assessed the fatty acid composition of mature BM in Congolese women. The authors stated that in comparison to profiles from other countries, a higher proportion of IMCSAFA were found in Congolese BM samples (25.97 ± 8.17% of total fatty acids). Typical values for this group of fatty acids range from 16% to 18% of total BM fatty acids (Anonymous 1998). The above average content of IMCSAFA found in their BM was attributed to their frequent ingestion of carbohydrate rich food items. The appearance of high levels of IMCSAFA in the BM of this group, was related to OA content, showing a strong negative correlation between OA and C12:0 as well as between OA and C14:0 ($r = -0.85$ and $r = -0.89$, respectively $p<0.01$). In support of the authors' findings, it has been long established that a high carbohydrate diet is associated with the biosynthesis of high levels of IMCSAFA (Insull *et al.* 1959; Read *et al.* 1965a; Read *et al.* 1965b; Hachey *et al.* 1989, Silber *et al.* 1988). The Congolese BM samples were also found to be rich in PUFA content, having 0.55 ± 0.22% of total fatty acids present in the form of DHA, 1.19 ± 0.46% as LNA, 0.44 ± 0.09% as AA and 13.65 ± 3.63% as LA. Adequate PUFA content was attributed to their frequent consumption of

fish, vegetable oil, and high fat fruit such as avocado and bushbutter. Therefore, the fact that dietary composition affects the fatty acid profile of BM was demonstrated in this investigation, most notably in terms of the effect of a high carbohydrate diet.

An investigation assessing the BM fatty acid profiles of 11 well nourished Bantu mothers living in a semi urban area in Tanzania, revealed a relatively high proportion of IMCSAFA (Muskiet *et al.* 1987). The authors speculated that the consumption of a carbohydrate rich, low fat diet, typical in this region and other developing countries, may be responsible for the increased levels of IMCSAFA (approximately 35%), in the BM samples. Content of EFA in the BM was within typical ranges, having 13.88 ± 5.32% present in the form of LA, 0.98 ± 0.44% as LNA, 0.60 ± 0.13% as AA, and 0.27 ± 0.11% as DHA. This shows that achievement of adequate EFA content in BM is possible, even when consuming a high carbohydrate, low fat diet.

2.13 Latin American and Carribean Countries

The fact that a high carbohydrate diet leads to the incorporation of a high proportion of IMCSAFA has been illustrated in Dominica. Van Beusekom *et al.* (1990), reported that 45 % of total milk fatty acids were comprised of IMCSAFA in Dominican women, while 24% of these fatty acids were found in the BM of women living in Belize investigated in the same study. Higher carbohydrate intakes in Dominica were held accountable for the increased levels of IMCSAFA as compared to the group from Belize. As in the investigation by Rocquelin *et al.* (1998), the incorporation of IMCSAFA in the Dominican group was found to take place at the expense of OA and to a lesser extent of the long chain saturated fatty acids, C16:0 and C18:0. Average levels of n-3 LCP were also found to be more than adequate in the Dominican group having a proportion of DHA equal to 0.91% of total fatty acids, while the group from Belize had only 0.21% present in their BM samples. The authors speculated that the high proportion of DHA in the BM from Dominica was due to consumption of DHA rich local fish, while no comment for DHA levels in Belize was made. However, a recent review categorized the diet of the group from Belize as approaching Westernized diets (Jensen 1996), which may be

responsible for the fatty acid profile of their BM. Therefore, it is apparent that a high carbohydrate intake leads to the incorporation of a high proportion of IMCSAFA in BM, which does not affect the appearance of DHA, provided there is an adequate intake of n-3 fatty acids.

Muskiet *et al.* (1987), assessed the fatty acid composition of well nourished women living in Curacao and Surinam. The proportion if IMCSAFA was higher than that typically found in Western countries, being 24% of total fatty acids in the group from Curacao and 28% of total fatty acids in the group from Surinam. This is in accordance with levels of these fatty acids found in other non Westernized countries, as mentioned in the previous paragraphs. Levels of EFA in the BM were within typical values for both Surinam and Curacoa with AA comprising $0.71 \pm 0.17\%$ and $0.56 \pm 0.15\%$ for each respective country, and DHA making up slightly over 0.4% of total fatty acids in both countries. As with the profiles described in the previous paragraph for the group from Dominica, it appears as if adequate DHA status is possible even with the consumption of a diet that is higher carbohydrate and lower in fat than in Western countries.

2.14 Western Countries

Serra *et al.* (1997), assessed the fatty acid profiles of BM in 20 Italian women over time. Samples of mature milk were collected at 2 to 4 weeks postpartum. The authors claimed that levels of all BM fatty acids for the group they investigated fell within the ranges reported for European countries, more closely reflecting the profiles of other Southern European countries. The level of monounsaturated fatty acids (MUFA) in the BM of the Italian women was on the higher end of the range, comprising almost 40% of total fatty acids, as was seen in a group of Spanish women (Pita *et al.* 1985). Claiming that European countries in Southern regions have similar dietary habits, the authors stated that similarity in lipid profiles of these groups may be due to high consumption of MUFA through olive oil, and low consumption of SAFA. Serra *et al.* (1997), found the LNA content in the BM of the group of Italian women they studied to be lower than that reported in other European countries, and DHA was also low at 0.12% of total fatty acids.

However, low levels of n-3 fatty acids in BM were not apparent in the Spanish study, where all EFA levels were within normal ranges. This leads to the assumption that high MUFA intake most likely did not have a negative impact on n-3 fatty acid levels. Dietary intake was not assessed in the Italian investigation, which can only lead to speculation for levels of EFA in BM samples. The impact of these findings on the visual and neurological development of BF infants in this Italian region may warrant attention due to low levels of n-3 fatty acids.

Sanders and Reddy (1992), investigated the effect of vegan and vegetarian diets on the fatty acid composition of human BM in England. The percentage of BM fatty acids present in the form of LA was significantly higher in the group of vegan women than in an omnivore control group. It is possible that the high LA levels could in part be attributable to the fact that the LNA present in vegans did not undergo ample conversion to DHA due to enzymatic competition. This is evident when observing the proportion of BM fatty acids present as DHA, which was found to be significantly higher in the omnivorous group than in the vegan group. Results from the dietary analysis of this study revealed that vegan women consumed a diet with an LA/LNA ratio of 20:1 while the dietary ratio for omnivorous women was 10:1. Also, vegan diets were totally devoid of LCP, making conversion from LNA the only possible source for the appearance of DHA in the milk of this group. The above study supports the fact that the adequacy of long chain n-3 PUFA in human milk, namely DHA, is contingent on the dietary presence of this LCP as well as the LA/LNA ratio of the diet.

Innis and Kuhnlein (1988), compared the BM fatty acid content of Inuit women consuming a traditional diet high in marine oils to Vancouver residents consuming a typical mixed North American diet. The Inuit women had significantly higher DHA levels in comparison to the Vancouver residents ($1.4 \pm 0.4\%$ vs. $0.4 \pm 0.2\%$), indicating that presence of DHA in BM varies with DHA intake. This study supports the fact that regular consumption of food items high in LCP such as DHA, are reflected in BM fatty acid levels, thereby indicating that the degree of the presence of this fatty acid in human milk is dependant on the degree of its consumption. In support of this, the effect of

maternal DHA supplementation through fish oil capsules has also been investigated, showing a strong dose dependant effect on BM levels of this fatty acid (Harris *et al.* 1984; Makrides *et al.* 1996)

The presence of trans fatty acids in human milk of Canadians was recently investigated (Ratnayake & Chen 1996; Chen *et al.* 1995). Breast milk profiles revealed the presence of *trans* fatty acids, averaging 7.19 ± 3.03 % of all fatty acids in milk samples of 198 Canadian women from across the country. The distribution of 18:1 *trans* isomers revealed that the major source of these *trans* fatty acids was from partially hydrogenated vegetable oils, leading to the conclusion that intake of food items containing these fatty acids in Canada is substantial (Ratnayake & Chen 1996). Despite normal levels of BM LA and LNA (10.47 ± 2.62% and 1.16 ± 0.37% of total fatty acids respectively), their long chain metabolites AA and DHA were low (0.35 ± 0.11% and 0.14 ± 0.10% of total fatty acids respectively). One possible reason could be that *trans* fatty acids may inhibit the degree of chain elongation and desaturation that occurs in their absence (Rosenthal & Doloresco 1984; Houwelingen & Hornstra 1994), which may lead to poor long chain EFA status and impaired growth in infants (Koletzko 1992). The amount of LA and LNA present in BM was found to be affected by the quantity of *trans* isomers, showing highly significant negative correlations between these variables. Therefore, other factors aside from ingestion of EFA may be important in the metabolism of these fatty acids and the degree of the presence of LCP in human milk.

The fact that *trans* fatty acids may have a negative effect on the health and growth of infants, prompted another investigation assessing levels of these fatty acids in BM from 15 German women (Koletzko *et al.* 1988). Median levels of total *trans* fatty acids in this group were found to be 4.4% of total fatty acids, while levels of AA and DHA were within typical values, but on the lower end of the scale. A group of French women also revealed the presence of *trans* fatty acids in their BM, but to a lesser degree than the German group (Chardigny *et al.* 1995). Content of DHA in the BM of the French women ranged from 0% to 0.82% of total fatty acids, having an average of 0.32 ± 0.2%. As averages for all milk samples were presented, the higher end of the DHA range may be

reflective of the different sampling times, which ranged from <5 days (colostrum) to ≥90 days postpartum. Therefore, average DHA content of mature milk may be lower than the average presented, as the proportion of LCP in BM is known to decrease as lactation progresses (Jensen 1989). Unfortunately, the authors of these 2 investigations did not consider whether an association between *trans* isomers and DHA or AA existed, which could have led to a hypothesis of whether the degree of presence of *trans* fatty acids resulted in a lowering of long chain EFA in BM for these groups. The complete absence of DHA in some samples and presence of *trans* isomers in all, validates further research evaluating the effect *trans* fatty acids and Western diets may incur on the nutritional quality of human milk and infant development.

2.15 Summary

The above mentioned investigations support the hypothesis that human BM fatty acid composition clearly reflects maternal diet. It has been found to vary among people of different cultures with differing dietary habits. Presence of abnormally high levels of IMCSAFA in human BM has been related to dietary patterns. Populations consuming a diet rich in high carbohydrate food items, typical of Third World countries, have been found to have dramatically higher levels of these fatty acids in comparison to persons consuming a typical Western diet. Higher levels of IMCSAFA have also not been found to affect the LCP content of BM, provided that dietary intake of EFA is adequate.

It appears as if inhabitants of non-Westernized countries typically have higher levels of LCP than those residing in industrialized countries. This finding is thought to be reflective of diet. The adequacy of DHA levels in BM appears to be reliant on DHA intake and/or LNA intake and LA/LNA ratio of the diet, the latter 2 points being of greatest significance in the absence of dietary DHA. The appearance of high levels of *trans* fatty acids in BM may also have an effect on the amount of EFA present, as was noted for Western countries (Ratnayake & Chen 1996; Chen *et al.* 1995; Koletzko *et al.* 1988) Therefore, it can be hypothesized that a population with an adequate proportion of DHA in their BM, have appropriate dietary EFA intake with respect to absolute amounts

and ratios, and most likely do not consume high amounts of *trans* fatty acids. This point warrants further attention in terms of the negative effects *trans* fatty acids, which are commonly found in Westernized diets, may have on the growth and neurological development of BF infants (Houwelingen & Hornstra 1994). The importance of EFA in the neurological development of infants has been under investigation for a number of years, showing benefits of BF in humans due to the presence of LCP in human milk.

2.2 Essential Fatty Acid Status and Neurological Development

2.21 Essential Fatty Acids and Central Nervous System Structure

The CNS can be thought of as the central processing unit of the human body, which has a highly specialized structure to allow for the proper coordination of various innate functions. In relation to other organs, the brain contains a very high concentration of lipids, which serve structural and functional (not energetic) purposes (Bourre *et al.* 1991). In terms of structure, EFA of the n-3 series are one of the largest biochemical components of the cerebral cortex and retina (Connor & Neuringer 1988). The most notable contributors of all EFA in the CNS are DHA and AA. However, AA is found in relatively large amounts in membranes of all tissues, while DHA is scarce in non-neuronal membranes (Bazan 1990). Docosahexaenoic acid has been found to account for about one third of cerebral cortex and 35%-60% of photoreceptor outer segment (part of the retina) fatty acids in certain phospholipid subclasses of various animals, including humans (Bazan 1990). The fact that nerve endings of retinal photoreceptor cells are so enriched with DHA, suggests that this fatty acid plays an important role in neuronal transmission and the visual process (Martinez 1991). Therefore, replacement of the dietary essential n-3 fatty acids in these tissues when they are not available for optimal levels of incorporation, leads to altered structure, which may have an impact on function. As early infancy is a known period of rapid growth, which is especially evident in the brain, adequate dietary intake of EFA is thought to be required for the attainment of

optimal fatty acid composition in the CNS. As function may be affected when optimal intakes are not possible, tests of CNS function, such as visual acuity development, can be performed to assess adequacy of development.

2.22 Tests of Rod Function and Visual Development

Various tests for assessing rod and visual function in infants, small children, and non verbal adults exist. In research assessing the impact of n-3 fatty acids on rod function and visual development, the rod electroretinogram (ERG), visual evoked potentials (VEP), and forced choice preferential looking acuity tests (FPL) are commonly used. The ERG gives an estimation of rod function (activity) by measuring the retinas response to a brief flash of light as detected by contact lens electrodes placed on the eye (Uauy *et al.* 1992). Several measurements of response output can be made by this method, including; amplitude threshold, which is the intensity of light required to elicit a criterion amplitude response peak, and implicit time or peak latency, which is the time it takes to obtain the response peak (Neuringer *et al.* 1994). In comparison to the other 2 methods, the ERG requires the most expensive equipment and specialized personnel, but has the advantage of rendering a result which is objective in nature.

A method of evaluating the neural integrity of the pathway from the retina to the primary visual cortex through an electrophysiologic response is the VEP (Anonymous 1998). In this method, infants view checkerboard or grating patterns of varying sizes, on a video display, which undergo contrast reversal, having each change evoke a response from the primary visual cortex which is detected by electrodes (Uauy *et al.* 1992). Based on amplitudes of response peaks and pattern size, acuity thresholds can be extrapolated. This method also requires highly specialized equipment and personnel.

Forced choice preferential looking acuity tests are a behavioral measure of visual acuity which relies on the maturation of systems beyond the visual cortex, thereby developing later than VEP acuity (Neuringer *et al.* 1994). It is considered to be a standardized measure of neurological maturation (Anonymous 1998). Investigations most often use the Teller Acuity Card (TAC) system, a set of cards with patches of high

contrast gratings (black and white vertical stripes of equal width) on one side and a luminescence matched gray background (Teller 1989). This procedure is subjective as it relies on an observers interpretation of an infants behavioural response to the series of gratings (Teller *et al.* 1986). Nevertheless, it has the advantage of requiring less expensive equipment than the previously mentioned tests. Cards are presented in order from coarser to finer gratings and the infant produces a behavioural/motor response, such as a look by turning of their head, or eyes, which is observed by the examiner through a small hole in the middle of the card. The examiner, who is unaware of which side the striped patch is on, makes a judgement about this based on the infants looking response (Uauy *et al.* 1992). As stripe widths decrease, an individuals visual acuity threshold is reached, after which both sides of the card appear the same (ie. the striped patch and gray side), no longer providing a stimulus for looking. Visual acuity threshold using this technique is defined as the last card for which an infant shows consistently correct looking behaviour. Age acuity norms for clinical assessment of infant visual acuity development using the TAC procedure have been recently reevaluated, and provide a basis for assessing functional normality of individuals and groups (Mayer 1995; Rios-Salomão & Fix-Ventura 1995). Ease of training of personnel and relatively inexpensive equipment are benefits of this method over the rod ERG and VEP tests, however, the latter 2 methods are more objective and less prone to human error and bias.

2.23 Neural Tissue Essential Fatty Acid Concentration and Retinal Function in Mammals

Clinical studies in mammalian infants have clearly demonstrated that n-3 fatty acid intake during fetal and early post natal development affects the fatty acid profile of the brain and retina (Ward 1996; Suh *et al.* 1996; Craig-Scmidt *et al.* 1996), which has been found to influence retinal function (Connor & Neuringer 1988; Pawlosky *et al.* 1997; Weisinger *et al.* 1996).

Artificial rearing of female rats and pups on an n-3 deficient formula led to depressed levels of brain DHA in 2 subsequent pup generations (Ward *et al.* 1996). The

first generation showed decreases in brain DHA content of more than 50% at 8 weeks postpartum, while the second generation revealed an even greater level of depression, being more than 90%. In both generations, the decrease in DHA content was balanced by an increase in long chain n-6 fatty acid content, mainly through docosapentaenoic acid (n-6), (DPA(n-6)). In a contrasting study, the effects of small amounts of supplemental LCP in the form DHA and/or AA on the rod outer segment (ROS) fatty acid content of retinal photoreceptors was investigated (Suh *et al.* 1996). Rats fed diets containing supplemental DHA and/or AA showed higher levels of LCP in ROS than groups fed only LA and LNA in adequate ratios. This indicates that their may be a need for LCP intake as opposed only to their dietary precursors for optimal accretion of these fatty acids in neural tissue. Dietary DHA alone was found to lead to increased DHA and AA levels in the ROS as compared to the groups receiving only their precursors. However, AA alone did not lead to increased amounts of ROS DHA. Comparable results were found for retinal phoshatidylcholine (PC) LCP content in piglets fed similar diets to the rats in the above mentioned study or sow milk (Craig-Schmidt *et al.* 1996). These 3 investigations illustrated the relation between dietary EFA intake and retinal LCP composition in 2 different animal models.

Connor and Neuringer (1988), produced an n-3 fatty acid deficiency in newborn monkeys by feeding a safflower oil based diet to mothers, beginning at 2 months pre conception. Another group which was fed a soy oil based diet served as a control. After birth, infant monkeys were fed diets similar to that which their mother previously received. The authors examined the relationship between diet and phospholipid content of plasma, erythrocytes, skin, liver, adipose tissue, the neural retina and cerebral cortex between birth and 22 months. Plasma levels of DHA and total n-3 fatty acids were significantly reduced in the deficient monkeys as compared to the control monkeys at birth, after which levels decreased until they were barely detectable in the deficient group. Other tissues in deficient monkeys were found to contain only 10% to 20 % of the DHA found in control monkeys (Connor & Neuringer 1988). At 22 months, cerebral cortex concentrations of DHA more than doubled in the control group, but remained the same in

the deficient group, indicating that these fatty acids are preserved once present in neural tissues (Connor & Neuringer 1988). Similar results were apparent for retinal DHA content, and in both neural tissues measured, the long chain n-6 fatty acids docosatetraenoic acid (DTA) and DPA(n-6) were found to replace DHA in the n-3 deficient group. The functional impact of altered DHA levels in neural tissues was evident as the deficient group had significantly longer ERG peak latencies and subnormal visual acuity as measured by a FPL test. This study illustrated the essentiality of n-3 fatty acids in the CNS of developing primates.

In another branch of the above study, n-3 deficient monkeys were supplemented with fish oil beginning at 10 months (Connor & Neuringer 1988). An increase in plasma and erythrocyte n-3 fatty acids, accompanied by a decrease in long chain n-6 fatty acids, occurred and stabilized after 12 weeks of fish oil feeding. Similar patterns of n-6 fatty acid replacement were found in the frontal cortex through dramatic increases in n-3 fatty acid content (mainly DHA), which was noted within one week of supplementation. An investigation assessing the effect of n-3 repletion on the fatty acid profiles of each phospholipid sub class in the same group of monkeys found parallel results (Connor *et al.* 1990). Cortex DHA levels in the repleted group even rose above those in the control soy oil group. Despite biochemical improvement as noted by DHA tissue profiles, retinal function as measured by ERG did not improve, even after 9 months of fish oil feeding. The authors speculated that due to the fact that supplementation began after the period of rapid brain development, irreversible alterations of retinal function could have occurred (Connor *et al.* 1988). Another reason could have been that excessively high levels of n-3 fatty acids in the retina may have led to increased susceptibility of oxidative or phototoxic damage, which has a negative impact on retinal structure and thus function (Weisinger *et al.* 1996).

Pawlosky *et al.* (1997), investigated the effect of maternal diet on developing felines. Females were fed one of six diets differing in their fatty acid profile beginning 1 month before mating and continuing throughout pregnancy and lactation. Diets 1 to 4 were practically devoid of n-3 EFA, containing only corn oil and partially hydrogenated

coconut oil, while the remaining 2 controls were supplemented with DHA and AA. Rod ERG were performed on the kittens at 8 weeks, and the group fed diets devoid of LCP had significantly longer response times than controls, indicating that retinal function is at least in part dependant on LCP and/or n-3 EFA intake (Pawlosky *et al.* 1997). Fatty acid analysis of the brain and ROS of the retina revealed that animals fed diets devoid of LCP (and n-3 PUFA), had significantly higher amounts of long chain n-6 PUFA and lower amounts of long chain n-3 PUFA than controls. The authors concluded that corn oil based diets were appropriate for the maintenance of AA in the developing brain and retina but that only the diets containing DHA could support a high accumulation of this fatty acid in neural tissues. Therefore, it is apparent that diets deficient in n-3 fatty acids lead to altered brain and retinal structure in felines, with ensuing functional abnormalities.

Weisinger (1996), assessed the effect of differing retinal phospholipid DHA levels on the ERG response of guinea pigs. Achievement of retinal phospholipid DHA levels ranging from 2.5 ± 0.23% to 30.82 ± 0.31% was feasible by feeding females diets containing different n-6/n-3 ratios for 2 generations, having the third generation continue on the specified diets for 6 to 9 weeks postpartum, when analyses were performed. Success of dietary manipulation was seen through direct correlations between retinal DHA content of third generation animals and both total n-3 supply and n-6/n-3 ratio of the diet. It was observed that long chain n-6 fatty acids were highest in the retinas of the n-3 deficient group, indicating a replacement of n-6 for n-3 fatty acids when the latter are not available through the diet. Electroretinogram responses showed better rod function in groups fed the diets containing moderate levels of n-3 fatty acids, which were devoid of LCP, than the other 2 groups. The n-3 deficient group had poorest retinal function responses, followed by a group fed a very high n-3 diet containing fish oil, with 12.74% of total fatty acids belonging to the n-3 series. The authors contended that increased oxidative stress caused by an abnormally high DHA content, may have led to altered photoreceptor function. They created an inverted U shaped model to explain the relation between retinal DHA content and retinal function as measured by ERG in guinea pigs. According to the model, as retinal DHA content increases, retinal function improves,

reaching its optimal level of functioning at 19% of total fatty acids in guinea pigs. Increases in DHA content beyond 19% of total fatty acids lead to decreased retinal function according to the model. Therefore, it appears as if abnormally high levels of n-3 fatty acids available through the diet lead to higher incorporation into retinal tissue, which may have a negative effect on the development and function of the retina.

2.231 Summary

Neural tissues appear to regulate the level of PUFA they contain, as in n-3 deficiency, long chain n-6 fatty acids appear to replace the long chain n-3 fatty acids which are normally found in abundance (Wards *et al.* 1996; Connor & Neuringer 1988; Weisinger *et al.* 1996). With diets containing very high levels of n-3 PUFA, lower levels of long chain n-6 PUFA have been noted in animal tissue, which may also have a negative impact on CNS development (Weisinger *et al.* 1996). Therefore, it appears as though an appropriate dietary balance of the 2 groups of EFA is required. It is clear that n-3 fatty acid deficiency during the period of rapid brain growth and development has a negative impact on the CNS function of various mammals. There is enough evidence to state that n-3 fatty acids are essential nutrients and their absence during periods of early development show a detrimental effect on the CNS. Therefore all efforts should be made to ensure that humans are not subject to diets containing negligible quantities of n-3 fatty acids, especially during fetal and early postnatal development.

2.24 Neural Tissue Fatty Acid Contents in Human Infants in Relation to Diet

There have been a limited number of investigations assessing the effect of diet on the EFA content of neural tissues in human infants. Direct evidence of diet affecting cerebral DHA content in human infants was demonstrated in a recent autopsy study by Farquarson *et al.* (1992). The fatty acids present in the cerebral cortex of human infants fed either human milk or infant formula were compared. The authors divided the infants into the following 4 groups (n=5 for each); (1) infants fed various formulas whose death occurred between 1 and 15 weeks; (2) BF infants whose death occurred between 5 and 16

weeks; (3) a group of infants whose death occurred after 15 weeks, fed a formula with an LA/LNA ratio of 10:1, and (4) a group of infants whose death occurred after 15 weeks fed a formula with an LA/LNA ratio of 40:1. As mentioned in the previous section on human BM fatty acid profiles, BM is known to contain LCP, while the infant formulas in this study only contained their precursors LA and LNA. When assessing differences between groups 1 and 2, the infants fed formula had significantly lower cerebral DHA levels in comparison to the BF group of similar age. This is thought to be due to the fact that BM is known to be a better source of n-3 fatty acids than formula, and indicates that the degree of the presence of cerebral DHA in infants, is dependant on dietary intake of preformed long chain n-3 fatty acids. No significant differences were found between the 2 formula fed groups of similar age. The authors attributed the lack of other significant relations to the low sample size of the study and reported that the older infants fed formula with an LA/LNA ratio of 40:1 had the lowest DHA level of all groups, being 32% less than the BF group. The older groups did, however, have a higher total cerebral PUFA content which was due to higher levels of long chain n-6 fatty acids. This shows that there is a tendency for cerebral DHA to be replaced by long chain n-6 PUFA in the absence of adequate dietary n-3 intake in humans. The authors conclusions support similar studies done on mammals but a small sample size limits the ability to interpret their results.

Differences in fatty acid composition of several tissues in healthy term infants that were BF or received formula devoid of LCP with an adequate LA-LNA profile were investigated by Makrides *et al.* (1994). Breast fed infants were found to have a greater percentage of DHA present in total lipids of erythrocytes and brain cortex when compared to those fed formula. Infants that were BF also had significantly higher erythrocyte AA levels than formula fed infants, but this relation of diet on AA levels was not seen in the other tissues measured. No significant differences in fatty acid composition of the retina were found between groups. The authors concluded that dietary LCP through BF, led to increased levels of DHA in erythrocyte total lipids, which was related to brain cortex DHA, but that retinal DHA contents were not reliant on dietary LCP intake in this group.

2.241 Summary

It appears as if dietary intake of EFA during early postnatal life may be reflected in the neural tissues of human infants. Those receiving BM, which contains LCP have been found to have higher levels of DHA in the brain or brain cortex when compared to formula fed infants. This was related to erythrocyte total lipid content in one investigation (Makrides *et al.* 1994). The investigation assessing retinal DHA content of human infants, found no differences in relation to feeding practices, despite significantly higher levels in erythrocyte total lipids and the cerebral cortex of BF infants.

2.25 The Effect of Diet on the Essential Fatty Acid Status of Term Infants

The EFA status of infants is clearly related to feeding practices. It has been noted that term infants are capable of forming LCP from their dietary precursors LA and LNA (Salem *et al.* 1996; Sauerwald *et al.* 1996; Koletzko *et al.* 1996). Whether the extent of their ability to do so allows for optimal accretion of these fatty acids into neural tissues remains unknown. Breast milk is known to contain LCP, while standard formula (SF) has what is considered to be adequate amounts of LA and LNA, but does not contain LCP. Therefore, most investigations assessing blood levels of these fatty acids in infants have found that those fed BM have significantly higher blood lipid DHA and AA levels than SF fed infants (De-Lucchi *et al.* 1988; Decsi *et al.* 1995). Addition of LCP to infant formula has been shown to change the fatty acid profile of plasma and erythrocytes in infants so that they resemble those of BF infants, more so than changing the amount of LNA and/or the LA/LNA ratio of infant formulas (Clark *et al.* 1992; Ponder *et al.* 1992; Jensen *et al.* 1996; Hayes *et al.* 1992; Agostini *et al.* 1994; Kohn *et al.* 1994), and supplementation of BF mothers (Henderson *et al.* 1992).

De-Lucchi *et al.* (1988), prospectively assessed the impact of BF over SF feeding on fatty acid levels of various red blood cell (RBC) phospholipid fractions during the first month of life. Blood samples were taken at birth, 7 days and 30 days. No differences existed between groups until 30 days, when infants fed SF had significantly lower total n-3 and n-6 fatty acid RBC phosphatidylethanolamine (PE) concentrations than BF infants.

In all other phospholipid fractions, BF infants had higher n-3 and n-6 fatty acid contents than those fed SF at 30 days, but not all differences were statistically significant. Differences in the EFA status of BF and SF fed infants was also demonstrated by Decsi *et al.* (1995). Plasma phospholipid analysis revealed significantly higher median values of DHA at 4 and 8 weeks, and AA at 2 and 4 weeks in a group of BF infants as compared to infants fed SF. The level of depression of DHA in the formula fed group was about 55%, showing the dramatic difference in this biochemical measure between groups. Both investigations support the notion that feeding SF rather than BM may induce changes in circulating LCP levels, but whether this induces functional changes in cell membranes of the developing CNS remains unknown.

Clark *et al.* (1992), prospectively investigated the fatty acid profiles of total lipids in RBC and plasma of infants fed BM or randomly assigned to SF with varying LA/LNA ratios. It was found that infants fed formula with the highest LA/LNA ratio of 19.2:1 were susceptible to a reduction in plasma and erythrocyte DHA levels when compared to groups fed 1 of 2 other formulas with lower LA/LNA ratios. However, none of the formula fed groups reached the DHA levels found in BF infants, nor did any of the groups reach levels of AA in the erythrocytes of BF infants. This shows that even though there was a substantial amount of dietary LA, the precursor to AA, in the group with the highest LA/LNA ratio, it could not be converted to AA at a level to give rise to an RBC profile matching or exceeding that of BF infants. The authors also used a set of equations to predict the LA/LNA ratio required to produce a level of long chain n-3 PUFA comparable to that of BF infants. They predicted that a dietary ratio of these fatty acids of 2:1 or less could attain tissue levels similar to that of BF infants, but that this would result in a reduction in AA levels. The authors also concluded that it is possible that dietary LA has its limitations in providing a source of AA to the developing infant, indicating the importance of including LCP in infant formula. Despite the fact that supplementing SF with LCP may improve the EFA status of infants, the importance of promoting BF among all populations should be considered a more appropriate and logical option.

Ponder *et al.* (1992), prospectively assessed the effect of randomly feeding infants

SF based on soy oil or corn oil for the first 2 months of life. The difference in the LA/LNA ratio of the formulas in this investigation was greater in magnitude than the previously mentioned investigation, being 7:1 for the soy oil group and 39:1 for the corn oil group. Despite this large difference, both groups of formula fed infants had similar levels of DHA in plasma phospholipids, and erythrocyte PE and PC at 1 and 2 months, while having lower values than a control group of BF infants in most blood fractions at most time points. The formula fed infants also showed higher levels of LA in all blood fractions at 1 and 2 months than BF infants. Both this investigation and the one previously mentioned by Clark *et al.* (1992), showed better DHA status in BF infants than formula fed infants who had no preformed source of LCP. In contrast to the above mentioned study, the investigation by Ponder *et al.* (1992), showed that large variations in LNA intake and LA/LNA ratio had no measurable effect on DHA levels. This may be due to the fact that each investigation measured different fractions of lipids in erythrocytes and plasma. Whether this led to differences in outcomes remains unknown.

Hayes and colleagues (1992), studied the effect of feeding SF or BM on the EFA profile of total lipids in plasma and erythrocytes of healthy term infants at 3 months. Infants that were not BF were randomly assigned to receive SF with an LA/LNA ratio of 10:1 or 29:1. No significant differences were found in DHA levels between the 2 formula fed groups in RBC or plasma. A significant difference was found between the 29:1 LA/LNA ratio group and BF infants for the proportion of DHA in erythrocytes, but the authors speculated that it was not of biological significance. No significant differences were found for plasma fatty acid levels of DHA or AA, nor for erythrocyte AA between groups. This may be due to the fact that the long chain n-3 PUFA content of the human milk fed to the infants in this study was low, having only $0.2 \pm 0.1\%$ of total fatty acids present as a combination of 2 long chain n-3 PUFA, DHA and eicosapentaenoic acid (EPA). The levels of both DHA and AA in erythrocytes were lower than that reported in the above mentioned study by Clark *et al.* (1992), for BF infants. In that investigation, the DHA content of BM accounted for 0.32% to 0.64% of total fatty acids and when combined with the other long chain n-3 PUFA identified, made up 0.69 to 1.42 % of total

BM fatty acids. Therefore, the lack of strong significance between groups may have been due to the fact that the BM did not contain a significant proportion of long chain n-3 PUFA. However, the authors concluded that the depressed levels of DHA and AA in their samples were due to peroxidative losses caused by a somewhat lengthy storage of frozen samples.

The effect of feeding infants with differing LA/LNA ratios on blood lipid profiles was recently assessed by Jensen *et al.* (1996). All infants were randomly assigned to receive 1 of 4 formulas as the sole source of nutrition for the first 4 months of life. All formulas contained 16% of total fatty acids in the form of LA, with the following variations in LNA content, 0.4%, 0.95%, 1.7% and 3.3%. Plasma fatty acid measures were related to diet, with infants receiving the highest LNA intake showing significantly higher levels of DHA in plasma phospholipids and plasma total lipids, and those receiving the lowest LNA intake showing significantly higher levels of AA at 21, 60 and 120 days. Despite relatively higher plasma AA and DHA levels in the above mentioned groups, levels of these fatty acids did not reach those reported in other studies for BF infants (Jensen *et al.* 1996). Analysis of erythrocyte phospholipids did not show any differences among groups, except for significantly higher DHA levels in the group receiving the highest LNA content at 120 days. For all groups RBC phospholipid DHA was lower than that reported for BF and enriched formula fed infants and similar to SF fed infants in the study to follow by Agostini *et al.* (1996). The authors concluded that the variations of LA/LNA ratio they provided could not produce levels of DHA or AA found in BF infants. This indicates the possible importance of including LCP in infant formula, but more importantly of promoting BF, as it naturally contains LCP as well as many other important constituents not found in formula.

Infants randomly fed SF or formula enriched with egg lipids providing long chain n-3 and n-6 PUFA showed differences in blood fatty acid profiles at 4 months (Agostini *et al.* 1994). The enriched formula group and a group of BF control infants had significantly higher levels of AA and DHA in plasma and erythrocyte phospholipids and plasma total lipids than did infants fed SF. In the former 2 groups, DHA levels were

more than double of that in the SF group in the fractions of blood analyzed. The authors concluded that accumulation of LCP was reliant on dietary intake, which was best met by supplying AA and DHA directly, either through BM or enriched formula in the group of term infants studied.

A prospective longitudinal investigation, also using egg lipids as a source of LCP in enriched infant formula, found similar results (Kohn *et al.* 1994). Infants whose parents elected not to BF, were randomly assigned to receive SF or the enriched formula, taking measures at baseline, day 7, 1 month and 3 months. Infants fed SF had significantly lower erythrocyte PC and plasma phospholipid DHA and AA than those receiving the enriched formula at 1 and 3 months and erythrocyte PE DHA at 3 months. Breast fed and enriched formula fed infants exhibited similar AA and DHA contents at 1 and 3 months in all blood fractions measured. Significant difference between the SF and BF groups were identical to those mentioned for the enriched and SF groups in terms of DHA content. However, significant differences between SF and BF infants were not apparent at 3 months for AA content in the blood fractions mentioned above. The authors concluded that the addition of LCP to infant formula, led to DHA and AA concentrations similar to those of BF infants, which were not achieved by those receiving SF, most likely due to limited enzyme activity in term infants (Kohn *et al.* 1994).

With the aim or examining the effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes, Henderson *et al.* (1992) supplemented lactating women with 6 g of fish oil daily for 21 days. Changes in BM LCP consisted of a significant increase in each long chain n-3 fatty acid, including DHA. Despite a trend for erythrocyte DHA to increase from baseline levels, (4.5% to 5.6% in mothers and 4.5% to 6.1% in infants) no significant changes were found. It is possible that a lack of statistical significance was due to the low sample size of 5. However, two other long chain n-3 fatty acids, EPA and docosapentaenoic acid (n-3) (DPA(n-3)) increased significantly in erythrocyte total lipids of mothers and infants. Therefore, it is possible to increase levels of long chain n-3 fatty acids in mothers and BF infants through maternal fish oil supplementation.

2.251 Summary

Investigations evaluating EFA levels in plasma and erythrocytes of infants subject to different feeding practices have shown that LCP levels are affected by the type and amount of EFA present in the diet. Decreasing the LA/LNA ratio in SF has been found to cause a shift favouring the production of DHA as seen in some investigations. Nonetheless, DHA and AA levels of BF infants are still significantly higher. Superior AA status in BF infants has been noted even when total LA content and LA/LNA ratio of formula were higher than in BM. However, when BM fatty acid concentrations of LCP were low, infants showed suppressed DHA and AA levels in comparison with BF infants in other investigations. Supplementation of SF with LCP at levels similar to that found in BM, has been found to lead to EFA blood profiles similar to that of BF infants. Therefore, it appears as if dietary intake of LCP, as opposed to only their precursors LA and LNA, leads to increased blood levels of DHA, which may have functional implications in the neurological development of human infants.

2.26 Essential Fatty Acids and Visual Development in Human Infants

Investigations relating visual acuity and cognitive development to feeding practices and infant EFA status have shown contradictory results. Some discrepancies may be due to age group studied, method of visual acuity assessment and degree of EFA sufficiency while others may be due to bias. Tests of visual acuity development tend to be of most significance when studying populations fed a diet with a poor n-3 fatty acid profile (Birch *et al.* 1993). Some investigations have clearly found an association between VEP acuity and infant EFA intake (Birch *et al.* 1998; Makrides *et al.* 1993) while the validity of other studies showing similar results with VEP or TAC methods are questionable (Makrides *et al.* 1995; Courage *et al.* 1998; Jørgensen *et al.* 1996). Many well designed recent investigations have failed to find an association between these measures in healthy term infants (Calrson *et al.* 1996; Innis *et al.* 1996; Innis *et al.* 1994; Gibson *et al.* 1997; Jensen *et al.* 1997). To try to understand the discrepancies in this field of research, some recent investigations used a multicenter approach with the aim of

decreasing potential bias (Innis *et al.* 1997; Auestad *et al.* 1997).

Birch *et al.* (1993), prospectively investigated the effect of feeding corn oil based formula, which has been used to produce n-3 fatty acid deficiency in animal models, on the retinal and neurological development of term and preterm human infants. A group of BF infants in each group (term and preterm), served as a control. At 4 months corrected age, term and preterm infants fed n-3 fatty acid deficient formula exhibited significantly poorer visual acuity development than their BF counterparts as assessed by VEP and the TAC procedure. Acuity was also strongly related to RBC total lipid DHA/DPA(n-6) ratio which the authors used as an index of n-3 fatty acid status. As term infants were followed for 36 months, long after formula ceased being the sole source of nutrients, authors maintained low levels of n-3 fatty acids in this group by supplying an LA rich supplement and stipulating that supplementary foods containing only low n-3 fatty acid content be fed. Levels of n-3 fatty acids were maintained after weaning in the BF group by providing egg yolk as a solid food source of n-3 fatty acids. The variety of visual and behavioural tests performed at 36 months, showed that children who received n-3 deficient diets had significantly poorer visual stereoacuities and letter matching ability than the BF group. Stereoacuity and letter matching scores were also significantly correlated to the DHA/DPA(n-6) ratio indicating that dietary differences led to biochemical differences which had an effect on CNS function up to 36 months (Birch *et al.* 1993). No statistically or clinically significant differences were found for visual acuity scores at 36 months. However, there was a trend for BF children to have better acuities than the n-3 deficient group. No significant differences were found between groups for picture naming or colour vision tests. The results of this investigation indicate that term and preterm infants require dietary n-3 fatty acids to attain optimal CNS function and that biochemical indices of DHA status are related to visual acuity development using both VEP and TAC. However, the formula used in the experimental group is no longer deemed acceptable due to its ability to produce n-3 fatty acid insufficiency, which may be why differences in visual acuity were so clearly defined between the formula fed and BF groups.

The impact of feeding formula with supplemental LCP on fatty acid profiles of RBC total lipids and visual acuity was recently assessed by Birch *et al.* (1998). Infants that were to be bottle fed were randomly assigned to receive one of the following 3 formulas at birth; SF, formula with 0.35% DHA, or formula with 0.36% DHA and 0.72% AA. A group of exclusively BF infants served as a control and all diets were mandatory until 17 weeks of age, after which infants were permitted to receive unspecified supplemental foods. No differences in RBC fatty acid levels were apparent between groups at birth. Infants receiving SF had significantly lower levels of DHA at all time points measured (17 and 57 weeks) which coincided with significantly lower visual acuity as measured by VEP, at 6, 17, and 57 weeks, but not at 26 weeks when compared to the 3 other groups. The authors explained that a plateau in visual acuity development occurs between 25 and 35 weeks, making differences more difficult to detect during this period (Birch *et al.* 1998). As lower visual acuity was consistent with lower DHA levels and persisted long after formula ceased being the sole food source, the authors hypothesized that adequate dietary DHA is critical during the early period of development, as it may lead to lasting changes in underlying neural structure or function (Birch *et al.* 1998). Regression analysis of erythrocyte lipids and VEP acuity also illustrated the influence of diet on visual acuity development, with DHA and n-3/n-6 ratio giving significant results at every time point except 26 weeks. The only biochemical difference noted among enriched formula groups were significantly lower AA levels in the group receiving formula containing only DHA as compared to infants receiving BM or formula with AA and DHA at 17 weeks, indicating that a preformed source of AA may be required in infant formula to maintain levels similar to that of BF infants. However, there were no differences among these groups in terms of growth or visual acuity, so the functional and physiological impact of lower AA levels is yet to be determined. Visual acuity was also assessed by the TAC procedure, but the authors stated that if differences existed among groups, they were too small to distinguish with this method.

A retrospective investigation by Makrides *et al.* (1993), also found a strong relation between visual acuity development and EFA status in 22 week old infants.

Breast fed infants were found to have significantly higher DHA and lower LA levels in RBC total lipids and significantly better VEP outcomes than infants fed SF. A strong correlation was found between VEP acuity and DHA levels indicating that poor DHA status may lead to impaired visual development (Makrides *et al.* 1993). A strong correlation between acuity and erythrocyte LA levels was also found, illustrating that high LA levels are related to poorer visual acuity development. However, the trend appeared to be more dependant on the SF fed infants than the BF group. Therefore, it may be that high LA levels in infants receiving SF led to lower DHA formation from its precursor LNA (their only source of DHA), and that the resulting poorer DHA status is what affected visual development. This may be the case, as the correlation line of LA and acuity score for the BF infants appeared to be zero. According to this investigation, biochemical indices of DHA status are strongly related to the functional outcome of visual acuity development, indicating the importance of creating infant formulas that more closely resemble human milk and more importantly, promoting BF.

Makrides and colleagues (1995), also found significantly better erythrocyte DHA status and visual acuity development in BF infants and infants receiving formula enriched with n-3 fatty acids than SF fed infants. Erythrocyte DHA levels were significantly higher in the enriched formula fed infants than exclusively BF infants at 16 and 30 weeks. The SF fed group had significantly lower DHA levels than the other 2 groups at both time points. These changes coincided with better visual acuity as measured by VEP in infants receiving the enriched formula or BM, when compared to the SF fed group at 16 and 30 weeks. The VEP findings at 30 weeks are in disagreement with the study by Birch *et al.* (1998), where differences in VEP acuity were not found during the 25-35 week visual acuity development plateau period. Lack of agreement between investigations may be due to the fact that differences in number of infants used in statistical analyses of the study by Makrides *et al.* (1995), varied by feeding group with poor explanations by the authors. The number of infants used in VEP analysis were 5/13 (38.5%) for the enriched formula group, 16/19 (84.2%) for the SF group, and 15/23 (65.2%) for the exclusively BF group, while almost all infants were included in the fatty acid analysis. The authors

claimed that there were no differences among groups in their ability to extrapolate acuity threshold, but it appears as if there were other reasons for differences in number of subjects used per group, which are not given. Therefore, the findings of this investigation are questionable in terms of bias that may have existed in completion and use of VEP tests by feeding group.

In the above investigation, 24 mothers who intended to exclusively BF, weaned infants onto formula before 16 weeks (Makrides *et al.* 1995). The authors therefore assessed differences in visual acuity development as a function of duration of BF. At 30 weeks, infants who ceased receiving BM before 16 weeks (n=5), had similar VEP measures as infants who were fed SF since birth, which was significantly poorer than infants who were exclusively BF (n=15) or BF for more than 16 weeks and less than 30 weeks (n=5). The fact that a significant difference in VEP acuity was found between the 2 groups of not exclusively BF infants during the visual acuity developmental plateau should have been discussed by the authors, as well as the DHA status of these groups. Surprisingly, average VEP at 16 weeks was poorer in the BF for <16 weeks groups than in term infants of comparable age fed an n-3 deficient corn oil diet in the study by Birch *et al.* (1993). This finding is questionable and the authors do not compare VEP scores to normative clinical values, stating only that differences between diet groups were within those found in other studies (Makrides *et al.* 1995). As 24 infants belonged to the non-exclusive BF group, and only 10 (5 per subgroup), were used in VEP statistical analyses, reasons for missing values should have been presented if the results of this investigation are to be taken seriously. Also, differences in blood lipid profiles between these groups are not shown, and averages are only present for the group of infants who were exclusively BF. Despite these discrepancies, which were not adequately discussed, the authors came to the conclusion that a continuous DHA supply may be necessary for optimum VEP acuity which is noted when exclusive BF continues past 4 months. Lack of inclusion of all groups (ie. non exclusively BF) in biochemical analyses and lower percentages of VEP inclusion in some feeding groups may also be the reason that regression analysis showed a relation between erythrocyte DHA and VEP acuity at 16 and

30 weeks (r^2=0.23, $p<0.001$ and r^2=0.12, $p<0.005$), which was stronger when the SF group was assessed alone (r^2=0.37, $p<0.001$ and r^2=0.17, $p<0.05$). Despite the number of significant relations found, the possibility of bias should be considered when assessing the results of this investigation.

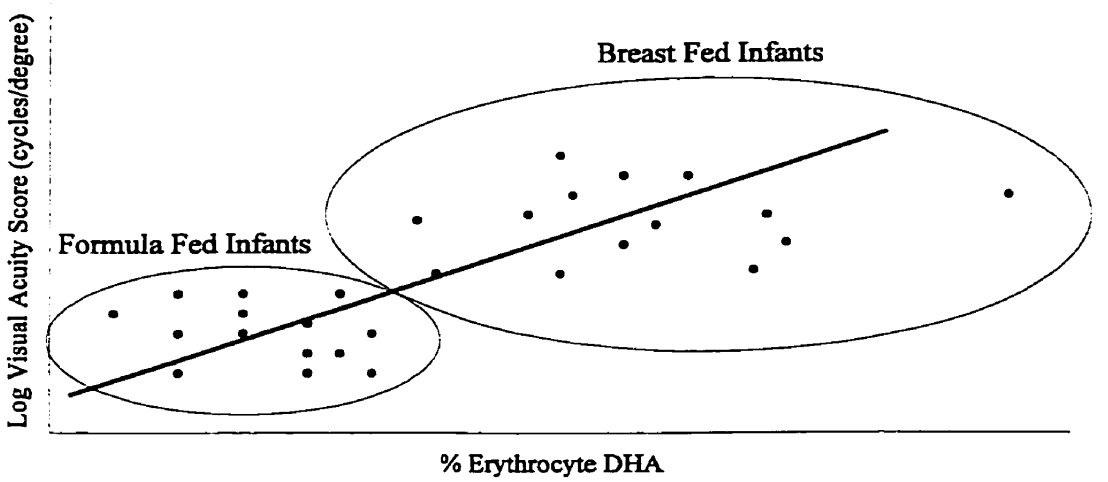
Courage *et al.* (1998), prospectively assessed erythrocyte PE fatty acid proportions and visual acuity development in infants receiving SF, evaporated milk or BM by parental choice. The highest levels of LCP were found in BM, followed by evaporated milk, while SF was devoid of all LCP, but had higher contents of their precursors LA and LNA. Fatty acid content of erythrocyte PE varied by diet group, showing a significantly higher proportion of DHA in BF infants at 3 and 6 months and higher AA at 3 months in comparison to the 2 other groups. Infants fed evaporated milk had significantly higher DHA levels than the SF fed group at 3 and 6 months, and significantly lower levels of LA. This may be due to the fact that the LA/LNA ratio of evaporated milk was very low (2.9:1), which was due to the low amount of LA (2.3% of total fatty acids). Surprisingly, AA levels were not affected by this abnormally low LA content and LA/LNA ratio, which may have been due to the presence of long chain n-6 fatty acids in this milk. Visual acuity was assessed with the TAC procedure, showing significantly better outcomes in the BF group as compared to the evaporated milk group at 3 and 6 months, but not at 18 months, which led the authors to conclude that earlier differences were transitory (Courage *et al.* 1998). The SF fed group had average visual acuity scores that fell between the 2 other groups, not being significantly different from either of them. This finding is questionable, as the SF group had the lowest DHA and highest LA levels at both time points, which have been related to poorer visual acuity development in artificially fed infants (Makrides *et al.* 1993). Reasons for this finding are unknown and left unexplained by the authors. Despite statistically significant differences between some of the diet groups, visual acuity averages fell within age norms for all groups, indicating that no clinical differences existed between groups (Courage *et al.* 1998). This findings of this investigation suggest that the TAC procedure may have limitations in assessing differences in visual acuity as related to DHA status.

Differences in erythrocyte DHA content and visual acuity were prospectively assessed in BF and SF fed infants at 1, 2 and 4 months (Jørgensen *et al.* 1996). Fatty acid analysis of erythrocyte PE and PC, showed higher DHA levels in BF infants than SF fed infants at 2 and 4 months. This coincided with significantly higher increases in visual acuity score over time as measured by the TAC procedure in the BF group. A strong correlation between DHA and visual acuity was observed for all infants (PC:$r = +0.65$, $p<0.001$; PE:$r = +0.48$, $p<0.01$). However, this relation ceased to exist when assessing the relation in each diet group. The authors contended that significant differences in visual acuity between feeding groups and the strong correlations mentioned above, could be due to higher breast milk DHA levels in this group than in similar investigations in other Western countries (Jørgensen *et al.* 1996). However, another reason for significant differences in this investigation could be due to the fact that the visual acuity tester was not blind to infant feeding status, leading to the possibility of biased results. This is evident as correlations between the biochemical parameter (DHA) and the functional measure (visual acuity) were non significant when feeding groups were assessed separately. If this were truly a relation based on level of DHA, it would not matter if the group were BF or SF fed, the level of DHA and visual acuity score would be the only issues of importance. However, it is possible the BF group, as a whole, could have received higher visual acuity scores than the SF fed group due to a biased tester. Therefore, the correlation may have simply be due to the creation of artificial differences between groups, which would have produced 2 clouds of points that were obviously in the direction of a positive correlation. An example of what is meant by the above speculation is presented in Figure 1. As a graphical representation of the correlation was not shown, the above theory cannot be pragmatically assessed, yet the fact that the tester was not blind to infant feeding status questions the validity of the results in this investigation.

Carlson *et al.* (1996), followed the visual acuity development and DHA status of infants randomly assigned to receive SF or formula enriched with 0.1% DHA and 0.43% AA, taking measures at 0, 2, 4, 6, 9 and 12 months. A group of BF infants who served as a control did not differ from the 2 other groups in terms DHA content of tissue measures

Figure 1

Possible Appearance of Relation Between DHA Status and Visual Acuity
In the Investigation by Jørgensen et al. (1996)



presented at any time point. However, the enriched formula group had significantly higher levels of DHA in plasma PC at 2, 4 and 6 months and erythrocyte PE at 4, 6 and12 months when compared to the SF group. Differences in visual acuity as assessed by the TAC procedure revealed differences only at the 2 month mark, with the SF fed group having significantly lower average scores than either of the other 2 groups.
No differences in visual acuity existed between groups at 4, 6, 9 or 12 months, despite significantly higher DHA status in the enriched formula group at the corresponding time points measured. Reasons for the lack of different visual acuity scores between groups after 2 months are unknown, but it may be that earlier differences were transitory (Carlson *et al.* 1996).

Innis *et al.* (1996), retrospectively investigated the visual function of term infants fed BM or SF. At 9 months, subjects were broken up into 1 of 7 groups related to the length of BF. They measured preferential looking acuity with the TAC procedure, as well as cognitive function using the Fagan Test of infant intelligence. The authors did not find any differences between any of the groups in terms of visual or behavioural measures, and

concluded that the absence of LCP in infant formula does not impose measurable deficits in the above mentioned tests of visual and cognitive function at 9 months, as long as adequate LNA was present. This finding is in accordance with the above study by Carlson *et al.* (1996), where acuity development as measured by TAC did not differ between feeding groups after 4 months.

In another investigation by Innis *et al.* (1994), a 3 month prospective design was used to investigate the visual acuity development of term infants that were fed SF or exclusively BF by parental choice. Acuity tests performed at 14 days and 3 months postpartum, showed no significant differences between the groups despite substantial differences in blood lipid DHA concentrations at 3 months. For example, at 3 months postpartum, formula fed infants had significantly lower levels of DHA in erythrocyte PE and PC and plasma phospholipids than BF infants. As 2 independent blind testers performed the test, and no differences were found between their results, the possibility of bias is unlikely. The lack of difference in visual acuity measures between groups may have been due to the fact that despite differences in blood lipid EFA parameters, both groups may have had adequate levels of DHA for optimal visual acuity development, as can be measured by the TAC method. Regression analysis indicated that visual acuity was not related to dietary intake or any blood lipid marker of EFA status as tested for the entire group of infants or only within the BF or the formula fed group of infants (Innis *et al.* 1994). The authors concluded that infants are capable of converting LNA into DHA, but that DHA levels are higher in infants who directly receive it in their diet. The lack of a relation between blood lipid markers of DHA status and visual acuity score also leads one to speculate that there may be a threshold level above which visual acuity, as measured by the TAC procedure, does not improve with increased blood lipid DHA levels, provided that the levels are not excessively high.

The effect of increasing the DHA content of BM through maternal DHA supplementation on infant blood lipid parameters and neural indices of exclusively BF infants was recently investigated (Gibson *et al.* 1997). Women were randomly supplemented with various levels of DHA (0, 0.2, 0.4, 0.9 or 1.3 g/day) for 12 weeks.

This produced DHA levels in BM ranging from 0.1% to 1.7% of total fatty acids. Breast milk DHA was related to infant plasma and erythrocyte phospholipid DHA concentrations in a curvilinear manner ($r=0.89$ and $r=0.88$, respectively, $p<0.001$). There were no apparent differences in visual acuity as measured by VEP between groups at 12 or 16 weeks postpartum. A relation between the DHA level in BM or infant blood and VEP was also not apparent. However, a test of infant mental and psychomotor development performed at 1 and 2 years of age showed a relation between this measure at 1 year and DHA status at 12 weeks. Despite this finding, the authors speculated that social and environmental influences may be more significant than the effect of this single dietary factor, as home stimulation score was also related to this test (Gibson *et al.* 1997). The authors concluded that maternal DHA supplementation resulted in increased levels of this fatty acid in BM, which correlated with infant DHA status, However, they argued that this increase did not coincide with improved visual function as measured by VEP at 3 or 4 months postpartum.

Circulating levels of n-3 and n-6 fatty acids, but not visual acuity development were related to infant diet in a prospective longitudinal random feeding study assessing the effect of dietary LA/LNA ratio (Jensen *et al.* 1997). Infants receiving formula with the highest LNA content (3.2%) and LA/LNA ratio (4.9:1), possessed the highest DHA plasma phospholipid concentrations at all time points. Visual acuity development was assessed by VEP at 4 and 8 months, with no differences among groups, despite better DHA status in the group which received the highest LNA content. Significantly higher plasma phospholipid AA concentrations were noted in the group receiving the lowest LNA content when compared to the highest dietary LNA content. This coincided with a significantly lower average weight at 4 months in the latter group. Due to possible negative effects of high LNA intake and/or low LA/LNA ratio on growth, and as no differences in visual acuity development existed between groups, the authors suggested that lower LA/LNA ratios recommended for SF should not be adopted until their effects on growth are more rigorously evaluated.

The following 2 investigations used a multicenter approach with larger sample

sizes than previous investigations and found no relation between visual acuity and DHA status in term infants (Innis *et al.* 1997; Auestad *et al.* 1997). In the longitudinal prospective study by Innis *et al.* (1997), infants either received BM or were randomly assigned to one of the 2 following SF; formula 1, containing 1.9% LNA (LA/LNA ratio 9.5:1), or formula 2 containing 4.7% LNA (LA/LNA ratio = 7.3:1). At 90 days, fatty acid analysis revealed significantly higher DHA and AA levels in plasma phospholipids and erythrocyte PC and PE of BF infants when compared to the 2 other groups. The group receiving formula 2 also had significantly higher plasma phospholipid DHA than infants fed formula 1, but this difference was not found in erythrocytes. As a measure of validity, the authors also stated that there were no differences in fatty acid profiles of any lipid fraction for any group between study centers. In spite of better DHA status, visual acuity as measured by the TAC procedure, did not differ among groups at 90 days. In an attempt to relate DHA content to visual acuity, correlation analyses were performed. However, no relation was found when assessing the group as a whole, or each group individually. Despite significant differences in AA status, anthropometrical measures between all groups at 3 months were similar. However, the LA/LNA ratios of the groups in this investigation were larger and the difference between them smaller than in the previously mentioned study by Jensen *et al.* (1997), where weight was lower in infants fed formula with the lowest LA/LNA ratio. Therefore, the authors concluded that feeding infants SF does not appear to affect visual development as measured by the TAC procedure and that the LA/LNA ratios they provided do not appear to alter growth.

A second longitudinal prospective random feeding multicenter investigation also found no relation between tissue fatty acid profiles and visual acuity assessed at various time points (Auestad *et al.* 1997). Infants were exclusively fed one of the 3 following formulas for a minimum of 4 months; SF with an LA/LNA ratio of 10:1, formula enriched with 0.12% DHA and 0.43% AA, or formula enriched with 0.23% DHA and 0.07% EPA. In the group of BF infants which served as a control, exclusive BF was mandatory for a minimum of 3 months, after which a SF (LA/LNA ratio of 7.1:1) was allowed. Fatty acid profiles of erythrocyte PC and PE at 4 and 12 months, showed similar AA and DHA

concentrations in infants fed BM and formula enriched with AA and DHA. However, at both time points, DHA concentrations in both phospholipid fractions were significantly lower in SF fed infants and significantly higher in the DHA + EPA group when compared to the other 2 groups. Despite these differences, the authors stated that averages for all groups were within the range of DHA concentrations found in infants fed human milk (Auestad *et al.* 1997). In spite of differences in DHA status, visual function tests performed at 2, 4, 6, 9 and 12 months using VEP and/or the TAC procedure (depending on study site), did not reveal any differences in acuity thresholds at any time point. No relation between DHA or DHA/DPA(n-6) ratio and visual acuity threshold was found either, leading the authors to conclude that RBC fatty acid levels may not always be correlated to visual function in healthy term infants as some earlier studies have noted. Levels of AA were also significantly lower in the SF group at 4 months when compared to the BF and DHA + AA group. However, the DHA + EPA group was found to have significantly lower AA levels than even the SF fed group, which may indicate a need to balance n-6 LCP content in long chain n-3 supplemented formulas. Notwithstanding, the variations in AA levels were not associated with any differences in anthropometry as assessed at 1, 2, 4, 6, 9, and 12 months indicating that the degree of the biochemical differences incurred by such diets did not affect infant growth. The authors concluded that only the formula with DHA + AA led to tissue levels similar to that of BF infants, but that this was not of functional or physiologic significance when considering visual acuity development or growth.

2.3 Conclusion:

Breast milk fatty acid profiles appear to be dependant on dietary patterns. In general, higher long chain n-3 fatty acid levels have been found in BM of women residing in non Westernized countries than in industrialized nations. Fatty acids of the n-3 series are thought to influence neurological development of infants, indicating the importance of adequate levels in BM. The necessity of adequate n-3 EFA ingestion during the period of rapid brain growth and development has been illustrated in investigations using animals

and humans. Diets deficient in n-3 fatty acid content have been found to lead to lower DHA levels in various fractions of blood which have been related to lower levels in the cortex and retina in various animal models. The lower long chain n-3 fatty acid contents in these animals have also been associated with altered rod function and visual acuity development. Animal models have clearly defined the essentiality of dietary n-3 fatty acids during early human development.

Human infants are rarely exposed to the n-3 deficient diets used in animal studies. However, it is possible that those fed SF may not be able to accumulate levels of DHA in neural tissue, which are equivalent of those of BF infants. Therefore, ideal EFA balance for optimal CNS development in this group warrants attention. In healthy term human infants, it is apparent that dietary intake affects LCP levels of blood including DHA and AA. Infants fed formula enriched with LCP and BF infants have been found to possess higher blood levels of LCP than those fed SF with various LA/LNA ratios. However, only a limited number of investigations relating feeding practices or erythrocyte DHA status and neural tissue DHA levels in human infants have been done. The effect of lower circulating levels of DHA on the CNS has been more frequently assessed through non direct measures using tests of visual acuity. Despite differences in DHA levels of plasma and/or erythrocytes, advanced scores for measures of visual acuity in infants fed enriched formula or BM over those fed SF were not always apparent. For infants receiving adequate but different forms of n-3 fatty acids, VEP acuity has been related to diet more so than acuity as measured by the TAC procedure. However, many investigations have found no relation between diet or DHA blood levels and visual acuity as assessed by either VEP or TAC. As some of the older investigations that found this relation have apparent biases or fed n-3 deficient formula to infants, the effect of feeding infants artificial formulas which are presently considered adequate, warrants further attention. Also, it has been argued that researchers in the field of nutrition may not have adequate expertise in measuring VEP acuity as it is a technically demanding task and raw data are not always interpreted optimally (Anonymous 1998).

Further, visual resolution acuity is only one aspect of infant development that may

be compromised due to poorer EFA status. Essential fatty acids play an important role in eicosanoid biosynthesis and dietary presence early in life may affect cholesterol metabolism later in life (Uauy-Dagach *et al.* 1998). Concerns about effects of early malnutrition on later human development which are not easily assessed, such as emotional responses to stressful events (Levitsky & Strupp 1995), may also be affected by poor EFA status early in life. As the impact that lower levels of circulating EFA may have on SF fed infants is presently unknown, the promotion of BF and healthy diets for BF women deserves more attention than trying to improve infant formula in the solitary aspect of LCPUFA content. Also, the inhabitants of many nations do not have access to appropriate infant formula, thereby making the need to encourage healthy diets and exclusive BF practices even more important in developing countries. As a concern regarding the EFA status of BF women residing in Cuba existed and EFA are known to play such an important role in the neurological development of infants, the current investigation was undertaken.

3. Research Objectives and Hypothesis

The purpose of this investigation was to assess the adequacy of the EFA status in mothers and healthy term infants as measured in total lipids of BM, plasma and erythrocytes, as well as to assess development of infant visual acuity at 2 months in Havana, Cuba. The study also presents a longitudinal assessment of growth and therefore energy intake, through anthropometrical measurements of infants and mothers at birth and 2 months postpartum. Following is a list of specific objectives:

1. To compare fatty acid profiles of total lipids in the following tissues to literature values:

- mature human breast milk
- infant plasma and erythrocytes
- maternal plasma and erythrocytes

2. To assess the relation of the essential fatty acid profiles in mature human breast milk, maternal and infant plasma and erythrocytes.

3. To assess the relation between infant visual acuity as measured by the Teller Acuity Card procedure and:

- clinical normative values
- maternal essential fatty acid profiles for exclusively breast fed infants
- infant essential fatty acid profiles in plasma and erythrocytes

4. To assess the adequacy of anthropometrical indices of the infants under investigation in comparison to reference populations at birth and 2 months postpartum.

The main null hypothesis of this investigation is that there will be no biochemical or physiological evidence of there being an essential fatty acid deficiency in the infant or maternal population residing in Havana, Cuba.

4. RESEARCH METHODS

4.1 Study Design and Sample Size

A prospective cohort study was assembled to assess the EFA status of Cuban women and their infants. The mother infant pairs were followed from birth until 2 months postpartum. Due to changes in the protocol, the study became a cross sectional nutrition survey of EFA status at 2 months postpartum as well as an investigation assessing anthropometrical indices in the same group as a cohort from birth to 2 months postpartum. The sample size was based on the inference for a mean calculation, where the results of this study were to be compared to known normal values. An average mean for visual acuity as measured by the TAC procedure in healthy 2.5 month old infants is approximately 2.16% cycles/degree (1.11 octaves), having a standard deviation (SD) of 0.43 (Mayer *et al.* 1995). A low average value for visual acuity development at this age was set at 1 card below the closest card to the average which was 1.7 cycles/degree (0.76 octaves) (Mayer *et al.* 1995). The calculation for sample size estimation when testing for the mean of a normal distribution for a one sided alternative was used with an α=0.01 and β=0.90 (Rosner 1995). The following calculation yielded the minimum required sample size:

$$n = \frac{\sigma^2(z_{1-\beta} + z_{1-\alpha})^2}{(\mu_0 - \mu_1)^2} = \frac{0.1849(3.61)^2}{(1.11-0.76)^2} = 43$$

Therefore, a minimum sample size of 43 was required to ascertain whether this population deviated from the normal average for visual acuity development. It was decided to recruit 70 mother-infant pairs in order to allow for drop outs due to difficulties in transportation.

4.2 Collaborating Partners

This project, conducted in Havana, Cuba, was a collaboration between the Instituto de Nutricion e Higiene de los Alimentos (INHA) in Havana, Cuba and the School of Dietetics and Human Nutrition of McGill University. Other collaborating

parties include America Arias Maternity Hospital, the Pediatric Hospital of Central Havana and Boston's Children's Hospital. INHA staff presented the original study protocol of maternal n-3 fatty acid supplementation to the Cuban Ministry of Health and obtained ethical approval for that project, which was used for the project described in this thesis. Organizational aspects of the investigation, such as delegation of tasks and assurance that data were adequately collected and samples adequately stored, were conducted by the principal collaborator Dr. Alejandrina Cabrera.

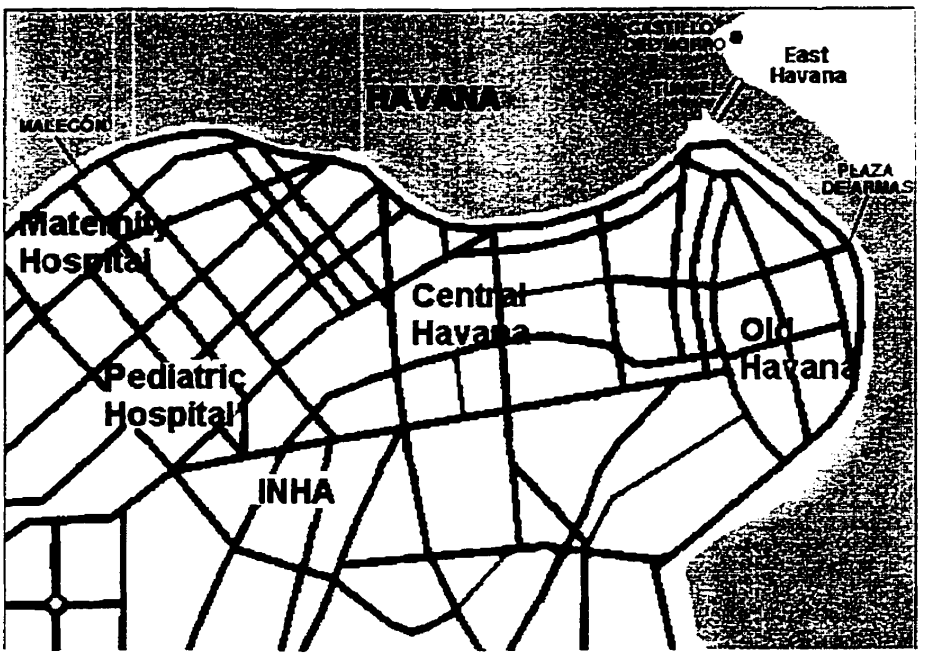
4.3 Subject Selection and Recruitment

Subject recruitment took place at the pre delivery room of America Arias Maternity Hospital, Monday through Thursday for 9 weeks starting in late December of 1997. These days of the week were chosen to accommodate the INHA staff responsible for dietary and anthropometrical assessments. INHA staff (3 persons), hospital staff (various persons) and the author all contributed to the task of subject recruitment. Upon arrival into the pre delivery room, the patient files of potential subjects were reviewed to assess whether they met the following inclusion criteria: (i) experiencing a normal pregnancy, having no medical risks which may affect fatty acid metabolism such as heart disease, kidney disease, diabetes (including gestational diabetes), hypertension, gall bladder disease, and thyroid disease; (ii) between 18 and 35 years of age; (iii) live in the regions of Central Havana or Old Havana; (iv) be free of pathological diseases such as HIV/AIDS and hepatitis, according to medical history charts.

The above criteria were in part adapted from recruitment criteria for BM donors for investigations assessing fatty acid composition as stipulated by Jensen (1989). The criteria used from this source were included, as the purpose of this investigation was to assess whether there was an insufficiency of EFA intake in the general population as described by EFA profiles, not in persons experiencing medical conditions that may alter fatty acid metabolism such as diabetes or cardiovascular disease. The purpose for including only subjects from Old and Central Havana was threefold; (i) the hospital which agreed to participate in the study, America Arias Maternity Hospital, caters mainly to

Figure 2

Map of Study Areas



these areas, (ii) these areas are geographically close to the Pediatric Hospital where the second part of the study took place (Figure 2), (iii) INHA indicated that these areas are known to have poorest indicators for living conditions/socioeconomic status in Havana and would most likely house a larger proportion of the population that may be suffering from poor nutritional status. Inclusion of subjects free of known pathological diseases was done for the safety of lab workers handling blood samples in the absence of proper conditions for working with and the disposal of infected blood samples.

After assessing whether a potential subject met the inclusion criteria, she was approached and informed of the study that was being done and what was involved if she participated. If the potential subject was comfortable with all techniques and agreed to the conditions of the study, written consent was obtained (Appendix B).

4.4 Data and Sample Collection at Birth

A questionnaire was filled out obtaining information on various socioeconomic, demographic and behavioural variables (Appendix C_1 and C_2). When available,

anthropometrical indices as measured and recorded by family doctors during pregnancy were also recorded from medical histories. At this time, a maternal venous blood sample (8 ml) was drawn by trained hospital or INHA staff and either directly collected into a vacutainer containing EDTA as an anticoagulant or into a syringe and immediately placed into a vacutainer containing EDTA and stored in a refrigerator which was assumed to be at 4°C. When the patient delivered, cord blood (8ml) was collected immediately after the cord was clamped into a tube containing EDTA as an anticoagulant. Samples were transferred from the Hospital to INHA on ice within 2 to 20 hours of collection for processing and storage. Infant birth weight was recorded as that measured by trained neonatology nurses immediately after birth, in the delivery room using a pan pediatric scale. One to two days following delivery, anthropometrical measurements of the mother and infant were taken by a team of 2 well trained anthropometrists from INHA. Forms used to collect anthropometrical data are presented in Appendix D_1 and D_2. Infants were weighed unclothed using a pan pediatric scale and infant recumbent length was measured with an infantometer. Mothers were weighed using a platform beam scale and their heights were measured without shoes, utilizing a standiometer. All circumferences were measured using a metric measuring tape and skinfolds were assessed with a calibrated skinfold caliper.

4.5 Data and Sample Collection at 2 Months

4.51 General Procedures

Subjects recruited at birth were visited in their homes 2-3 days prior to the scheduled day for collection of data 2 months after delivery. They were informed/reminded of the appointment they had at the Pediatric Hospital of Central Havana and encouraged to attend. As travel to the hospital by public transport for a mother and infant was a difficult task, transportation by car was provided for all women living at a distance to the hospital that was not achievable on foot starting at the second week of this phase of the study.

Once at the hospital, maternal venous blood (8 ml) and infant blood (1-5 ml) was drawn by INHA staff. It was collected directly into a vacutainer containing EDTA as an anticoagulant or into a syringe and immediately placed into a tube containing EDTA , placed on ice and brought to INHA within 2 hours for processing and storage.

After blood samples had been taken, mothers and infants had various anthropometrical measurements made on them (Appendix D_1 and D_2.) by the same team of anthropometrists that had taken the measurements at birth, as per previously mentioned methods. Other data including infant feeding practices were collected by INHA staff (Appendix D_1 and D_2.), and a full medical examination was performed on each infant by a neonatology resident after the initial part of the BM sampling procedure.

4.52 BREAST MILK SAMPLING

Breast milk sampling was done with the aim of obtaining a representative sample for the day. The person assisting with the BM sampling was required not to use hand creams in order to avoid contamination with lipids and mothers were asked not to use creams for the same reason on that day. Originally, the entire contents of one breast were to be extracted at mid morning and mid afternoon with a Medela electric breast pump. A 24 hour sample is the best method for BM collection but the above method is considered adequate and is not as bothersome to the subjects and was therefore stipulated for use. However, it was not possible to obtain an afternoon sample due to inability to house the mothers for the entire day at the available location. Therefore, only a mid morning sample was taken. This method was used as initially, a separate part of the project was to investigate the volume of BM ingested, for which a representative sample of total lipids from milk was required to obtain the amount of each fatty acid ingested per day by exclusively BF infants.

The following procedure was adapted from Jensen (1989); the mother was asked to nurse her infant on the breast of her hand of dominance (ie. if she is right handed, she fed from her right breast) until the infant was satisfied. If for some reason this could not occur, it was stipulated that this information be recorded for future reference. The mother

was then assisted in applying the breast pump to the dominant breast to ensure complete emptying of the breast. The milk extracted at this stage was not used for analysis. After 1.5 hours of the infants morning feed, the dominant breast was washed with distilled water. The breast pump was held on the dominant breast for approximately 8 minutes or until the milk no longer flowed out evenly. Whenever possible, the baby was simultaneously nursed on the other breast as there are differences in maternal prolactin levels caused by artificial expression and infant suckling (Jensen 1989). The baby's simultaneous nursing aids in letdown and stimulates a normal prolactin response helping to ensure that a complete milk sample is obtained (Jensen 1989). It was observed that some mothers were not producing much milk for the sample and not all infants were feeding while the sample was extracted. Therefore, the importance of recording whether the infant was simultaneously feeding and total volume of milk expressed was remarked. All samples were placed on ice after removal, and small samples of 1 and 3 ml, were transferred into a labeled cryotubes containing 3 drops of 0.01% BHT in methanol. This storage container was then transferred to a -70°C freezer within 2 hours of sample collection until departure back to Canada. As dry ice was not available, samples were transferred back to Canada using coolers and blue ice packs. Upon arrival in Canada, samples were placed on dry ice until transferred to a -80°C freezer where they remained until they were analyzed, 5 months later.

4.53 Visual Acuity Testing

Anytime between the infants' first nursing and before the subjects left for the day, a test of preferential looking acuity was administered using the TAC procedure. Details regarding set up and training for the visual acuity test are presented in the next section, followed by procedural details of the test in study infants.

The set of TAC, stage and other materials were kindly donated by Vistech Consultants Inc., Dayton, OH. The Teller apparatus was set up by an expert in the field, Dr. Luisa Mayer, from Boston Children's Hospital and standardized in terms of lighting and positioning, securing the apparatus in a fixed place. Two clip on lamps with 60 watt

bulbs, were positioned on the top of each of the 2 side wings of the screen to attain adequate luminescence, as the test room had poor lighting. The author, who was the tester was thoroughly trained by Dr. Mayer before commencing this investigation. Training began by having the trainee read the TAC Handbook (Teller 1989), and then observe the trainer use the procedure on a number of infants. This was followed by having the trainee test 19 non study infants between the ages of 1 and 6 months. Once the study began, the test distance was set and secured at 38 cm, which is appropriate for the age group under investigation. In 6 of the practice infants and 4 of the study infants (10 in total), the trainer was able to conduct a separate test to assess success of training. In 5 of the retested infants the results between trainer and trainee agreed perfectly, a 1 card difference existed for 4 of the infants and a 2 card difference in the other infant, yielding a difference of one card or less 90% of the time. Reliability between tester and trainer was adequate, as agreement between testers is thought to be optimal when there is a difference of 1 card or less 90% of the time (Mayer *et al.* 1995).

The person assigned to be the holder of the infants, was also trained by Dr. Mayer to ensure this aspect of the test would be carried out appropriately. Due to time constraints, no other holders could be properly trained by Dr. Mayer, even though another one was required. As such, after Dr. Mayer left, the tester trained another holder.

For study infants, the tester was kept blind as to whether the infant was BF exclusively or not, so as not to bias the resulting visual acuity scores. Every morning, before testing began, the test distance (38 cm) was measured and adequate luminescence was confirmed against the blank card using a light meter. The start card which was used for all infants was the 0.32 cycles/cm card. Each card was presented at least 3 times to each infant. Raw threshold acuity was defined as the card with the finest grating that the infant could reliably and repeatedly resolve as shown by consistently correct looking behavior, corresponding to correct observer judgments (Teller 1989). As the tester's capability of judging the infants ability to detect the side on which the gratings appear becomes more difficult as gratings become finer, the acuity threshold card was always confirmed (Mayer & Dobson 1997). Raw scores were recorded as the spatial frequency

of the stripes written on the back of the card (cycles/cm), which was converted to actual acuity (cycles/degree), using the conversion chart provided in the Teller Acuity Card Handbook for a test distance of 38 cm (Teller 1989). Confidence ratings on a scale of 1 to 3, with 1 representing low confidence, were recorded by the tester for each infant. Whenever possible, if the infant was not too tired or disagreeable, a second test was done on the infant to assess the degree of intra-observer reliability.

4.6 Laboratory Methods:

4.61 Processing and Storage of Blood Samples

Plasma and RBC from all blood samples were separated by centrifugation at 3500 rpm at 4°C for 15 minutes. The plasma (1ml) was withdrawn and placed directly into a labeled cryotube, containing 3 drops of 0.01% BHT in methanol, flushed with N_2 and frozen at -70°C until departure back to Canada, or placed into a -20°C freezer until the RBC were processed, after which they were transferred to the -70°C freezer. On some occasions, plasma samples were left outside, at room temperature until the RBC samples were finished being processed, however, the extent of the occurrence of this was not known. After removal of the plasma, the RBC's were washed 3 times with 0.15 M NaCl, 1 mM EDTA. The RBC (1 ml) samples were then transferred into labeled cryotubes containing 0.01% BHT, flushed with N_2 and stored at -70°C until departure to Canada. Maternal samples were stored in triplicate, 2 cryotubes per person were to be transferred to Canada and 1 was to remain in Cuba. Therefore, different trays in the freezer were specified for each group of samples. However, as infant samples were most often small, and only 1 cryotube of plasma and/or RBC were available on some occasions, samples were to be stored in a way which clearly identified the complete set to be transferred to Canada for analysis. It was only possible to store the samples from the first 2 weeks of sample collection after birth under N_2 as there was not any available for sale in the city after that time. Transfer to Canada occurred as per BM samples.

4.62 Loss of Blood Samples

Blood samples from 55 mothers and 45 infants were obtained and recorded in the log of blood samples drawn and stored. All maternal samples arrived and were present for analysis in Canada. However, not all of the infant samples were. Upon close assessment of the log and the days on which samples were stored, it appeared as if samples on certain days were stored in the wrong location (ie. Cuba tray vs. Canada tray), and must have remained in Cuba, as they were not within the set of samples to be analyzed in Canada. This appeared to be the situation, as for samples recorded in the log on Friday, March 20, 1998, only 1 RBC sample arrived in Canada. This sample was for an infant who had 2 cryotubes of RBC and 1 of plasma recorded in the log, while neither plasma nor RBC of other infants that were drawn on the same day, which only had 1 cryotube available for each, arrived in Canada. Similar occurrences on Thursday, March 12, Friday, March 13, and Friday April 10, 1998, led to a decrease in the number of samples available for analysis. On other days, when only 1 cryotube of RBC and plasma per infant were available, both arrived in Canada, indicating that the person preparing and storing the samples in the freezer did not do so consistently, or that labels on the trays were moved around on certain occasions. A flow chart of reasons for loss of infant and maternal blood samples are presented in Figures 3 and 4, respectively.

4.63 Lipid Extraction and Preparation of Fatty Acid Methyl Esters

4.631 Blood Samples

A modified Folch extraction was used to extract total lipids from the samples (Folch *et al.* 1957) and the fatty acids methylated as per the procedure by Al Makdessi *et al.* (1985). Packed RBC, (approximately 1g when available), or plasma (approximately 1 ml when available) were placed into a 50 ml culture tube and MeOH (4 ml, containing 0.01% BHT) was added to the sample. The culture tube was then heated to 55°C in a water bath for 15 minutes. A solution of hexane:chloroform (12 ml of 4:1 v/v) was then added and placed in a wrist action shaker for 15 minutes. Millipore water (2 ml) was added and the sample put on the wrist action shaker for an additional 10 minutes. The

Figure 3

Reasons for Loss of Infant Blood Samples

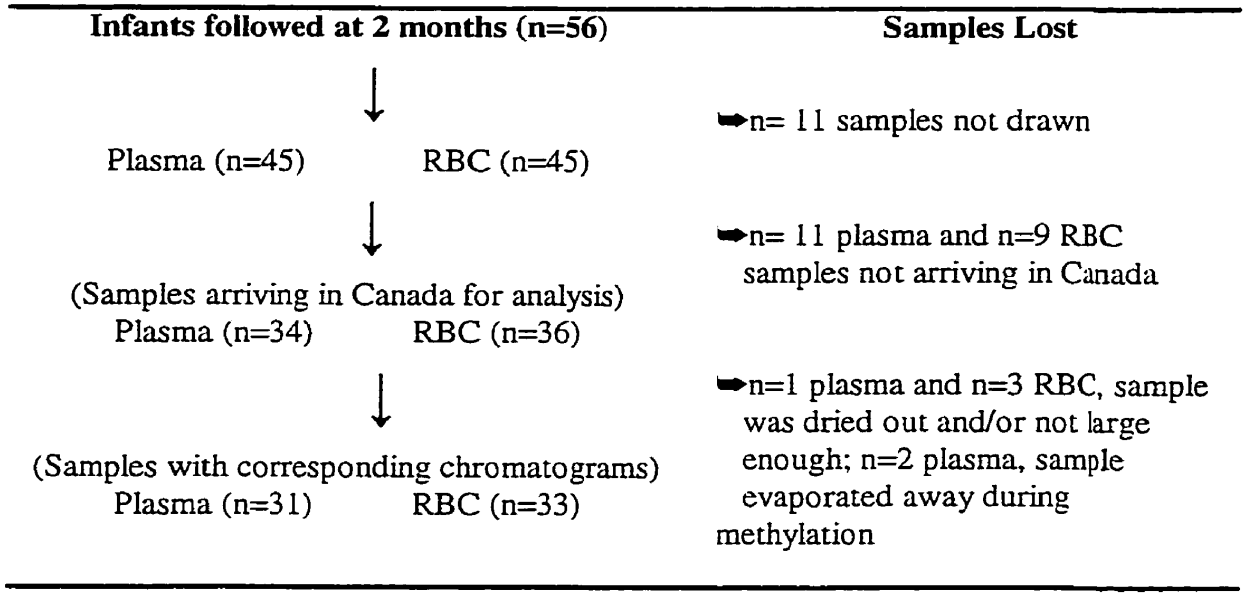
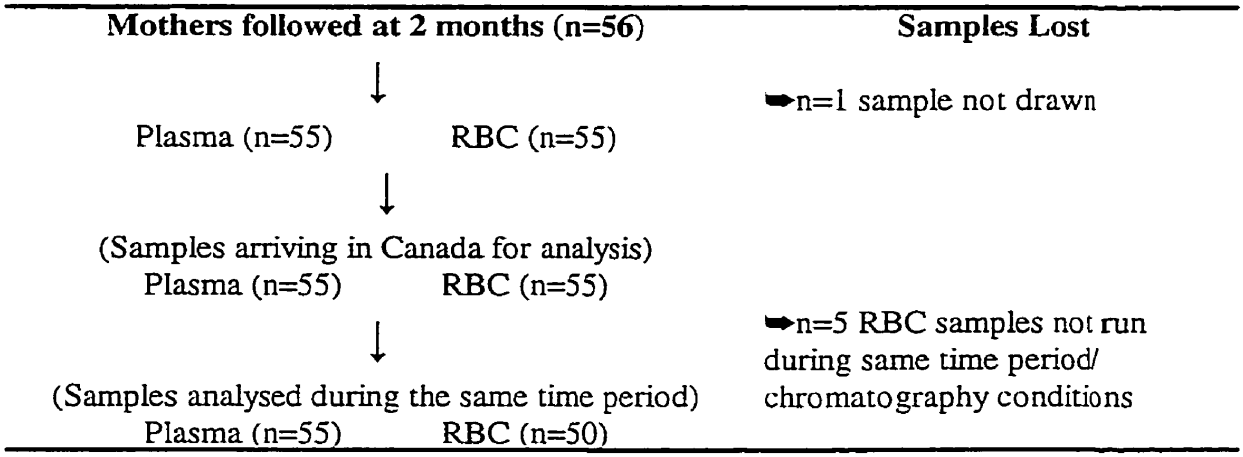


Figure 4

Reasons for Loss of Maternal Blood Samples



sample was then centrifuged at 1500 rpm at 4°C for 15 minutes and the organic supernatant phase transferred to 30 ml culture tube and dried under N_2 at 45°C. The aqueous layer was then re-extracted by adding 12 ml of hexane:chloroform and shaking the sample for 15 minutes. It was then centrifuged at 1500 rpm and the supernatant added to the first extraction and dried down. Methylating reagent (0.5 ml of 7:6:7 BF_3MeOH:benzene:MeOH) was then added to the sample. The tube was flushed with N_2, sealed with Teflon tape and vortexed. The tubes were then heated at 100°C for 55 minutes and allowed to cool in tepid water. Hexane (1 ml) and millipore water (0.5 ml) were then added to the sample, after which it was vortexed and centrifuged for 5 minutes at 2500 rpm. The top layer was transferred to a 1.5 ml crimp seal vial dried down under N_2 and 1 ml of chloroform added. As infant samples were often small for optimal GC analysis, they were concentrated in 100 µl polypropylene vial inserts.

4.632 Breast Milk Samples

The lipase present in human milk was inactivated by thawing frozen milk samples in cold water with subsequent rapid heating to 80°C for 1.5 minutes (Innis & Kuhnlein 1988). The lipids present in lipase inactivated milk were then isolated and methylated using the same procedure as that for the blood samples mentioned above, starting with the addition of methanol to placement in to crimp seal vials. Total weight of the fatty acid extract per ml of sample was also measured and recorded to assess the total fat content of milk in grams per 100 ml.

4.64 Gas Chromatography

The composition of fatty acid methyl esters of plasma, RBC and BM were determined in duplicate using a Hewlett-Packard 5890 gas liquid chromatograph (GC), (Palo Alto, CA), equipped with a 30m x 0.2 mm SP 2330 column (Supelco, Bellefonte, PA), flame ionization detectors and automated injection. The oven temperature was held at 100°C for 1 minute and increased to 190°C at a rate of 3°C/minute, after which it was held at this temperature for the remainder of the run. The injector temperature was set at 210°C and the detector temperature at 250°C. Helium was used as a carrier gas. Fatty

acid methyl esters were identified based on the retention time of known standards (Supelco, Belfonte, PA). All results were expressed as percent of total fatty acids by weight (wt/wt%).

4.7 Presentation and Statistical Analyses of Results

4.71 STANDARDIZATION OF ANTHROPOMETRICAL DATA

Assessment of adequacy of infant growth was done by calculation of Z scores for weight, length, and head circumference. The Z scores were calculated using the following formula:

$$\text{Z score} = \frac{\text{Subject's value - (median reference value)}}{\text{SD of reference}}$$

WHO reference standards by gender (WHO 1983), which are presented as medians at monthly intervals, were used for weight and length measures. Since age ranges at the second time point for measurements were between 54 and 73 days postpartum, median values were calculated on a per day basis, assuming linear growth from 1 to 3 months in the reference standards. As such, it was possible to assess adequacy of growth on a per day rather than a per month basis, giving more accurate assessment of growth for age. Head circumference reference data by gender compiled by Roche *et al.* (1987), were used as reference standards for this measure. As per the WHO standards, daily means were calculated from the monthly means assuming linear growth from 1 to 3 months, to allow for Z score calculations as assessed per age in days, rather than months.

The other anthropometrical measures taken of the mother and infant (Appendix D_1 and Appendix D_2), do not have appropriate reference data for comparison purposes. Therefore, this data will be presented to describe the population, and ascertain whether differences existed between the subjects who came to the visit at 2 months and those who did not.

4.72 EXCLUSION OF SUBJECTS IN STATISTICAL ANALYSES AND CREATION OF GROUPS

There were some problems with the resolution of the γ-linolenic acid (γ-LNA) and LNA peaks in some of the chromatograms of maternal plasma and erythrocytes. All subjects that had abnormally high values for these fatty acids due to a merging of peaks with background noise, were excluded in the calculation of means and statistics involving these fatty acids. Grouping of infants and mothers into feeding groups for certain statistical analyses were done making the following categories; (1) exclusively BF infants and (2) infants that were not exclusively BF.

4.73 DATA MANAGEMENT AND STATISTICAL ANALYSIS

Prior to statistical analyses, all visual acuity data were $\log_2$ transformed, to obtain octave rather than linear values, as visual acuity in humans follow a logarithmic scale and statistics must be performed using log transformed values (Teller 1989). As per convention, the presentation of the mean and SD were done as follows, the average of the $\log_2$ values for acuity were transformed back to the linear value in cycles/degree, and the SD remained in the $\log_2$ transformed scale of octaves (Teller 1989). A one sample t-test for the mean of a normal distribution was used to assess whether the average of the visual acuity tests was different from age norms and was calculated using methods from Rosner (1995).

All statistics were performed using SAS Version 6.12 (Cary, NC). Presence of outliers and normality of all continuous data were assessed. This procedure was done for each variable in the entire group, as well for each feeding group mentioned in the previous section, and for baseline data of the group that was not followed after birth and the group that was followed. Variables for which potential outliers were indicated on box plot print outs were further assessed using the Extreme Studentized Deviate procedure for the detection of single or multiple outliers (Rosner 1995). In order to assess whether the lack of normality for some variables was due to the presence of outliers, the test for normality was run after the outliers were removed. Variables were considered non normal if the Wilke Statistic had a $p<0.05$. As some variables were not normally

distributed, even after outliers were removed, analyses of certain variables were performed using nonparametric methods. In the following sections, correlations in non normally distributed data were performed using Spearman Correlations and Wilkoxon Rank Sum Tests were used in lieu of t-tests for non normally distributed variables (Rosner 1995).

Unpaired student t-tests were used to compare differences in continuous descriptive factors between the group that came to the 2 month appointment, and the group that did not. Differences among these groups for discrete measures such as gender and residential area were analyzed using the Chi Square test. For analyses using 2 by 2 contingency tables, the Fisher's Exact Test Statistic was used to determine whether differences existed between the groups. For analyses using 2 by 3 contingency tables, the Chi Square Statistic was used to ascertain whether there were apparent differences between the groups. A significance level of $p=0.05$ was set to determine whether differences existed among these 2 groups.

Pearson correlations were run in order to assess whether the presence of any individual or groups and ratios of fatty acids within a given tissue were related to others. Correlation analyses were also performed for certain EFA between tissues of the same subject (ie. mother's milk and mother's plasma), and for mother-infant pairs of exclusively BF infants (ie. mother's milk and infant plasma) to ascertain whether n-3 fatty acid status was consistent between tissues. The relation between EFA status and infant visual acuity was assessed using correlations against infant EFA profiles, assessing the entire group together and each group alone by feeding status. Individual EFA and groups and ratios of EFA in infant plasma and erythrocytes were also correlated to age and gender standardized anthropometric measures to assess whether any EFA was related to growth. Visual acuity and standardized infant anthropometric measures were also correlated to EFA profiles of maternal tissues for exclusively BF infants.

To assess whether the total fat content of BM samples was related to maternal energy status, correlations between BM fat content and various anthropometric measures were made. Total fat content of BM was also correlated to age and gender standardized

infant anthropometrical indices. For variables that had outlying values as assessed by the Extreme Studentized Deviate procedure (Rosner 1995), all correlations were calculated both with and without those individuals to assess whether they were influential in nature (Draper & Smith 1966). Due to the large number of correlations that were performed, significance was set at 0.01, in order to minimize type I error.

To further explore whether exclusive BF had an impact on measures made, unpaired t-tests were performed between the feeding groups mentioned in section 4.72. Variables which were assessed between groups were levels of fatty acids in infant plasma and erythrocytes, visual acuity development scores, and age/gender standardized anthropometric measures. A significance level of $p=0.05$ was set for this group of tests.

To assess whether any descriptive measures of the sample population were related to rates of exclusive BF, Chi square analyses were performed for discrete variables such as infant gender and ethnic background, and t-tests between continuous variables such as maternal anthropometry.

To assess whether the ethnic makeup of the group was similar to that of the region's, a data set with a sample size of 53 was created based on proportions in the population. In Cuba, the ethnic make up is approximately, 66% white, 12% black and 22% of mixed heritage ("Cuba" 1999). Havana is known to have the same proportions of ethnic groups as the rest of the country, being composed mainly of persons of Spanish ancestry, with a large minority of people of African ancestry and those of mixed heritage referred to as mullattoes or meztisos ("Havana" 1999). A Chi Square test between the group investigated and the created data set based on expected proportions was thus performed.

5. RESULTS

5.1 General Information of the Study Population:

5.11 DESCRIPTIVE DATA AND ANTHROPOMETRY

Of the 73 mothers who gave written consent to participate in this investigation, 56 returned to the visit at 2 months postpartum. Data describing the 2 groups of women, followed and not followed, at baseline are presented in Table 1. No differences were found between the groups, except for significantly higher maternal heights in the group that was followed (1.60 ± 0.06m vs. 1.56 ± 0.07m, $p<0.05$) and a difference in the proportion of women from different ethnic backgrounds (Table 1). However, the proportion of subjects from different ethnic groups proved to be similar to that of expected values as assessed by a Chi Square test. This indicated that the group which returned at 2 months was representative of the region. Also, even if a difference from the proportions in the population had been found, access to factors which often relate to health and nutritional status, such as access to health care and education, do not differ among ethnic groups in Cuba (Nodal 1986).

It was not possible to obtain descriptive data or measures for all subjects. As data pertaining to maternal weight gain was obtained from medical histories, not all subjects had complete data on this topic. When initial weights were recorded past 15 weeks gestation (1 followed, 4 not followed), when there was no last weight before birth recorded (1 followed), or when no pregnancy weight gain data was recorded at all (2 followed), women were not included in averages of these measures. For the group that was not followed, subject 31 was also excluded as data pertaining to weight gain did not make sense, having a weight at 12 weeks of 51 kg and before delivery of 52 kg, while infant birth measures were all normal, and the person who recruited this subject and recorded the information scratched out various numbers on the sheet, which led to confusion about the actual situation.

Of the infants that were followed, 3 did not have any baseline anthropometrical

Table 1

Comparison of Descriptive Baseline Data[a] of All Subjects

Gestational Age (weeks)	56	40.4 ± 1.5	17	40.2 ± 1.1
Maternal Age (years)	56	26.8 ± 4.0	17	26.9 ± 5.3
Maternal Height (m)	56	1.60 ± 0.06	17	1.56 ± 0.07*
Maternal Weight at Birth (kg)	54	70.2 ± 10.9	15	66.7 ± 8.7
Pregnancy Weight Gain (kg)	52	12.9 ± 4.3	12	12.9 ± 3.4
Maternal MUAC (cm)[c]	51	25.8 ± 2.9	15	25.4 ± 2.5
Maternal Tricep Skinfold (mm)	52	16.8 ± 7.3	16	15.0 ± 4.7
Maternal Subscapular Skinfold (mm)	52	19.1 ± 9.9	16	19.8 ± 10.2
# Low Birth Weight Infants (%)	56	1 (1.8%)	17	1 (5.9%)
# Macrosomic Weight Infants (%)	56	3 (5.4%)	17	2 (11.8%)
Infant Birth Weight (g)[c]	56	3296 ± 408	16	3107 ± 397
Infant Birth Length (cm)[c]	53	49.3 ± 1.7	16	48.8 ± 1.3
Infant MUAC (cm)[c]	52	9.8 ± 0.7	16	9.6 ± 0.6
Infant Head Circumference (cm)	53	34.0 ± 1.4	17	34.0 ± 1.4
Infant Birth Weight Z Score[c]	56	0.06 ± 0.9	16	-0.31 ± 0.9
Infant Birth Length Z Score[c]	53	-0.45 ± 0.7	16	-0.62 ± 0.5
Infant Head Circumference Z Score	53	-0.25 ± 0.9	17	-0.20 ± 0.9
Parity (# with 0/1/≥2 children)	55	29/20/6	16	8/6/2
Gender (# of males)	56	38 (67.8%)	16	8 (50%)
Number of Smokers	56	10 (17.9%)	17	1 (5.9%)
Residence (# Living in Central Havana)	56	29 (51.8%)	17	8 (47.1%)
Ethnic Background (#Black/White/Mullato or Chinese)	53	12/25/16	16	7/8/1‡
Number Receiving Overseas Monetary Help	53	18 (34.0%)	17	7 (41.2%)
Education (# less than high school)	53	2 (3.8%)	17	0 (0.0%)
Monthly Income Per Person in Family (#<100 pesos)	46	28 (60.9)	13	6 (46.2%)

[a] mean ± SD, frequency, or percentage
[b] n refers to number of subjects used in averages and statistical tests
[c] one mother in the followed group and one macrosomic infant in the not followed group excluded as outliers
* and ‡ significantly different from followed group ($p<0.05$); * by t-test and ‡ by Chi Square analysis

measures recorded, other than birth weight. This was most likely due to the fact that some subjects left the hospital before the anthropometrists were able to take measures. Also, one infant did not have his MUAC recorded for no specified reason. For the mothers that were not followed, one MUAC measure was not used as it was recorded as 12 cm, which is not physiologically possible given that all other anthropometrical measures were normal. As various persons were involved in recruitment, the following measures were not recorded, but specific reasons are unknown; ethnic background for 3 followed and 1 not followed; overseas monetary help for 3 followed, and income for 10 followed and 4 not followed.

Data describing mothers and infants at 2 months are presented in Appendix D. Of the subjects that were followed, one mother infant pair did not have any anthropometry recorded as they arrived at the hospital late, after the anthropometrists had left. Measures that did not make sense were also not used in the calculation of descriptive statistics, such as a head circumference for 1 infant, recorded as 307 and a maternal MUAC of one mother, recorded as 086. For all infants that were followed, box plots of percentiles for Z scores of length, weight, and head circumference are presented in Figure 5. This figure illustrates that the infants exhibited adequate growth for age and by gender, as a group.

5.12 Infant Feeding Practices

According to the method in which infant feeding practices were recorded, infants were divided into 1 of 3 feeding groups presented in Table 2. The level of exclusive BF in the group studied was 55% at 2 months. This is similar to the level found in the same region of the country in 1996, when 58% of the infants were exclusively BF at 4 months (Aliño-Santiago *et al.* 1997). Whether the group investigated in this thesis will continue to have similar rates of exclusive BF at 4 months remains unknown. Data describing types of supplemental milks/formulas fed and time of onset are presented in Tables 3 and 4. Preparation of milk varied, with some mothers adding different quantities of sugar, syrup, corn starch and water, as well as feeding different quantities daily. Unfortunately, accurate data were not recorded on this topic for all subjects. Time of onset of receiving

Table 2

Infant Feeding Practices

Exclusively Breast Fed	31	55%
Mixed (Breast Fed and Bottle Fed)	21	38%
No Breast Milk at 2 Months	4	7%

Table 3

Distribution of Supplemental Milks Fed to Infants

	Evaporated Milk	Cow's Milk	Yogurt	Yogurt and Rice Water	Formula Basal	Other Specified
Mixed Fed (n=21)	5	13	0	1	0	2
No Breast Milk (n=4)	1	1	1	0	1	0

[a] a home made liquid formula of malanga (a tuber), oil, beef or chicken, and sugar.

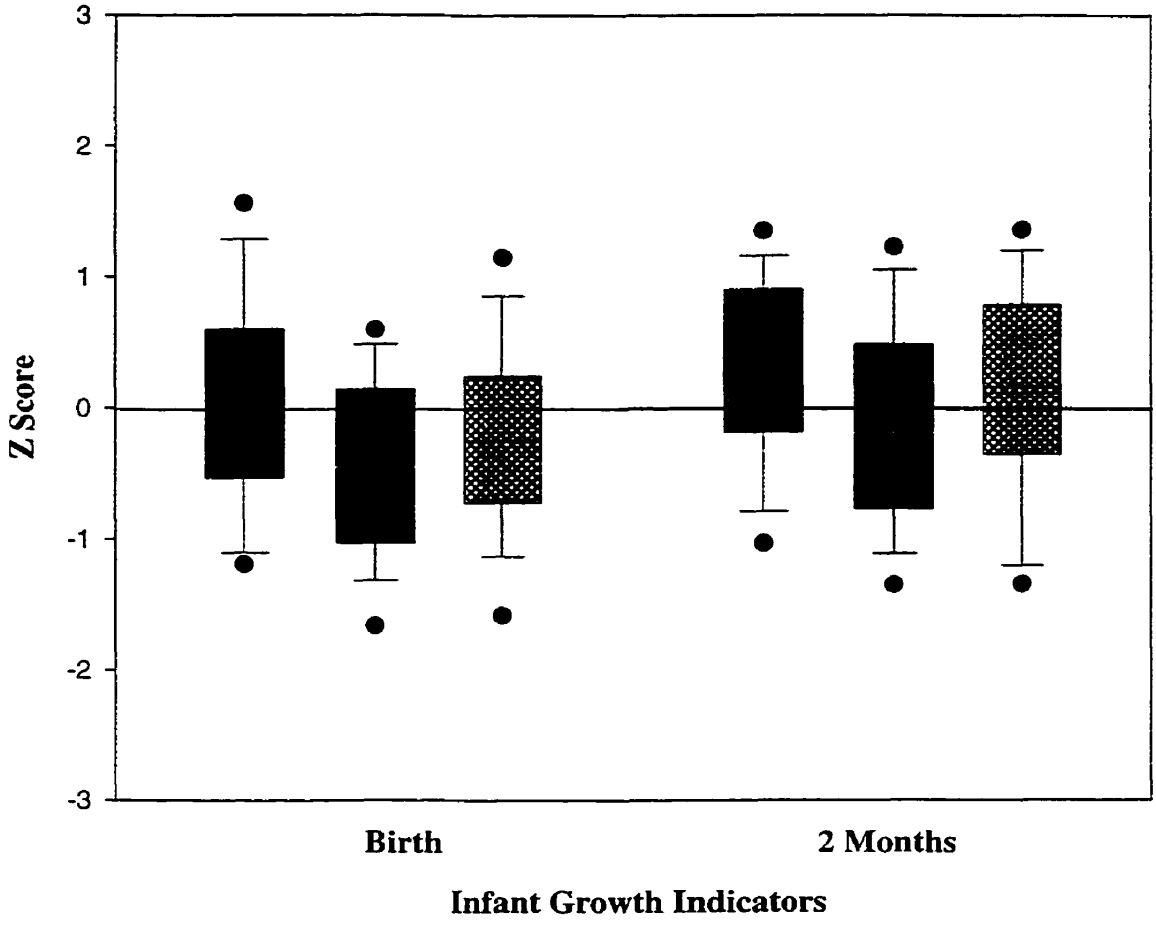
Table 4

Length of Time Receiving Supplemental Milk

	[illegible]	[illegible] weeks	[illegible]	[illegible]
Mixed Fed (n=21)	1	11	2	7
No Breast Milk (n=4)	0	1	1	2

Figure 5

Percentiles for Z Scores of Infant Anthropometrical Measures by Gender and Age



Infant Weight (n=56 at birth and n=55 at 2 months)
Infant Length (n=53 at birth and n=55 at 2 months)
Infant Head Circumference (n=53 at birth and n=54 at 2 months)

The horizontal line inside the box represents the mean.
The boundries of the box represent the 25^{th} and 75^{th} percentiles.
The vertical bars represent the 10^{th} and 90^{th} percentiles.
The circles represent the 5^{th} and 95^{th} percentiles.

supplemental milk varied, but this information was not recorded for one third of the subjects who were weaned (Table 4).

5.2 Fatty Acid Profiles of Breast Milk Samples

A total of 52 mature human milk samples, drawn at 63.1 ± 4 days postpartum, were available for analysis of total fat content and fatty acid profile. Of the 52 mothers who were lactating, it was possible to obtain 51 samples, as one mother refused to, due to fear of the milk extractor. Another sample was obtained from a mother who said her infant had not received BM for over a month. This was possible as she had been letting him nurse prior to bottle feeding, and falsely assumed that she was no longer producing milk, but as she was, a sample was obtained for analysis. Therefore, in reality, there were only 3 infants that were not receiving BM at 2 months.

Boxplot analyses revealed 2 subjects who had consistent outlying values for most fatty acids of the n-3 series, as well as other subjects who had 1 or 2 outlying values. Upon viewing the chromatograms of these subjects, and as outlying values were consistent among fatty acids of the same series for individuals, it was apparent that outlying values were due to dietary and/or biological variations and not technical error as in γ-LNA and LNA of some maternal plasma and erythrocyte samples. It does not appear as if the subjects with outlying values of n-3 fatty acids artificially increased the means of these fatty acids for the group investigated, but they greatly added to the variation in this measure. For example, the mean and SD for DHA in BM with the 2 outliers was 0.43 ± 0.26% of total fatty acids while it was 0.39 ± 0.18% without them. Mean levels, ranges and medians are presented for the 18 fatty acids identified, as not all were normally distributed (Table 5). However, levels of EFA coincided with normal values. To illustrate the fact that normal levels of EFA and IMCSAFA were present in the BM of the Cuban women investigated, graphs juxtaposing their values to values from various other countries, were prepared (Appendix G through Appendix K). Total lipid content of the milk samples was slightly high at 5.29 ± 1.81 g/100ml having a large range of 3.51 g/100ml to 13.97 g/100ml. This value was however close to typical values, and when

Table 5

Fatty Acid Composition (wt/wt%) of Total Lipids in Mature Human Milk (n=52)

Fatty Acid	Mean ± SD	Min	Max	Median
C8:0	0.17 ± 0.05	0.06	0.29	0.17
C10:0	1.57 ± 0.34	0.81	2.30	1.58
C12:0	7.81 ± 1.99	3.65	12.09	7.73
C14:0	8.97 ± 2.89	4.46	16.44	8.30
C16:0	19.39 ± 2.29	13.40	25.25	19.16
C18:0	4.62 ± 0.82	3.00	6.69	4.48
C16:1n-7	4.07 ± 1.04	2.38	7.89	4.05
C18:1n-9 & C18:1n-7	29.68 ± 4.14	21.11	42.03	29.08
C20:1n-9	0.51 ± 0.13	0.27	0.89	0.50
C18:2n-6 (LA)	19.37 ± 4.58	10.14	28.86	18.86
C18:3n-6	0.92 ± 0.27	0.56	2.08	0.88
C20:3n-6	0.47 ± 0.14	0.24	0.95	0.46
C20:4n-6 (AA)	0.67 ± 0.15	0.32	1.02	0.64
C22:4n-6	0.15 ± 0.07	0.32	0.41	0.13
C18:3n-3 (LNA)	0.92 ± 0.24	0.53	1.81	0.88
C20:5n-3 (EPA)	0.12 ± 0.07	0.03	0.35	0.09
C22:5n-3	0.15 ± 0.07	0.07	0.43	0.14
C22:6n-3 (DHA)	0.43 ± 0.26	0.09	1.55	0.33
Total SAFA	42.54 ± 4.66	32.52	52.81	41.29
Total IMCSAFA (C8:0 - C14:0)	18.53 ± 4.92	9.17	29.86	17.94
Total MUFA	34.25 ± 4.61	24.67	47.21	34.12
Total PUFA	23.20 ± 4.68	13.61	33.40	22.66
Total n-6	21.58 ± 4.74	11.83	30.98	21.20
Total n-3	1.62 ± 0.54	0.82	4.11	1.50
Total n6/n3	14.59 ± 5.56	4.94	27.69	13.76
ΣLC n-6[a]	1.30 ± 0.29	0.72	1.94	1.25
ΣLC n-3[b]	0.70 ± 0.37	0.21	2.30	0.57
ΣLC n-6/n-3	2.20 ± 0.94	0.69	5.33	2.20
LA/LNA	22.73 ± 9.13	7.86	46.66	21.26
AA/DHA	1.99 ± 1.00	0.50	5.30	1.80
P:S	0.56 ± 0.16	0.31	0.96	0.54
Total Fat (g/100 ml)	5.29 ± 1.81	3.51	13.97	4.86

[a] sum of all long chain (C_{20} and C_{22}) n-6 fatty acids

[b] sum of all long chain (C_{20} and C_{22}) n-3 fatty acids

outliers were removed, it approximated normal levels even more closely, being 4.92 ± 0.97 g/100 ml (Akre 1989).

5.3 Fatty Acid Profiles of Blood Samples

The fatty acid profiles of maternal and infant samples are presented in Tables 6 and 7, indicating the different number of subjects used in the calculation of averages. Once again, as some fatty acids were not normally distributed, the range and median are also presented. The γ-LNA peak of 4 maternal erythrocyte and 5 maternal plasma samples as well as the LNA peak of 4 maternal erythrocyte and 4 maternal plasma samples were not included due to technical error. Other fatty acid peaks that had outliers that were not due to technical error remained in the calculation of descriptive statistics. For maternal plasma and erythrocyte and infant plasma samples, 15 different fatty acids were identified, whereas 14 were identified for infant erythrocytes. A sample chromatogram of maternal erythrocytes is presented in Appendix P, illustrating the retention times of the fatty acid peaks identified. Averages of fatty acid profiles were not indicative of n-3 fatty acid deficiency, and results of certain fatty acids were juxtaposed with literature values in similar age groups, in Appendix L through Appendix O.

5.4 Visual Acuity data

Many infants were sleepy during visual acuity testing due to the various procedures that they were subject to prior to the visual acuity test. However, attempts to test all infants for visual acuity were made, but due to persistent vomiting, (1 infant) or persistent sleepiness (1 infant), it was not possible to complete a test for 2 of the 56 infants. Scores for all infants tested fell within the 99% prediction limits for 2.5 month old infants (Mayer *et al.* 1995), and were normally distributed (Figure 6). The average of the group investigated was 2.00 ± 0.68 cycles/degree, which coincided with large sample population age norms upon t-test analysis for infants of the same age from Brazil, which were set at 2.02 cycles/degree (Rios-Salomão & Fix-Ventura 1995), and was slightly lower than acuity norms of 2.5 month old infants from the US, which were set at 2.16 ± 0.43 cycles/degree (Mayer *et al.* 1995).

Table 6

Fatty Acid Composition (wt/wt%)[a] of Total Lipids in Maternal Plasma and Erythrocytes

Fatty Acid	Maternal Plasma (n=50)				Maternal Erythrocytes (n=50)			
	Mean ± SD	Min	Max	Median	Mean ± SD	Min	Max	Median
C14:0	0.73 ± 0.28	0.4	1.6	0.7	0.48 ± 0.19	0.2	1.1	0.4
C16:0	20.53 ± 2.34	14.8	28.1	20.7	23.95 ± 1.34	21.3	29.0	23.9
C18:0	7.53 ± 1.08	5.0	12.4	7.5	13.28 ± 2.24	9.2	17.7	13.8
C16:1n-7	2.39 ± 0.93	0.4	5.3	2.4	0.45 ± 0.16	0.2	0.9	0.4
C18:1n-9 & C18:1n-7	18.99 ± 2.52	12.8	24.7	18.4	15.54 ± 1.43	13.2	20.0	15.6
C20:1n-9	0.19 ± 0.04	0.1	0.3	0.2	0.42 ± 0.15	0.2	1.2	0.4
C18:2n-6	32.96 ± 5.32	22.2	44.7	32.9	11.50 ± 1.91	5.9	15.9	11.6
C18:3n-6[b]	0.68 ± 0.32	0.2	1.8	0.6	0.29 ± 0.24	0.1	1.0	0.2
C20:3n-6	1.99 ± 0.62	1.0	4.4	1.8	2.05 ± 0.47	1.4	4.2	2.0
C20:4n-6	8.74 ± 1.69	5.2	12.1	8.8	15.67 ± 1.48	12.2	20.3	15.7
C22:4n-6	0.73 ± 0.21	0.3	1.7	0.7	5.79 ± 1.57	1.1	3.3	2.2
C18:3n-3[c]	0.40 ± 0.13	0.2	0.8	0.4	0.35 ± 0.12	0.2	0.9	0.3
C20:5n-3	0.62 ± 0.36	0.2	1.7	0.5	0.63 ± 0.27	0.3	1.4	0.6
C22:5n-3	0.54 ± 0.12	0.3	1.0	0.5	2.42 ± 0.34	3.3	8.4	5.6
C22:6n-3	2.56 ± 0.84	0.9	4.8	2.3	6.80 ± 1.24	4.1	9.5	6.7
Total SAFA	28.80 ± 3.22	20.3	41.1	28.9	37.71 ± 2.50	32.8	46.9	37.8
Total MUFA	21.57 ± 3.06	14.0	28.2	20.7	16.41 ± 1.49	14.0	21.3	16.3
Total PUFA[d]	49.49 ± 4.95	39.8	58.8	50.3	45.62 ± 1.92	39.0	49.3	45.6
Total n-6[b]	45.46 ± 5.09	34.4	55.3	45.7	35.35 ± 2.10	28.6	39.2	35.7
Total n-3[c]	4.09 ± 1.16	2.1	10.0	3.9	10.20 ± 1.36	7.2	13.2	10.0
n6/n3[d]	12.17 ± 3.72	5.8	19.8	11.6	3.51 ± 0.56	2.2	4.6	3.6
ΣLC n-6	11.46 ± 1.67	7.8	15.4	11.6	23.50 ± 1.73	19.7	27.4	23.4
ΣLC n-3	3.69 ± 1.14	1.7	6.9	3.3	9.68 ± 1.52	6.4	12.9	9.5
ΣLC n6/n3	3.37 ± 1.09	1.8	6.2	3.1	2.50 ± 0.46	1.7	3.7	2.4
LA/LNA[c]	92.10 ± 34.15	31.7	198.0	89.2	34.82 ± 12.11	11.5	69.0	37.4
AA/DHA	3.78 ± 1.44	1.5	7.7	3.5	2.39 ± 0.52	1.6	3.9	2.3

[a] mean ± SD

[b] n=50 for plasma and n=46 for erythrocytes, omits outliers due to merging peaks

[c] n=51 for plasma and n=46 for erythrocytes, omits outliers due to merging peaks

[d] n=50 for plasma and n=45 for erythrocytes, omits outliers due to merging peaks

Table 7

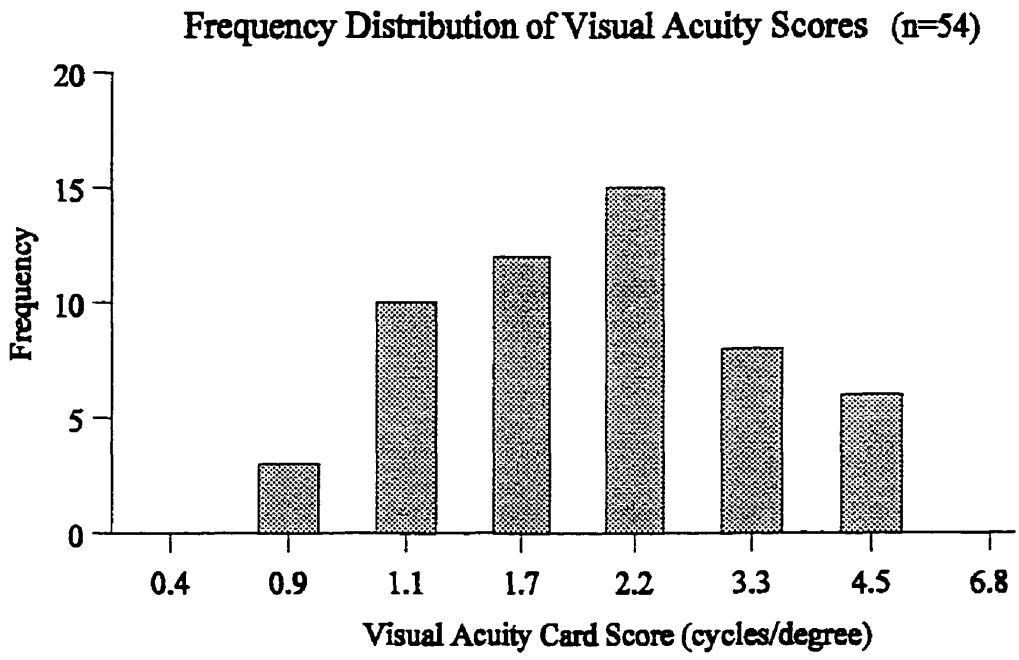
Fatty Acid Composition (wt/wt%)[a] of Total Lipids in Infant Plasma and Erythrocytes

Fatty Acid	Infant Plasma (n=38)				Infant Erythrocyte (n=33)			
	Mean ± SD	Min	Max	Median	Mean ± SD	Min	Max	Median
C12:0	0.56 ± 0.56	0.0	2.0	0.5	nd[b]	-	-	-
C14:0	3.28 ± 1.13	1.4	5.4	3.4	0.83 ± 0.26	0.3	1.6	0.8
C16:0	22.98 ± 2.84	18.3	29.7	22.5	24.59 ± 1.42	20.1	27.0	24.8
C18:0	7.03 ± 1.17	5.0	9.1	7.0	11.88 ± 1.32	9.0	15.3	11.8
C16:1n-7	3.68 ± 1.96	1.5	11.0	2.9	1.01 ± 1.16	0.4	7.2	0.7
C18:1n-9 & C18:1n-7	26.03 ± 5.47	18.7	35.0	25.7	17.49 ± 2.73	14.3	26.3	16.8
C18:2n-6	23.11 ± 7.71	6.7	36.3	25.2	10.33 ± 1.93	6.0	14.2	10.5
C18:3n-6	0.48 ± 0.21	0.1	1.0	0.4	0.29 ± 0.10	0.1	0.6	0.3
C20:3n-6	1.27 ± 0.40	0.6	2.2	1.2	2.09 ± 0.49	1.5	3.3	2.0
C20:4n-6	6.35 ± 1.81	3.1	9.7	6.9	16.48 ± 1.82	10.4	19.0	17.0
C22:4n-6	1.06 ± 0.57	0.4	2.3	0.9	5.60 ± 1.13	2.7	8.1	5.4
C18:3n-3	0.56 ± 0.35	0.2	1.7	0.5	0.36 ± 0.15	0.1	0.8	0.4
C20:5n-3	0.41 ± 0.17	0.1	0.9	0.4	0.43 ± 0.23	0.2	1.0	0.4
C22:5n-3	0.29 ± 0.17	0.1	0.7	0.2	1.03 ± 0.37	0.6	2.2	0.9
C22:6n-3	2.82 ± 0.84	1.1	4.9	2.8	7.41 ± 1.16	5.3	9.4	7.4
Total SAFA	34.08 ± 3.91	28.1	42.5	33.5	37.30 ± 1.29	33.1	40.7	37.2
Total MUFA	29.90 ± 7.19	20.8	44.2	27.8	18.50 ± 3.62	14.8	33.5	17.4
Total PUFA	35.91 ± 9.54	17.3	46.8	39.0	44.04 ± 4.02	28.2	49.6	45.0
Total n-6	31.84 ± 9.58	13.3	44.3	35.0	34.80 ± 3.72	20.8	39.9	35.4
Total n-3	4.08 ± 0.92	2.4	6.4	4.1	9.25 ± 1.20	7.4	11.8	9.4
n6/n3	8.28 ± 3.49	2.5	18.5	8.1	3.82 ± 0.64	2.8	5.3	3.8
ΣLC n-6	8.66 ± 2.10	4.6	12.1	8.7	24.17 ± 2.52	14.7	28.7	24.5
ΣLC n-3	3.52 ± 0.35	1.9	6.1	3.6	8.88 ± 1.20	7.0	11.0	9.0
ΣLC n-6/n-3	2.57 ± 0.72	1.4	4.5	2.4	2.76 ± 0.43	2.0	3.6	2.7
LA/LNA	60.2 ± 41.14	8.5	149.0	62.8	34.51 ± 17.97	8.6	83.0	64.5
AA/DHA	2.41 ± 0.80	0.8	4.2	2.3	2.26 ± 0.37	1.7	3.0	2.2

[a] mean ± SD

[b] nd = not detectable

Figure 6

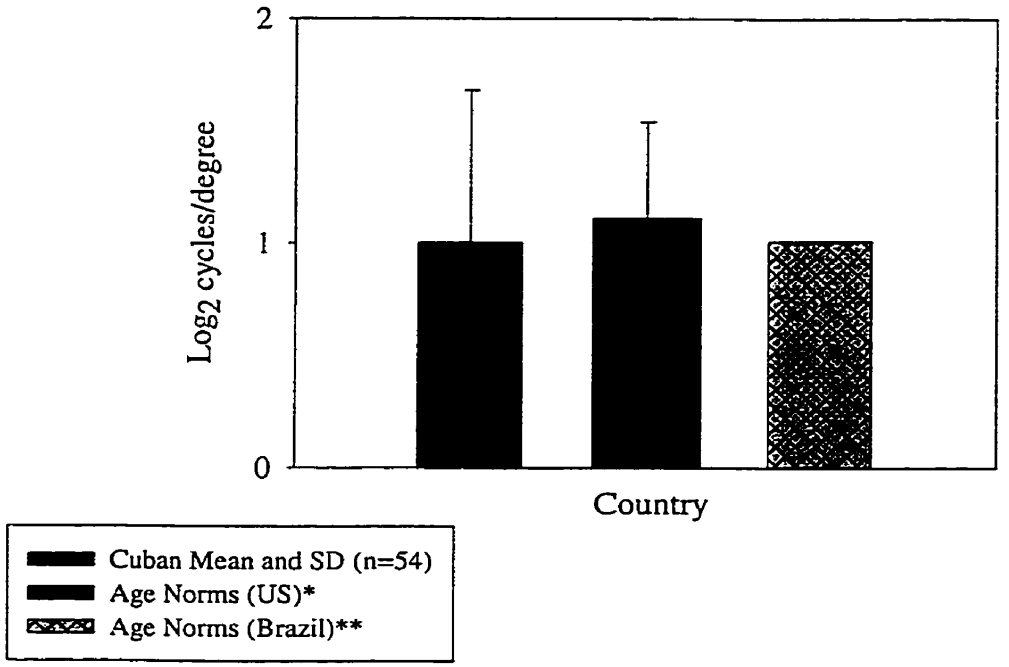


5.41 INTRA OBSERVER RELIABILITY

Of the 54 infants tested for this study, it was possible to retest 12 in order to assess intra observer reliability. Eleven of the12 infants were retested within 2 hours of the initial test and one returned the following week to be retested. Test scores were the same upon retest for 6 of the 12, a one card difference was obtained for 5 of the infants and a 2 card difference for 1 infant, having 92% of the scores fall within 1 card (0.5 octaves) of the initial test result. Therefore, no systematic differences were found between test and retest scores, as the norm is that 90% of the retests fall within 0.5 octaves (a 1 card difference) of the initial test (Mayer *et al.* 1995).

Figure 7

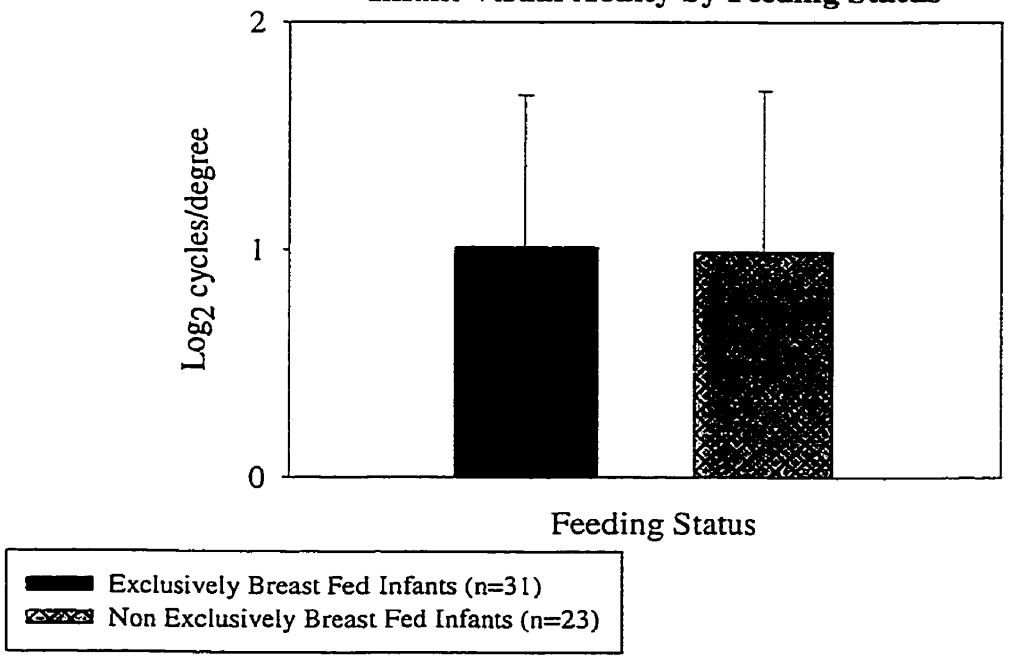
Visual Acuity Scores of Cuban Infants Compared to Age Matched Norms



*Mayer et al. (1995) 2.5 month old infants
**Rios-Salomão & Fix-Ventura (1995) 2 month old infants

Figure 8

Infant Visual Acuity by Feeding Status



5.6 Correlation Analyses

5.61 Relations Within Samples

Relations between certain fatty acids and groups of fatty acids in BM samples are presented in Tables 8 and 9, showing significant relations of varying degrees within and between fatty acid families and groups of fatty acids. As variables which had outliers were not influential in nature, correlations with and without them were similar. Therefore, correlations for the entire group are presented in all correlation tables. To distinguish between the type of correlation performed, Spearman or Pearson, a separate column in each graph contains this information when applicable. In the following text, Pearson correlations are presented with a subscript P and Spearman's correlations with a subscript S. To highlight significant findings, results that were significant at the 0.01 level are presented in bold characters, while those that revealed a trend towards significance are presented in italicized characters in all correlation tables. The highest positive correlations for fatty acids in BM samples were between DHA and EPA (r_s=+0.69, p=0.0001), C16:0 and C18:0 (r_p= +0.44, p=0.001), C18:0 and C18:1 (r_p= +0.39, p=0.004). The strongest negative correlations in this tissue were between IMCSAFA and C18:1 (r_p= -0.76, p=0.0001), C16:0 and LA (r_p= -0.70, p=0.0001), and C18:0 and LA (r_p= -0.47, p=0.0004). A slight trend towards a positively significant relation was seen between the sum of all long chain n-6 fatty acids (ΣLC n-6) and the sum of all long chain n-3 fatty acids (ΣLC n-3) (r_s= +0.25, p=0.05).

Similar results are presented for maternal and infant erythrocyte and plasma fatty acids in Tables 10 and11. Once again EPA and DHA exhibited a strong positive relation, as seen in plasma and erythrocytes of maternal samples (r_s= +0.49 and r_s= +0.42, respectively, p<0.001). The LA/LNA ratio of plasma samples was also positively related to the presence of AA (r_p= +0.34, p=0.01), while LA revealed a trend of being negatively related to the presence of both AA and the ΣLC n-6 in maternal erythrocytes. The content of LA was also found to negatively relate to DHA in maternal erythrocytes (r_s= -0.35, p=0.01). Infant plasma samples showed a fair number of correlations between

Table 8

Pearson Correlations[a] of Fatty Acids in Mature Human Milk (n=52)

Fatty Acid	C16:0	C18:0	C18:1	C18:2 n-6
IMCSAFA	*-0.32*	**-0.34**	**-0.76**	-0.09
(C8:0 to C14:0)	*0.02*	**0.01**	**0.0001**	NS[b]
C16:0		**+0.44**	**+0.39**	**-0.70**
		0.001	**0.004**	**0.0001**
C18:0			**+0.51**	**-0.47**
			0.0001	**0.0004**
C18:1				**-0.42**
				0.002

[a] r value over p value
[b] NS = not significant

Table 9

Spearman and Pearson Correlations of Essential Fatty Acids in Human Milk (n=52)

x	y	T[a]	r	p
C18:2 n-6	C18:3 n-3	**S**	**-0.44**	**0.001**
C18:2 n-6	C20:4 n-6	**P**	*+0.29*	*0.04*
C18:2 n-6	ΣLC n-6	**P**	+0.25	NS
C18:2 n-6	C22:6 n-3	**S**	-0.08	NS
C18:3 n-3	C22:6 n-3	**S**	+0.18	NS
C18:3 n-3	C20:5 n-3	**S**	+0.01	NS
C18:3 n-3	ΣLC n-3	**S**	+0.19	NS
C20:5 n-3	C22:6 n-3	**S**	**+0.69**	**0.0001**
ΣLC n-6	ΣLC n-3	**S**	+0.25	*0.05*
LA/LNA	C20:4 n-6	**S**	+0.25	NS
LA/LNA	C22:6 n-3	**S**	-0.18	NS

[a] T= type of correlation, S= Spearman; P= Pearson

Table 10

Spearman and Pearson Correlations of Essential Fatty Acids in Maternal Plasma and Erythrocytes

		Erythrocytes (n=50)			Plasma (n=55)		
x	y	T[a]	r	p	T[a]	r	p
C18:2 n-6	C18:3 n-3[b]	**S**	*-0.34*	*0.02*	**S**	*-0.27*	*0.06*
C18:2n-6	C20:4 n-6	**P**	*-0.29*	*0.04*	**P**	-0.08	NS
C18:2n-6	ΣLC n-6	**P**	*-0.32*	*0.02*	**S**	-0.19	NS
C18:2 n-6	C22:6 n-3	**P**	**-0.35**	**0.01**	**S**	-0.12	NS
C18:2 n-6	C20:5 n-3	**S**	-0.22	NS	**S**	*-0.32*	*0.02*
C18:3n-3[b]	C22:6 n-3	**S**	-0.11	NS	**S**	+0.15	NS
C18:3n-3[b]	C20:5 n-3	**S**	+0.10	NS	**S**	*+0.31*	*0.03*
C18:3n-3[b]	ΣLC n-3	**S**	-0.03	NS	**S**	*+0.26*	*0.06*
C20:5 n-3	C22:6 n-3	**S**	**+0.47**	**0.007**	**S**	**+0.49**	**0.0001**
ΣLC n-6	ΣLC n-3	**S**	-0.02	NS	**S**	+0.06	NS
LA/LNA[b]	C20:4 n-6	**P**	*-0.30*	*0.04*	**P**	+0.25	NS
LA/LNA[b]	C22:6 n-3	**P**	-0.10	NS	**S**	-0.17	NS

[a] T= type of correlation, S= Spearman; P= Pearson

[b] for correlations with C18:3 n-3: n=51 for plasma and n=46 for RBC, omits chromatograms with merging peaks

Table 11

Spearman Correlations of Essential Fatty Acids in Infant Plasma and Erythrocytes

		Erythrocytes (n=33)		Plasma (n=31)	
x	y	r	p	r	p
C18:2 n-6	C18:3 n-3	-0.29	NS	**-0.59**	**0.0005**
C18:2 n-6	C20:4 n-6	+0.01	NS	**+0.54**	**0.002**
C18:2 n-6	ΣLC n-6	+0.18	NS	**+0.57**	**0.0008**
C18:2 n-6	C22:6 n-3	+0.13	NS	+0.09	NS
C18:2 n-6	C20:5 n-3	*-0.40*	*0.02*	**-0.47**	**0.007**
C18:3n-3	C22:6 n-3	-0.21	NS	-0.29	NS
C18:3n-3	C20:5 n-3	+0.11	NS	+0.27	NS
C18:3n-3	ΣLC n-3	-0.13	NS	-0.20	NS
C20:5 n-3	C22:6 n-3	0.00	NS	+0.22	NS
ΣLC n-6	ΣLC n-3	+0.15	NS	*+0.38*	*0.04*
LA/LNA	C20:4 n-6	*+0.37*	*0.03*	**+0.75**	**0.0001**
LA/LNA	C22:6 n-3	+0.21	NS	+0.24	NS

fatty acids having LA relate positively to its long chain metabolites AA and ΣLC n-6 in the plasma fraction. The LA content showed a strong negative relation with LNA content in plasma of infants. However, no highly significant relations were noted in the erythrocyte fraction of infant blood.

5.62 Relations Between Samples

The relationship between EFA profiles of different maternal tissues are presented in Table 12, showing highly significant, strong positive relations for all LCP for which a potential relation was assessed. Relations between infant plasma and erythrocytes were also apparent, and are presented in Table 13. Relations of infant plasma and RBC of exclusively BF infants with maternal samples are presented in Tables 14 and 15, showing very few significant relations.

5.63 Relations Between Fatty Acids, Visual Acuity Development and Standardized Anthropometry

Significant correlations between visual acuity score or age and gender standardized anthropometrical measures of infants and any EFA in infant tissues were not found to exist when assessing the entire group together or each group alone by feeding status. No relation between EFA or IMCSAFA profiles of maternal tissues for exclusively BF infants and visual acuity development, or infant standardized anthropometrical measures, were found either.

5.64 Other Relations

The content of fat in human milk samples was not found to relate to maternal weight, BMI or MUAC at 2 months. The milk fat content was also not found to relate to age and gender standardized anthropometrical measures of exclusively BF infants.

Table 12

Spearman and Pearson Correlations of Essential Fatty Acids Between Maternal Tissues

Fatty Acid		Erythrocytes			Plasma vs.			Erythrocytes vs.	
	T[a]	r	p	T[a]	r	p	T[a]	r	p
C18:2 n-6	**P**	**+0.41**	**0.004**	**P**	**+0.59**	**0.001**	**P**	**+0.40**	**0.004**
C20:4 n-6	**P**	*+0.29*	*0.05*	**P**	**+0.45**	**0.001**	**P**	**+0.37**	**0.009**
C18:3 n-3[b]	**S**	+0.03	NS	**S**	+0.19	NS	**S**	-0.19	NS
C20:5 n-3	**S**	**+0.46**	**0.001**	**S**	**+0.43**	**0.002**	**S**	**+0.70**	**0.0001**
C22:6 n-3	**S**	**+0.55**	**0.0001**	**S**	**+0.65**	**0.0001**	**S**	**+0.64**	**0.0001**
ΣLC n-3	**S**	**+0.60**	**0.0001**	**S**	**+0.64**	**0.0001**	**S**	**+0.61**	**0.0001**

[a] T= type of correlation, S= Spearman; P= Pearson

[b] not including chromatograms with merging peaks for C18:3 n-3: n=40 for Erythrocytes x Milk, n=46 for Plasma x Milk, and n=43 for Erythrocyte x Plasma

Table 13

Spearman Correlations of Essential Fatty Acids Between Infant Plasma and Erythrocytes

Fatty Acid	Erythrocytes vs. Plasma (n=26)	
	r	p
C18:2 n-6	**+0.33**	**0.0001**
C20:4 n-6	+0.45	*0.02*
C18:3 n-3	*+0.45*	*0.02*
C20:5 n-3	+0.60	**0.001**
C22:6 n-3	+0.07	NS
ΣLC n-3	+0.06	NS

Table 14

Spearman and Pearson Correlations of Milk and Infant Essential Fatty Acids for Exclusively Breastfed Infants

Fatty Acid	Milk vs. Erythrocytes (n=18)			Milk vs. Plasma (n=16)		
	T[a]	r	p	T[a]	r	p
C18:2 n-6	**P**	+0.28	NS	**S**	**+0.76**	**0.0007**
C20:4 n-6	**S**	+0.22	NS	**P**	-0.06	NS
C18:3 n-3	**S**	-0.20	NS	**S**	+0.13	NS
C20:5 n-3	**S**	**+0.64**	**0.0004**	**S**	+0.39	NS
C22:6 n-3	**S**	+0.42	NS	**S**	+0.25	NS
ΣLC n-3	**S**	+0.42	NS	**S**	+0.31	NS

[a] T= type of correlation, S= Spearman; P= Pearson

Table 15

Spearman and Pearson Correlations Between Maternal and Infant Essential Fatty Acids for Exclusively Breastfed Infants

Fatty Acid	Erythrocytes (n=15)			Plasma (n=17)		
	T[a]	r	p	T[a]	r	p
C18:2 n-6	**P**	**+0.69**	**0.004**	**S**	*+0.48*	*0.05*
C20:4 n-6	**S**	+0.34	NS	**P**	+0.19	NS
C18:3 n-3[b]	**S**	-0.10	NS	**S**	+0.24	NS
C20:5 n-3	**S**	*+0.50*	*0.06*	**S**	**+0.62**	**0.008**
C22:6 n-3	**S**	+0.19	NS	**S**	+0.43	NS
ΣLC n-3	**S**	+0.29	NS	**S**	+0.25	NS

[a] T= type of correlation, S= Spearman; P= Pearson
[b] for correlations with C18:3 n-3 (LNA): n=14 for Erythrocytes, n=16 for Plasma, omits chromatograms with merging peaks

5.7 Differences Between Exclusively Breast Fed Infants and Weaned Infants

No differences were found between the 2 feeding groups for infant standardized anthropometrical measures (Table 16). As Z scores for infant length at 2 months were not normally distributed in the weaned group, the range and median are also presented for this variable. There were a fair number of significant differences between feeding groups for fatty acids of the n-6 series (Tables 17 and 18). Differences were more prominent in the plasma fraction than in the erythrocyte fraction. There were no notable differences between feeding groups for visual acuity scores as per an unpaired student's t-test (Figure 8).

Table 16

Infant Standardized Anthropometry at 2 Months by Feeding Status

	Weight Z Score	Head Circumference Z Score	Length Z Score			
			Mean ± SD	Min	Max	Median
Exclusively Breast Fed (n=30)[a]	+0.36 ± 0.75	+0.04 ± 0.69	-0.18 ± 0.85	-1.50	+1.39	-0.46
Not Exclusively Breast Fed (n=25)	+0.22 ± 0.82	+0.19 ± 1.00	-0.21 ± 0.82	-2.41	+1.29	-0.11

[a] n=29 for head circumference Z score

Table 17

Fatty Acid Composition of Total Lipids in Plasma of Infants by Feeding Group

Fatty Acid	Exclusively Breast Fed (n=17) Mean ± SD	Min	Max	Median	Not Exclusively Breast Fed (n=[illegible]) Mean ± SD	Min	Max	Median
C18:2n-6[a]	**27.91 ± 4.11**	19.7	36.3	29.2	**18.61 ± 7.27****	6.7	30.6	18.5
C20:4n-6	**7.31 ± 1.45**	4.3	9.7	7.7	**5.19 ± 1.53****	3.1	7.9	5.1
C18:3n-3[a]	**0.37 ± 0.13**	0.2	0.6	0.4	**0.75 ± 0.40****	0.3	1.7	0.7
C22:6n-3	2.85 ± 0.72	1.5	3.9	2.8	2.77 ± 1.01	1.1	4.9	2.9
Total n-6[a]	**38.06 ± 3.72**	30.5	44.3	39.2	**25.86 ± 9.48****	13.3	42.8	25.0
Total n-3	3.96 ± 0.83	2.4	5.1	4.0	4.21 ± 1.04	2.7	6.4	4.2
n-6/n-3	**9.88 ± 3.52**	3.4	18.5	8.9	**6.34 ± 2.36****	2.5	10.7	5.7
AA/DHA	*2.72 ± 0.83*	1.3	4.2	2.5	*2.04 ± 0.61**	0.8	2.8	2.0

Table 18

Fatty acid Composition of Total Lipids in Erythrocytes of Infants by Feeding Group

Fatty Acid	Exclusively Breast Fed (n=18) Mean ± SD	Min	Max	Median	Not Exclusively Breast Fed (n=15) Mean ± SD	Min	Max	Median
C18:2n-6	**11.28 ± 1.47**	8.3	14.2	11.1	**9.20 ± 1.82****	6.0	12.5	9.9
C20:4n-6[b]	16.87 ± 1.52	14.7	18.9	17.4	16.37 ± 1.48	14.5	19.0	16.3
C18:3n-3[a]	*0.30 ± 0.12*	0.1	0.5	0.3	*0.41 ± 0.12*‡	0.2	0.7	0.4
C22:6n-3	7.66 ± 1.00	6.0	9.4	7.8	7.12 ± 1.30	5.3	8.8	6.8
Total n-6[c]	**36.60 ± 2.02**	32.9	39.9	36.8	**33.49 ± 0.72****	27.4	37.9	33.2
Total n-3	9.29 ± 1.28	7.4	11.8	9.3	9.19 ± 1.14	7.4	11.3	9.6
n-6/n-3	*4.02 ± 0.67*	2.9	5.3	4.0	*3.58 ± 0.53**	2.8	4.6	3.5
AA/DHA	2.24 ± 0.36	1.7	2.9	2.2	2.28 ± 0.40	1.7	3.0	2.2

[a] one outlying subject in the exclusive BF group excluded from analysis
[b] one outlying subject in the exclusive BF group and one from the not exclusive BF group excluded from analysis
[c] one outlying subject in the not exclusive BF group excluded from analysis
* and ** and‡; significantly different from the exclusively BF group, *p≤0.05 and **p≤0.01 by T-test and ‡p<0.05 by Wilkoxon Rank Sum Test

5.8 Other Tests

Rates of exclusive BF were not found to be dependant on maternal anthropometrical measures at 2 months as assessed by an unpaired t-test (Table 19). The following variables were also not found to be associated with rates of exclusive BF by Chi Square analysis; infant gender, receiving overseas monetary help, residential area or ethnic background.

Table 19
Maternal Anthropometry at 2 Months by Feeding Status

[illegible]	[illegible]	[illegible]
Maternal Weight (kg)	61.79 ± 10.0	59.67 ± 9.37
Maternal BMI (kg/m^2)	24.22 ± 3.42	23.23 ± 3.75
MUAC (cm)	26.29 ± 3.22	25.34 ± 3.22

[a] n=29 for MUAC

6. DISCUSSION

6.1 Breast Milk Fatty Acids

Fatty acid analysis of the 52 BM samples revealed normal composition, as averages and ranges of all fatty acids fell within values found in other countries. Comparison of certain EFA with those found in a variety of other populations can be seen in Appendix G through Appendix K. One fatty acid which was particularly high in the BM samples of this group was LA, making up almost 20% of total fatty acids. This is not abnormal however, as similar levels have been found in other groups (Muskiet *et al.* 1989; Kneebone *et al.* 1985; Budowski *et al.* 1994; Innis & Kuhnlein 1988; Henderson *et al.* 1992), while substantially higher levels were found in a variety of investigations (Chulei *et al.* 1995; De Tomas *et al.* 1994; Van der Westhuyzen *et al.* 1988; De Lucchi *et al.* 1988). It also appeared as if the content of LA in BM samples was considerably homogeneous in this group, as the coefficient of variation (CV) was 24%, indicating rather uniform intakes of this fatty acid in the Cuban population. The high level of LA in Cuban BM samples appeared to be replacing OA, as levels of OA were moderately lower than that found in other countries. Actually, the average LA and OA proportions found in BM samples in this investigation were almost identical to those found in the study by Budowski *et al.* (1994), indicating that intake of these 2 fatty acids may be similar in both populations. Despite differences in the above mentioned fatty acids, levels of AA and DHA did not seem to be affected by the high content of LA in BM samples.

Arachidonic acid content of BM samples was normal, averaging almost 0.7% of total fatty acids. The CV for AA was 23%, which is similar to that found in other investigations (Budowski *et al.* 1994; Rocquelin *et al.* 1998). This is indicative of uniformity in the content of this fatty acid among BM samples in this population. It has been suggested that the content of AA in BM is controlled and thus not affected by large variations in AA intake (Chen *et al.* 1997), which explains the moderate CV of this fatty acid. Levels of DHA in BM averaged $0.43 \pm 0.26\%$ of total fatty acids, ranging from 0.09% to 1.55%, which is higher than that found in many Western countries (Chen *et al.*

1995; Serra *et al.* 1997; Sanders & Reddy 1992). It does not appear as if the subjects with outlying values of n-3 fatty acids artificially increased the means of these fatty acids for the group investigated in a manner which is of clinical significance, however, they did influence the variance to a greater extent. The CV without the outliers was 45.45%, while it was 60.41% with them. A large CV was also found for EPA, being slightly over 60%. It is conceivable that this is due to the fact that unlike the AA content of BM, levels of DHA and EPA have been found to vary with dietary intake to a great degree (Innis & Kuhnlein 1988; Chulei *et al.* 1995; Makrides *et al.* 1996). The EFA levels present in BM samples of this group are indicative that there was no apparent problem of low n-3 fatty acid status in this sample of BF women. The presence of the above mentioned levels of DHA in BM are not likely to occur in a group suffering from poor n-3 fatty acid intakes or an excessively unbalanced ratio of dietary n-6/n-3 fatty acids. If this group of women were suffering from poor n-3 fatty acid status for a prolonged period of time, as argued by INHA staff, it would be expected that EFA profiles would parallel those of other nations experiencing poor n-3 fatty acid intakes, as was seen in rural South African women (Van der Westhuyzen *et al.* 1988). In that investigation, a poor n-3 fatty acid intake was evident through fatty acid composition of BM samples. The BM fatty acid profiles of this group revealed low levels of LNA and DHA ($0.1 \pm 0.1\%$ and $0.1 \pm 0.2\%$ of total fatty acids, respectively), which were indicative of poor n-3 fatty acid status.

To address the argument that high levels of n-6 fatty acids negatively affect the presence of DHA in BM, it should be noted that this effect has been found in populations with excessively low n-3 fatty acid intakes, and negligible quantities of dietary LCP (Van der Westhuyzen *et al.* 1988; Sanders & Reddy 1992). The availability of food items containing long chain n-3 fatty acids in the diet of the Cuban population, such as fish and eggs, may be responsible for the fact that DHA levels were not negatively affected by the slightly elevated content of LA in BM samples. Normal and high levels of DHA in BM were also seen in other populations, despite even higher levels of LA in BM samples when a number of dietary sources of n-3 fatty acids were available (Chulei *et al.* 1995).

Ratios of BM fatty acids also fell within normal ranges. The LA/LNA ratio of BM

samples suggest that consumption of vegetable oils other than soy oil exist in the Cuban population. For women residing in Israel, where soy oil makes up the greatest proportion of dietary fat, the LA/LNA ratio of BM was found to be almost identical to soy oil, being 13:1 (Budowski *et al.* 1994). However the LA/LNA ratio of the BM samples in this investigation was almost 23:1, indicating that an LA rich and LNA poor oil must also be consumed rather frequently in Cuba. The LA/LNA ratio of BM in this investigation was however adequate, as it fell within the typical range of 8:1 to 34:1 (Anonymous 1998). If it were the case that the group under investigation consumed an excessively high amount of n-6 fatty acids relative to n-3 fatty acids, it would be expected that this would be reflected in the BM ratio of LA/LNA and AA/DHA. In the investigation by Van der Westhuyzen *et al.* (1988), rural women consuming an n-3 fatty acid poor diet were found to have a LA/LNA ratio of 157:1 and an AA/DHA ratio of 10:1, in BM samples, far surpassing those found in this investigation (Table 5).

The ratio of long chain n-6/n-3 fatty acids was in accordance with values found in other countries (Budowski *et al.* 1994; Koletzko *et al.* 1988; Chen *et al.* 1997), suggesting an acceptable dietary balance for BF infants. The presence of high levels of PUFA resulted in a high P:S ratio for BM fatty acids. The P:S ratio of BM in the group of Cuban women investigated was 0.56:1, being identical to that found in Israeli women who consumed a diet rich in PUFA (Budowski *et al.* 1994).

Content of IMCSAFA, which includes C8:0 through C14:0, fell in between values for Western countries and developing countries, being more closely related to the higher end found in populations consuming Westernized diets (Appendix K). This group of fatty acids are known to be produced de novo within the mammary gland, and the degree of their presence increases with increasing levels of carbohydrate in the diet, and decreasing fat intake (Insull *et al.* 1959; Read *et al.* 1965a; Read *et al.* 1965b; Hachey *et al.* 1989). It has also been noted that levels of IMCSAFA increase with lower energy intakes (Kneebone *et al.* 1985). Therefore, it can be thought that the diet of the lactating women under investigation was not as high in carbohydrate, or as low in energy and fat as is typical in many developing countries. Yet, as it was on the higher end of values found in

Western countries, it must most likely be on the higher end for carbohydrate intake, and lower end for fat intake, in these nations. Adequate maternal energy status was also seen through anthropometrical measures such as BMI and MUAC (Appendix D). The presence of ample quantities of IMCSAFA is seen as a desirable quality of BM by some nutritionists, since they are easily absorbed and are a rich energy source for growing infants (Rocquelin *et al.* 1998). They may also play an important role in sparing EFA from oxidation for energetic purposes (Uauy-Dagach *et al.* 1998). However, recommendations for levels of this group of fatty acids in BM are currently not clear (Anonymous 1998). As the IMCSAFA levels of the group investigated are neither excessively high nor excessively low, it can be argued that this may be optimal based on current knowledge.

Correlation analysis of fatty acids in BM also showed a fair number of highly significant relations between certain fatty acids and groups of fatty acids. Most investigations do not assess the relations between groups of fatty acids to the extent done in these analyses. Therefore, comparisons can be made only with a limited number of investigations. The relation between LA and LNA found in Cuban BM samples was negative and highly significant (r_s= -0.44, p=0.001). The only other investigation which found a relation between these 2 fatty acids is in opposition to the results of this study (Budowski *et al.* 1994). The authors of that investigation found a highly significant positive correlation between these 2 fatty acids (r= +0.80, p=0.0001) in the BM of Israeli women. This is most likely due to the fact that soy oil was the main source of LA for the group of Israeli women (Budowski *et al.* 1994), which has an ample quantity of LNA, implying that as LA levels increased, LNA levels would have increased as well. However, other LA rich and LNA poor oils, such as sunflower oil, are probably consumed in Cuba, as seen through this negative correlation and higher LA/LNA ratio than in Israeli samples. This is most likely the situation as the 3 oils Cuba primarily imports are soy oil, palm oil and sunflower oil (AAWH 1997). It would thus appear that women who have access to other oils beyond that which is received through the food distribution system usually opt for those with a higher n-6 content. As other nations also consume LA rich

LNA poor oils, it is likely that this relation may have existed in other investigations, but as correlations between fatty acids in BM are not regularly assessed, this information remains unknown. The fact that the relation found in the study by Budowski *et al.* (1994), was so highly significant may also be due to the fact that they used 2 samples from each person in the calculation of the correlation. Therefore, it is likely that the degree of the correlation is due to a combination of an actual relation among all subjects, as well as the obvious relation within subjects.

A slight positive trend for an association between the ΣLC n-6 and the ΣLC n-3 fatty acids was also noted (r_s= +0.25, p=0.05). This, however, was lower than that found in the investigation by Budowski *et al.* (1994), where the relation between these groups of fatty acids was somewhat stronger and more significant (r= +0.44, p=0.001). This again may have been due to the fact that the authors of that investigation appeared to have used 2 samples from each subject in correlation analyses which may have artificially increased the significance of their results. One other investigation did find a strong positive relation between these 2 groups of fatty acids (r= +0.80, p<0.05) (Koletzko *et al.* 1988). The degree of this association may have been due to the fact that *trans* fatty acids were present in differing degrees in the BM samples of the investigation by Koletzko *et al.* (1988). As *trans* fatty acids are thought to limit the capacity of desaturation and elongation for the formation of LCP from parent EFA's (Rosenthal & Doloresco 1984; Houwelingen & Hornstra 1994; Koletzko 1992), it may be that this relation would be more prominent in a society with varying degrees of enzyme activity for the formation of LCP from both the n-6 and n-3 series, due to these potential inhibitors. It is possible that the lack of a strong relation between long chain n-6 and long chain n-3 fatty acids in the Cuban population is due to the consumption of long chain n-3 fatty acids which would alter the composition of BM in terms of these fatty acids, more so than intake of food items with high levels of long chain n-6 fatty acids (Chen *et al.* 1997). If one were relying on parent precursors for the formation of LCP, it is possible that the relation between these 2 fatty acids would be more pronounced than in a society where they may be consumed through the diet directly.

As in other investigations, the parent EFAs, LA and LNA were not found to

correlate to their longer chain metabolites AA and DHA or ΣLC n-6 and ΣLC n-3 (Rocquelin *et al.* 1998; Koletzko *et al.* 1988). However, a study by Budowski *et al.* (1994), did find a positive relation between LA and the ΣLC n-6 fatty acids, and LNA and ΣLC n-3 fatty acids ($r = +0.43$, $p=0.002$ and $r = +0.30$, $p=0.03$, respectively), while LA was not found to be related to AA nor LNA to DHA. Therefore, it may be that the longer chain metabolites in the BM of Cuban women, were obtained from preformed LCP ingested through the diet. As comments regarding ingestion of food items with LCP in the group of Israeli women were not made in the investigation by Budowski *et al.* (1994), whether the hypothesis is potentially valid, is unknown.

The relation between IMCSAFA and other groups of fatty acids were in accordance with other investigations. Correlation analysis illustrated a similarity between the studies by van Beusekom *et al.* (1990), and Rocquelin *et al.* (1998), in that this investigation exhibited a strong negative and highly significant correlation between IMCSAFA and OA, IMCSAFA and C18:0, and a trend towards a negative correlation between IMCSAFA and C16:0 (Table 8). This exemplifies the fact that when the level of de novo fatty acid synthesis in the mammary gland decreases, the IMCSAFA are mainly replaced by OA, and to a lesser extent by the saturated long chain fatty acid that is present in circulation, C18:0 and C16:0. The 3 fatty acids which related negatively to IMCSAFA, namely C18:0, C18:1, and C16:0, were all found to relate positively to one another (Table 8). This indicated that mothers who had higher levels of IMCSAFA, possibly due to ingestion of higher carbohydrate diets, had lower amounts of long chain saturated fatty acids and MUFA incorporated into BM from circulation. Levels of LA were also negatively related to the 3 previously mentioned fatty acids. This implies that higher dietary PUFA may be related to lower dietary SAFA and MUFA in the women investigated. However, levels of these fatty acids fell within typical values. Therefore, the biological importance of this finding for the growing infant is not considered of significance according to current knowledge.

Fat content of the milk samples was slightly higher than the range found in mature BM at 2 months, being 5.29 ± 1.81 g per 100 ml (Akre 1989). This may be due to the fact

that due to procedural difficulties, milk was only extracted at mid morning, which is when lipid content is supposed to be at its highest (Rocquelin *et al.* 1998). The range of fat content was also large, having one subject with a particularly high amount of total milk fat being 13.97 g per 100 ml. Levels exceeding 10 g per 100 ml have been noted previously (Jensen 1989), however, it is quite rare for a healthy individual to produce milk with such a high fat content. Reasons for the high fat content of this individual are unknown, but factors such as maternal dietary habits, or disease are most likely involved (Jensen *et al.* 1996). Unfortunately, the person obtaining the breast milk samples did not record information which could have explained such a high level, such as volume removed. However, it is assumed that the contents of an entire breast were removed as stipulated by procedures.

Measures of maternal anthropometry were not related to fat content of BM samples. This is in accordance with investigations which assessed groups of women from Western nations with average BMI's of at least 21 kg/m^2, while countries where average BMI's were less than 19 kg/m^2 often showed positive relations between milk fat content and increased weight or fatness (Brown & Dewey 1992). Based on the results of the fatty acid analysis of BM, it is quite obvious that the population of BF women residing in Havana produce milk with adequate fat, EFA and IMCSAFA contents to support the optimal development of BF infants.

6.2 Maternal and Infant Fatty Acid Profiles

The fatty acid profiles of total lipids in plasma and erythrocytes of maternal samples were highly related to profiles of BM. This suggests that EFA are taken in from circulation to be incorporated into BM and that the adequate status found in BM samples was found in these tissues as well. Table 12 shows a variety of highly significant, strong positive relations between maternal blood and BM EFA levels. These relations were slightly stronger between plasma and BM than erythrocyte and BM in most cases. This is most likely due to the fact that both plasma and BM fatty acid profiles are indicative of more recent intakes while RBC profiles have a longer turnover rate and are indicative of

past EFA intake (Nettleton 1995). Of interest is the fact that AA in BM was significantly related to AA in plasma (r= +0.51, p=0.001), and the noted trend for a relation of AA between erythrocytes and BM (r= +0.34, p=0.02). The reason this relation brings about curiosity is because AA has been found to remain rather stable despite wide variations in AA intake (Chen *et al.* 1997). However, aside from IMCSAFA, most fatty acids present in milk are taken in from circulation (Collier 1985). Therefore, it appears logical to see such a relation. The fact that long chain n-3 fatty acids were highly related between BM and plasma, BM and erythrocytes, as well as plasma and erythrocytes, implies that both recent and past intakes of these fatty acids were similar. Also levels of LCP present in erythrocytes and plasma of maternal samples were higher than those found in adults consuming typical Western diets (Appendix L and Appendix M). Therefore, it is conceivable to conclude that n-3 fatty acid deficiencies must be exceedingly rare, if they exist at all, in the population of BF women residing in Havana.

Fatty acids present in the total lipid fraction of maternal plasma and erythrocytes were similar to levels found in women living in Western societies supplemented with fish oil capsules (Appendix L and Appendix M). This may be due to the fact that *trans* fatty acids present in Western diets may inhibit the formation of LCP from their precursors (Koletzko 1995), which can be normalized through the direct consumption of LCP as through fish oil supplementation. Therefore, the levels of DHA found at the end of supplementation in the groups presented in Appendix L and Appendix M, may be indicative of normal status, while the baseline levels may not be as adequate. The level of DHA in plasma total fatty acids of 2 Inuit populations consuming a traditional diet high in marine animals was found to far surpass levels in individuals consuming typical Western diets, while the values from lactating Cuban women fell between these 2 groups (Appendix M). This indicates that the Cuban population is by no means consuming high levels of seafood noted among Northern Native populations, but either are receiving higher levels of dietary LCP or have a better capacity to form LCP from their precursors than people consuming typical Western diets.

Relations of fatty acids within maternal plasma and erythrocytes were less

frequent than in BM samples. As in BM samples, EPA and DHA were found to relate positively in both plasma and erythrocytes (r_s= +0.49 and r_s=0.42, respectively, $p<0.001$). A negative relation was found between LA and DHA (r_p= -0.34, p= -0.01), and a trend toward a negative relation was found between LA and AA (r_p= -0.36, p=0.04), and LA and ΣLCn-6 fatty acids (r_p=-0.32, p=0.02), in maternal erythrocytes. This is in accordance with an investigation assessing relations between fatty acids in Native populations, which found even stronger negative relations between these fatty acids in adults, but not children (Parkinson *et al.* 1994). These relations are explicable as high levels of LA can limit the amount of LNA that is converted to DHA, and compete with DHA for incorporation into phospholipid membranes. Competition for incorporation into membrane phospholipids between LA and its longer chain metabolite AA, is the most probable explanation for the negative relation found between these 2 fatty acids.

Average levels of all EFA fell within normal ranges seen in BF infants from other nations, and were higher than those fed SF (Appendix N and Appendix O). However, some infants were weaned on other milk, and showed different levels of certain fatty acids than those exclusively BF (Tables 17 and 18). This was mainly seen for levels of n-6 fatty acids, showing more pronounced differences in plasma than erythrocytes. This may have been due to the fact that in most recorded cases, weaning occurred rather recently (Table 4). As weaning progresses in such a group, the degree of depression of n-6 fatty acids in erythrocytes may parallel those of plasma. The higher levels of n-6 fatty acids in exclusively BF infants is logical since the proportion of LA in the cow milk preparation was substantially lower than in BM samples (12% vs. 19% of total fatty acids). Also, levels of LA in other dairy products fed to infants, such as evaporated milk are known to be even lower (Courage *et al.* 1998), which could be responsible for differences between groups.

Linoleic acid was found to be significantly lower in both plasma and erythrocytes of non exclusively BF infants, while AA levels were lower only in plasma. One reason this difference was not seen between these groups was that one subject receiving yogurt had a low outlying value for erythrocyte AA, and was thus removed from analysis. Also,

since weaning did occur rather recently in most cases, it would be expected to see more pronounced changes in plasma than in erythrocytes. The content of DHA did not appear to be affected by the practice of weaning, which is most likely due to a combination of factors. One possible reason is that weaned infants had significantly higher levels of LNA, the precursor to DHA, in both plasma and erythrocytes (Tables 17 and 18). This can be explained by the higher LNA content of the cow milk preparation as compared to the average found in BM (1.58% vs. 0.92% of total fatty acids). Another possible reason is the lower LA content and LA/LNA ratio of the cow milk formulation. This was reflected in a significantly lower n-6/n-3 ratio in the blood of weaned infants (Tables 17 and 18), which could favour the formation of DHA. Other dairy products fed to infants such as evaporated milk are known to have an even lower LA content and LA/LNA ratio (Courage *et al.* 1998), which is below that recommended by experts (Anonymous 1998). It is possible that with prolonged feeding of evaporated milk or other dairy products, significant differences may be seen for AA levels in erythrocytes, as was found in infants fed formula with a low LA content (3.47±0.03% of total fatty acids), after 10 weeks of feeding (Clark et al. 1992)

The differences in the level of certain fatty acids in infant plasma and erythrocytes may be masking a larger difference that would potentially exist between groups receiving different milks. Unfortunately, due to incomplete information regarding feeding practices for some infants (Tables 3 and 4), and loss of some infant blood samples (Figure 4), it was not possible to take different types of milk fed, or the length of time receiving supplemental milk into account. It could be assumed, however, that in terms of fatty acid profile, the cow's milk preparation would be better suited to infant needs than evaporated milk, as fatty acid levels in the former preparation more closely reflect current recommendations for infant formulas (Anonymous 1998). The availability of a product which possesses a fatty acid profile in accordance with current recommendations reduces the possibility that weaned infants in Cuba will develop an EFA deficiency as compared to those in other Latin American countries (De Tomas *et al.* 1994), as long as the proper milk is given. Also, since a high proportion of the infants are exclusively BF or partially

BF, infants are not at a high risk for suffering from n-3 fatty acid deficiency. The possibility of poor AA status in infants fed other dairy products, such as evaporated milk or yogurt should, however, be addressed. A discussion regarding the effect of BF and weaning practices on the health of infants in Cuba will be presented in a later section.

6.3 Infant Visual Acuity

The visual acuity test using the TAC procedure which was carried out in this investigation, can be used to assess functional normality in individuals and groups (Fielder *et al.* 1992; Mayer *et al.* 1995). As many investigators have related visual acuity to EFA status, it was assumed that a deficiency of n-3 fatty acids would have functional implications that could be detected by the TAC procedure (Birch *et al.* 1993; Birch *et al.* 1998; Carlson *et al.* 1995). However, many investigations have not found differences among groups with different n-3 fatty acid levels using the TAC procedure (Innis *et al.* 1996; Innis *et al.* 1997; Auestad *et al.* 1997). Results that were most prominent, were seen when a deficiency of n-3 fatty acids existed or potentially existed (Birch *et al.* 1993; Connor *et al.* 1988). Therefore, the main purpose of this test was to assess whether the infant population was suffering from a deficient intake of n-3 fatty acids which could lead to abnormal visual acuity development below age norms.

It was apparent that the visual acuity development of the group under investigation was adequate. There were no statistically significant differences between the group investigated or infant age norms from 2 different countries as per a one sample t-test ($p>0.1$) (Figure 7). This indicates that visual acuity development is similar to that found in normal infants from other countries. Also, none of the visual acuity scores fell below the lower 99% prediction limit for this age group, indicating that all infants that were tested exhibited adequate acuity development.

As many other studies have noted, there were no relations between levels of any EFA in infant plasma or erythrocytes and visual acuity development as assessed by the TAC system (Innis *et al.* 1996; Innis *et al.* 1997; Auestad *et al.* 1997). Relations between maternal BM or blood levels of EFA and visual acuity development for exclusively BF

infants were also not apparent. Reasons for the lack of relation may be that this test may not be sensitive enough to detect differences among infants who possess a proportion of DHA above some unknown critical value. The only investigation which reported the fatty acid profile of retinas for BF or SF fed healthy term infants (Makrides *et al.* 1994), may be of help in understanding this. The investigation showed the retinal DHA content to be similar in both groups, despite lower levels of erythrocyte total lipid DHA content in the SF fed group. Therefore, different levels of circulating n-3 fatty acids may not affect retinal DHA content, so long as they are above some critical level, leading to similar visual acuity responses. However, functional implications of lower blood levels of DHA should not be disregarded, as the investigation by Makrides *et al.* (1994), did find lower levels of DHA in the cortex of SF fed infants, which may impose other developmental problems. No significant differences were found between feeding groups for visual acuity development in the present investigation (Figure 8). This may have been due to the fact that there were no significant differences among groups in terms of circulating DHA levels.

6.4 Adequacy of Infant Anthropometry

It can be assumed that energy intakes of this group of infants were adequate as all age and gender standardized measures were similar to international reference populations (Figure 5). The lower average Z scores for infant birth length than birth weight, may be due to the fact that the former assessment is measured less precisely than the latter in newborn infants (WHO 1995). Adequate growth in infants may be in part due to the fact that it appears as though BM of Cuban women has an adequate fat content and fatty acid profile. This is most likely related to maternal energy status as noted in various maternal measures made at 2 months (Appendix D).

As seen in the t-tests among the 2 feeding groups described previously, no differences were seen in infant growth among groups either (Table 16). One possible reason may be that weaning occurred rather recently in most cases. In other developing nations, early weaning of infants is often associated with inferior growth. This is due to a

combination of factors, one of which is illness caused by inadequate quality of water for the preparation of milk formulas (Anonymous 1989; Mondal *et al.* 1996; Brown *et al.* 1989). However, after spending some time in Cuba, it became apparent, that there are various efforts to educate the public about the boiling of water to eliminate the likelihood of water borne disease. This is of great importance at present, since access to water treatment chemicals have decreased, leading to a reduction in the availability of safe drinking water (AAWH 1997). A short commercial type program, which is run frequently on the television, overviews the techniques for successfully eliminating the presence of harmful bacterial and viral agents in water. To assess whether this is an appropriate means of reaching the general population, the number of televisions in use in Cuba play an important role. It was estimated that 1.85 million televisions were in use in 1993 ("Cuba" 1997). With a population of 10 904 932, as estimated in 1993, this relates to a about one working television per every 6 persons. Therefore, this can be thought of as an appropriate means for such a message to be delivered. Other potential sources of such information include family doctors. Also, upon visiting the homes of subjects to inform them of their appointment, it was noticed that almost all households had a gas stove, which would enable them to boil water as required. Two of the major water borne diseases occurring in Cuba at present are typhoid fever and viral hepatitis (AAWH 1997), which are included in the 12 vaccinations all infants in Cuba receive free of charge. Therefore, even long after weaning of infants occurs, it may be that impaired growth due to illnesses incurred by water of poor quality, such as diarrhea caused by a number of infectious agents (Anonymous 1989; Mondal *et al.* 1996; Brown *et al.* 1989), may not be prominent in this society. Therefore, the adequate growth observed in this group of infants regardless of feeding status, suggests adequate energy intakes, as well as limited illness which may provoke growth failure.

6.5 Possible Implications of Weaning Practices

The best strategy to ensure optimal EFA status in this, and all populations, appears to be through the promotion of BF. This is due to the fact that infants that were BF

showed adequate growth, and fatty acid profiles of milk samples were in accordance with those found in other countries. As BF has various associated benefits, this is the logical choice. However, there appeared to be a trend of early weaning in various subjects, having almost half of them introduce other milks before 2 months postpartum. Concern over the lack of information regarding weaning practices for mothers is warranted, as details regarding the best choice and method of preparation may be lacking. This investigation uncovered the fact that the cow's milk preparation has a better fatty acid profile than evaporated milk, as it falls within current guidelines for North American infant formula (Anonymous 1998). As long as the fat used to supplement the cow's milk before it is rationed continues to possess a desirable profile, this can be thought of as the best option, since dairy products usually contain lower LA/LNA ratios than are acceptable. One investigation predicted that an LA/LNA ratio of 2:1, which is similar to that found in evaporated milk, would favor the formation of DHA in infants, possibly leading to levels similar to those found in BF infants (Clark *et al.* 1992). However, the authors of that investigation stated their concern over such a ratio leading to poor long chain n-6 fatty acid status which could affect growth.

Unfortunately, it was not possible to assess whether difference existed between cow and evaporated milk fed infants due to a limited sample size. However, when looking at the averages of AA in these 2 groups, it is possible that an effect of dietary LA may be present. Linoleic acid levels in the plasma of evaporated milk fed infants (n=2), were 12.15 ± 4.31%, while they were 20.43 ± 6.72% in cow milk fed infants (n=9). This may have affected the plasma levels of AA which averaged 5.68 ± 1.44% of total fatty acids in cow's milk fed infants, and 3.60 ± 0.71% for those weaned on evaporated milk. Arachidonic acid levels in erythrocytes showed a negligible magnitude of difference with cow milk fed infants having 16.36 ± 1.46% and evaporated milk fed infants having 14.95 ± 0.64% of total fatty acids. Once again, this may be due to the length of time infants had been receiving supplemental milks. As supplementary feeding progresses and amount of BM fed lessens, infants fed evaporated milk may be at risk of poor long chain n-6 fatty acid status which could have an impact on growth and development. It would be

unethical to continue studying this population in terms of the effects of different available milks on EFA status of infants, since it is known that one of them is better and everyone has access to it through the food distribution system. Also, this milk is received every other day from an extremely nearby location for every citizen. Therefore, the possibility that the cow milk formulation is not used by all mothers is most likely not due to a lack of availability or proper storage conditions. Therefore, education and further promotion of BF as well information regarding the use of the cow milk formulation over other available dairy products warrants special attention. The fact that improper information regarding weaning of infants may exist in Cuba should be considered and addressed.

A recent investigation found that many women living the same region of Cuba as in the current investigation, but from a different neighborhood, had poor knowledge of infant feeding practices (Garcia-Diaz 1997). The investigation assessed infant feeding practices and maternal knowledge of proper practices during the first year of life in 150 women attending one polyclinic. It was found that 58% of the mothers had poor knowledge regarding supplementary feeding of their infants, and that information was most often obtained from observing popular daily practices in the community and advice from peers, relatives or neighbors (Garcia-Diaz 1997). On the other hand, information regarding BF was mainly obtained from family doctors and the mass media, which is most likely due to the implementation of UNICEF's Friendly Baby Hospital Initiative throughout the country.

It is understandable that in a desire to maintain high levels of exclusive BF, information regarding weaning practices are not often relayed to mothers by the family doctors at such an early stage. Rates of early weaning were apparent in both the present investigation and the one by Garcia-Diaz (1997). Therefore, it may be that different strategies for the maintenance of exclusive BF after hospital discharge need to be employed, and that proper information regarding infant weaning practices be made available to the population. Reasons for discontinuing exclusive BF were not assessed in the present investigation, but the investigation by Garcia-Diaz (1997), showed that the main reason for this was the self diagnosis of hypogalactia in 91% of the 150 women

investigated. Given the adequacy of anthropometrical measures found in this investigation and of other groups in the same region (Cabrera-Hernandez *et al.* 1997: Garcia-Ferrera *et al.* 1996), it is unlikely that such a large proportion of BF women would be suffering from insufficient BM production. This contention revolves around the fact that the basis of the diet comes from the food distribution system, which is similar among different areas in the country. Therefore, it appears as if other strategies for maintaining high levels of exclusive BF need to be adopted. It is important to address the issue of proper infant weaning practices regarding supplementary feeding through similar media as that of BF, but that it be done in a way so as not to discourage BF, and only to impart accurate information. Upon speaking to various health care professionals, it appears as though knowledge regarding adequate infant weaning practices are not optimal. Many regarded evaporated milk as desirable and did not see the error of giving young infants sugar laden food items. The fact that some infants received large quantities of sugar also raised concern in the investigation by Garcia-Diaz (1997). Therefore, in order for infants in Cuba to have optimal nutritional intakes, it appears as if supplementation of mothers with EFA is not an important issue, while imparting adequate knowledge regarding infant feeding practices is.

6.6 Observations Regarding Dietary Habits and Health

The fact that local investigators feared the Cuban population was presently experiencing a diet that is abnormally low in fat and energy and high in carbohydrate exemplifies the fact that cultural norms may be taking a priority over adequate nutrient recommendations in Cuba. Based on anthropometrical measures and fatty acid composition of human milk samples, it is apparent that the group under investigation was not suffering from either a low energy or excessively high carbohydrate and low fat intake. It is logical to assume that the general population whom may not have the knowledge of nutrition experts come to the conclusion that the level of fat intake is abnormally low. However, when those making dietary recommendations for the country have these opinions, and wish to base nutrient recommendations on them, clarifications

regarding normal ranges for healthy nutrient intakes should be made.

Before the onset of the "Special Period", the rates of obesity and patterns of chronic disease were similar to those in developed nations (Cabrera-Hernandez *et al.* 1997). Upon speaking with various citizens of Havana from all walks of life, it became apparent that previous levels of fat intake must have been quite high. People would often complain that they were not eating, yet upon closer assessment of their statements, it became apparent that this contention was in reference to the fact that they were no longer eating in a way which they were accustomed prior to the onset of the "Special Period". The main reason people seemed to assert this was because they were no longer able to eat meat in the large quantities they were used to. Also, it appeared as though the favorite method of food preparation in the country is that of deep frying foods, and since the amount of oil available for frying is no longer comparable to that in the pre 1990's era, people asserted that they were not eating, when in reality they were.

It is understandable that the combination of not having meat at every meal and not knowing how to prepare food items available for consumption in a manner that is culturally acceptable, may lead a population to believe that they have nothing to eat. However, in difficult economic situations, such as the one Cuba has been experiencing since the fall of the Soviet Bloc, it would be logical to make efforts to increase the acceptability of nutritious food items that are available. It would be logical that an organization such as INHA would plan or partake in efforts to help in the transition from the countries previous dietary habits. Instead, the institute's staff was in accordance with the general populations lack of desire to accept new food items despite their excellent quality both in terms of nutrition and palatability. One example of this was with soy products. Upon learning that a yogurt was made out of soy beans in the country, it was suggested that this could be a good source of n-3 fatty acids for pregnant women if need be. The response was one of negativity, insisting that people don't like it, therefore, it should not be considered, as it is not "real" food, suggesting that more oil for the population would be preferable. However, such an option is not sustainable, and it would be better to use that which is available in the country. It seems rather illogical to use

foreign aid to provide a population with food items to replace those they do not want to eat, when there are other nations whose people are truly suffering from inadequate nutrient intakes and starvation.

Some suggestions for improving acceptability of food items available for consumption at present, would be to use the media and culinary schools. There is a culinary school in Havana which could be used as an educational resource, by having students create new recipes utilizing food items available to the general population. Outreach programs, could have chefs or others trained in the preparation of new recipes visit social centers, schools, and other appropriate locations. Through such efforts, people may learn how to prepare food items in different and acceptable ways. If people can learn how to use the food that is available to them in a way that is new, but acceptable, it may be that they will be happier to eat them, thereby improving the nations food security.

Since the staff at INHA appeared to have a concern that people do not want to eat n-3 rich food items available, it would be logical for them to make an effort in promoting their importance, especially during the period of pregnancy and BF. In this way, women can learn the importance of eating their fish for the optimal neurological development of their infant. It may be that if people are shown a good reason for adopting a behaviour, they may be more willing to accept it. As the general population in Cuba is adequately educated to understand messages of this nature and possess a literacy rate above 95% ("Cuba" 1999), there are various media through which such educational information can be passed. When food availability is limited, it is important to instill the need to accept that which is accessible. Other tactics, aside from finding new and acceptable methods for preparation, can be used for those who still do not enjoy eating certain provisions. Food items such as the fish provided through the governments food distribution system, could also be viewed as a medication which should be taken by pregnant and BF mothers for the optimal neurological development of their infants. In this way, mothers to be can be convinced of the importance of taking this food item, and can break it down into weekly doses. If educational measures are taken through a combination of the above suggested methods, the potential for the acceptance of new food items and adequate

dietary habits, at least during critical periods of the life cycle, may prove worth while.

Recommendations regarding the use of cooking oil in amounts the population was accustomed to before 1990, could actually put Cuba in a worse situation, as far as optimal health is concerned. From conversations with various people from all walks of life in Havana, it appeared that people like to use vegetable oil for deep frying. This practice may have negative health implications. This is due to the fact that vegetable oils used in Cuba, namely soy and sunflower oil (AAWH 1997) have a high unsaturated fatty acid content. High temperatures, such as those employed in deep frying, are known to lead to the formation of various compounds including *trans* isomers of fatty acids, especially when highly unsaturated oils are used (Sébédio & Chardigny 1996; Kitts 1996). In relation to the present investigation, *trans* fatty acids have been implicated in poorer birth weight in infants (Kozeltko 1995). Concerns regarding its presence in BM of women consuming diets which contain *trans* fatty acids have also been noted (Houwelingen & Hornstra 1994). Preliminary data from animal studies have shown *trans* fatty acids to be present in the retinas of rats fed diets containing *trans* isomers and altered ERG responses of retinas exposed to *trans* fatty acids (Sébédio & Chardigny 1996). Also, consumption of *trans* fatty acids has been implicated in a variety of diseases, such as increasing the presence of risk factors for heart disease (Mensink & Katan 1990; Nestel *et al.* 1992). It is possible that rates of this disorder would return to those of the pre "Special Period" era if the amount of oil available for deep frying returns to the levels during that time period. A recent investigation no longer detected the relation between fat intake and heart disease observed in the late 1980's (Cabrera-Hernandez *et al.* 1997). It may be that the decrease in the availability of oil for deep frying has played a role in this phenomena. These issues combined with the fact that the availability of medical supplies in this nation is poor, due to the breach of international law by the US (Kirkpatrick 1996; AAWH 1997), could lead to a health care crisis. Even in a country which does not have the added burden of an embargo on medical supplies, providing adequate health care services and medical treatments for the population have proven difficult when rates of chronic disease are high, (Pedersen 1998a; Pedersen 1998b; Hunter 1997; Struzik 1997; Pedersen1997).

Therefore, it is possible that such a crisis could occur in Cuba if eating habits return to those of the past and the availability of medicine remains as it is at present. Although this is truly unfair to inhabitants of Cuba, it is a reality that must be considered. As such, it would be logical to implement public health programs aimed at teaching the population how to prepare food items available in a way that is both culturally acceptable and healthy, not only for pregnant and BF women, but for the entire population.

The use of vegetable oils in a manner which maintains their desirable qualities of maximum levels of EFA and minimum *trans* fatty acids should be encouraged. Adding small amounts of unheated vegetable oil to typical Cuban dishes such as rice and beans, or to recipes that encorporate cooked vegetables, are some ways that EFA can be ingested without excessive heating. This could also be important in increasing the acceptance of vegetables in this population. It was noticed that many people did not eat vegetables, or used them as a condiment, which was attributed to lack of a cultural preference for them. Late introduction of vegetables to infants could also be corrected by efforts aimed at promoting vegetable consumption in adults. The investigation by Garcia-Diaz (1997), found that 53% of the 150 infants had not received any vegetables after 1 year of life, and that mothers did not see them as a source of vitamins and nutrients. Vegetables were the only group of foods that were not introduced in this group, having all 150 infants receive tubers, fruits, cereals and meat products, but only 71 being introduced to vegetables in the same time frame. Therefore, education regarding the importance of including vegetables in the diet are also needed in this country. It would be preferable that such educational measures be taken before availability of food items in levels which are potentially detrimental to the health and well being of this society return. By taking such efforts at this point, it may instill better habits at a stage when people may be more willing to accept them out of necessity. As availability of vegetable oil for deep fat frying increases in Cuba with the changing economic situation, such efforts may lead to improved nutritional and health status in the population. For infants, it may decrease their exposure to the potentially harmful properties of *trans* fatty acids. For the general population, it may mean that chronic diseases which are thought to be related to *trans* fat intake may not

escalate back to pre "Special Period" rates. The above mentioned preventative measures may be considered beneficial for a society with a limited availability of medicine and equipment for medical procedures due to the imposition of an embargo.

6.7 Limitations

The main limitation of this investigation was that no record was kept of eligible women who chose not to participate. As many people were involved in subject recruitment, it was difficult to obtain this information. Some of the doctors and nurses at the hospital would recruit women to be in the study when they had a free moment and the opportunity was available. Therefore, not all women were approached as it would have been impossible for some of those recruiting to approach all women with respect to this investigation as they had other more important tasks to perform. Therefore, it is not known if selection bias existed. In keeping with this theme, one of the main people responsible for recruitment expressed her enthusiasm on obtaining "thin" women for the study as she believed "thinner" women would be more interesting to investigate. It had already been explained that all women, regardless of weight status were to be approached for inclusion, and upon hearing such comments, the message was repeated. This may be an example of selection bias against women who were not "thin". It is not known if such behaviour led to an over representation of "thinner" women, as the characteristics of eligible women not participating in the investigation were not recorded.

Another limitation was that some infant blood samples were not drawn. This was a limitation from an ethical point of view more so than from a scientific point of view. When blood is drawn by venipuncture from infants, it is critically important that an entirely competent and experienced person, such as a senior qualified nurse, be used to ensure the highest level of safety and to decrease the level of trauma (Whitehead 1998). In the present investigation, a person with experience in drawing blood from adults and older children was used. As such, many of the children had the needle inserted into them various times and some were left with unnecessary bruising and hematomas. This practice was strongly discouraged, yet there was little that could be done by an outsider.

From a scientific point of view, the power decreased for some of the tests that were done using infant blood fatty acid levels. However, statistical analysis such as t-tests between feeding groups were done as an after thought and were not essential to answering the main research question which was to establish whether an EFA deficiency existed in BF women and infants in Cuba. The maternal BM and blood samples were sufficient to answer this question, as in most cases, infants were receiving BM. The concern was that there were no dietary sources of EFA and low levels of dietary fat which would produce a BM poor in EFA content and lead to poor EFA status in infants. The BM was found to be adequate in its EFA content. Therefore, if there still are concerns over children not obtaining enough n-3 fatty acids, the promotion of BF should be targeted, by adding to or changing current promotion strategies.

7. CONCLUSIONS AND RECOMMENDATIONS

Infant anthropometry reflected adequate growth and therefore ingestion of energy in the group investigated. The presence of an n-3 fatty acid deficiency in the population investigated was not apparent. Fatty acid profiles of BM and relations found within this tissue were in accordance with a population eating a balanced diet in terms of carbohydrate, energy, and fat. Adequate EFA status of infants was also evident, as was visual acuity development of the infants under investigation. One problem that may be of importance to tackle is the lack of adequate knowledge regarding proper eating practices which may exist among health care professionals and the general population. This should be considered of significance when addressing the population of BF women. One main recommendation would be that soy oil, or a blend of oils providing an LA/LNA ratio and LNA content similar to that of soy oil continue to be used in the preparation of the cow milk formulation given to infants and children. The promotion of the use of the cow milk preparation over evaporated milk or other dairy products is also recommended. Proper information regarding supplemental feeding of infants may also prove beneficial in Cuba. It also is recommended that approaches similar to those mentioned in the last section of this thesis be adopted with the aim of increasing food security in the population by helping the inhabitants of Cuba accept the new and possibly healthier food items that are now present in the country.

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Appendix A

Content (wt/wt%) of Identified Fatty Acids Present in the Cow Milk Preparation

	wt%
C8:0	0.32
C10:0	0.81
C12:0	1.00
C14:0	4.31
C16:0	17.76
C18:0	8.84
C16:1n-7	0.77
C18:1n-9 & C18:1n-7	45.19
C18:2n-6	12.49
C18:3n-3	1.58
C18:2 n-6/C18:3n-3 (LA/LNA)	7.9:1

Appendix B

Autorización Materna de Participación

Yo,_____autorizo mi participación y la de mi hijo en el trabajo de evaluación del efecto de mi dieta en el desarollo de mi hijo.
Me comprometo a:

- permitir que los empleados del Insituto de Nutrición e Higiene de los Alimentos esten presentes en el nacimiento de mi hijo y que colecten sangre del cordón al momento del nacimiento
- tomar muestras de mi sangre el dia después del parto, y a los 2 meses después del parto a mi y a mi hijo
- hacer las encuestas dietéticas según las orientaciones
- evaluaciones antropométricas para mí y para mi hijo
- de tomar agua marcada y dar pequeñas muestras de leche materna y de orina de mi hijo
- asistir a la consulta médica creada para este fin a los 2 meses de nacido
- tomar muestras de leche materna a los 2 mese de nacido
- realizar prueba de agudeza visual al niño a los 2 meses de nacido

_____ Fecha:_____
firma de la madre

Maternal Authorization of Participation (English translation)

I, _____authorize the participation of myself and my child in a study to evaluate the effect of my diet on my child's development.
I agree to:

- allow INHA staff to be present during my child's birth, and to collect a sample of cord blood
- give a blood sample the day following birth, and for myself and my child to give a blood sample 2 months after birth
- participate in the dietary history assessment
- have anthropometric measures done on my child and me
- drink doubly labeled water and give small samples of breast milk and my child's urine
- attend the medical examination created for this study 2 months after birth
- give breast milk samples 2 months after birth
- allow my child to have a visual acuity test 2 months after birth

_____ Date:_____
mother's signature

Appendix C_1

Datos Básicos de la Madre

Información General
Fecha:_____
Número de Indentificación:_____
Apellido: _____ Nombre:_____
Edad: _____
Región de Salud: _____
Dirección Residencial: _____
_____
_____
Número de Teléfono: _____
Dirección de Trabajo: _____
_____
_____
Número de Teléfono: _____

Número de hijos (embarazos):_____
Raza: _____

Información Socioeconómico:
Nivel de Educación:_____
Categória de Trabajo:_____
Número de personas en la casa: _____
Número de niños en la casa: _____
Número de personas con empleo que viven en la casa: _____
Ingreso mensual en la casa: _____
Número de parientes que viven en el extranjero: _____
Recibe Ud. ayuda monetario de ellos? Si ☐ No ☐

Datos Antropométricos:
Datos antes del embarazo: peso:_____ kg Talla:_____m BMI:_____Kg/m^2

Datos al parto_____semanas: Peso:_____kg

Has fumado durante los últimos dos meses? Si☐ No☐
Si fumo, cuántos cigarrillos por día: <5 ☐ 5-10 ☐ 10-20 ☐ >20 ☐

Appendix C_2

Maternal Baseline Information Sheet (English Translation)

General Information:
Date: _____
Identification number: _____
Family Name: _____ Given Names: _____
Age: _____
Health Area: _____
Residential Address: _____
_____
_____
Phone #: _____
Work Address: _____
_____
_____
Phone #: _____

Parity (pregnancies):_____
Race:_____

Socioeconomic Information:
Educational level:_____
Job Category:_____
Number of people living in residence: _____
Number of children living in residence: _____
Number of Employed people living in your residence: _____
Monthly Household Income: _____
Number of Relatives living overseas: _____
Do you receive monetary help from overseas relatives? Yes ☐ No ☐

Anthropometric Data:
Pre-pregnancy: weight:_____ kg ht.:_____ m BMI:_____ kg/m^2

Data at delivery_____ weeks: weight_____kg

Did you smoke during the past 2 months? Yes ☐ No ☐
If yes, how many cigarettes per day: <5 ☐ 5-10 ☐ 10-20 ☐ >20 ☐

Appendix D_1

Datos de la Madre y el Bebé al Parto

Fecha: _____
Número de Identificación: _____
Apellido: _____ Nombre: _____

Información de la Madre:
Peso_____ Kg Talla_____cm
MUAC _____ cm Tricep SF_____ Subscapular SF_____

Información del Niño:
Apellido: _____ Nombre: _____
Sexo: V□ H□
Peso al Nacer: _____ g Medida de Largo:_____cm Peso_____kg
Edad Gestaciónal: _____ semanas Circunferencia de la cabeza: _____ cm.
MUAC: _____ cm.

Datos Generales de la Madre y el Niño a los 2 Meses

Fecha: _____
Número de Identificación: _____
Apellido: _____ Nombre: _____

Información de la Madre:
Peso_____ Kg Talla _____cm IMC_____kg/cm^2
MUAC_____ cm Tricep SF_____ Subscapular SF_____

Has fumado durante los últimos dos meses? Si□ No□
Si fumo, cuantos cigarrillos por día: <5 ■ 5-10 ■ 10-20 □ >20 □

Alimentación del Nino:_____
_____
_____

Información del Niño:
Peso:_____ g. Medida de largo: _____ cm. Circ. de Cabeza:_____ cm.
MUAC: _____ cm.

Número de días con diarrea durante los 2 meses anteriores: _____ días
Número de infeciones repiratorios durante los 2 meses anteriores: _____ infeciones
Número de reaciones alergicas de la piel durante los 2 meses anteriores: _____ reaciones

Appendix D_2

General Maternal and Infant Information at Birth (English Translation)

Date: _____
Identification Number: _____
Family Name: _____ Given Names: _____

Maternal Anthropometrical Information
Weight _____kg Height_____cm
MUAC _____cm Tricep Skinfold_____ Subscapular Skinfold_____

Infant Information
Gender: M ☐ F ☐
Birth weight: _____ g Birth Length: _____ cm Weight:_____kg
Gestational age: _____ weeks Head Circumference: ____cm MUAC: ____cm
APGAR Score: _____

General Information and Anthropometry at 2 Months

Date: _____
Identification Number: _____
Family Name: _____ Given Names: _____
Maternal Information:
Weight_____ kg Height _____ cm BMI _____ kg/cm^2
MUAC _____cm Tricep Skinfold_____ Subscapular Skinfold _____

Did you smoke during the past 2 months? Yes ☐ No ☐
If yes, how many cigarettes per day: <5☐ 5-10☐ 10-20☐ >20☐

Infant Feeding:_____
_____
_____

Infant Information
Weight: _____ g Length: _____ cm Head circ.: _____ cm
MAC: _____ cm

Number of days with diarrhea in past 2 months: _____ days
Number of respiratory infections in the past 2 months: _____ infections
Number of allergic skin reactions in the past 2 months: _____ reactions

Appendix E

Descriptive Data of Infants and Mothers at 2 Months

Visual Acuity (cycles/degree)	54	2.00 ± 0.68
Infant Age (days)	56	63.1 ± 4.0
Maternal Weight (kg)	55	60.8 ± 9.7
Maternal Weight Loss (kg)	50	4.1 ± 2.7
Maternal BMI (kg/m^2)	55	23.8 ± 3.6
Maternal MUAC (cm)	54	25.9 ± 3.2
Tricep Skinfold (mm)	55	18.3 ± 7.9
Subscapular Skinfold (mm)	55	19.4 ± 9.0
Infant Weight (g)	55	5355 ± 723
Infant Length (cm)	55	57.8 ± 2.1
Infant Weight Increase (g)	55	2083 ± 560
Infant Daily Weight Increase (g)	55	32.9 ± 8.8
Infant MUAC (cm)	55	12.3 ± 1.2
Infant Head Circumference (cm)	54	38.8 ± 1.4
Infant Weight Z Score	55	0.30 ± 0.78
Infant Length Z Score	55	-0.19 ± 0.83
Infant Head Circumference Z Score	55	0.11 ± 0.84
Number (Percent) of Smokers	56	17 (30.4%)

[a] n refers to the number of subjects used in averages

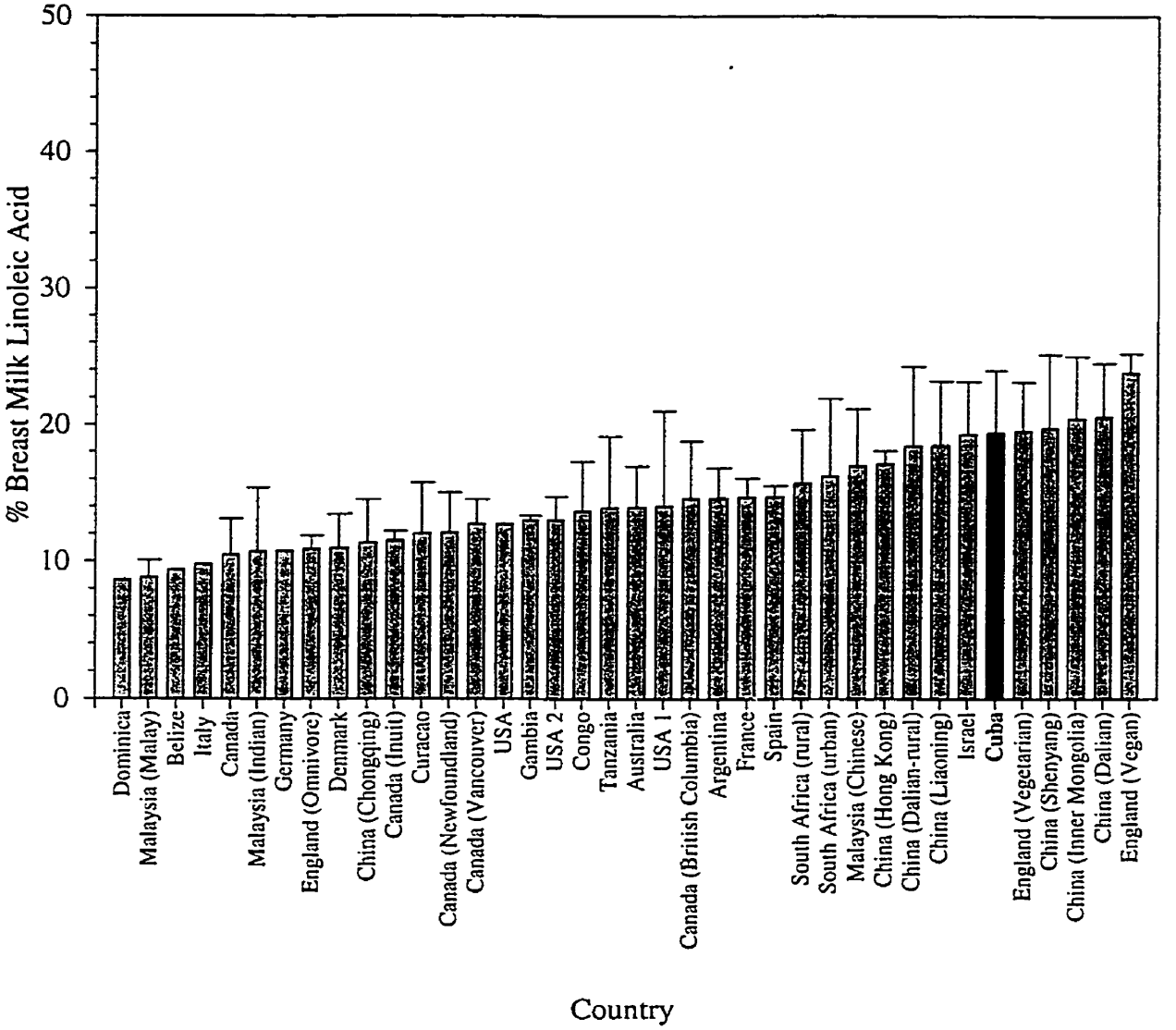
Appendix F

List of References for Countries in Appendix G Through Appendix K

Argentina	De Tomas et al. 1994
Australia	Makrides et al. 1995
Belize	Van Beusekom et al. 1990
Canada	Chen et al. 1995
Canada (British Columbia)	Innis et al. 1997
Canada (Inuit)	Innis & Kuhnlein 1988
Canada (Newfoundland)	Courage et al. 1998
Canada (Vancouver)	Innis & Kuhnlein 1988
China (Chongqing)	Chen et al. 1997
China (Dalian)	Chulei et al. 1995
China (Dalian-rural)	Chulei et al. 1995
China (Hong Kong)	Chen et al. 1997
China (Inner Mongolia)	Chulei et al. 1995
China (Liaoning)	Chulei et al. 1995
China (Shenyang)	Chulei et al. 1995
Congo	Rocquelin et al. 1998
Cuba	Present Investigation
Curacao	Muskiet et al. 1989
Denmark	Jørgensen et al. 1996
Dominica	Van Beusekom et al. 1990
England (Omnivore)	Sanders & Reddy 1992
England (Vegan)	Sanders & Reddy 1992
England (Vegetarian)	Sanders & Reddy 1992
France	Chardigny et al. 1995
Gambia	Prentice et al. 1989
Germany	Koleztko et al. 1988
Israel	Budowski et al. 1994
Italy	Serra et al. 1997
Malaysia (Chinese)	Kneebone et al. 1985
Malaysia (Indian)	Kneebone et al. 1985
Malaysia (Malay)	Kneebone et al. 1985
South Africa (rural)	van der Westhuyzen et al. 1988
South Africa (urban)	van der Westhuyzen et al. 1988
Spain	De Lucchi et al. 1988
Surinam	Muskiet et al. 1989
Tanzania	Muskiet et al. 1989
USA	Birch et al. 1998
USA 1	Hayes et al. 1992
USA 2	Henderson et al. 1992

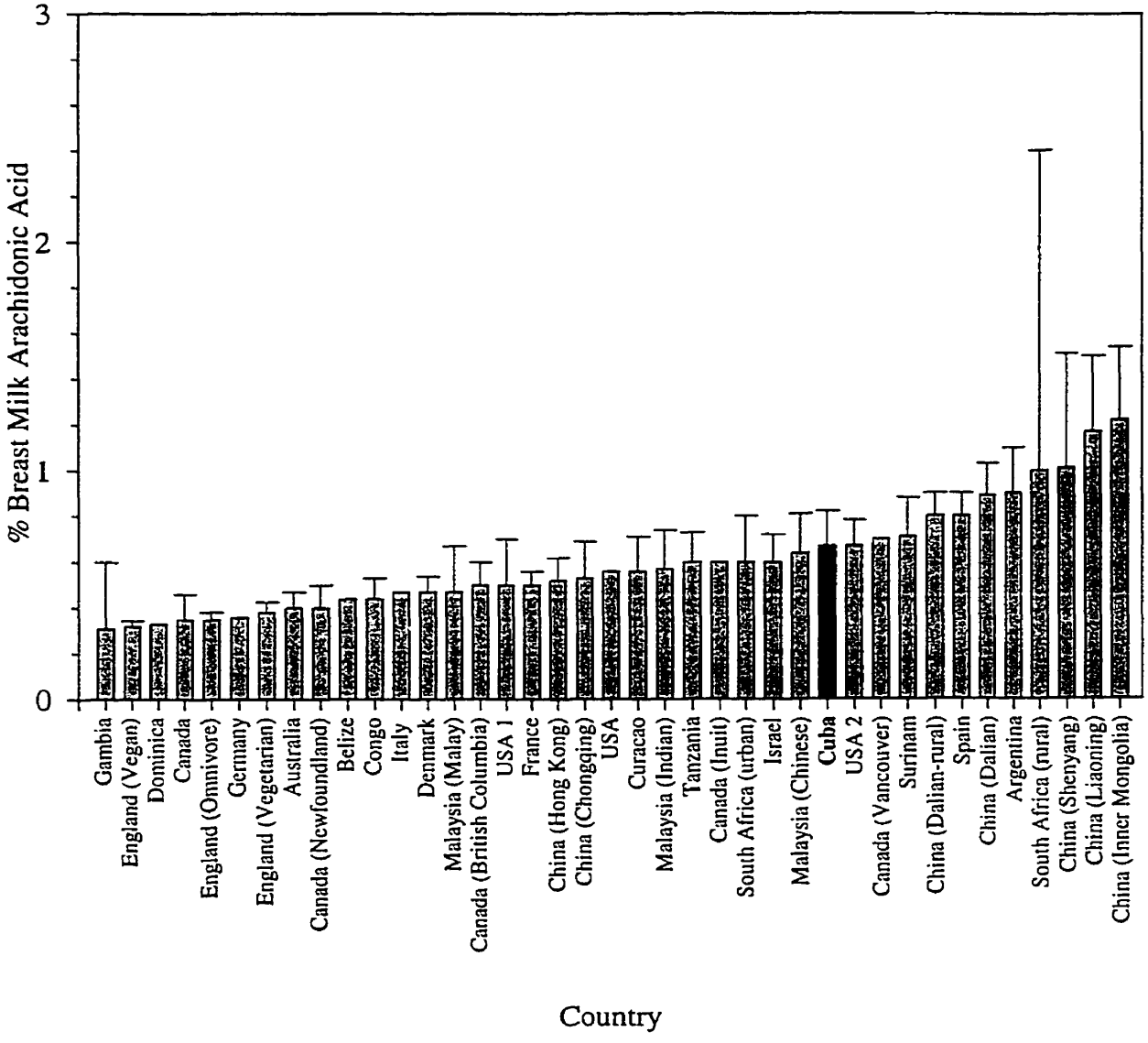
Appendix G

Breast Milk Linoleic Acid Content in Various Countries



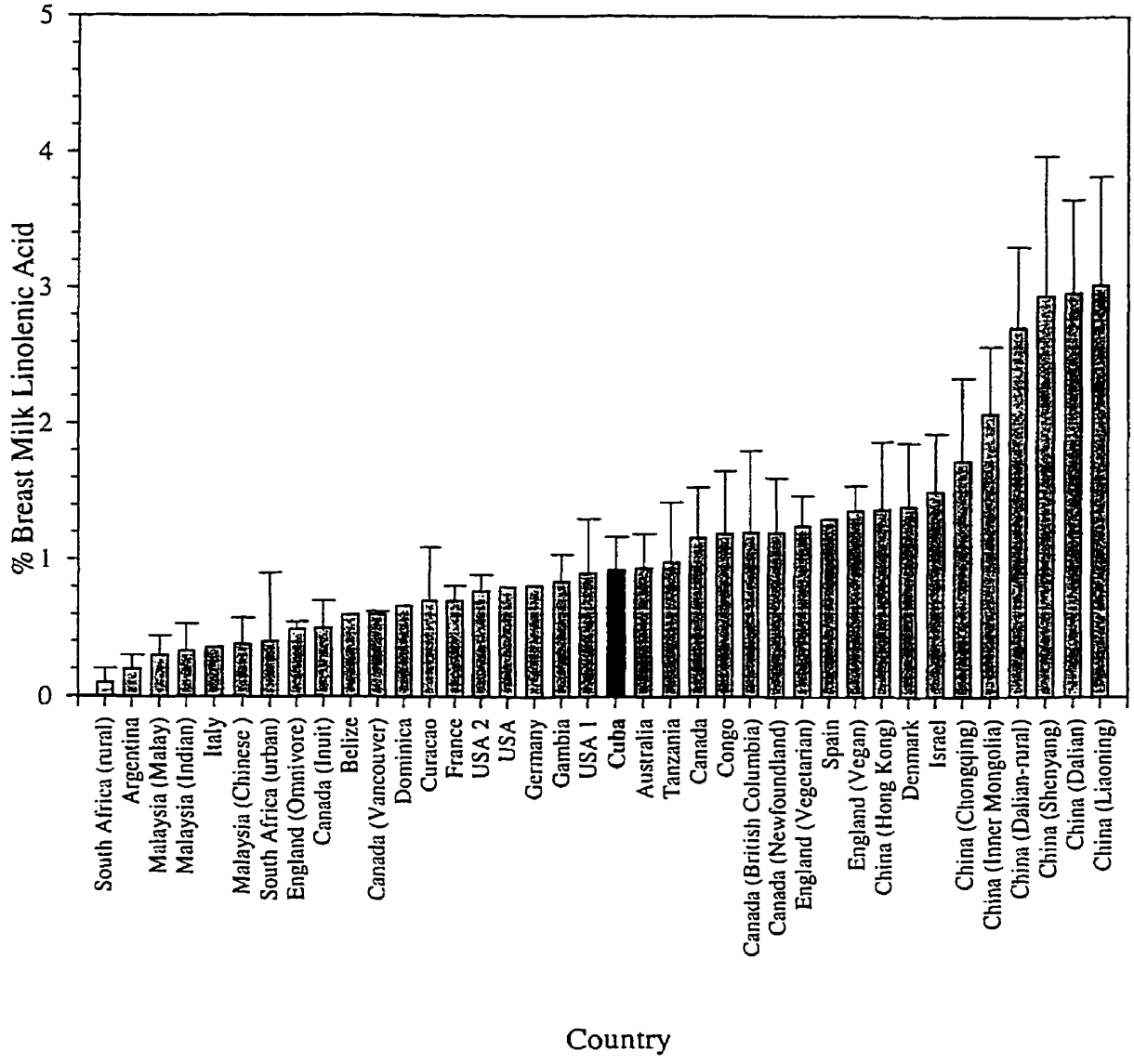
Appendix H

Breast Milk Arachidonic Acid Content in Various Countries



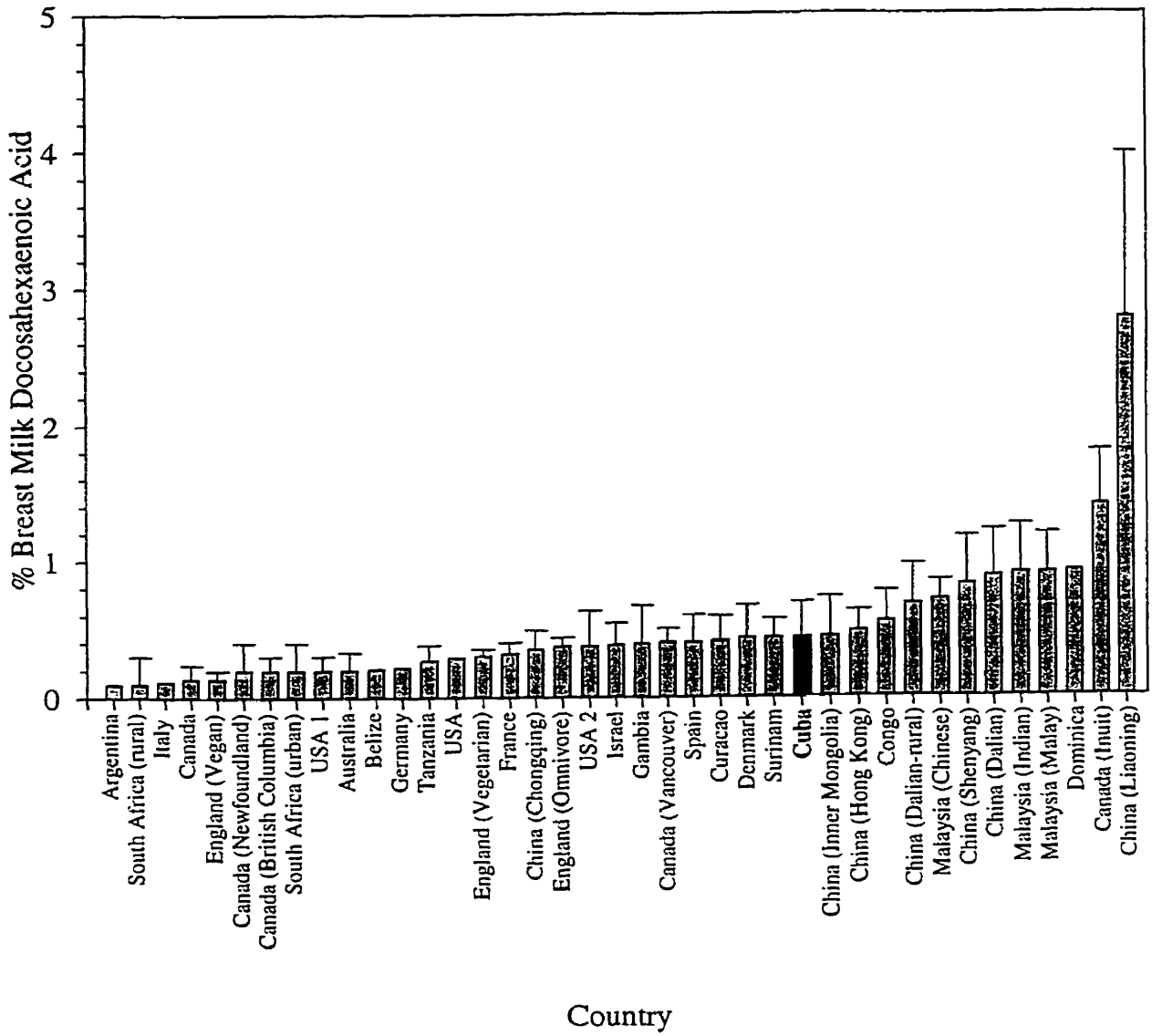
Appendix I

Breast Milk Linolenic Acid Content in Various Countries



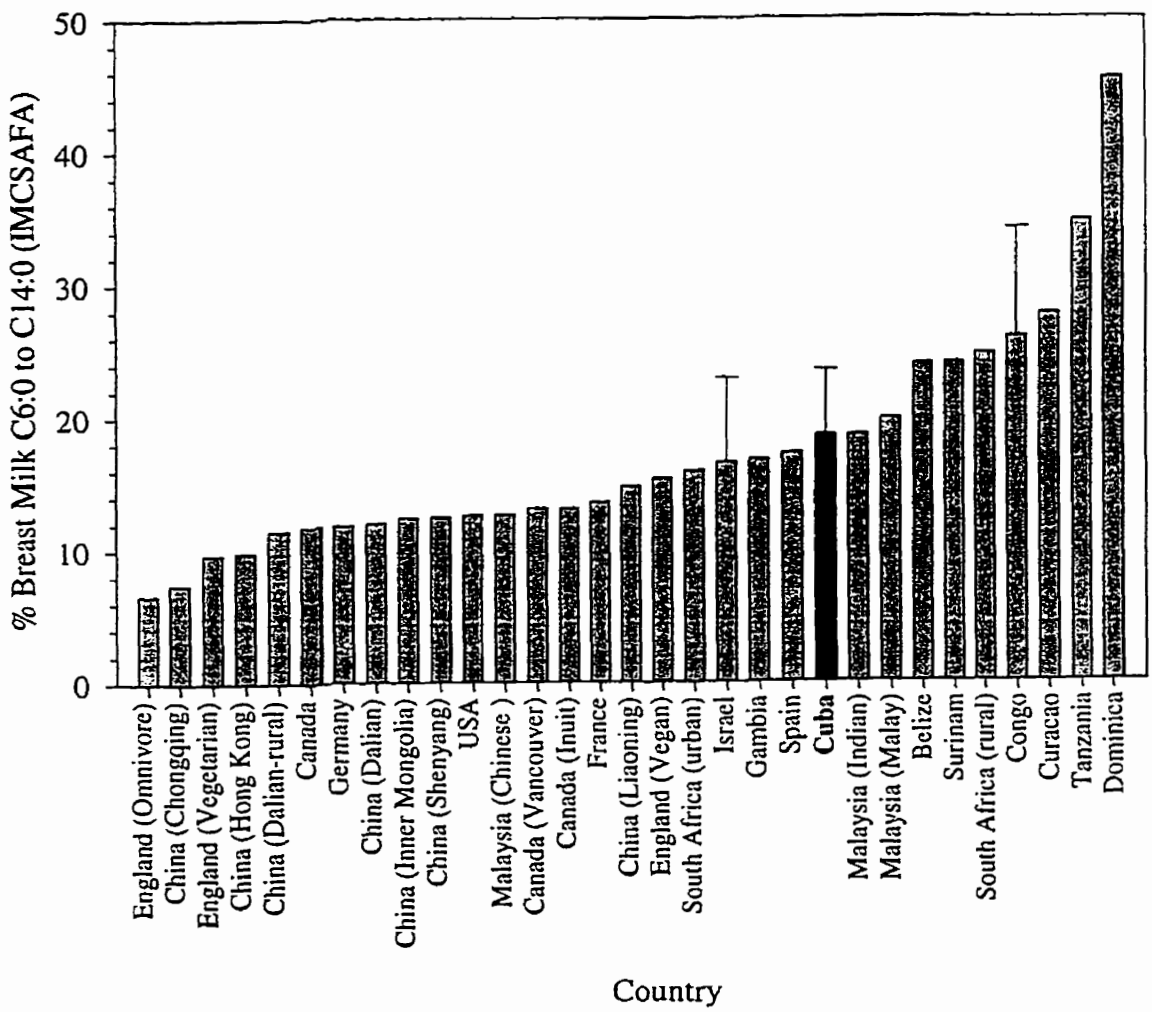
Appendix J

Breast Milk Docosahexaenoic Acid Content in Various Countries



Appendix K

Breast Milk C6:0 to C14:0 (IMCSAFA) Content in Various Countries



Appendix L

Comparison of Selected Fatty Acids (wt/wt%)[a] in Erythrocyte Total Lipids of Cuban Mothers to Literature Values

C18:2n-6	11.50 ± 1.91	8.2 ± 2.6	9.6 ± 1.2	n/a	n/a
C20:4n-6	15.67 ± 1.48	12.9 ± 5.2	14.6 ± 1.3	n/a	n/a
C22:4n-6	5.79 ± 1.57	3.8 ± 1.4	4.1 ± 0.3	n/a	n/a
C18:3n-3	0.35 ± 0.12	0.09 ± 0.06	0.03 ± 0.04	n/a	n/a
C20:5n-3	0.63 ± 0.27	0.2 ± 0.2	1.4 ± 0.4	n/a	n/a
C22:5n-3	2.42 ± 0.34	1.4 ± 0.5	2.2 ± 0.3	n/a	n/a
C22:6n-3	6.80 ± 1.24	4.5 ± 1.7	5.6 ± 1.1	4.08 ± 1.13	5.97 ± 1.19
Total n-6	35.35 ± 2.10	26.7 ± 9.2	30.0 ± 0.8	n/a	n/a
Total n-3	10.20 ± 1.36	6.2 ± 2.2	9.2 ± 1.6	n/a	n/a
n6/n3	3.51 ± 0.56	4.3 ± 0.3	3.3 ± 0.6	n/a	n/a

[a] mean ± SD

[b] baseline; before fish oil supplementation

[c] after fish oil Supplementation

[d] unsupplemented mothers at delivery

[e] fish oil supplemented mothers at delivery

Appendix M

Comparison of Selected Fatty Acids (wt/wt%)[a] in Plasma Total Lipids of Cuban Mothers to Literature Values

C18:2n-6	32.96 ± 5.32	32.92 ± 4.75	36.25 ± 4.91	30.57 ± 7.52	n/a	n/a
C20:4n-6	8.74 ± 1.69	5.97 ± 1.20	4.12 ± 1.05	5.27 ± 2.01	n/a	n/a
C22:4n-6	0.73 ± 0.21	n/a	n/a	n/a	n/a	n/a
C18:3n-3	0.40± 0.13	0.57 ± 0.18	0.51 ± 0.14	0.41 ± 0.12	n/a	n/a
C20:5n-3	0.62 ± 0.36	0.46 ± 0.32	3.03 ± 2.04	6.41 ± 4.11	n/a	n/a
C22:5n-3	0.54 ± 0.12	n/a	n/a	n/a	n/a	n/a
C22:6n-3	2.56 ± 0.84	1.49 ± 0.55	3.98 ± 1.30	5.22 ± 1.56	1.47 ± 0.29	2.35 ± 0.69
Total n-6	45.46 ± 5.09	42.03 ± 4.36	41.97 ± 4.52	37.27 ± 5.99	n/a	n/a
Total n-3	4.09 ± 1.16	3.19 ± 0.89	8.65 ± 3.38	13.44 ± 5.46	n/a	n/a
n6/n3	12.17 ± 3.72	13.98 ± 3.97	5.79 ± 2.77	3.53 ± 2.22	n/a	n/a

[a] mean ± SD
[b] non Native adults
[c] Inuit adults living in a river village
[d] Inuit adults living in a coastal village
[e] unsupplemented mothers at delivery
[f] fish oil supplemented mothers at delivery

Appendix N

Comparison of Selected Fatty Acids (wt/wt%)[a] in Plasma Total Lipids of Cuban Infants to Literature Values

C18:2n-6	23.11± 7.71	26.9 ± 5.7	26.3 ± 3.7	22.7 ± 4.7	25.48 ± 4.22	23.78 ± 3.49	25.92 ± 1.43	13.26 ± 1.20
C20:4n-6	6.35 ± 1.81	7.0 ± 1.5	4.4 ± 1.1	8.5 ± 2.4	7.68 ± 1.36	4.06 ± 0.82	4.22 ± 0.70	2.98 ± 0.67
C22:4n-6	1.06 ± 0.57	n/a	n/a	n/a	0.34 ± 0.17	0.19 ± 0.04	0.15 ± 0.03	0.12 ± 0.04
C18:3n-3	0.56 ± 0.35	n/a	n/a	n/a	0.43 ± 0.29	0.35 ± 0.05	1.40 ± 0.32	0.66 ± 0.06
C20:5n-3	0.41 ± 0.17	0.18 ± 0.09	0.21 ± 0.15	0.50 ± 0.30	0.24 ± 0.10	0.24 ± 0.06	0.55 ± 0.13	0.69 ± 0.14
C22:5n-3	0.29 ± 0.17	n/a	n/a	n/a	0.54 ± 0.15	0.45 ± 0.15	0.77 ± 0.14	0.62 ± 0.09
C22:6n-3	2.82 ± 0.84	2.1 ± 0.6	0.6 ± 0.1	2.7 ± 0.8	2.53 ± 0.68	1.01 ± 0.25	1.57 ± 0.44	1.20 ± 0.22
n6/n3	8.28 ± 3.49	14.7 ± 5.15	28.7 ± 7.5	9.1 ± 2.9	n/a	n/a	n/a	n/a
ΣLC n-6	8.66 ± 2.10	9.6 ± 2.1	6.8 ± 1.7	11.9 ± 3.5	10.28 ± 1.74	6.24 ± 1.13	6.09 ± 1.04	4.66 ± 0.92
ΣLC n-3	3.52 ± 0.35	2.5 ± 0.6	1.1 ± 0.3	3.8 ± 1.1	3.31 ± 0.80	1.70 ± 0.44	2.89 ± 0.57	2.50 ± 0.38

[a] mean ± SD

[b] infants fed formula + AA, DHA and EPA at 4 months

[c] infants fed standard formula at 4 months

[d] breast fed Infants at 4 months

[e] breast fed infants at 10 wks

[f] infants fed formula with a high LA/LNA ratio at 10 wks

[g] infants fed formula with a low LA/LNA ratio and high LNA content at 10 wks

[h] infants fed formula with a low LA/LNA ratio and low LNA content at 10wks

Appendix O

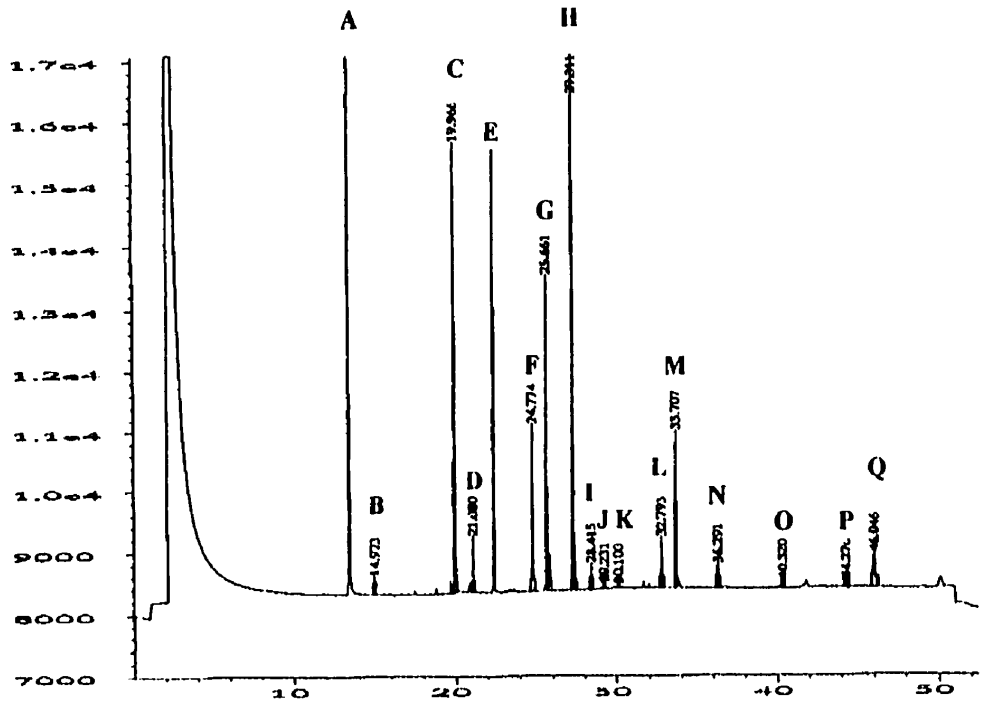
Comparison of Selected Fatty Acids (wt/wt%)[a] in Erythrocyte Lipids of Cuban Infants to Literature Values

C18:2n-6	10.33 ± 1.93	8.7 ± 0.9	11.7 ± 1.0	10.5 ± 0.6	8.44 ± 1.08	11.02 ± 0.86	10.52 ± 0.93	6.43 ± 0.31
C20:4n-6	16.48 ± 1.82	15.1 ± 1.4	12.9 ± 1.3	10.2 ± 1.0	17.15 ± 0.90	14.81 ± 0.99	14.53 ± 0.76	13.37 ± 1.05
C22:4n-6	5.60 ± 1.13	n/a	n/a	n/a	4.21 ± 0.86	3.70 ± 0.38	3.29 ± 0.33	2.62 ± 0.29
C18:3n-3	0.36 ± 0.15	n/a	n/a	n/a	0.11 ± 0.08	0.24 ± 0.04	0.44 ± 0.07	0.37 ± 0.04
C20:5n-3	0.43 ± 0.23	0.3 ± 0.1	0.2 ± 0.1	1.9 ± 0.2	0.27 ± 0.12	0.39 ± 0.07	0.80 ± 0.13	1.28 ± 0.17
C22:5n-3	1.03 ± 0.37	n/a	n/a	n/a	1.77 ± 0.31	2.11 ± 0.39	3.40 ± 0.47	3.45 ± 0.45
C22:6n-3	7.41 ± 1.16	5.5 ± 1.0	2.5 ± 0.3	6.3 ± 0.7	6.55 ± 1.23	3.47 ± 0.46	4.78 ± 0.45	4.48 ± 0.49
ΣLC n-6	24.17 ± 2.52	n/a	n/a	n/a	24.71 ± 1.73	22.24 ± 1.21	20.92 ± 0.62	19.52 ± 1.03
ΣLC n-3	8.88 ± 1.20	n/a	n/a	n/a	8.59 ± 1.58	5.97 ± 0.76	8.98 ± 0.65	9.30 ± 0.95

[a] mean ± SD
[b] breastfed infants at 4 months
[c] infants fed standard formula at 4 months
[d] infants fed enriched formula at 4 months
[e] breast fed infants at 10 wks
[f] infants fed formula with a high LA/LNA ratio at 10 wks
[g] infants fed formula with a low LA/LNA ratio and high LA content at 10 wks
[h] infants fed formula with a low LA/LNA ratio and low LNA content at 10wks

Appendix P

Sample Chromatogram of Maternal Erythrocytes



Identified Peaks:

A= Butylated Hydroxy Toluene; B=C14:0; C=C16:0; D=C16:1; E=C17:0 (internal standard); F=C18:0; G=C18:1n-9 and C18:1n-7; H=C18:2n-6; I=C18:3n-6; J=C18:3n-3; K=C20:1; L=C20:3n-6; M=C20:4n-6; N=C20:5n-3; O=C22:4n-6; P=C22:5n-3; Q=C22:6n-3