Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding

by

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Doctor of Philosophy

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The most elegant and compelling example of the symbiotic co-evolution between producer and consumer is the milk synthesised by a mammalian mother for her infant. Hinde and German (2012)

Artificial feeding of infants is, in fact, the largest uncontrolled *in vivo* experiment in human history. Minchin (1989)
Abstract

The focus of this body of research was to investigate the potential associations between breastfeeding and fatty acid deficiencies in Autism Spectrum Disorder (ASD). As research progressed, the mediational role of fatty acid deficiency in the relationship between breastfeeding duration and ASD diagnosis was examined.

Breastmilk is a bioactive substance common to all mammals and consists of three stages: colostral milk, transitional milk and mature milk. One aim of this thesis is to reconceptualise colostrum as an ‘immunological and functional instruction manual’ rather than a source of nutrition for the neonate. It is accepted in animal research that colostrum assists with gut maturation and that a lack of colostral feeding affects the offspring’s immune function. Thus, a proposition of this thesis is that it is crucial that the baby receives colostrum as soon as possible after birth and that the absence of colostral feeding leaves a neonate critically exposed to environmental insult.

This thesis investigates some of the currently accepted pre- and peri-natal risk factors for ASD and reconceptualises them as variables that may contribute to suboptimal breastfeeding; specifically, a lack of breastfeeding during the first hour after birth and a reduction in breastfeeding duration.

Inspired by research linking breastfeeding and ASD, Paper 1 is a commentary focusing on fatty acid deficiencies in ASD as a potential result of a lack of breastfeeding. The animal research model is considered and the lack of first hour
breastfeeding data in any paper is noted. This paper also discusses the inconsistencies in regard to what is coded as breastfeeding in many research papers.

Paper 2 is a review paper inspired by Horrobin’s ‘Membrane phospholipid hypothesis of schizophrenia’. It takes the evidence that Horrobin presented for his hypothesis; that dysregulated fatty acid metabolism interrupts cell signalling, and investigates the possibility that the same or a similar mechanism could be operating in ASD.

Paper 3 reports on the results of a pilot study conducted with parents of children with an ASD diagnosis. It investigates the utility of a clinical fatty acid deficiency scale that is non-invasive and reportable by parents. The paper focuses on first hour breastfeeding and controls for the effect of family grouping.

Paper 4 reports on a large study investigating the link between pre-natal risk factors identified in the literature as associated with an ASD diagnosis, breastfeeding duration, fatty acid status and the potential for fatty acid status to be a mediating factor between breastfeeding duration and ASD diagnosis.
Chapter 1 – Autism Spectrum Disorder, fatty acids and colostrum

Introduction – what is ASD?

ASD is one of the 21st century’s biggest medical mysteries. Despite over 100 years of research, we are no closer to a definitive cause of autism. Parenting style, environmental toxins, genetics, testosterone and the pre- and post-natal environment have all been posited as causal agents, however there is still no clear etiology nor path to prevention or cure. With an estimated Autism diagnosis being given to a child approximately every 20 minutes, it has never been more crucial to find new avenues to explore.

Autism Spectrum Disorder is a disorder usually diagnosed in childhood that is characterized by a continuum of social and communicative irregularities and deficits. In contrast to their normally developing peers, those diagnosed with a mild Autism Spectrum Disorder often respond inappropriately in conversations, may not maintain cultural norms regarding eye contact and can have difficulty developing friendships or show a reduced interest in being socially connected. Those with severe Autism Spectrum Disorder are sometimes entirely non-verbal, engage in repetitive behaviours, and show little or no interest in developing relationships with others.

Recent changes in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (APA, DSM-5) mean that children previously diagnosed with Asperger’s Disorder, Childhood Disintegrative Disorder or Pervasive Developmental Disorder, are now to be diagnosed with Autism Spectrum Disorder or another DSM-5 diagnosis.
Health burden of ASD

ASD is a catastrophic life-long condition in which the majority of diagnosed individuals are severely limited in their ability to engage in fundamental activities such as work and relationships, with many never able to function independently without ongoing physical, emotional and financial support (Austin and Shandley, 2010). The burden that an ASD diagnosis places on families, health and educational systems and welfare agencies cannot be underestimated.

ASD prevalence continues to rise, with a recent study by the Centre for Disease Control (CDC) estimating that as many as 1 in 88 children (1 in 54 males) have an ASD diagnosis, an increase of 23% in two years (Centers for Disease Control, 2014). ASD represents a significant public health concern and definitive understandings as to its causation, prevention, treatment and control remains elusive.

Comorbidity

For much of its history, ASD has been considered a discrete psychological disorder with treatment solely confined to the realm of behavioural interventions. The diagnostic criteria updated in the DSM-5 remain firmly rooted in the triad of social impairment, speech/communication deficits and repetitive behaviours and restricted interests – the hallmark of diagnostic criteria since Autism’s inclusion in the DSM-III in 1980. However, studies have highlighted ASD’s high level of comorbidity with other neurodevelopmental disorders such as ADHD, dyslexia, dyspraxia and epilepsy (Richardson & Ross, 2000).
A conceptual framework presented by Walter Kaufmann at the 2012 Autism Consortium in regard to the DSM-5, includes an acknowledgement of the growing evidence of ASD’s biological underpinnings. Firstly, Kaufmann (2012) noted ASD’s comorbidity with ADHD, Social Anxiety and Intellectual Disability. Secondly, a suite of biological symptomatology known to be extremely common to those with ASD was referenced: Gastro-intestinal dysfunction, sleep disturbance, Motor problems, Epilepsy-EEG abnormalities and Immune dysfunction. Whilst certainly not the acknowledgement of ASD as the disease state that some researchers have called for (Shandley & Austin, 2010), this conceptualisation represents an acceptance of the growing evidence of biological disturbances in ASD.

Genetics

With emerging evidence of a familial association and the disproportionate number of boys than girls being diagnosed (4:1), researchers began envisaging a single gene explanation of ASD. The comorbidity of ASD with known genetic conditions such as Fragile X and Rett’s syndrome highlighted the potential of ASD to be genetically predetermined (Budimirovic & Kaufmann, 2011). However the percentage of these cases turned out to be small, accounting for only 6-15% of ASD cases (Rossignol & Frye, 2012) and thus far ASD research has not been able to unearth a single gene or chromosomal disorder that conclusively explains the development of ASD (Rossignol & Frye, 2012). Nevertheless, a promising avenue of research has identified specific genetic polymorphisms associated with both a reduction and increase in ASD risk. A recent meta-analysis of polymorphism studies found MTHFR gene polymorphisms associated with ASD; the C677T gene (particularly in a homozygotic configuration
TT or CC) was found to be associated with elevated ASD risk and the homozygotic A1298C gene was found to be protective (Pu, Shen & Wu, 2013).

**Environment**

Whilst historically biological research into ASD has focused on the brain as the potential location of pathology, there is more compelling evidence that ASD is a pathology of the whole body system that is influenced by an individual’s genetics and their idiosyncratic exposure to various environmental factors (Herbert, 2010).

With the more compelling evidence for an underlying biological and/or environmental mechanism to exist, in addition to genetics, research has dispersed into five main areas of concern: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and exposure to environmental toxicants (Rossignol & Frye, 2012).

In the last five years, the role of mitochondrial dysfunction in ASD has become a research focus and many researchers have proposed that ASD is a mitochondrial disorder (Clark-Taylor & Clark-Taylor, 2004). Recently, Rossignol & Frye (2012) stated that due to the mitochondria’s important role in lipid metabolism, this may explain the lipid abnormalities found in ASD.
Chapter 2: The intersection of genetics and environment: Phospholipids, fatty acids and metabolism

Lipids

Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. For many years lipids were considered only to be important as sources of energy and the building blocks of membranes. However, in recent times, virtually every lipid class has been found to have some unique biological role that is distinct from the above.

Phospholipids are lipids that contain phosphor and are major structural components of neuronal cell membranes. They are comprised of a glycerol three-carbon atom backbone, with a phosphor in the third position and fatty acids of many different types attached to the first two carbons (Figure 1). The synthesis and breakdown of phospholipids are involved in the growth and restructuring of synaptic connections (Horrobin, 1998).

Figure 1: Phospholipid structure
**Fatty acids**

Fatty acids are comprised of an organic acid group known as carboxylic acid and a long chain (aliphatic) tail of carbon and hydrogen. The aliphatic tail of a fatty acid can lack double carbon bonds or contain up to six, and the number of double bonds determines the saturation of the fatty acid. Thus, an absence of carbon bonds allows the fatty acid to be saturated with hydrogen and creates a straight, inflexible structure (a saturated fatty acid) whilst one carbon bond creates a fatty acid which is curved (monosaturated) and multiple double bonds create flexible ‘U’ shaped structures known as Polyunsaturated Fatty Acids (PUFAs) (Figure 2). PUFAs are incorporated into the nerve cell membranes of the brain and retina and contribute to the functional maturation of the Central Nervous System (Alessandri et al., 2004).

![Figure 2: Saturated and unsaturated fatty acid structure](image)

Fatty acids are required for phospholipid metabolism and both saturated and monosaturated fatty acids can be synthesized within the body. However PUFAs are classed as Essential Fatty Acids (EFAs) because they cannot be synthesized by the body endogenously and must be provided by the diet (Vancassel et al., 2001b)
Fatty acid metabolism begins with two precursor fatty acids: Linoleic Acid [LA] (18:2 \( n-6 \)) an Omega-6 fatty acid, and \( \alpha \)-Linolenic acid [ALA] (18:3 \( n-3 \)) an Omega-3 fatty acid. Fatty acid metabolism involves an elongation and de-saturation process which means that at each iteration, the aliphatic tail of the PUFAs become longer and collects more carbon double bonds thus becoming more unsaturated (Figure 3).

Figure 3: Fatty acid metabolism pathway
The PUFAs with the most highly unsaturated tails are Eicosapentaenoic acid [EPA] (20:5, \(n\)-3), Docosapentaenoic acid [DPA] (22:5, \(n\)-3) and Docosahexaenoic acid [DHA] (22:6, \(n\)-3). EPA, DPA and DHA are known as Highly Unsaturated Fatty Acids (HUFAs) or Long Chain Polyunsaturated Fatty Acids (LCPUFAs).

The brain phospholipids are exceptionally rich in HUFAs (Horrobin, 1998; Vancassel et al, 2001). In fact, a unique feature of neurons is the smaller amounts of the precursors LA and ALA and the higher amounts of their metabolites: AA and DHA (Horrobin, 1998; Bourre, 2007). AA and DHA account for 20% of the dry brain weight (Alessandri et al., 2004; Meguid, Atta, Gouda, & Khalil, 2008; Vancassel et al., 2001a).

Also present in phospholipids are DGLA(20:3, n-6), Adrenic Acid(22:5, n-6), EPA(20:5, n-3) and DPA(22:5, n-3) but they are not as prolific as AA and DHA (Horrobin, 1998; Bourre, 2007). DHA is known to be involved in cell signalling and cell proliferation (Salem, Litman, Kim, & Gawrisch, 2001) and has an important structural role in the brain (Richardson, 2001) whilst AA is crucial for brain growth. EPA is not know to have a structural role, but is considered vital for the regulation of brain function (Richardson, 2001).

Since the discovery that AA is a precursor of prostaglandins that are involved in inflammation and other disease states, the metabolism of EFAs have been linked with visual acuity, neurodevelopment, ADHD, schizophrenia, depression and more recently, ASD.
**Fatty acids in breastmilk**

The history of fatty acid research is inextricably linked to infant feeding. Early studies by Hansen (Hansen, Haggard, Boelsche, Adam, & Wiese, 1958) discovered that deficiencies in LA and AA were implicated in infant eczema due to the common practice of feeding infants skim milk and sugar as a human milk substitute. Until more sophisticated artificial milks were introduced, infantile eczema was a common medical problem in the 1940s and 1950s (Holman, 1998). It is now firmly established that eczema may result from abnormal fatty acid metabolism in regard to converting LA to GLA. GLA is implicated in production of PG1s which are anti-inflammatory and offer immune support and suppress inflammation that can come from an over abundance of PG2s.

Human milk contains significant amounts of AA and DHA (Marszalek & Lodish, 2005). However, until 2002, infant formulas used in the US contained considerable amounts of 18:2, n-6 and 18:3, n-3, but no AA or DHA (Voigt et al., 2001) and infants have limited capacity to convert LA to AA and LNA to DHA (Marszalek, 2005). Infants are at risk of fatty acid deficiency if they do not have access to breastmilk or formula milk that is supplemented with LCPUFAs.
Chapter 3: ASD and infant feeding

Suboptimal breastfeeding (such as delaying the initiation of breastfeeding, early weaning or formula supplementation) has previously been established as a risk factor for health conditions such as asthma and atopic dermatitis (van Odijk et al., 2003), atopy, diabetes mellitus and childhood obesity (E. E. Stevens, Patrick, & Pickler, 2009).

Over the last decade, suboptimal breastfeeding is gradually being recognised as an ASD environmental risk factor (Al-Farsi et al., 2012; C. M. Brown, D. W. Austin, & L. Busija, 2014; Schultz et al., 2006; Tanoue & Oda, 1989). Following the finding that children with ASD were more likely to have been weaned within one week of the instigation of breastfeeding (Tanoue & Oda, 1989), Schultz et al. (2006) surveyed 861 parents of children with ASD and 123 parents of children without ASD, and found that the children with ASD were significantly less likely to have been breastfed. The absence of breastfeeding significantly increased the odds of a later ASD diagnosis and Schultz et al (2006) found that formulas supplemented with fatty acids (‘Gold’ formulas) were less likely to be associated with an ASD diagnosis. Brown, Austin & Busija (2014) and Al-Farsi (2012) found that first hour breastfeeding was negatively associated with autism. In addition, Brown et al (2014) found some evidence for fatty acid deficiency in the children with autism.

Suboptimal breastfeeding is also correlated with birth interventions and neonatal risk factors such as low birthweight and prematurity, and many of the suboptimal breastfeeding risk factors are common with risk factors identified with ASD. A neonate with low birthweight, hypoxia, delivered by caesarean section or any other
risk factors commonly associated with elevated ASD risk, is more likely to receive suboptimal breastfeeding. Donath and Amir (2008) found that neonates with gestational ages under 40 weeks were less likely to be breastfed. Breastfeeding initiation and maintenance is compromised after caesarean sections independent of the age of the mother (Fisher et al., 2013). Karlström, Lindgren, and Hildingsson (2013) found breastfeeding difficulties in women who had both emergency and elective caesareans.

If ASD and suboptimal breastfeeding share some risk factors, it may be argued that birthing risk factors such as caesareans, artificial rupture of membranes (AROM), inductions and epidurals may cause an environment where the initiation and duration of breastfeeding is compromised.

**Breastfeeding**

Research has shown that breastmilk hydrates, provides beneficial gut bacteria and immunological protection to an infant, but the exact composition of breastmilk is still unknown (Hinde, 2013).

The World Health Organization (WHO) recommends exclusive breastfeeding: that is, the infant only receives breast milk without any additional food or drink, not even water, on demand day and night for the first six months of life and that initiation of breastfeeding takes place within the first hour of life (WHO/UNICEF, 2003). WHO estimates that the achievement of universal coverage of optimal breastfeeding could prevent 13% of deaths in children under 5 (WHO, 2009).
A paediatric cost analysis in the U.S. estimated that if exclusive breastfeeding was attained for six months by 80-90% of families, the saving would be approximately $13 billion dollars per year by reducing costs associated with medical conditions such as otitis media, gastroenteritis, infectious diseases, necrotizing enterocolitis, asthma and atopic dermatitis (Bartick & Reinhold, 2010). At this time, it is estimated that exclusive breastfeeding rates are only 34.8% worldwide (WHO, 2009).

**Breastmilk**

In humans, and all mammals, lactation has three distinct stages: colostrum, transitional milk and mature milk, and at each stage the composition of the milk differs. Genetic analysis has found stage specific transcripts for immune defence in colostrum, milk protein synthesis in the transitional milk, and lipid production in mature milk (Lemay et al., 2013) indicating that each stage has a specific functional role.

**Colostrum**

Colostrum is a specialized milk produced in the first days after birth (Steimer & Klagsbrun, 1981) and due to the tight junctions in the mammary epithelium being open, many immunologically derived protective components from the mother’s circulation are transported into the colostrum (Rodriguez, Meier, Groer, & Zeller, 2009). The tight junctions close over the first days post partum and transitional milk occurs (Rodriguez et al, 2009).
Colostrum at a concentration of 2%, contains more growth factor activity than mature milk at a concentration of 20% (Steimer & Klagsbrun, 1981) and in transitional milk protein levels fall as much as fivefold with lactose increasing by about 50% (Casey, 1989).

Colostral milk has been shown to support epithelial cell proliferation more than mature milk does and has been shown to be selective: supporting the growth of epithelial cells but not fibroblasts (Steimer and Klagsbrun, 1981). In contrast, transitional milk (obtained one week post parturition) is completely inactive in terms of epithelial growth factors (Miller, 2008). The growth factors in colostrum seem to be involved in controlling the early growth and maturation of mammary epithelial cells of the neonatal intestine (Miller, 2008).

Colostral milk contains immunoglobulins, predominantly IgA, which play an important role in gut mucosal immunity (Vassilev & Veleva, 1996) but it also contains IgG (Miller, 2008) and IgM (Koenig, de Albuquerque Diniz, Barbosa, & Vaz, 2005). IgM is found in low concentrations in colostrum as the newborn is fully capable of producing IgM endogenously (Koenig et al, 2005). In most species, immunoglobulins are absorbed through the neonatal intestinal epithelium for only a few hours post parturition and absorbed immunoglobulins enter the intestinal lymphatic system and then enter the neonatal blood circulation. Absorbed IgA is secreted onto mucosal surfaces of the neonate (Wagstrom, Yoon, & Zimmerman, 2000) especially in the gut where it is generally thought to form an ‘immunopaint’ to prevent exposure of the mucosa and hence of the immature immune system, to environmental pathogens and other antigenic proteins (Casey, 1989). Meanwhile, IgG remains in circulation (Wagstrom et al, 2000). The mean IgA concentration in
colostrum is significantly higher in mothers of very premature infants (less than 32 weeks gestation) and decreases less rapidly than for term infants (Koenig et al, 2005). In this way, colostral composition seems to differ in response to the gestational age of the infant (Koenig et al, 2005; Rodriguez et al, 2008). Unfortunately, extremely low birth weight infants are often exposed to prolonged periods of nil by mouth and antibiotic exposure, leading to intestinal atrophy and an abnormal pattern of intestinal colonization (Rodriguez et al, 2008).

Leukocytes are cells of the immune system that defend the body against both infectious disease and foreign materials and colostrum contains ten times the amount of leukocytes than mature breast milk. Most of these leukocytes are macrophages and neutrophils, which remove microbial pathogens. Lymphocytes, including T cells, natural killer cells, and antibody-producing B cells, make up 10% of the leukocytes in human breast milk (Jackson & Nazar, 2006).

Calves deprived of maternal colostral leukocytes are found to lag behind in immune development by 1-2 weeks (Reber et al., 2008) and it is believed that the provision of maternal colostral leukocytes immediately after birth stimulates the development of the neonatal immune system and in this way is a part of the neonatal immune system (Reber et al, 2008).

The regulation of metabolic homeostasis depends on nutritional status, energy expenditure and hormonal signals (Ben-Jonathan, Hugo, Brandebourg, & LaPensee, 2006). The pituitary hormone, Prolactin (PRL) has more functions than all the other pituitary hormones combined (Ben-Jonathan et al, 2006). High concentrations of PRL are found in colostrum and fall rapidly in the days following birth: 157 ng/ml on the
third day compared with 24 ng/ml 13 days postpartum (Healy, Rattigan, Hartmann, Herington, & Burger, 1980). In addition to the role it plays in initiating and maintaining lactation, PRL regulates enzymes and transporters that are associated with glucose and lipid metabolism in other target organs. With some genetic studies of ASD implicating the PRL system at the genetic level and the PRL pathway as a whole (Yrigollen et al., 2008), further study is warranted into the potential bioactivity of PRL in colostrum and breastmilk and whether it may be protective in regard to ASD.

**Breastmilk, colostrum, formula and EFAs**

The percentage content of Long Chain Polyunsaturated Fatty Acids (LCPUFAs) are twofold higher in colostrum when compared with mature milk and this is consistent in all lactating women in all parts of the world (Fidler & Koletzko, 2000). The fat globules in colostrum are larger than in transitional, mature and artificial milks (Michalski, Briard, Michel, Tasson, & Poulain, 2005). It is theorized that the larger fat globule size of colostrum in the first two days postpartum could be an adaptation to the immature digestive system of the neonate contributing to an efficient and rapid digestion of colostrum lipids (Michalski, 2005). New homogenization techniques in use in infant formula since 1984 use the emulsification of vegetable oils which means that the fat globules are much smaller in artificial milk than in human colostrum and mature milk (Michalski, 2005) but it is not known how this may affect the infant.
**Research implications**

Colostrum profiles very differently to transitional and mature milk and seems to have a different function. At this time, there is no artificial colostral substitute and infants that are fed artificially are fed formula based on the composition of mature milk.

The commentary paper synthesises the findings outlined in these chapters and outlines the potential links between fatty acid deficiency and ASD, with a particular focus on the unique role of colostrum. The paper is a suggestion to researchers to differentiate between colostral milk and mature milk in human research the way it is expressed in animal research. The task of differentiating between milk stages is complex, however, with the discovery that the different milks are signified by a differentiation in potassium and sodium levels, rather than day of lactation (Lemay et al., 2013).

The WHO breastfeeding guidelines recommend breastfeeding in the first hour due to the establishment of a breastfeeding relationship between the mother-infant dyad, but crucially, because it has been found that neonatal deaths in populations where the mortality rates are high, could be reduced by 22% if infants were fed within the first hour (Edmond et al., 2006). In the Western world, the neonatal mortality rate is less than what is found in other parts of the globe, but the question remains: What does colostrum do in that first hour? A key proposition of the commentary paper is that colostral feeding in the first hour may assist an infant to lay down an immunological and metabolic pathway in a critical window of development.


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If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

As the HDR thesis author, I researched the literature for this paper, wrote and designed the paper, revised it critically and was responsible for the final draft.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: 18/1/2015

Christine Brown

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Commentary: Fatty Acids, Breastfeeding and Autism Spectrum Disorder

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Abstract

Fatty acid deficiencies are linked to Autism Spectrum Disorder. This commentary discusses the protective role of breastfeeding and the urgency of research into the human infant’s intake of colostrum to prevent fatty acid deficiency.

Keywords: Autism; Breastfeeding; Fatty Acids; Colostrum

Introduction

Autistic Spectrum Disorder (ASD) has been growing in prevalence over the last decade and new estimates suggest that rates in Australia could now be as high as 1 in 160 children (Williams, MacDermott, Ridley, Glasson, & Wray, 2008). Whilst there is no general agreement as to the reason(s) for the increase, with the number of children diagnosed with ASD growing, research into the disorder gathers even more urgency and the burden on families, health, education and welfare services ever greater.

Research has shown fatty acid deficiencies to be over-represented in Attention Deficit Hyperactivity Disorder (ADHD), Schizophrenia, Depression, Pervasive Developmental Disorder, Developmental Coordination Disorder, Epilepsy and, more recently, ASD (Bell et al., 2004; Freeman et al., 2006; Richardson, 2004; Vancassel et al., 2001). Often found in the ASD population are the physical signs of fatty acid deficiency including excessive thirst, frequent urination, keratosis pilaris on the upper arms or upper thighs, dandruff, and atopic tendencies (Bell et al., 2004), as are lower plasma levels of fatty acids and elevated cytosolic PLA2 enzymes compared with matched controls (Bell et al., 2004; Richardson, 2004). Some researchers, based on clinical reports and correlational studies, have gone so far as to propose a causal role for a disorder of fatty acid metabolism in ASD (Clark-Taylor & Clark-Taylor, 2004; Richardson & Ross, 2000).

If we are to accept that fatty acid deficiency (or a fatty acid metabolism impairment) may be relevant in ASD (whether as a causal agent, risk factor or epiphenomenal biological presence), then it is critically important to ensure that an infant has adequate supplies of fatty acids in utero and post-natally. The long chain polyunsaturated omega-3 and omega-6 fatty acids are so essential for the developing foetus that circulating levels double in maternal plasma (Crawford, 2000). An increase in omega-3 rich seafood in the maternal diet during pregnancy has been found to be correlated with optimum outcomes for prosocial behaviour, fine motor, communication and social development scores in children aged from 6 months to 3.5 years of age (Hibbelen et al., 2007). Omega-3 and omega-6 fatty acids also account for the majority of fats found in breast milk (Gibson & Kneebone, 1981). Given this fact, it seems reasonable to expect that breastfeeding would serve to protect the child from neurodevelopmental conditions such as ASD. Indeed, this is what studies have found (Schulz et al., 2006; Tanoue & Oda, 1989).

Schulz et al. (2006) surveyed 861 parents of children with ASD and 123 parents of children without ASD, and found that the children with ASD were significantly less likely to have been breastfed. The absence of breastfeeding significantly increased the odds of a later ASD diagnosis (OR 2.48, 95% CI 1.42, 4.35). The apparent link between ASD and breastfeeding was first cogently addressed by Tanoue and Oda (1989), who found that a significant number of infants subsequently diagnosed with an ASD had been weaned within one week of the commencement of breastfeeding with the duration of breastfeeding proposed as a protective factor. Similarly, the odds of being diagnosed with ASD also reduced with the duration of breastfeeding in the Schultz et al study, but not significantly. One major limitation of both of these studies is that colostrum intake was not accounted for.

In humans, and all mammals, lactation has three distinct stages: colostrum, transitional milk and mature milk, and at each stage the composition of the milk differs (Davis et al., 2007). Colostrum milk contains immunoglobulins such as IgA, which play an important role in gut mucosal immunity (Vassilev & Veleva, 1996), and a higher level of protein than mature milk.

Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding


(Davis et al., 2007). Colostrum also contains twice the long chain polyunsaturated fatty acids of mature milk and this is consistent in all lactating women in all parts of the world (Fidler & Koletzko, 2000). Hence colostrum can supply the breastfed neonate with significant amounts of preformed fatty acids during the first few days after birth even though the total milk intake is less than a mature milk feed (Fidler & Koletzko, 2000).

The World Health Organization (WHO) definition of exclusive breastfeeding is that the infant only receives breast milk without any additional food or drink, not even water, on demand day and night for the first six months of life (WHO, 2009). The remainder of the WHO guidelines lead to a critical issue for researchers in this field: that is, the recommendation that initiation of breastfeeding takes place within the first hour of life (italics added). Adherence to the WHO recommendation ensures that infants are able to access and utilise the preformed and highly digestible omega-6 and omega-3 fatty acids found in colostrum immediately after birth. As the timing of the establishment of breast feeding varies due to a number of circumstances, especially in premature babies, it is critical to determine whether infant fatty acid status and/or metabolism can be compromised by a lack of colostrum in the early stages after birth. An indication of the seriousness with which this issue is being taken is provided by numerous animal studies that have demonstrated fatty acid metabolism impairments and fatty acid deficiencies to be linked to low colostral intake. For example, the best protection for newborn piglets, calves and horses is immediate colostral feeding followed by an extended period of suckling over many weeks (Baxter, Baxter, & MacCormack, 1983; Gerrard, 1974). One study of neonatal calves found that delaying their colostral intake by just 24 hours impaired their fatty acid, carotene, retinol and alpha-tocopherol status (Blum, Hadorn, Salimann, & Schuep, 1997). Concentrations of fatty acids and fat-soluble vitamins in colostrum decrease as the time since parturition increases, and the infant calves’ absorptive capacity is decreased due to gut closure. In animals, there is clear evidence of a critical timeframe (24 hours post-birth) where colostrum must be accessed to ensure optimal fatty acid metabolism (Blum et al., 1997).

In stark contrast to the well accepted norms of animal management, the importance of colostrum ingestion in human infants is largely overlooked. If a human infant does not receive the fatty acid rich colostrum in a timely fashion, does this interrupt the establishment of a functional fatty acid metabolism as it seems to for animals? It would strike us as unusual if it didn’t but, at this point, there is no way of answering the question definitively. One human study (Vukavic, 1984) found that if the initiation of breastfeeding is postponed, spontaneous gut closure may not take place within the first 30 hours leaving the infant exposed to potential environmental contaminants (Blum et al., 1997). As colostrum is known to be implicated in the process of gut closure (Blum et al., 1997; Vukavic, 1984), infants who do not receive colostrum may have their health compromised by a delay in gut closure and a reduction in essential fatty acids.

This issue may be particularly important for premature infants who are invariably fatty acid depleted at birth due to the bulk of Docosahexaenoic Acid (DHA) accretion into the brain and central nervous system (CNS) occurring via the placenta in the last trimester of pregnancy (Makrides et al., 2009). Makrides et al. found that premature infants supplemented with a high level of DHA performed better at 18 months of age on the Mental Development Index of the Bayley Scales of Infant Development than non-supplemented peers. No such effect was found for males, however. Unfortunately, no information is given in regard to infant colostral intake. Of interest is the finding that females, in comparison to males, metabolise fatty acids at a rate of 4 to 1 (Burdge, 2004) seemingly due to the fact that testosterone can inhibit fatty acid synthesis (Marra & de Alaniz, 1989) and that both prematurity and male gender are independent risk factors for ASD (Kolevzon, Gross, & Reichenberg, 2007).

Pragmatic and/or medical issues prevent breastfeeding to WHO guidelines in all issues. Therefore, a critical research question relates to whether the current practice of not attempting to substitute for colostrum and moving directly to a mature milk replacement (formula) is the most effective approach to take for infants unable to be breastfed. Until 2002, if fatty acids were found in formula they were in the form of Linoleic Acid (LA) the omega-6 precursor to Arachidonic Acid (AA) and alpha Linoleic Acid (ALA) the omega-3 precursor to DHA (Alessandri et al., 2004). This led Alessandri et al. to make the observation that as infants have a limited capacity to synthesise DHA from ALA (which reduces with gestational age), artificially fed human infants are the only mammals that do not receive 20 and 22 carbon polyunsaturated fatty acids during their first months of life (Alessandri et al., 2004). Since 2002, DHA and AA have been included in selected infant formulas, although unsupplemented formulas are still widely available.

Infants fed unsupplemented formula have been shown to have lower DHA in their CNS, plasma and red blood cells, and autopsy studies have shown that these infants show depletion of their adipose tissue DHA stores by six months of age, whilst breastfed infants have maintained their DHA levels (Sarkadi-Nagy et al., 2004). Simply adding fatty acids to formula is a complex process, however, as dietary fatty acids synthesise via metabolic competition and a dietary source of one can deplete the synthesis of another (Sarkadi-Nagy et al., 2004). In a study of term and

preterm baboon neonates designed to measure the effects of supplementing formula with DHA and AA, supplemented formula was shown to partially restore the biosynthesis of DHA/AA to lower breastfed levels but the commonly available formula DHA concentrations were found to be inadequate to ensure a complete match of breastfed levels (Sarkadi-Nagy et al., 2004). The use of infant formula without DHA or AA supplementation versus exclusive breastfeeding was also significantly associated with an increase in ASD in the Schultz et al. (2006) study (OR 4.41, 95% CI 1.27, 15.7). Despite the limitations of the study (non-random sample and self-reported infant feeding practices) there appears to be mounting evidence that fatty acids serve a protective function in regard to the development of a number of neurodevelopmental disorders (Crawford, 2000; Richardson, 2004).

Clinical trials of fatty acids as a treatment in ASD are in their infancy and clear protocols regarding form of fatty acid, dose and agreement on adequate placebos in research trials require further clarification (Richardson, 2004). Furthermore, research in this field in the future would be greatly improved by ensuring thorough documentation of fatty acid intake beyond superficial examinations of intake in supplemental form. Specifically, researchers must examine colostral and mature milk intake separately, and also consider the source of any fatty acid supplementation and the relative ratios of the major fatty acid types (particularly of omega-3 to omega-6). With a more thorough understanding of fatty acid metabolism and its role in development, new and effective preventative and treatment measures are possible, not only with relevance to ASD, but other developmental conditions such as learning disorders and ADHD.

Acknowledgments
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References


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**Research Profiles**
Ms Christine Brown is a registered psychologist and graduated from the University of Melbourne with a first class Honours degree in Psychology. Christine commenced her PhD with SABRI in 2008 and is exploring the relationship between autism and early infant feeding and nutrition.

Associate Professor David Austin is a clinical psychologist and Associate Professor in the Faculty of Life and Social Sciences, Swinburne University of Technology, and the founding Director of SABRI. David has published widely on autism in international peer-reviewed journals and has very quickly established SABRI as the leading research group in Australia examining the biological bases of autism.

Chapter 4: ASD and phospholipids

A disruption of membrane phospholipid metabolism was originally proposed by Horrobin, Glen, and Vaddadi (1994) as the possible etiological basis of schizophrenia and is known as the membrane hypothesis of schizophrenia. The concept proposes that brain phospholipid metabolism is altered because of an increased rate of loss of DGLA(20:3, n-6), EPA(20:5, n-3), AA(20:4, n-6) and DHA(22:6, n-3) from the Sn2 position of phospholipids. This loss leads to changes in the functioning of the membrane-associated proteins and of the cell signalling systems. One explanation for this is thought to be overactivity of one or more of the PLA2 group of enzymes that remove these EFAs from the Sn2 position. This review paper investigates the possibility that underlying phospholipid dysregulation may also be operant in ASD. The paper also hypothesises that breastfeeding initiation and duration could be associated with fatty acid deficiency.
Paper 2: Autistic disorder and phospholipids: A review. Reprint of original article


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As the HDR thesis author, I researched the literature for this paper and was responsible for the overall concept and design of the paper. I revised it critically and was responsible for the final draft of the paper.

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Autistic disorder and phospholipids: A review

Christine M. Brown, David W. Austin*

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Abstract

Dysregulated phospholipid metabolism has been proposed as an underlying biological component of neurodevelopmental disorders such as autistic disorder (AD) and attention-deficit/hyperactivity disorder (ADHD). This review provides an overview of fatty acid and phospholipid metabolism and evidence for phospholipid dysregulation with reference to the membrane hypothesis of schizophrenia. While there is evidence that phospholipid metabolism is at least impaired in individuals with AD, it has not been established whether phospholipid metabolism is implicated in causal, mechanistic or epigenomological models. More research is needed to ascertain whether breastfeeding, and specifically, the administration of colostrum or an adequate substitute can play a preventative role by supplying the neonate with essential fatty acids (EFAs) at a critical juncture in their development.

Regarding treatment, further clinical trials of EFA supplementation are essential to determine the efficacy of EFAs in reducing AD somatotype and whether supplementation can serve as a cost-effective and readily available intervention.

Keywords:
Autism
Autistic disorder
Phospholipids
EPA
Breastfeeding
Colostrum

1. Introduction

Autistic disorder (AD) is a lifelong neurodevelopmental disorder that has been growing in prevalence over several decades [1]. AD is now a more prevalent childhood disorder than Type 1 diabetes, Down Syndrome and childhood cancer combined [2]. In the UK, 1% of children aged between 5- and 9-years of age have an existing diagnosis on the autistic spectrum and for every three cases that are diagnosed, there may be a further two cases that remain undiagnosed [3]. These figures represent a twelve-fold increase in AD over the last 30 years [3]. In Australia, prevalence is estimated at 1 in 160 children [4]; however, this figure is based on data from 2003/2004 and therefore may underestimate current prevalence. Although there is no general agreement as to the reason(s) for this continuing increase in prevalence, with the number of children diagnosed with AD growing, research into the disorder gathers even more urgency and the burden on families, health, education and welfare services ever greater.

For much of its history, AD has been considered a discrete psychological disorder and largely managed with behavioural interventions. However, many studies have outlined the dimensionality of AD in regard to its comorbidity with other neurodevelopmental disorders such as ADHD, dyslexia, dyspraxia [5] and epilepsy [6]. In addition to this, the fact that all of the aforementioned disorders affect a disproportionate number of males than females and that there is a strong familial association has led to a re-conceptualisation of these disorders [5]. A new proposition is that there may be an underlying biological component that is modified by an individual’s genetic constitution and exposure to environmental factors [7]. Researchers are now theorising that one of the underlying biological components in all these neurodevelopmental disorders involves dysregulated phospholipid metabolism [5,8,9].

The brain phospholipids are exceptionally rich in highly unsaturated fatty acids (HUFAs) [10,11] and in contrast to other bodily tissue, a unique feature of neurons is the smaller amounts of the precursors LA(18:2, n-6) and ALA(18:3, n-3) and the higher amounts of their metabolites: AA(20:4, n-6) and DHA(22:6, n-3) [11–13]. The two major PUFAs in all vertebrates are AA and DHA [14] and account for 20% of the dry brain weight [10,15]. Also present in phospholipids are DGLA(20:3, n-6), arachidonic acid(20:4, n-6), EPA(20:5, n-3) and DPA(22:5, n-3) but they are not as prolific as AA(20:4, n-6) and DHA [11,12]. DHA is known to be involved in cell signaling and cell proliferation [16] and has an important structural role in the brain [17] whilst AA is crucial for brain growth. EPA(20:5, n-3) seemingly has no structural role, but it is considered vital for the regulation of brain function [17].

Phospholipases are enzymes that break down phospholipids and particular interest is taken in the phospholipases A2 (PLA2) enzymes which are upstream regulators of many inflammatory processes. PLA2 recognizes the Sn2 bond in phospholipids and catalytically hydrolyzes the bond releasing AA(20:4, n-6), EPA(20:5, n-3) and DHA(22:6, n-3) [18]. AA is then modified in...
active compounds called eicosanoids (prostaglandins, leukotrienes and thromboxanes) which are local hormones that participate in a number of physiological as well as pathophysiological conditions such as the activation of immune cells, platelet aggregation and parturition initiation [19]. EPA and DHA are metabolized to resolvins and protectins that have important roles in the resolution of inflammation [18].

Phospholipids are a unique intersection of the environment and genes as the final structure of each phospholipid molecule depends on an interaction between genetic and environmental factors [20]. That is, the enzymes involved in the synthesis and breakdown of phospholipids are under genetic control, but the key essential fatty acids (EFAs) of neuronal phospholipids must come exogenously, from the diet. Strictly speaking, EFAs are solely the precursor fatty acids: LA(18:2 n-6) and ALA(18:3 n-3). However, as the EFAs cannot be synthesized de novo, if they are unavailable they will be replaced by non-essential fatty acids, such as saturated fats, thus changing the phospholipid structure [11]. For example, during dietary deprivation of ALA(18:3 n-3), DHA(22:6 n-3) is replaced by EPA(20:5 n-3) in the retina and brain of animals [21]. As this fatty acid is the most like DHA, this seems to suggest the existence of a compensatory mechanism [21]. Consequently, for the purpose of this review, all the polyunsaturated fatty acids (PUFAs) are referred to as EFAs.

A disruption of membrane phospholipid metabolism was originally proposed by Hornbin et al. [22] as the possible etiological basis of schizophrenia and is known as the membrane hypothesis of schizophrenia. The concept proposes that brain phospholipid metabolism is altered because of an increased rate of phospholipid breakdown in the brain of unmedicated schizophrenics [31,32].

1. Increased circulating levels of PLA2 enzymes in the bloodstream [23–25].
2. Reduced levels of AA(20:4, n-6) and DHA(22:6, n-3) in red cell membrane phospholipids [26–29], perhaps due to oxidative stress [30].
3. 31-phosphorus neurospectroscopy (31P MRS) indicating an increased rate of phospholipid breakdown in the brain of unmedicated schizophrenics [31,32].
4. A diagnosis of schizophrenia being associated with a reduced flushing response to oral or topical niacin indicating that the amount of available AA(20:4, n-6) is reduced [33].
5. Reduced ERG response to light stimuli [34–36] indicating reduced DHA(22:6, n-3) availability [11,35].
6. Two different genetic abnormalities found on chromosome 1 in the vicinity of the gene for PLA2 [37].
7. Clozapine raising the red cell phospholipid AA and DHA levels in schizophrenic patients [38] and perhaps accounting for some of the therapeutic effects [11].
8. Resistance to arthritis and other inflammatory diseases, resistance to pain and improvement in psychosis which frequently occurs in response to fever [22,23,40].

With evidence mounting that irregular phospholipid metabolism was, at least, concomitant with schizophrenia, research commenced into other neurodevelopmental disorders with the finding that fatty acid deficiencies are also over-represented in attention-deficit/hyperactivity disorder (ADHD), depression, pervasive developmental disorder, developmental coordination disorder and epilepsy [10,41–43]. Further, dysregulated lipid metabolism is now accepted as a pathogenic factor in many neurological disorders such as bipolar disorder and neurodegenerative diseases such as Alzheimer’s, Parkinson’s, Niemann-Pick and Huntington diseases [18]. Altered lipid metabolism is also believed to be a key event which contributes to central nervous system (CNS) injuries such as stroke [18].

Research into phospholipid in AD is in its infancy and began with the observation that many children with AD have visible signs of fatty acid deficiencies such as excessive thirst, frequent urination, keratosis pilaris on the upper arms or upper thighs, dandruff, and atopic tendencies [43].

2. The evidence for a phospholipid pathogenic component in autism spectrum disorder

2.1. Increased circulating levels of PLA2 enzymes in the bloodstream

Bell et al. [43] identified significantly increased red blood cell type IV PLA2 activity in patients with regressive autism, classical autism and Asperger’s disorder which is consistent with previous findings of elevated PLA2 in schizophrenia, depression, bipolar disorder and dyslexia [44]. An unexpected finding was that the HUFA composition of the regressive autism group reduced dramatically by between 50% and 82% following 6 weeks of storage at –20 °C. This unexpected loss of red blood cell PUFAs was also found in a study of schizophrenic patients [45]. At –20 °C, the decay rates of schizophrenic patients’ red blood cell PUFAs were nearly twice that of non-patient control participants. Both studies theorized that elevated levels of PLA2 activity may account for the increased degradation [43,45] although Bell et al. also considered a possibility of increased lipid peroxidation in the affected sample.

2.2. Reduced levels of AA and DHA in red cell membrane phospholipids

A study comparing plasma fatty acid levels of children with AD and children with mental retardation and no autism diagnosis found that DHA(22:6, n-3) levels were reduced by 23% in autistic children [19]. AA(20:4, n-6) levels were also lower (but not significantly) and the total PUFAs levels were reduced by about 20% in the total plasma of children with autism [10]. A significant increase in the Omega 6/Omega 3 ratio values in children with AD was found. There was also a tendency to reduced levels of EPA(20:5, n-3) but not significantly. It was noted that the children with mental retardation may not serve as a true control group given that they also may have fatty acid deficiencies so the differences between groups may have been reduced [10]. In another study, significantly lower levels of AA were found in patients with regressive autism compared to controls [43].

A case study of an 8-year-old male found reduced levels of DHA and EPA, despite a normal blood level of their precursor, ALA(18:3 n-3). Omega-6 fatty acids were at normal levels [46].

Bu et al. [47] were not able to replicate the findings of Bell et al. [43] although a few alterations of red blood cell membrane fatty acids were found in regressive autistic children. Bu et al. did not find an increased AA(20:4, n-6)/EPA(20:5, n-3) ratio in children with classic or regressive autism. Whilst EPA levels in both classic and regressive autistic groups had wider variations compared to the children with developmental disabilities and the neurotypical control group, the variation was not significant. However, Bu et al. also reported that two PUFAs: eicosapentaenoic acid and eicosadienoic acid, were elevated in children with regressive autism compared to the
neurotypical control group. As eicosapentaenoic acid, the acid in question must be, in fact, eicosapentadecanoic acid (20:2, n-6). Eruic acid (22:1, n-9) is a monounsaturated acid so the elevated level of erucic acid (22:1, n-9) should not be of significance in regard to the membrane phospholipid hypothesis. A significantly higher level of eicosapentaenoic acid (20:2, n-6) was found in children with regressive autism when compared to the group of children with early onset autism. Bu et al. did not find any evidence that Omega-3 fatty acids were decreased in children with AD. A larger study of 153 cases of autism and 97 general population controls [48] found an overall trend for those with an autism diagnosis to have lower DHA (22:6, n-3) levels; however, lipid compositions varied sufficiently to overlap with the distributions in the control group. Interestingly, significantly lower levels of AA (20:4, n-6) were found, but only in the females with autism compared with the female controls.

2.3. 31-Phosphorus neurospectroscopy ($^{31}$P MRS) and phospholipid breakdown

In 1993, a pilot study [49] found evidence (albeit described as tentative), for alterations in phospholipid metabolism in eleven high-functioning autistic and adolescent young men after controlling for age and IQ effects.

2.4. Niacin response

Puri and Singh [50] found no significant difference between the mean volumetric niacin response in patients with autism and the mean volumetric niacin response in the control group. They concluded that the fatty acid abnormalities in autism are likely to differ from those that occur in schizophrenia.

2.5. Reduced ERG response

Reduced ERG b-wave amplitude was found in 48% of individuals with AD [91] and four of their first-degree relatives [52]. ERG responses were altered in a group of autistic children when compared with a normal reference range of responses and the alterations positively correlated with the clinical severity of the autism [53].

2.6. Genetic abnormalities on the PLA2 gene

One case study has reported a genetic site linked to autism on chromosome 8q22 in the proximity of the gene for secretory soluble PLA2 [54,55]. Given the important role the PLA2 enzyme has in hydrolyzing the sn-2 fatty acids in phospholipids it is theorized that this enzyme may have an important role in the etiology of autism [56].

2.7. Clozapine

A single case study of long-term treatment of autism in an adult male, showed that over a 5-year treatment period with Clozapine, there was a marked improvement in levels of aggressiveness and social interaction [57]. No data exists in regard to the effect of Clozapine on red cell phospholipid PUFAs levels in AD, but the possibility exists that some of the improvement in symptomatology may be due to changes in PUFAs levels after administration of Clozapine as per the effect in those with schizophrenia reported by Horrobin [11].

2.8. Pain and fever response

A study of pain reactivity in children with autistic disorder, whilst based on parent report, found abnormally low levels of pain reactivity when compared with non-autistic children when matched for age, sex and socio-economic level [58].

Curran et al. [59] conducted a study in response to the anecdotal evidence of AD symptom improvement during fever. When a child’s temperature was over or equal to 38.0 °C, fewer aberrant behaviours were found on the domains of irritability, hyperactivity, stereotypy and inappropriate speech.

Currently, more investigation is required in order to fully understand any possible etiological role of phospholipids in AD. Nevertheless, there appears enough evidence to suggest that fatty acid disruptions or deficiencies are at least a predictable biological presence in AD and, as such, warrant urgent and detailed investigation. Horrobin [11] proposed that increasing the availability of EFAs in the diet would improve symptoms in schizophrenia; however, those with the rate of synthesis and incorporation into phospholipids would be reduced in males and those with an excess of oxidants.

3. Does an increased availability of EFAs attenuate AD symptomatology?

Johnson and Hollander [60] supplemented an 11-year-old boy with a history of autism with fish oils containing EPA which was increased over a period of 4 weeks to 540 mg per day. Both his parents and clinician reported a complete resolution of anxiety and agitation after a week on that dosage and improvements continued for 8 months of follow-up. A significant improvement was noted in the patient’s quality of life.

Amminger et al. [61] gave 1.5 g per day of Omega-3 EFAs (84 g EPA, 72 DHA) to seven autistic males (5–17 years) over 6 weeks. A blinded placebo condition (n–6) received coconut oil. Children in the Omega-3 condition were found to improve on measures of hyperactivity and stereotypy, each with a large effect size. A non-significant trend towards improvement on hyperactivity was also found. This result is particularly impressive given the short period of supplementation and the very low power of the analyses due to the small sample size. It remains somewhat curious, however, that the researchers chose to use a lipid (coconut oil) as a placebo as it would be reasonable to speculate that such a material would be unlikely to have a purely neutral effect on the subjects’ fatty acid metabolism.

Meguid et al. [15] analyzed the plasma PUFA levels of 30 autistic children and found that LA, DHA, 1n-6A and AA levels were all significantly lower in the autistic children compared with the control group (n–30). However, the mean ratio of AA/DHA was significantly higher in autistic children compared with the control group. Over a 3-month period the clinical group was supplemented with Omega-3 and Omega-6 fatty acids (60 mg DHA, 12 mg GLA, 13 mg EPA and 5 mg AA) and Vitamin E. 66% showed clinical and biochemical improvement. From blood analyses, the supplemented group showed elevated levels of DHA and LA.

Politi et al. [62] studied the effects of Omega-3 (0.03 g EPA and DHA) supplementation in an open label study of 19 young adults (18–40 years old) with severe autism (CARS > 40) over a period of 6 weeks. During this period, the researchers also administered 5 mg of Vitamin E to minimize lipid peroxidation. Behaviour was rated using the Rossaio Behavioural Checklist and the inter-rater reliability of caregivers was established at or above 90% at baseline. Both problem frequency and average severity scores were obtained at pre-treatment, during-treatment and post-
4. Is the rate of EFA synthesis sex dependent in AD?

In dyslexia, dyspraxia and attentional disorders without hyperactivity, twice as many males as females are diagnosed [63]. For the more disruptive forms of ADHD and AD the ratio is closer to 5:1 [63]. It is estimated that females, in comparison to males, metabolise fatty acids at a rate of 4:1 [64] seemingly due to the fact that testosterone can inhibit fatty acid synthesis [65]. Oestrogen appears to attenuate fatty acid metabolism issues and the relative lack of oestrogen in males makes them more vulnerable than females to deficiencies in HUFAs [63]. Being male is a well-established risk factor for AD [66].

Wiest et al. [40] theorized that the lower AA:20:4 n-6) concentration in the autistic females may be due to the differences in how autism arises in males and females or due to dietary factors. It would be pertinent to explore the role of oestrogen in AA metabolism to shed further light on this finding.

5. Oxidative stress and AD

Lipid peroxidation is increased in the plasma of children with autism when compared with their neuropathical siblings [56]. Oxidative stress markers such as reduced levels of glutathione, decreased catalase, abnormal iron and copper levels and increased nitric oxide, have all been found in individuals with an AD diagnosis [56]. A review of oxidative stress in psychiatric disorders found evidence for increased oxidative stress in disorders including autism, mental retardation, Rett's disorder, ADHD and schizophrenia [67]. Oxidative stress is associated with increased lipid peroxidation as PUFAs are particularly vulnerable to peroxidation by oxysradicals [67].

6. Fatty acid intake and neurodevelopment

Individuals with schizophrenia are significantly less likely to have been breastfed than controls [86,68]. The protective effect of breastfeeding may be due to breast milk being a rich source of DGLA, AA, EPA and DHA, which may attenuate any adverse consequences of impaired fatty acid synthesis [11]. As infants, especially pre-term neonates, are not able to convert dietary LA and ALA precursors at an adequate rate to the EFA's important in brain development [70,71], it is crucial that preformed DHA and EPA are supplied by diet.

Prenatally an increase in Omega-3 rich seafood in the maternal diet during pregnancy has been found to be correlated with optimum outcomes for prosocial behaviour, fine motor, communication and social development scores in children aged from 6 months to 3.5 years of age [72]. A study of postnatal EFA supplementation found that premature infant girls supplemented with a high level of DHA(22:6, n-3) performed better at 18 months of age on the Mental Development Index of the Bayley Scales of Infant Development than their non-supplemented peers [73].

The importance of EFAs to neurodevelopment is highlighted by the findings of Salem et al. [16], who demonstrated that when an adult mammal consumes a diet low in DHA(22:6, n-3) and its Omega-3 precursors, the DHA levels in the nervous system is much less altered than are other DHA levels in other organs. The implication being that once neural development has occurred, DHA is "tenaciously retained" [16, p. 945]. In contrast, animal studies have shown that when Omega-3 fat sources are inadequate during early neural development, levels of brain and retinal DHA decline [16]. This points to the potential for a critical window when EFA status needs to be consolidated to ensure adequate levels in the infant.

7. Is a lack of breastfeeding a risk factor for AD?

Schultz et al. [74] surveyed 861 parents of children with AD and 123 parents of children without AD, and found that the children with AD were significantly less likely to have been breastfed. The absence of breastfeeding significantly increased the odds of a later AD diagnosis (OR 2.48, 95% CI 1.42, 4.35). The apparent link between AD and breastfeeding was first cogently addressed by Tanoue and Oda [75], who found that a significant number of infants subsequently diagnosed with an AD had been weaned within 1 week of the commencement of breastfeeding with the duration of breastfeeding proposed as a protective factor. Similarly, the odds of being diagnosed with AD also reduced with the duration of breastfeeding in the Schultz et al. study, but not significantly. One major limitation of both of these studies is that colostrum intake was not accounted for as colostrum contains twice the PUFAs as transitional and mature milk [76]. At this stage, only in animal research is the importance of early fatty acid consumption in the form of colostrum acknowledged and accepted. A study of neonatal calves found that delaying their colostral intake by just 24 h impaired their fatty acid, carotene, retinol and alpha-tocopherol status [77] at least for the first week of life.

8. Conclusion

A review of the literature suggests that there is compelling evidence that phospholipid metabolism is at least impaired in individuals with AD. However, at this stage, it has not been established whether phospholipid metabolism is implicated in causal, mechanistic or epiphenomenological models. More research is needed to ascertain whether breastfeeding and specifically, the administration of colostrum or an adequate substitute can play a preventative role by supplying the neonate with EFAs at a critical juncture in their development.

In regard to treatment, further clinical trials of EFA supplementation are essential to, at worst, eliminate the possibility of the efficacy of EFAs in reducing AD symptomatology and at best, determine whether supplementation can serve as a cost-effective and readily available intervention. If so, it is critical to determine which EFAs are most effective and in what ratios.

New non-invasive tests such as the measurement of volatiles in breath [78] need to be rigorously applied to investigate the possibility of establishing a reliable biological diagnostic determinant of AD.
Acknowledgement

The authors wish to acknowledge the Friends of SABRI.

References


Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding


Chapter 5: Observable fatty acid markers

In 1995, Stevens et al. (1995) published a study of boys with a diagnosis of ADHD. This paper outlined seven clinical fatty acid deficiency markers: excessive thirst, frequent urination, dry hair and skin, brittle or soft nails, dandruff and rough, bumpy skin (keratosis pillaris) and the association of these signs with plasma polar lipid fatty acid levels. The authors found that a high fatty acid deficiency (FAD) score, as measured by the seven observable signs, was significantly associated with low levels of LCPUFAs, in particular, DHA. Additionally, those boys without an ADHD diagnosis were significantly less likely to have been breastfed (81% versus 45%) and the breastfeeding duration in the neurotypical cohort was significantly longer than in the boys with ADHD (6.5 months versus 2.5 months).

In 2001, Vancassel (2001) found that children with an ASD diagnosis had significantly lower levels of plasma fatty acids, in particular, 20% less DHA than controls. Amminger (2006) found that \( n-3 \) fatty acid supplementation attenuated ASD symptomatology and Antalis (2006) again found a correlation between observable fatty acid deficiency and low DHA plasma phospholipid. Whilst this study showed plasma levels deficiency in ASD, blood levels of EFA were not significantly different to controls. This may have indicated a fatty acid conversion issue, but it was not explored in the paper.

In 2004, Bell found significant differences in the fatty acid deficiency (FAD) markers in individuals with autism, Asperger’s and the control group. However, in 2007, Sinn published a paper that found that although the FAD score predicted lower fatty acid
levels and a lower rate of breastfeeding in individuals with ADHD, it was not a reliable way of identifying those who could benefit from fatty acid supplementation. This study seems to be the most recent paper published that reported using the FAD measure.

An alternative measure of fatty acid status is organic acid testing, which is a technique that uses liquid chromatography tandem mass spectroscopy to test for organic acids in urine. Tandem mass spectroscopy is used to assess for inborn errors of metabolism such as phenylketonuria (PKU) (Ozben, 2013), however organic acid testing seeks to measure subclinical levels of organic acids. After researching, investigating and analysing data on Organic Acid Testing as a potential biomarker for fatty acid metabolic dysregulation or deficiency, several issues were identified. Firstly, there were issues with regards to accepted reference ranges and also their specificity to detect dysregulation associated with issues of fatty acid metabolism. Furthermore, the test was found to be more onerous for parents and children as a first morning urinary sample must be supplied. Ultimately, the decision was made to use the observable signs of fatty acid deficiency: the FAD score (Bell, 2004).

The following paper concentrated on breastfeeding and FAD scores. The intent was to ascertain whether first hour colostral feeding would be significantly associated with an ASD diagnosis and whether the seven observable fatty acid deficiency signs would correlate with a diagnosis of ASD as they had in the study by Bell (2004).
Paper 3: Observable fatty acids and Autism spectrum disorder, reprint of original article

**AUTHORSHIP STATEMENT**

1. **Details of publication and executive author**

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If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

As the HDR thesis author, I conceived of the project, designed the methodology, collected the data via telephone interviews, set up the data file in SPSS, analysed the data (with assistance), wrote the paper, reviewed it critically and was responsible for the final draft of the paper.

*I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.*

| Signature and date | 18/1/15 |

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<tr>
<td>Christine Brown, Deakin University</td>
<td>Conception, design, data collection, statistical analysis, drafting manuscript</td>
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<tr>
<td>David Austin, Deakin University</td>
<td>Critical revision and editing</td>
</tr>
<tr>
<td>Lucy Busija, Deakin University</td>
<td>Assistance with statistical analysis and design, revision</td>
</tr>
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Observable essential fatty acid deficiency markers and Autism Spectrum Disorder

ABSTRACT
Autism Spectrum Disorder (ASD) has been associated with essential fatty acid (EFA) deficiencies, with some researchers theorising that dysregulation of phospholipid metabolism may form part of the biological basis for ASD. This pilot study compared observable signs of fatty acid status of 19 children with an ASD diagnosis to 23 of their typically developing siblings. A pregnancy, birth and breastfeeding history was also obtained from their parents, which included a measure of infant intake of fatty acid rich colostrum immediately post-partum. When considered within their family group, those infants not breastfed (with colostrum) within the first hour of life and who had a history of fatty acid deficiency symptoms were more likely to have an ASD diagnosis. Other variables such as formula use, duration of breastfeeding, gestational age and Apogar scores were not associated with group membership. The results of this study are consistent with previous research showing a relationship between fatty acid metabolism, breastfeeding and ASD such that early infant feeding practices and the influence this has on the fatty acid metabolism of the child may be a risk factor for ASD.

Keywords: essential fatty acids, Autism Spectrum Disorder, breastfeeding, colostrum, FAD score, EFA, ASD

Breastfeeding Review 2014; 22(2): 21–26

INTRODUCTION
Autism Spectrum Disorder (ASD) represents a continuum of social and communication deficits generally diagnosed in childhood (Diagnostic and Statistical Manual of Mental Disorders, 5th edition, American Psychiatric Association). ASD prevalence has risen dramatically over recent decades with the latest estimates suggesting that as many as 1 in 88 children (1 in 54 males) have an ASD diagnosis: an increase of 23% in 2 years (Rice et al 2012). ASD represents a significant public health concern and definitive understandings as to its causation, prevention and treatment remain elusive.

There is an emerging acknowledgement that both the brain and whole body systems are implicated in ASD (Herbert 2010). In addition to evidence for genetic factors, there is growing evidence to suggest that immune dysregulation (Ashwood & Van de Water 2004) and inflammation (Vargas et al 2005), oxidative stress (Adams et al 2011), mitochondrial dysfunction (Weissman et al 2008) and environmental toxicant exposures (Austin 2008, Palmer, Blanchard & Wood 2009; Roberts et al 2007) may also be implicated (Rossignol & Frye 2012).

At a unique intersection of both genes and the environment are phospholipids: a class of lipids that are the major component of cell membranes (Horrobin 1998). Brain phospholipids, rich in highly unsaturated fatty acids, are known to be essential for normal neurological function (El-Ansary, Ben Bacha & Korb 2012). Although the enzymes involved in the breakdown of phospholipids are under genetic control, the essential fatty acids (EFAs) that determine the phospholipid structure must be provided exogenously through the diet. Proposed by Horrobin (1998) as a possible etiological basis of schizophrenia, dysregulated phospholipid metabolism has also been implicated in both ASD and Attention Deficit Hyperactivity Disorder (ADHD) (Bell et al 2004).

Fatty acid metabolism is influenced by sex hormones, with testosterone inhibiting and oestrogen stimulating the conversion of the essential fatty acids precursors...
Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding

(linoleic acid, LA and alpha-linolenic acid, ALA) into their metabolites (arachidonic acid, AA and docosahexaenoic acid, DHA) (Burdge 2004; Deisi & Kennedy 2011). Some researchers have theorised that this may, at least partly, explain the elevated rates of autism found in males compared to females (Schuckardt et al 2010).

A disruption to phospholipid metabolism is thought to reduce the availability of circulating fatty acids and thus result in fatty acid deficiencies (Horrobin 1998). Indeed, studies have found reduced fatty acid levels in the plasma of children with an ASD diagnosis (Antalis et al 2006; Bell et al 2004; Vancassel et al 2001). Although not all studies have found consistently reduced levels of fatty acids in children with an ASD diagnosis (Bu et al 2006), there has been a consistent finding of an undesirable ratio of omega-6 to omega-3 fatty acids (El-Ansary et al 2012; Bell et al 2010).

Another emerging body of research is revealing the impact of suboptimal breastfeeding practices on infant development and its association with ASD (Al-Farsi et al 2012; Schultz et al 2006). Early weaning (Tanoue & Oda 1989) and an entire absence of breastfeeding have been associated with an increased risk of an ASD diagnosis. One consequence of suboptimal breastfeeding practices is to decrease the likelihood of an infant receiving fatty acid rich colostrum, particularly when the initiation of breastfeeding is delayed (Al-Farsi et al 2012). In our previous review (Brown & Austin 2011) we theorised that colostral intake could reduce the risk of an ASD diagnosis by increasing the availability of EFAs to the infant at a critical developmental stage. Indeed, it may be more cogent to view colostrum not as a food source per se, but as a fundamental exogenous facilitator of a neonate’s metabolic development.

Although EFA status can be determined via biological testing of blood samples (El-Ansary, Bacha & Al-Ayahdi 2011) and cheek cells (Kirby et al 2010), a non-invasive, observational test known as a Fatty Acid Deficiency score also has utility in the assessment of EFA status. In 1930, Burr and Burr observed that rats on a fat-free diet developed excessive thirst and dandruff, reversible only with the addition of linoleic acid, a long chain EFA, in their diet. Observable symptoms of EFA deficiency in humans were first described by Colquhoun and Bunday (1981) who noticed that hyperactive children showed more signs of thirst than their undiagnosed peers. Later research by Stevens et al (1995) compared the incidence and severity of seven observable signs of EFA deficiency in boys with ADHD: excessive thirst, dry hair, dry skin, brittle nails, dandruff, frequent urination and keratosis pilaris (rough, dry bumpy skin on the upper arms and/or legs). Results showed that blood plasma concentrations of AA and DHA were significantly lower in those with the highest observable EFA deficiency score. These findings were replicated in ASD populations (Bell et al 2004; Vancassel et al 2001) and Bell et al formalised these same seven symptoms in a questionnaire from which a Fatty Acid Deficiency (FAD) score could be derived.

The intent of this study was to ascertain whether FAD scores were elevated in a sample of children with ASD when compared to their normally developing siblings. Additionally, we aimed to determine if breastfeeding factors such as colostral intake and exclusivity of breastfeeding were associated with a diagnosis of ASD.

**METHOD**

**Participants**

Following receipt of ethics approval from Swinburne University Human Research Ethics Committee, 54 families who were members of a database of supporters of autism research at Swinburne University in Melbourne, Australia, were contacted via email. Consent was given by 19 families who volunteered to participate in this study. Telephone interviews were conducted with parents (18 mothers, 1 father) in regard to their 42 children. Of these children, 19 had a diagnosis of ASD, whilst the remaining 23 were typically developing. There was one set of dizygotic twins, with the male twin having a diagnosis of ASD and the female twin typically developing.

Survey items recorded the pregnancy histories of the mothers and the early feeding histories of the children. The feeding history of the children specifically asked whether the children had been breastfed in the first hour after birth and whether or not they had been exclusively breastfed as per World Health Organization (WHO) guidelines (no other liquids or nutrition for the first 6 months). Prenatal maternal fatty acid intake was measured by how many times per week fish was consumed during pregnancy.

The FAD measured seven observable signs of fatty acid deficiency: excessive thirst, frequent urination, dry skin, dry hair, soft or brittle nails, dandruff and rough, dry, bumpy skin. Parents were asked if their child had ever displayed any of these symptoms. Responses were scored as 0 (symptom absent) or 1 (symptom present) and summed to produce a total FAD score for each child, with higher scores indicating more observable signs of fatty acid deficiency.

**Statistical analyses**

Given the modest sample size, potentially relevant predictors of ASD were identified using chi-square tests for categorical variables and independent samples t-tests for interval variables. Following recommendations of Mickey and Greenland (1999), variables with p values <0.20 were subsequently included in a multivariate logistic regression model to assess their combined effect on ASD status. A cut-off value of p=0.20 is recommended for covariate selection as this conservative criterion tends
Table 2. Means (and standard deviations) for number of ultrasounds, gestation period, birthweight, Apgar and FAD scores for all children.

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¹ Indicates significant difference in group means at p<0.05
² 10-point Apgar score assessing prognosis and condition of newborn (Apgar 1953)

critical factor may not be exclusive breastfeeding in a general sense but, specifically, whether or not an infant has access to fatty acid-rich colostrum within the first hour (or hours) after birth. In this regard, it is essential to understand that the function of colostrum is not as a food for the neonate, but an important catalyst of the establishment of the biological mechanisms of the infant’s fatty acid metabolism. Consistent with this, a recent study by Al-Farsi et al (2012) found that both delayed colostrum intake and a reduced length of exclusive breastfeeding (up to 3 months versus up to 4 months) were significantly associated with an ASD diagnosis.

There are several limitations to this study. First, a small sample size limited the statistical power of the analyses, thus increasing the likelihood of type 2 error. Second, the telephone interviews necessitated self-reported birth and infant feeding histories. Although research has shown that the recall of birth characteristics reported by mothers is highly correlated with medical records (Sou et al 2006), it remains subject to recall error. Third, the breastfeeding history was recorded as a dichotomy (the presence or absence of exclusive breastfeeding). Many studies have shown a dose effect of breastfeeding (Al-Farsi et al 2012; Schultz et al 2006; Tanoue & Oda 1989). In future research it would be desirable to more accurately assess breastfeeding duration and to account for within-subject variability such as intermittent bouts of formula-feeding. It is also important to note that the FAD measure used in this study did not include a frequency, duration or severity measure, but rather recorded whether or not the child had ever displayed any of the FAD symptoms. Due to the robust finding that FAD scores were elevated in the ASD group, it would be important to modify the FAD measure to include frequency, duration and severity variables in future research and account for maternal fatty acid intake pre- and post-partum. Finally, a less homogeneous group than the group recruited for this study would be desirable. As previously outlined, in this group, most children were born in a hospital setting, many had medical interventions and most mothers were aged in their 30s at the time of their child’s birth. However, a broader cross-section of parental and birthing characteristics would allow other factors to be accounted for, such as home-birthing and the associated low levels of medical interventions that typically occur in such settings. Several risk factors associated with ASD are medicalised birth interventions such as caesarean section (Larsson et al 2005) and are the same risk factors associated with a reduced rate of breastfeeding (Donath & Amir 2008; Jordan et al 2005).

Table 3. Results of logistic regression analysis (with odds ratios) examining the association between ASD diagnosis and FAD score, with child’s sex and first hour breastfeeding as covariates.

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient B (SE)</th>
<th>Odds ratio</th>
<th>95% CI for odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.53 (0.72)</td>
<td>1.69</td>
<td>0.41 (0.92, 6.99)</td>
<td>0.463</td>
</tr>
<tr>
<td>Sex</td>
<td>-1.37 (0.90)</td>
<td>0.25</td>
<td>0.01 (0.04, 1.50)</td>
<td>0.130</td>
</tr>
<tr>
<td>First hour breastfeeding</td>
<td>-1.36 (0.64)</td>
<td>0.26</td>
<td>0.06 (0.07, 0.89)</td>
<td>0.033*</td>
</tr>
<tr>
<td>FAD score</td>
<td>1.02 (0.39)</td>
<td>2.77</td>
<td>1.28 (1.28, 5.99)</td>
<td>0.009**</td>
</tr>
</tbody>
</table>

*p<0.05
**p<0.01.
This study highlights the potential utility of observable fatty acid symptomatology in an ASD population in terms of both the quest to better understand the pathophysiology of the condition and also as a potential avenue for exploring supportive nutritional early intervention. This refers to both the examination of breastfeeding practices as a protective measure against ASD, as well as the possible clinical utility of fatty acid supplementation both pre- and postnatally. Clearly though, more research is needed to better understand the importance of the availability of fatty acids, primarily via colostrum, to the neonate and any potential role this may play in reducing an infant’s risk of an ASD diagnosis.

This research was supported by the Swinburne Bio-Research Initiative (SABRI), Swinburne University Alumni and Development, and the Friends of SABRI.

REFERENCES


Chapter 6 – Breastfeeding initiation and duration

In the previous study (Brown, Austin, & Busija, 2014), a very strong relationship between fatty acid deficiency (FAD) scores, first hour breastfeeding and ASD was found. Due to these findings, a study was devised to address some of the limitations in the pilot paper.

Firstly, a larger sample size was obtained and a more heterogeneous sample was recruited by advertising for participants from the general population and also at a home birth conference. Women who plan homebirths are often older than women in hospital settings and are less likely to be first time mothers, but crucially, are significantly more likely to have a spontaneous onset of labour (no induction), no epidural or spinal analgesia, no forceps, vacuum extraction or caesarean section (Homer et al., 2014). It was thought that this population would provide a measure of what Homer et al. defined as ‘normal labour and birth’.

The FAD measure was re-designed to improve sensitivity by gathering responses via a Likert scale, rather than asking for the presence or absence of a given symptom. A breastfeeding duration measure was included and pre-natal risk factors commonly associated with ASD were also included in the survey.

Subsequent to the publication of Autistic Disorder and Phospholipids: A review (Brown & Austin, 2011) and the commentary paper, Fatty Acids, Breastfeeding and Autism Spectrum Disorder (Brown & Austin, 2009) calling for research into the relationship between first hour colostral feeding and ASD, researchers published a study that found that first hour breastfeeding and breastfeeding duration were
significantly associated with ASD diagnosis (Al-Farsi et al., 2012). This paper, entitled ‘Effect of suboptimal breast-feeding on occurrence of autism: a case control study’ (Al-Farsi et al., 2012), included a measure of breastfeeding delay to account for colostral intake. Although the researchers did not find a significant association between ASD diagnosis and breastfeeding delay, this variable was included in this larger study to assess whether a larger and more heterogeneous sample would reveal any effect.

This paper was designed to extend the findings of the pilot paper, ‘Observable essential fatty acid deficiency markers and Autism Spectrum Disorder’ (C. M. Brown et al., 2014) and to continue in the research direction of Al-Farsi et al. (2012). With a population in the analysis more likely to have a normal labour and birth, the study was designed to assess whether birth interventions could be risk factors for ASD due to their role in the disruption of the initiation and duration of breastfeeding. Further, whether a lack of breastfeeding in the first hour and a shorter duration of breastfeeding would impact the fatty acid status of an infant and potentially increase the risk of an ASD diagnosis.

A cluster adjusted path analysis was used to test the model to ascertain whether evidence suggested that the FAD scale (as a measure of fatty acid deficiency) could be a mediating factor between breastfeeding duration and a diagnosis of ASD, after accounting for within-family clustering. It was hypothesised that the impact of obstetric risk factors such as caesareans would be less influential in the model when breastfeeding factors were included.
Paper 4: Staying the Course: Breastfeeding duration as a protective factor in ASD and fatty acid deficiency.

Brown, C.M., Busija, L., Shandley, K., Austin, D.W., Staying the Course: Breastfeeding duration as a protective factor in ASD and fatty acid deficiency.
## AUTHORSHIP STATEMENT

### 1. Details of publication and executive author

<table>
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<th>Publication details</th>
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<td>Staying the Course: Breastfeeding duration as a protective factor in ASD and fatty acid deficiency</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
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<td>School of Psychology</td>
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### 2. Inclusion of publication in a thesis

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<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
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<th>If Yes, please complete Section 3 If No, go straight to Section 4.</th>
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### 3. HDR thesis author’s declaration

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<th>Name of HDR thesis author if different from above. (If the same, write “as above”)</th>
<th>School/Institute/Division if based at Deakin</th>
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<tr>
<td>As above</td>
<td>School of Psychology</td>
<td>Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

As the HDR thesis author, I conceived the project, designed the methodology, wrote and set up the online survey, publicised the survey at the homebirth conference, wrote and placed the advertising in the parenting publications and schools, set up the data file (with assistance) and analysed the data (with assistance). I wrote the manuscript and was responsible for the final draft of the paper.

_I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below._

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
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<tbody>
<tr>
<td>Christine Brown, Deakin University</td>
<td>Conception, design, data collection, analysis, manuscript</td>
</tr>
<tr>
<td>David Austin, Deakin University</td>
<td>Design and revision</td>
</tr>
<tr>
<td>Lucy Busija, Deakin University</td>
<td>Statistical analysis and design, revision</td>
</tr>
<tr>
<td>Kerrie Shandley, Deakin University</td>
<td>Data analysis, design and revision</td>
</tr>
</tbody>
</table>
5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

xvi. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
xvii. that there are no other authors according to these criteria,
xviii. that the description in Section 4 of my contribution(s) to this publication is accurate,
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If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further
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<table>
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<tr>
<th>Name of author</th>
<th>Signature*</th>
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<td>Lucy Busija, Deakin University</td>
<td>Busija</td>
<td>18/1/15</td>
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<td>Kerrie Shandley, Deakin University</td>
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<td>18/1/15</td>
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<tr>
<td>David Austin, Deakin University</td>
<td>David Austin</td>
<td>18/1/15</td>
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6. Other contributor declarations

I agree to be named as a non-author contributor to this work.

<table>
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<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

*If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author

7. Data storage

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

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<th>Storage Location</th>
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<th>Name of custodian if other than the executive author</th>
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<td>Electronic</td>
<td>Password protected secure file, Deakin University</td>
<td>1/1/15</td>
<td>A/Prof David Austin</td>
</tr>
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Abstract

There is evidence that Autism Spectrum Disorder (ASD) is associated with essential fatty acid (EFA) deficiencies. This study investigated the relationship between breastfeeding duration, exclusivity and first hour colostral feeding, fatty acid deficiency and ASD diagnosis. A survey was conducted with biological mothers of 3,570 children aged between 3-13. When considered within their family group, a dose effect of breastfeeding was found with children receiving a longer duration of breastfeeding being significantly less likely to have an ASD diagnosis. Fatty acid deficiency was found to have a mediating effect between breastfeeding duration and ASD diagnosis. First hour breastfeeding, and exclusive breastfeeding were both significantly correlated with reduced fatty acid deficiency symptoms whilst a delay in breastfeeding initiation was significantly correlated with increased fatty acid deficiency symptoms. When fatty acid status, breastfeeding and family characteristics were accounted for in the model, low birth weight, pre-term birth, artificial rupture of membranes, birth by caesarean section and epidurals were all found to be non-significant in predicting ASD diagnosis. The results of this study indicate that breastfeeding factors may have a protective role in ASD and that the initiation and continuation of breastfeeding may play a crucial role at a critical juncture in infant development.
Introduction

ASD is a complex disorder that affects the social, behavioural, emotional and biological functioning of individuals [Shandley & Austin, 2010]. ASD is normally identified in children before their third birthday and by the age of eight, the Centers for Disease Control and Prevention now estimate that as many as 1 in 68 have an ASD diagnosis [Centers for Disease Control, 2014]. This statistic, based on data from 2010, is a 123% increase since 2002 [Centers for Disease Control, 2014]. Greater efficiencies in identification and diagnosis seem less and less plausible as an explanation for the entirety of the increase [Yeargin-Allsopp et al., 2003; Rice et al., 2012; Schieve et al., 2014].

Currently, it is generally accepted that ASD is likely determined by both genetic and environmental factors [Grafodatskaya et al., 2010] although there is very little agreement as to what these factors might be and how they might interact. One study [Hallmayer et al., 2011] estimated that environmental influences may explain up to 55% of the variation in ASD diagnoses over and above genetics. An important consideration is that the effects of some genes are conditional on environmental exposures [Caspi et al., 2007].

One environmental risk factor identified in ASD is suboptimal breastfeeding. Breastfeeding duration has repeatedly shown a relationship with ASD; that is, the longer the duration of breastfeeding, the less likelihood of an ASD diagnosis [Tanoue
Autism Spectrum Disorder, Essential Fatty Acids and Infant Feeding

Tanoue and Oda [1989] found that if infants had been breastfed for less than a week, then they were more likely to have an ASD diagnosis. Schultz [2006] and Al-Farsi [2012] both found ASD to be significantly associated with the duration of breastfeeding in the infant and previous research has also found that the likelihood of an ASD diagnosis increases if an infant is not breastfed within the first hour of birth [Al-Farsi et al., 2012; Brown et al., 2014].

One of the reasons suboptimal breastfeeding may be a risk factor for ASD is a reduction in access to Long Chain Polyunsaturated Fatty Acids (LCPUFAs) at a crucial time in the infant’s development. A class of lipids, LCPUFAs influence health and development throughout the lifespan [Janssen & Kiliaan, 2014]. The ability to metabolise LCPUFAs is inherited, but the precursor Essential Fatty Acids (EFAs) LA(n-6) and ALA(n-3), must be provided via dietary sources. For much of their biological activity, the EFAs LA and ALA require conversion by Delta6 desaturase enzymes into LCPUFAs: AA, EPA, DPA and DHA [Gibson et al., 2011].

The LCPUFA that has gained importance in the literature is the n-3 fatty acid, DHA. DHA is involved in brain development, gene expression and function [Crawford et al., 2014] and there is now wide agreement that DHA has a unique and irreplaceable role in brain lipid and photoreceptor structure [Guesnet & Alessandri, 2011]. DHA also influences growth, metabolism and immune outcomes in childhood [Gibson & Makrides, 2000].

Human milk is a crucial source of DHA and breastfed infants show higher levels of DHA when compared with infants receiving formula unsupplemented with DHA [Makrides et al., 1994]. Cortical levels of DHA have been found to be dependent on
breastfeeding duration [Makrides et al., 1994; Farquharson et al., 1995] and infant formula containing only the precursor fatty acids LA and ALA may not be effective in meeting the full EFA requirements of the infant [Simmer et al., 2008]. Alterations in neurotransmission, learning and development that are caused by fatty acid deficiencies in the pre- and post-natal period may be irreversible and not able to be remediated with supplementation [Guesnet & Alessandri, 2011].

As individuals diagnosed with ASD have been shown to have significantly lower LCPUFA levels than controls [Vancassel et al., 2001; Bell et al., 2004; Bu et al., 2006; Bell et al., 2010; El-Ansary et al., 2011] there is the potential that the length of breastfeeding an infant receives may impact their fatty acid status and thereby increase their risk of ASD. A number of studies have found that seven observable symptoms of fatty acid deficiency correlate with low LCPUFA levels in plasma [Stevens, Zentall, & Deck, 1995; Bell et al., 2004; Antalis et al., 2006]. Dry hair, dry skin or skin that is rough and bumpy, brittle nails, dandruff, frequent thirst and urination are all clinical signs of EFA deficiency [Bell et al., 2004]. Stevens, Zentall, Deck, et al. [1995] found that these seven signs also correlated with breastfeeding duration in a study of boys with a diagnosis of ADHD.

Environmental risk factors associated with suboptimal breastfeeding include prematurity [Donath & Amir, 2008], low birth weight and caesarean birth [Sutherland et al., 2012; Brown & Jordan, 2013; Fisher et al., 2013]. Giving birth by caesarean section often separates the mother and child for an extended time and a critical window is missed for the establishment of breastfeeding [Cakmak & Kuguoglu, 2007; Brown & Jordan, 2013]. Epidural anaesthesia has also been associated with breastfeeding cessation within one month [Dozier et al., 2013].
Despite the somewhat varied and inconsistent nature of the findings from ASD risk factor research, suboptimal breastfeeding and ASD share risk factors such as advancing parental age [Gentile et al., 2013], caesarean and preterm birth [Langridge et al., 2013] and low birthweight [Gardener et al., 2011]. It is possible that the contribution of pre- and post-natal risk factors commonly associated with ASD may be due to the creation of a suboptimal breastfeeding environment that contributes to an increased risk of an ASD diagnosis.

This study sought to investigate the relationship between ASD diagnosis, breastfeeding duration and fatty acid deficiency. Birth interventions and perinatal factors that were found in the literature to be associated with both ASD diagnosis [Froehlich-Santino et al., 2014; Schieve et al., 2014] and suboptimal breastfeeding [Limperopoulos, 2009; Mamidala et al., 2013] were included in the analysis as covariates.

The two main foci of this study were to determine the role of breastfeeding duration in ASD, after controlling for relevant confounders and to determine whether fatty acid deficiency may have a mediational role in the relationship between breastfeeding duration and ASD diagnosis.

Method

Participants

Following receipt of ethics approval from Swinburne University Human Research Ethics Committee, an online health survey was advertised in an Australian parenting magazine, on Autism support websites at special education schools and was also publicised at a homebirth conference in Echuca (Victoria, Australia) in May 2010.
The survey asked for the participation of biological mothers of children from 3 to 13 years of age. The survey was referred to as a ‘Pregnancy, birth, infant feeding and child health outcome’ survey or a ‘Health Survey’ and at no time made reference to autism.

Data were collected between 2011 and 2012. In total, 2,473 biological mothers of 4,306 children consented to take part in the survey. The survey responders provided data on 3,570 children, a completion rate of just over 80%. The remaining 736 survey respondents gave their demographic details, but did not complete details for their children and these data were therefore not included in the analyses.

Measures

Health survey

The Pregnancy, birth, infant feeding and health outcome survey was designed for the present study. It comprised four main sections:

1. The demographic and health information of the biological parents;
2. The pregnancy histories of the biological mothers;
3. The birth and early feeding history of each participating child; and
4. The health outcomes of each participating child.

Mothers were asked if their child was born at home, in hospital or at a birthing centre and whether or not they had birth interventions such as caesareans, artificial rupture of membranes, epidurals or an induction of labour. Both hospital births and birthing centre births were coded as having been born in a hospital.

The feeding history of the children gathered information specific to the first hour after
birth and also gauged breastfeeding duration and exclusivity. The question in regard to the exclusivity of breastfeeding was phrased, ‘Was your child exclusively breastfed? (That is, no formula, no water, no other liquids or substances until solid foods introduced?)’. Mothers were also asked how many days after their infant’s birth they began breastfeeding and then how many days or weeks elapsed before they discontinued. Breastfeeding duration was coded into months for the analysis.

The presence or absence of an ASD diagnosis was ascertained by the questions, ‘Has your child been diagnosed with Autism, Asperger’s Syndrome or an Autism Spectrum Disorder (such as Pervasive Developmental Disorder)? And ‘What diagnosis did your child receive?’ The answers given were collapsed into ASD consistent with DSM-5 classification.

**Fatty Acid Deficiency (FAD) status**

Fatty Acid Deficiency (FAD) was measured using the seven clinically observable signs: excessive thirst, frequent urination, dry skin, dry hair, soft or brittle nails, dandruff and rough, dry, bumpy skin. The FAD scale has been used by researchers to determine fatty acid deficiency in a non-invasive way and shown to correlate with plasma fatty acid levels [Stevens, Zentall, & Deck, 1995; Bell et al., 2004; Sinn, 2007].

The FAD questions were phrased, ‘In general, please rate your child’s tendency to suffer from the following’ and listed the seven signs with a Likert rating scale:

0 = Not at all 1 = Sometimes 2 = Frequently 3 = Always

The minimum FAD score was 0 with a maximum score of 21.
Statistical analysis:

The relationship between FAD status, breastfeeding duration (in months) and ASD was tested using path analysis [Muthén & Muthén, 2012] with observations clustered within families. Path analysis tested direct effects of breastfeeding duration and FAD status on ASD, controlling for prognostic factors such as gestational age, birthweight and birth interventions. The model also specified an indirect effect from breastfeeding duration to ASD through FAD status. FAD status was modelled as a latent variable, with the seven symptoms of FAD used as indicators. The path analysis used mean and variance adjusted weighted mean squares (WLSMV) estimator for all parameters. Given that this study collected data on all children from participating families, child per family, path analysis utilised cluster-adjusted standard errors to account for the within-family clustering of the children. Path analysis was carried out in Mplus Version 7.11 (Muthen & Muthen, 1998-2013, Mplus software. Los Angeles, CA).

Missing data ranged from 0.9% on the fatty acid deficiency variable excessive thirst to 9.6% on induction of labour. Missing data were assumed to be missing at random and were handled with pairwise deletion (a default approach to missing data handling in Mplus software when WLSMV estimator is used).

Results

Demographic details, pre- and post-natal interventions and feeding details of study participants can be found in Table 1. The significance levels in Table 1 were determined by One-way analysis of variance (ANOVA) and Chi-Square tests.
Table 1: Demographic, intervention and feeding details of children with and without a diagnosis of Autism Spectrum Disorder

<table>
<thead>
<tr>
<th></th>
<th>Autism Spectrum Disorder (ASD) (n=335)</th>
<th>No Autism Spectrum Disorder (non-ASD) (n = 3235)</th>
<th>Total (n=3570)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>M 31.10 (SD 5.7)</td>
<td>M 30.15 (SD 5.1)</td>
<td>M 30.24 (SD 5.16)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Paternal age</td>
<td>M 33.53 (SD 6.09)</td>
<td>M 32.61 (SD 6.00)</td>
<td>M 32.70 (SD 6.01)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Total FAD score</td>
<td>M 3.53 (SD 3.30)</td>
<td>M 1.71 (SD 2.02)</td>
<td>M 1.88 (SD 2.22)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>79 (23.6%)</td>
<td>1609 (49.7%)</td>
<td>1749 (40.6%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- Male</td>
<td>256 (76.4%)</td>
<td>1618 (50.0%)</td>
<td>1940 (45.1%)</td>
<td></td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>37 (11%)</td>
<td>176 (5.4%)</td>
<td>220 (5.1%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Low birthweight (&lt;2,500 gms)</td>
<td>35 (10.4%)</td>
<td>164 (5.1%)</td>
<td>205 (4.8%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Place of birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Home</td>
<td>14 (4.2%)</td>
<td>797 (24.6%)</td>
<td>841 (19.5%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- Hospital</td>
<td>320 (95.5%)</td>
<td>2395 (74.0%)</td>
<td>2851 (66.2%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Birth interventions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Induction</td>
<td>166 (49.6%)</td>
<td>1135 (60.5%)</td>
<td>1371 (31.8%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- AROM a</td>
<td>86 (25.7%)</td>
<td>751 (23.2%)</td>
<td>879 (20.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>- Epidural</td>
<td>106 (31.6%)</td>
<td>735 (23.2%)</td>
<td>873 (20.3%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- Caesarian</td>
<td>68 (20.3%)</td>
<td>512 (15.8%)</td>
<td>602 (14%)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Postnatal</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- Jaundice</td>
<td>154 (46%)</td>
<td>991 (30.6%)</td>
<td>1173 (27.2%)</td>
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<tr>
<td>- First Hour resus</td>
<td>11 (3.3%)</td>
<td>106 (3.3%)</td>
<td>118 (2.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>- First Hour NICU b</td>
<td>39 (11.6%)</td>
<td>162 (5.0%)</td>
<td>205 (4.8%)</td>
<td>&lt;.01</td>
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<tr>
<td>- First Hour h/crib</td>
<td>44 (13.1%)</td>
<td>181 (5.6%)</td>
<td>232 (5.4%)</td>
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<td>Breastfeeding</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Duration (months)</td>
<td>11.23 (SD 10.36)</td>
<td>18.00 (SD 12.38)</td>
<td>M 17.00 (SD12.37)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- First hour</td>
<td>205 (61.2%)</td>
<td>2548 (78.8%)</td>
<td>2820 (65.5%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- Exclusive</td>
<td>146 (43.6%)</td>
<td>2092 (64.7%)</td>
<td>2289 (53.2%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>- Delay (&gt;3 days)</td>
<td>11 (3.3%)</td>
<td>37 (1.1%)</td>
<td>48 (1.1%)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

a AROM: Artificial Rupture of Membranes
b NICU: Neonatal Intensive Care Unit
Fit between the path model and observed data was assessed with a range of fit indices. Non-significant chi-square goodness-of-fit (p>0.05), comparative fit index (CFI) and Tucker-Lewis index (TLI) values >0.95, and root mean square error of approximation (RMSEA) values <0.06 were interpreted as indicating good fit of the path model (Yu, 2002). Model goodness-of-fit statistics are reported in Table 2.

Table 2: Model fit statistics for path analysis

<table>
<thead>
<tr>
<th>Model Fit Information</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square Test of Model Fit</td>
<td>1148.250</td>
</tr>
<tr>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>134</td>
</tr>
<tr>
<td>p-value</td>
<td>0.00</td>
</tr>
<tr>
<td>Root Mean Square Error of Approximation (RMSEA)</td>
<td>0.044</td>
</tr>
<tr>
<td>90% Confidence Interval</td>
<td>0.042-0.047</td>
</tr>
<tr>
<td>Probability RMSEA &lt;= .05</td>
<td>1.00</td>
</tr>
<tr>
<td>CFI/TLI</td>
<td></td>
</tr>
<tr>
<td>CFI</td>
<td>0.861</td>
</tr>
<tr>
<td>TLI</td>
<td>0.636</td>
</tr>
</tbody>
</table>

The path model explained 39.5% of the variation in ASD diagnosis and 4.2% of the variation in FAD status.

After accounting for demographic characteristics and birth-related factors, breastfeeding duration was significantly associated with ASD diagnosis both directly and indirectly through FAD status.

Results showed that ASD diagnosis was more likely to be present with shorter duration of breastfeeding and more FAD symptomatology. Longer breastfeeding duration was significantly associated with lower FAD symptomatology and
consequently, lower risk of ASD diagnosis. Male gender was significantly associated with an increased risk of ASD diagnosis, as was older maternal age.

Hospital birth was significantly associated with an ASD diagnosis. However, birth interventions such as inductions, artificial rupture of membranes (AROM), caesarean section, and epidurals were not significantly associated with ASD.

First hour resuscitation, use of a humidicrib in the first hour after birth, admission into the Neonatal Intensive Care Unit (NICU) after birth, low birth weight, jaundice, and preterm birth were all non-significant in the model. Paternal age was also non-significant in the model. Maternal education level just reached significance in the model, with a higher level of maternal education associated with a reduction in the likelihood of an ASD diagnosis.

The probability of an ASD diagnosis did not increase significantly if a child did not receive breastmilk in the first hour after birth was not exclusively breastfed or if breastfeeding was delayed. However, first hour breastfeeding was significantly negatively correlated with FAD symptomatology as was exclusive breastfeeding, whilst a delay in breastfeeding was significantly positively correlated with FAD symptomatology.

Whilst birth interventions were not significantly associated with an ASD diagnosis directly, significant correlations were found between birth interventions and breastfeeding duration. Inductions, AROM, epidurals, caesareans first hour humidicrib and first hour admission to the NICU were all negatively correlated with breastfeeding duration. Being born in a hospital setting was also negatively correlated with breastfeeding duration.
Significant positive correlations were found between breastfeeding in the first hour after birth and exclusive breastfeeding with an increase in breastfeeding duration.

Standardised and unstandardised regression coefficients, confidence intervals and $p$ values for the path analysis can be found in Table 3 and a diagram of the path analysis can be found in Figure 1.

**Table 3: Standardised and unstandardised regression coefficients, confidence intervals and $p$ values for path analysis**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Unstandardised regression coefficient</th>
<th>Unstandardised 95% Confidence Intervals</th>
<th>$p$</th>
<th>Standardised regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid Deficiency (FAD)</td>
<td>1.748</td>
<td>1.447 - 2.050</td>
<td>0.000</td>
<td>0.347</td>
</tr>
<tr>
<td>Breastfeeding duration</td>
<td>-0.011</td>
<td>-0.016 - 0.005</td>
<td>0.001</td>
<td>-0.130</td>
</tr>
<tr>
<td>Breastfeeding delay (&gt;3 days)</td>
<td>-0.227</td>
<td>-0.592 - 0.138</td>
<td>0.306</td>
<td>-0.026</td>
</tr>
<tr>
<td>Breastfeeding first hour</td>
<td>-0.103</td>
<td>-0.281 - 0.074</td>
<td>0.338</td>
<td>-0.044</td>
</tr>
<tr>
<td>Exclusive breastfeeding</td>
<td>-0.031</td>
<td>-0.155 - 0.093</td>
<td>0.681</td>
<td>-0.015</td>
</tr>
<tr>
<td>Born in hospital</td>
<td>0.602</td>
<td>0.377 - 0.827</td>
<td>0.000</td>
<td>0.253</td>
</tr>
<tr>
<td>Induced</td>
<td>0.115</td>
<td>0.000 - 0.230</td>
<td>0.101</td>
<td>0.056</td>
</tr>
<tr>
<td>Artificial Rupture of membranes</td>
<td>-0.142</td>
<td>-0.263 - 0.020</td>
<td>0.055</td>
<td>-0.060</td>
</tr>
<tr>
<td>Epidural</td>
<td>-0.044</td>
<td>-0.173 - 0.086</td>
<td>0.579</td>
<td>-0.018</td>
</tr>
<tr>
<td>Caesarean</td>
<td>-0.180</td>
<td>-0.371 - 0.012</td>
<td>0.122</td>
<td>-0.066</td>
</tr>
<tr>
<td>Low birth weight (&lt; 2500 gms)</td>
<td>0.074</td>
<td>-0.201 - 0.348</td>
<td>0.659</td>
<td>0.017</td>
</tr>
<tr>
<td>Preterm (&lt; 37 weeks)</td>
<td>-0.089</td>
<td>-0.367 - 0.188</td>
<td>0.596</td>
<td>-0.021</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.539</td>
<td>-0.633 - 0.446</td>
<td>0.000</td>
<td>-0.269</td>
</tr>
<tr>
<td>First hour humidicrib</td>
<td>0.115</td>
<td>-0.977 - 1.206</td>
<td>0.863</td>
<td>0.028</td>
</tr>
<tr>
<td>First hour NICU$^a$</td>
<td>0.109</td>
<td>-0.135 - 0.353</td>
<td>0.462</td>
<td>0.025</td>
</tr>
<tr>
<td>First hour resuscitation</td>
<td>-0.241</td>
<td>-0.513 - 0.031</td>
<td>0.145</td>
<td>-0.042</td>
</tr>
<tr>
<td>Jaundice</td>
<td>0.097</td>
<td>-0.006 - 0.200</td>
<td>0.120</td>
<td>0.045</td>
</tr>
<tr>
<td>Father's age</td>
<td>0.005</td>
<td>-0.007 - 0.016</td>
<td>0.503</td>
<td>0.028</td>
</tr>
<tr>
<td>Mother's age</td>
<td>0.024</td>
<td>0.011 - 0.036</td>
<td>0.002</td>
<td>0.123</td>
</tr>
<tr>
<td>Mother's education level</td>
<td>-0.169</td>
<td>-0.301 - 0.037</td>
<td>0.035</td>
<td>-0.061</td>
</tr>
<tr>
<td>Regression of FAD on b/feeding duration</td>
<td>-0.006</td>
<td>-0.007 - 0.004</td>
<td>0.000</td>
<td>-0.205</td>
</tr>
<tr>
<td>Indirect effect of b/feeding duration on ASD diagnosis through FAD</td>
<td>-0.010</td>
<td>-0.013 - 0.007</td>
<td>0.000</td>
<td>-0.010</td>
</tr>
</tbody>
</table>

$^a$ NICU: Neonatal Intensive Care Unit
Figure 1: Cluster adjusted path analysis. Note: AROM: Artificial Rupture of Membranes, NICU: Neonatal Intensive Care Unit, B/F: Breastfeeding, LBW: Low birthweight, MAT/PAT: Maternal/Paternal, Edu: Education, Induct: Induction.
Standardised correlations and significance values for breastfeeding duration and other feeding conditions, interventions and FAD symptomatology can be found in Table 4.

Table 4: Standardised correlations and significance for breastfeeding, fatty acid symptomatology and interventions.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breastfeeding duration with:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>-0.218</td>
<td>0.000 **</td>
</tr>
<tr>
<td>AROM</td>
<td>-0.136</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Epidural</td>
<td>-0.213</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Caesarean</td>
<td>-0.113</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Low Birth Weight</td>
<td>-0.108</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Preterm</td>
<td>-0.122</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Hospital birth</td>
<td>-0.395</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Gender</td>
<td>0.018</td>
<td>0.268</td>
</tr>
<tr>
<td>First hour humidicrib</td>
<td>-0.087</td>
<td>0.000 **</td>
</tr>
<tr>
<td>First hour NICU</td>
<td>-0.057</td>
<td>0.002 *</td>
</tr>
<tr>
<td>First hour resus</td>
<td>-0.012</td>
<td>0.474</td>
</tr>
<tr>
<td>Jaundice</td>
<td>-0.081</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Paternal age</td>
<td>0.096</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.085</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Maternal education level</td>
<td>0.031</td>
<td>0.143</td>
</tr>
<tr>
<td>First hour breastmilk</td>
<td>0.200</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Exclusive Breastfeeding</td>
<td>0.458</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Delay in breastmilk &gt;3 days</td>
<td>-0.098</td>
<td>0.059</td>
</tr>
</tbody>
</table>

| **FAD symptomatology with:** |          |       |
| Induction                  | 0.053    | 0.048 * |
| AROM                       | 0.053    | 0.022 * |
| Epidural                   | 0.050    | 0.029 * |
| Caesarean                  | 0.008    | 0.734 |
| Low Birth Weight           | 0.026    | 0.297 |
| Preterm                    | 0.051    | 0.028 * |
| Hospital birth             | 0.083    | 0.001 ** |
| Gender                     | 0.005    | 0.810 |
| First hour humidicrib      | 0.083    | 0.000 ** |
| First hour NICU            | 0.076    | 0.000 ** |
| First hour resus           | 0.043    | 0.026 * |
| Jaundice                   | 0.108    | 0.000 ** |
| Paternal age               | -0.002   | 0.942 |
| Maternal age               | -0.044   | 0.095 |
| Maternal education level   | -0.043   | 0.107 ** |
| First hour breastmilk      | -0.098   | 0.000 ** |
| Exclusive Breastfeeding    | -0.079   | 0.000 ** |
| Delay in breastmilk >3 days | 0.124 | 0.000 ** |
Discussion

This research found that a longer duration of breastfeeding was significantly associated with lower FAD symptomatology and less likelihood of a diagnosis of ASD. Fatty acid status was found to have a mediating effect between breastfeeding duration and ASD.

Breastfeeding duration

These findings support the findings of previous studies that breastfeeding duration may act as a protective factor for both ASD [Tanoue & Oda, 1989; Schultz et al., 2006; Al-Farsi et al., 2012] and fatty acid status [Makrides et al., 1994; Stevens, Zentall, & Deck, 1995; Koletzko et al., 2001]. The lack of a significant association between first hour breastfeeding and ASD contradicts previous research [Al-Farsi et al., 2012; Brown et al., 2014], but the finding that breastfeeding delay is not significantly associated with ASD diagnosis replicates previous research [Al-Farsi et al., 2012]. However, given the strong correlation between both first hour breastfeeding, exclusive breastfeeding and fatty acid deficiency symptomatology, more research is needed into the timing of breastfeeding and its potential health benefits.

Microarray studies of the RNA of human milk have found that the stages of milk production appear to have different foci [Lemay et al., 2013]. The first stage, colostral milk, produces gene activity that encodes immunological factors. The second stage, transitional milk, contains gene activity that turn off the hormone that degrades protein and in the third stage, mature milk, gene activity focuses on lipid synthesis [Lemay et al., 2013]. Given this evidence for the ‘staged’ functionality of breastmilk,
it may be that in the beginning, first hour colostrum provides specific and crucial immunological instructions to the neonate. As high levels of LCPUFAs circulate in the full term neonate after placental transfer in the last trimester, perhaps an infant uses its lipid stores in the first few days whilst adapting and calibrating its immune system to life outside the womb. When the mature milk arrives, the gene activity in the milk that encourages lipid synthesis may be to assist in the neonate’s neurogenesis. This could be an explanation as to why it is longer exposure to breastmilk that seems to have the protective effect on ASD in susceptible individuals. As our results indicate, longer duration of breastfeeding significantly decreased the probability of ASD diagnosis.

Risk factors

In this study, when fatty acid status, breastfeeding and family characteristics were accounted for in the path model, low birth weight, a gestational age of less than 37 weeks, and birth interventions such as AROM and epidurals were all non-significant. These findings differ from those reported in the literature, with low birth weight and pre-term birth [Gardener et al., 2011] found to be significant predictors of ASD. This study also contradicted the findings of Gregory et al. [2013] with induction of labour not significantly associated with ASD.

However, risk factors traditionally associated with ASD were significantly correlated with breastfeeding duration. That is, pre-term birth, jaundice, induction, AROM, epidurals, caesareans and spending the first hour of life in the NICU or a humidicrib were significantly correlated with a reduction in breastfeeding duration. This lends support to the notion that it may not be useful to view interventions as risk factors per se, but as disruptors of breastfeeding initiation and maintenance.
In contrast, first hour and exclusive breastfeeding were both significantly correlated with longer periods of breastfeeding. In light of the strong association found in this study between breastfeeding duration and ASD, birth interventions that disrupt the initiation and maintenance of breastfeeding may contribute to an ASD diagnosis immunologically and nutritionally because a critical window for establishing optimal breastfeeding is missed.

The significant association between hospital birth and reduction in breastfeeding duration may be due to the reduced number of birth interventions in homebirths and therefore an environment more conducive to first hour breastfeeding and the establishment of a breastfeeding routine. Homer et al. [2014] found that women who birthed their infants at home were significantly more likely to have a spontaneous onset of labour, have no epidural, spinal analgesia, caesarean or episiotomy. The obvious point here is that women birthing at home may also have less pregnancy complications and risk factors requiring hospital intervention.

*Gender and maternal age*

This study found that female infants and children born to younger mothers had lower probability of ASD diagnosis, however older mothers were more likely to breastfeed for longer. Despite the longer breastfeeding duration, it is possible that an older woman’s fatty acid stores are more depleted than a younger female meaning less available fatty acids in breastmilk for the breastfeeding infant.

The finding in this study that boys were significantly more likely to have an ASD diagnosis is consistent with the extensive body of literature into ASD prevalence. When considering gender from a fatty acid perspective, it is worth considering that females may have an advantage when synthesising DHA from precursor fatty acids
A recent review by Lohner et al (2013) of 51 studies found significantly higher levels of AA and DHA in the plasma total lipids and plasma phospholipids of females [Lohner et al., 2013]. Both human and animal studies show compelling evidence of sex differences in both DHA status and capacity to convert LA to DHA [Sibbons et al., 2014]. Caspi et al. [2007] found allelic variations in the FADS2 gene, which indicate that individuals differ in their genetic ability to metabolise Polyunsaturated Fatty Acids (PUFA) and this polymorphism has been proposed as the reason why PUFA supplementation studies obtain mixed results [Janssen & Kiliaan, 2014]. And, at least in animal models, the mRNA expression of FADS2 is higher in the liver of females suggesting an enhanced ability of females to process fatty acids [Sibbons et al., 2014].

In a supplementation study, a higher level of DHA supplementation significantly improved the mean cognitive development of girls, but no such effect was seen in boys [Makrides, 2013]. It has been suggested that future studies looking at the associations between ASD and LCPUFA deficiency need to control for gender [Lohner et al., 2013].

A breastmilk compositional study has shown that in primates, the milk composition for male offspring can differ to the milk for female offspring with a higher level of fats versus sugar [Hinde, 2007]. Perhaps male offspring require higher levels of LCPUFAs due to a decreased ability to metabolise fatty acids.

In this study, the age of the mother was a significant predictor of ASD diagnosis, but the age of the father was not. Not only do older mothers have a higher likelihood of birth interventions [Carolan et al., 2011], but if they do indeed have depleted fatty acid stores, it would be worthwhile prioritizing older mothers as a cohort for
LCPUFA status assessment and supplementation if needed.

In this study, higher maternal education was significantly predictive of ASD diagnosis. Breastfeeding rates in older mothers are contradictory in the literature [Scott & Binns, 1998], however there is a consistent relationship between a higher educational level and a greater likelihood of optimal breastfeeding occurring [Scott & Binns, 1998; Ladomenou et al., 2007], which was supported in this study.

This study has a number of limitations. Firstly, it relied on a non-biological measure of fatty acid deficiency, the seven clinical signs of FAD. Although this measure of fatty acid status has been used in other studies [Stevens, Zentall, & Deck, 1995; Bell et al., 2004; Antalis et al., 2006; Sinn, 2007] and correlated with plasma fatty acid levels, it would be highly preferable to measure fatty acid status biologically.

Secondly, the collection of data on birth histories was through self-report, which may be susceptible to recall bias. However, while self-report and recall studies are less reliable than direct collection of data, it has been shown that self-report correlates with hospital records [Sou et al., 2006]. Nonetheless, it would be more optimal to gain access directly to hospital records or maternal health nurse records, even if just to confirm the self-report findings.

Whilst this study shows correlational effects between breastfeeding factors, fatty acid status and ASD, longitudinal studies are needed to corroborate the potential for causative effects of breastfeeding duration on both the fatty acids and ASD diagnosis.

This study did not take into account the fatty acid status of the mothers. In the Western world, we are becoming more deficient in $n$-3 fatty acids such as ALA and DHA [Gibson et al., 2011]. A baby breastfed in the 1980s would have had access to
higher levels of DHA via breastmilk than a baby today [Gibson et al., 2011] due to their mother’s higher levels of LCPUFAs. A study by Xie and Innis [2008] found that mothers that show genetic variance in the FADS2 gene have lower fatty acids in their mammary gland and breastmilk, which may indicate that altered fatty acid metabolism in the mother has an impact on the infant’s supply of LCPUFAs.

The strengths of this study were a large, heterogeneous sample that had good representation of hospital and home births and thorough coverage of known risk factors for ASD diagnosis. The large sample size and comprehensive coverage of prognostic factors allowed for adequate control of confounding, providing a clearer representation of pertinent risk factors for ASD. Additionally, the cluster-adjusted path analysis used to document the association between ASD diagnosis and known risk factors allowed us to account for the biological and environmental effects of being in the same family.

*Future directions*

It is envisaged that the significant relationship between breastfeeding duration and fatty acid deficiency found in this study may encourage future research to consider breastfeeding factors as an important factor implicated in ASD risk. As Caspi et al. [2007] stated; genome wide scans that look for direct effects of the phenotype regardless of environmental influences, will miss those genetic effects that are conditional upon the effects of the environment. The link that was previously found between genetic variation on the FADS2 gene and resultant IQ could have only been found against the background of breastfeeding that was “universal before formula feeding” [Caspi et al., 2007, p. 18863].

In regard to formula feeding, around the world, infant formulas are modelled on the
composition of breast milk of women in the U.S: that is, high in levels of \( n\)-6 LA with low amounts of \( n\)-3 LCPUFAs such as DHA [Gibson et al., 2011]. This is not indicative of LCPUFA levels in the breastmilk of women from other cultures [Huang et al., 2013], nor is it an ideal level in general. It is simply reflective of the degradation of the Western diet over time in regard to LCPUFAs. More research is needed to determine optimal and safe levels of LCPUFAs in all artificial milk substitutes when breastmilk is unavailable directly from the infant’s mother or from a human milk bank. Further investigation is warranted into differentiating LCPUFA levels in infant formulas for male and female infants.

Further investigation and validation of the fatty acid deficiency scale (FAD) is reasonable and if it continues to show itself to be a reliable measure of fatty acid deficiency, it may prove to be a first line, non-invasive observable biomarker that assist health professionals to be alert to the potential consequences of fatty acid deficiency in both infants and adults (especially pregnant women), and signal a need for supplementation.

In addition to providing a source of pre-formed LCPUFAs, human milk profoundly affects the function and integrity of the gastrointestinal tract [Colome et al., 2007], which is known to have both immunological and neurological functions [de Theije et al., 2014]. Human milk is a highly complex bioactive substance that delivers nutritional, health and protective benefits to an individual in ways that are currently not well understood [Hinde & German, 2012] and the breastfeeding period may be a critical window to set down developmental pathways [Hinde & German, 2012]. More precise and detailed breastfeeding research is required in order to more fully understand the unique bioactive substance that is human milk and how it interacts and alters in response to the human it is feeding.
The findings in this study indicate that the duration of breastfeeding may have the potential to alter the fatty acid status of infants. With the addition of breastfeeding factors in the model, this study found that some risk factors such as low birth weight and pre-term birth that are commonly associated with an elevated ASD risk became non-significant when family and breastfeeding variables were accounted for. The results of this study indicate that the continuation of breastfeeding may have a protective role in ASD via the provision of essential fatty acids at a critical time in an infant’s development.

Abbreviations:

Autism Spectrum Disorder, ASD, Fatty Acids, Fatty Acid Deficiency, FAD, Long-chain Polyunsaturated Fatty Acids, LCPUFA, FADS2, Artificial Rupture of Membranes, AROM, mRNA, DHA, PUFA, MPLUS, NICU, LA, ALA, EFA, EPA, DPA
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Chapter 7: Discussion

This body of research investigated the potential associations between breastfeeding and fatty acid deficiencies in Autism Spectrum Disorder (ASD) and the mediational role of fatty acid deficiency in the relationship between breastfeeding duration and ASD diagnosis.

The first published paper of this thesis titled, ‘Fatty acids, breastfeeding and autism spectrum disorder’ (Brown and Austin, 2009), focussed on the role of colostrum in ASD and theorised that breastfeeding within the first hour of life, as per World Health Organisation (WHO) guidelines, may have a protective function in ASD. The paper discussed the placental transfer of LCPUFAs, the role of colostrum in animal management and suggested the research practice of making a theoretical separation between colostral feeding and mature milk feeding.

The second paper of this thesis titled, ‘Autistic disorder and phospholipids: A review’ (Brown and Austin, 2011), suggested that dysregulated fatty acid metabolism may function in ASD the way it had been theorised to function in schizophrenia by Horrobin (1998). Evidence was gathered to synthesise the membrane hypothesis of schizophrenia by Horrobin (1998) with the evidence for a ‘membrane hypothesis of ASD’. The paper found evidence to support the notion that dysregulated fatty acid metabolism could be an underlying causative agent in both schizophrenia and ASD. It called for more research into ASD that accounted for colostral intake and breastfeeding. This paper has since been cited in several peer reviewed journal articles
(Kroll, 2013; Pecorelli, 2013; Hong, 2014; Ciccoli, 2013; Al-Farsi, 2012, Liu, 2011, Chen, 2013) and Al-Farsi et al (2012) referenced and researched some of the questions posed in the review paper. With a modest sample size, Al-Farsi et al (2012) found that first hour breastfeeding and breastfeeding duration was significantly associated with a diagnosis of ASD. To account for colostral intake, Al-Farsi (2012) tested the associated between breastfeeding delay and ASD, which was subsequently found to be non-significant after adjusting for other pertinent prognostic factors. The breastfeeding delay variable was included in the larger study to ascertain whether a larger and more heterogeneous sample would reveal any effect however, the non-significant result was consistent.

The third paper of this thesis titled, Observable essential fatty acid deficiency markers and autism spectrum disorder (Brown, Austin & Busija, 2014), was inspired by the findings of Stevens (1995) and Bell (2004) that deficiencies in EFAs were observable by behavioural and physiological signs: brittle nails, dry hair and skin, dandruff, rough skin, excessive thirst and frequent urination. Although the measure has limitations (Sinn, 2006), the fatty acid deficiency (FAD) signs showed such a strong significant association with ASD in the pilot paper, that it was used again in the fourth and final paper of this thesis. After accounting for shared family environment, first hour breastfeeding and the FAD score showed a highly significant relationship with a diagnosis of ASD. In this study (Brown, Austin & Busija, 2014), gender did not reach significance in the relationship with ASD diagnosis, however with the larger sample size of the last paper (Brown, Busija, Shandley & Austin, 2015), male gender was significantly associated with ASD diagnosis.
As an addendum, this research for the third paper was conducted over the phone with mothers (and one father) of children with an ASD diagnosis. The incredible challenges faced by parents with a child suffering from a condition with no known cause or cure, highlighted the importance of the continued research effort to understand ASD.

The fourth and final paper of this thesis titled, ‘Staying the Course: Breastfeeding duration as a protective factor in ASD and fatty acid deficiency’ (Brown, Busija, Shandley & Austin, 2015), investigated the relationship between birth interventions and ASD, with breastfeeding duration as a potential protective factor in ASD and a mediating effect of fatty acid status. The results showed a significant association between breastfeeding duration and ASD, and the mediating effect of fatty acid status was supported. Birth interventions such as inductions, epidurals and caesareans were found to be significantly correlated with a reduction in the duration of breastfeeding. This study utilised a large and heterogeneous sample and a sophisticated statistical approach and provides evidence for the protective role of optimal breastfeeding in ASD. It also supports the notion that neonatal risk factors and birth intervention risk factors for ASD such as low birthweight and caesareans may be viewed as disruptors of optimal breastfeeding, rather than risk factors in isolation.
Implications of research

Breastmilk and breastfeeding

The research presented in this thesis supports the continued investigation into breastmilk composition and function. The calls to action in breastfeeding research have been summarised as follows (Neville et al., 2012):

1. The urgent need to define the precise interaction and function of human and other milks.
2. Infant nutrition and breastfeeding to be viewed as an entire process from pre-conception to post-natal life.
3. High-risk populations given priority when measuring the impact of breastfeeding.

In the context of this research, it could be argued that the call to action to prioritise high-risk populations could be as simple as focussing on the impact of breastfeeding on infant males. Considering the huge variation in ASD diagnoses between the sexes, it is warranted to focus research on LCPUFA status and the potential impact of breastfeeding in that specific cohort.

Gender

Males are over-represented in ASD populations by about 4:1 (Abrahams & Geschwind, 2008) and this pattern was replicated in this research also. Baron-Cohen has theorised that ASD is an artefact of an extreme male brain: neurocircuitry that systematises rather than empathises (Baron-cohen, 2002). It may well be very useful to codify ASD as a product of extreme ‘maleness’. Perhaps the utility of viewing metabolic systems such as the fatty acid metabolic pathways as more likely to be dysregulated in males will assist future research. Particularly, research is warranted into whether FADS2 gene variance is more likely to be found in males. The discovery
that primate milk is comprised of different elements depending on the sex of the infant and that the milk for sons of primiparous mothers are richer in fats than sugars and proteins (Hinde, 2007), may suggest a compensatory mechanism for fatty acid systems that are ‘known’ not to function as optimally in male infants.

**Colostrum**

One data paper in this research found a significant relationship between first hour breastfeeding and ASD and another found a significant relationship between first hour breastfeeding and breastfeeding duration, which was in turn associated with ASD. There may well be an advantage to studying the effect of colostrum separately in humans as it is in animal research. Animal studies consistently recommend colostral feeding and show that health outcomes are optimised with immediate colostral feeding. In animals, Lecce and Morgan (1962) found that the cessation of the absorption of macromolecules (gut closure) in the gut of the newborn animal was a function of the feeding regimen. Specifically, the piglet is born with an immature intestinal epithelium and the colostral milk of the sow provides proteins that contribute to the serum protein profile and factors that induce the closure of the gut (Lecce, Morgan, Matrone, & Carolina, 1964). This process is described as “delicate and synchronous” by Lecce and Morgan (1964, p. 47), as by the time the neonate has absorbed sufficient protein, the epithelial cell is mature and cannot absorb macromolecules any longer. This process is estimated to be approximately 86 hours in pigs and 32 hours in calves (Vukavic, 1984). In puppies, the intestinal barrier remains open for the first 12 hours after birth, but with a sharp decrease in absorption after four hours (Chastant-Maillard et al., 2012). Can we infer from these findings in animals that a similar process takes place in humans and if so, can this explain some
of the gastrointestinal issues found in some children with ASD (Russo & Andrews, 2010)?

Animal studies emphasise the role of colostral feeding in immuno-development also. For foals, the recommended timing of colostral feeding is within 12 hours as the amount of the immune factor IgG significantly reduces when colostrum is delayed (Raidal, McTaggart, & Penhale, 2005). In calves, research shows that passive immunity is achieved best with immediate colostral feeding, but that colostral feeding also enhances clinical, metabolic and endocrine traits (Hadorn & Hammon, 1997). For puppies, the optimization of passive immune transfer requires that maternal suckling be given very early after birth (Chastant-Maillard, 2012).

It is important to further research in humans what is already well established in animal studies. In the animal literature, colostrum seems to be synonymous with immunity rather than nutrition. Once again, it may be more theoretically sound to envisage colostrum as an important ingredient of passive immunity and gut closure, rather than as a source of nutrition in the infant. In fact, colostrum has been described as the “most potent natural immune booster known to science” (Uruakpa, Ismond, & Akobundu, 2002). In the first three days after birth, a human infant fed colostrum will consume as much IgA as is produced in the mucosa of an adult (Uruakpa, 2003). What are the consequences if this does not occur? Further, if there is a critical window of colostral feeding, what if any, is the effect of an infant not being supplied with colostrum in that critical period?
Limitations

Self-report

The research reported in this thesis was subject to a number of limitations. Firstly, the reliance on self-report for breastfeeding histories, health outcomes and diagnosis is potentially problematic. Hospital records are considered the gold standard (Sou et al, 2006) and it would be optimal for studies to utilise the data collected at the time of birth. Nevertheless, research has shown that the correlation between medical records and maternal recall for specific outcomes such as birth weight and gestational age is around 0.9 and obstetric complications of sufficient severity, such as caesareans, are reliable (Sou et al, 2006).

Biological measures

Secondly, a biological measure of fatty acid deficiency in the infants and the mothers, would lend considerable veracity to the findings. Also, with no measure of the fatty acid status of the mothers, it is difficult to ascertain the effect of maternal LCPUFA levels on the fatty acid status of their infants. With the discovery that polymorphism in the FADS2 gene can affect both LCPUFA uptake, metabolism (Caspi, 2006) and milk production (Xie and Innis, 2008), these studies are hampered by their inability to gauge the influence of this seemingly important genetic variance.

Gender

The studies listed in this discussion have raised the question of gender differences in LCPUFA status, but these differences have not been addressed it statistically in this thesis. There is growing evidence that gender influences fatty acid metabolism and fatty acid levels in adults. A systematic review of 51 publications by Lohner, Fekete,
Marosvolgyi, and Decsi (2013) found such differences in the fatty acid profiles of men and women that they have called for LCPUFA studies to control for gender. It remains to be seen whether the apparent differences in LCPUFA metabolism will influence further research into LCPUFA deficiencies and health outcomes.

**Challenges**

*Birth cohort*

Breastfeeding research is a complex area due to birth cohort effects. In the following, research is presented that isolates at least three different cohorts in regard to breastfeeding:

Breast-fed versus formula fed: Makrides et al (1994) conducted a study that compared the retinal and cortical levels of DHA in breast-fed infants when compared to formula-fed infants. Post-mortem studies on infants found that cortex DHA in formula fed infants did not increase with age (7% total fatty acids) whereas breastfed infant levels continued to increase (10% total fatty acids) until 48 weeks. They found that the length of breastfeeding was the major factor influencing the proportion of cortex DHA in infants.

Breastfed versus breastfed: Gibson et al (2011) have noted that the breastmilk that a breastfed child in 2010 will receive will have a different nutritional profile than the breastfed child did in the 1980s due to falling levels of \( n-3 \) LCPUFAs in the Western diet. This also raises the challenge of comparing breastfed children who are brought up by mothers consuming a typical Western diet, versus breastfed infants from different cultural backgrounds. A study by of Taiwanese women found DHA levels in their breastmilk of 98% of the total fats in the breastmilk (Huang, 2013). These results
were even higher than those found in a cross-cultural review which showed DHA between ranges between 0.32 +/- 22% (Brenna et al., 2007).

Breastfed versus formula fed (supplemented differently): Around the world infant formulas are modelled on the high LA and low LCPUFA levels found in mothers who eat a Western diet (Gibson, 2011). As research discovers more about the components of breastmilk, the artificial milk formulas change. For example, whey-dominant formulas appeared in the 1970s, in the 2000s the protein profile changed. In 1913, formula contained cod liver oil, then added calcium and now sn-2 palmitate is a focus (Sidnell & Greenstreet, 2011)

Another consideration is dietary practices of the time. The practice of giving formula fed babies cod liver oil mixed with orange juice in the 1970s, just may have prevented some serious LCPUFA deficiencies from emerging at that time (Minchin, 2009). With the Western diet becoming more and more LCPUFA deficient, some of the older approaches to diet may have provided a protective effect that masked the deficiencies that researchers are finding now.

Future directions

Further research is needed into the significance of a common pattern of events referred to in ASD research. If being male makes an infant susceptible, perhaps due to less efficient fatty acid metabolism or a FAD2 genetic variation, then if that male infant does not receive any first hour colostrum, will they be immunologically compromised? If then they are not breastfed for long or at all, could they be more prone to ear infections? Some research suggests that breastfeeding has an inverse
relationship with ear infections (Ladomenou, Moschandreas, Kafatos, Tselentis, & Galanakis, 2010). Due to ear infections, will an infant be more exposed to antibiotics? Adams, Johansen, Powell, Quig, and Rubin (2011) showed an increase in ear infections and antibiotic use in individuals with ASD. Would a greater use of antibiotics affect the infant’s microbiome? Reports of high antibiotic exposure and GI dysfunction in the ASD population has encouraged research into disruption of the microbiome in ASD with particular attention paid to propionic acid (MacFabe, 2012). Will ear infections and immune dysfunction lead to more medication being given to a child? And could medications commonly used to reduce fever be associated with ASD (Becker & Schultz, 2010)? Will a dysfunctional microbiome and higher exposure to medication further compromise the infant’s ability to deal with environmental toxins? Will an infant develop ASD in response to a chain of risk?

A number of studies, including Dietert and Dietert (2008), have investigated the possibility of early life immune insults that begin the chain of risk for an infant, but they rarely start with the breastfeeding status of the infant. As Caspi et al. (2007) explain in their study, breastfeeding can be viewed as the evolutionary background against which some effects are measured. Without the evolutionary backdrop, genetic variations that depend on environment will be missed (Caspi, 2007). This research indicates that breastfeeding needs to be at least considered as a factor by more researchers in study development.

Further research is required to determine if there is a chain of risk in regard to the development of ASD. To conclude, the wider implications of the findings in this research are discussed.
Breastfeeding support for mothers

A U.S. study (Bonuck et al., 2014) found that pre- and post-natal support, provided as a routine intervention to mothers, increased breastfeeding intensity and duration by more than 80% at three months. These very positive effects were gained by a lactation consultant with an average time investment of three hours per mother. Bonuck et al (2014) estimate that their intervention could be delivered to more than 600 mother-infant dyads per year. The health benefits and cost savings in these types of intervention warrant further investigation. A key finding was that the intervention worked particularly well as it was delivered routinely to all mothers and not just to the mothers that actively sought help with breastfeeding. Further research is warranted into how many millions could be saved in health care with a fully resourced lactation consultancy provided routinely and governmental investment into a paid maternity leave scheme that would allow women the opportunity to breastfeed within WHO guidelines.

Maternal screening for fatty acid deficiencies and potentially for the FADS2 gene implicated in hindering the conversion of EFAs into LCPUFAs could be used a diagnostic biomarker for ASD risk and early dietary intervention in both the mother and the neonate. Recently Bell et al. (2013) were investigating the possibility of a rapid whole blood finger tip analysis to analyse LCPUFA composition. This is a promising development for use in maternal and neonate screening and for those who already have a diagnosis of ASD.
Health professionals

More research is warranted into the investigation of the observable signs of fatty acid deficiency. While they have been reported to not been a useful screening tool for those that may benefit from supplementation (Sinn, 2007), there is still scope to investigate the signs as a potential early warning sign for LCPUFA metabolism dysregulation or dietary deficiency. If the fatty acid signs can be validated into an empirically recognisable scale, there is the potential for clinicians and maternal health practitioners to have a non-invasive screening tool and an awareness of what the seemingly benign symptomatology may be indicating.

Conclusions

It is envisaged that research will continue into the relationship between breastfeeding, fatty acid status and ASD. This research supports the inclusion of comprehensive breastfeeding measures in the design of studies. Whilst acknowledging the limitations of this body of research, there are implications that fatty acid deficiency may be linked with breastfeeding duration and that birth interventions may be associated with ASD via disruption to the initiation and continuance of breastfeeding.

For low-risk women in childbirth, the introduction of an epidural, augmentation of labour or induction contributes to an increase in the use of forceps, episiotomies and emergency caesareans (Tracy & Tracy, 2003). This is often described as the ‘cascade of interventions’ in childbirth. To be considered is whether this cascade of intervention in labour and childbirth, through its effect on breastfeeding initiation and
maintenance, contributes to a reduced availability of LCPUFAs and thus exposes the infant to other environmental or phenotypical consequences.

The findings in this paper suggest a chain effect of risk in regard to an ASD diagnosis. That is, birth interventions or low birthweight or prematurity may disrupt the ability of a mother to feed her infant within the first hour of birth which may leave an infant immunologically exposed, and if the breastfeeding duration that follows is of a short duration, deficient in fatty acids. This may not be a causative pathway, but could conceivably contribute to the risk profile of an infant in regard to other environmental insults.
References


