UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Human Development and Health

Developmental influences and Infantile Atopic Eczema

by

Sarah El-Heis

Thesis for the degree of Doctor of Medicine

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ABSTRACT

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DEVELOPMENTAL INFLUENCES AND INFANTILE ATOPIC ECZEMA

Sarah El-Heis

Atopic eczema is a highly prevalent condition that can have a significant impact on affected infants and their families. Evidence that it partly originates in utero is increasing, where genetic predisposition and environmental exposures act together in determining the risk of developing this multifactorial, chronic skin condition.

The aim of this research was to examine early life developmental influences on infantile atopic eczema at ages 6 and 12 months. Maternal serum concentrations of tryptophan and related metabolites, maternal stress and low mood, and fetal and infant growth patterns were studied in relation to infantile atopic eczema in the well characterised Southampton Women's Survey mother-offspring cohort.

Lower maternal concentrations of the tryptophan metabolites nicotinamide and anthranilic acid during late pregnancy, and greater preconception perceived stress were associated with an increased risk of infantile atopic eczema at the age of 12 months. Postnatally, infants with eczema were shorter; with evidence of linear growth faltering that commenced in utero.

The findings demonstrated impacts of maternal micronutrient status and psychological wellbeing on infantile atopic eczema. The impaired linear growth of infants with atopic eczema was shown to start in utero, prior to the clinical onset of the condition. The findings provide new evidence that atopic eczema partly originates during development before birth, and point to potential interventions to optimise maternal diet and improve psychological wellbeing beginning prior to conception to ultimately reduce the risk of infantile atopic eczema.

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Academic Thesis: Declaration Of Authorship

I, Sarah El-Heis

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Developmental Influences and Infantile Atopic Eczema

I confirm that:

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   Barton S, Ngo S, Costello P, Garratt E, El-Heis S, Antoun E, Clarke-Harris R, Murray R, Bhatt T, Burdge G, Cooper C, Inskip HM, van der Beek EM, Sheppard A, Godfrey KM, Lillycrop KA and The EpiGen Consortium. DNA methylation of Th2 lineage determination genes at birth is associated with allergic outcomes in childhood. Clin Exp Allergy. 2017 Jul. Accepted Author Manuscript. doi:10.1111/cea.12988.

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Presentation abstracts

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Definitions and Abbreviations

3-HAA: 3-hydroxyanthranilic acid

3-HK: 3-hydroxykynurenine

5-HT: 5-hydroxytryptamine (serotonin)

ACTH: adrenocorticotropic hormone

AGE: advanced glycation end products

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

BMI: body mass index

cAMP PDE: cyclic adenosine monophosphate phosphodiesterase

CI: confidence intervals

CRL: crown-rump length

DAG: direct acyclic graph

DARC: Danish Allergy Research Centre

DNA: deoxyribonucleic acid

DOHaD: Developmental Origins of Health and Disease

EAACI: European Academy of Allergy and Clinical Immunology

EPDS: Edinburgh Post-natal Depression Scale

FGR: fetal growth restriction

FL: femur length

GC-MS: gas chromatography-mass spectrometry

GHQ-12: 12 item General Health Questionnaire

GUSTO: Growing Up in Singapore Towards healthy Outcomes

HPA: hypothalamic-pituitary-adrenal

HPLC: high performance liquid chromatography

IFN-γ: interferon-gamma

IgE: Immunoglobulin E

IGF-1: Insulin-Like Growth Factor 1

IL: Interleukin

iNOS: inducible nitric-oxide synthase

IQR: Interquartile range

ISAAC: The International Study of Asthma and Allergies in Childhood

KA: kynurenic acid

LC-MS/MS: Liquid chromatography- tandem mass spectrometry

LT: lymphotoxin

MS/MS: tandem mass spectrometry

NAD: nicotinamide adenine dinucleotide

NADP: nicotinamide adenine dinucleotide phosphate

NCD: non-communicable disease

NEFA: non-esterified fatty acid

NiPPeR: Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health

OR: odds ratio

pCRH: placental corticotropin-releasing hormone

PHQ-9: Patient Health Questionnaire- 9

PRAP-1: poly-ADP-ribose polymerase 1

PUFA: polyunsaturated fatty acids

QA: quinolinic acid

RNA: ribonucleic acid

SD: standard deviation

SF-36: Short Form (36) Health Survey

SNP: single-nucleotide polymorphism

SWS: Southampton Women's Survey

TDO: Tryptophan 2,3-dioxygenase

Th: T helper

TNF-α: tumour necrosis factor-alpha

Tregs: regulatory T cells

UKWPDC: UK Working Party Diagnostic Criteria

XA: xanthurenic acid

Chapter 1. Introduction and background

1.1 Atopic eczema

Atopic eczema (synonym atopic dermatitis (Johansson et al., 2004)) is a complex, multifactorial, inflammatory skin condition. It often has a chronic course with a remitting – relapsing pattern, and may have a considerable impact on quality of life and a significant financial burden (Gupta et al., 2004). There is increasing evidence that atopic eczema partly originates in utero, where genetic susceptibility and environmental exposures can result in immune dysregulation, influencing the risk of developing the condition. Better understanding of such early life influences could help identify preventative strategies.

1.1.1 Prevalence

The prevalence of atopic eczema in children from 56 countries has been reported to vary from 0.3% to 20.5%, with a consistent marked increasing trend over recent decades in both developed and developing countries (The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee, 1998, Asher et al., 2006). In children aged 1–2 years, the prevalence of the condition is reported to be 17.6% and is comparable across the world (von Kobyletzki et al., 2012).

1.1.2 Clinical presentation and impact

The clinical features of atopic eczema vary at different ages and different stages of the disease (Williams, 2005). In infants, the cheeks are usually affected, with the flexures, the nape of the neck and the hands and feet becoming involved in childhood. Xerosis (dry skin) and itching are hallmarks of the condition at all stages. Acutely affected areas are erythematous patches that can ooze, crust, or have eroded vesicles or papules. More long standing areas appear thicker and are excoriated, becoming lichenified and slightly pigmented with time.

Figure 1. Clinical presentation of atopic eczema

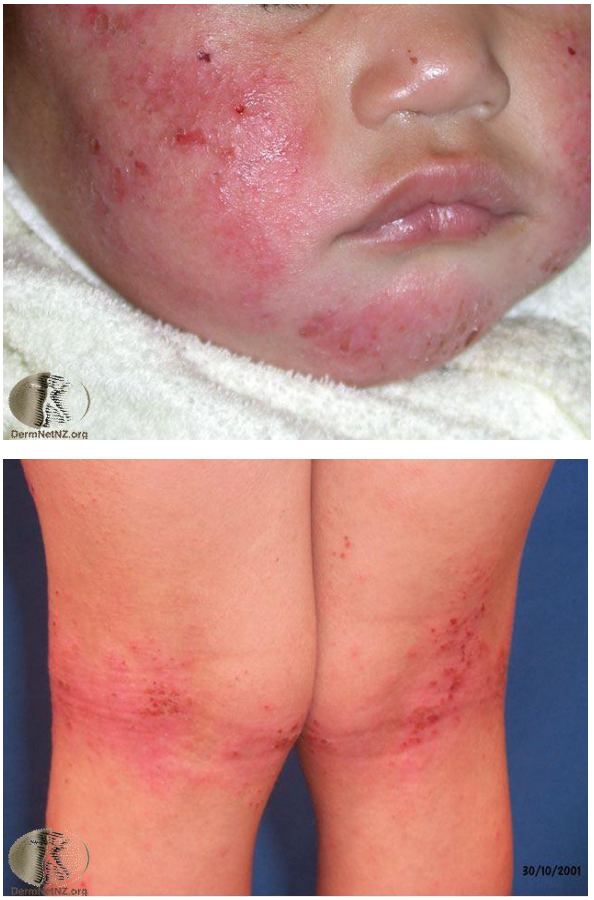


Image source DermNet.org

Diagnosis of atopic eczema is made on the basis of the clinical features. A number of diagnostic criteria have been developed and validated, with the UK Working Party Diagnostic Criteria being one of the most validated (Williams et al., 1994a, Williams et al., 1994b, Williams et al., 1996, Brenninkmeijer et al., 2008) and is described in detail in Chapter 2.

Atopic eczema often becomes clinically apparent in early infancy and childhood; with reports of 85% of cases in those aged 3-11 starting before the age of 5 years; 60% of which developed the first year of life and 45% in the first 6 months (Spergel, 2005, Kay et al., 1994). Some children 'outgrow' their disease; however, this is not always the case. There is evidence that by age 7 years less than 50% of cases show resolution and 60% by adulthood (Spergel and Paller, 2003, Spergel, 2005, Spergel, 2010). Early and severe onset of atopic eczema, early allergen sensitization and parental history of the condition are thought to predict prognosis (Peters et al., 2010, Illi et al., 2004). The mechanisms by which resolution occurs are not known but are likely to be multifactorial (Ricci et al., 2006, Brenninkmeijer et al., 2008).

The physical, psychological and psycho-social impact of atopic eczema on the lives of affected children and their families can be profound (Lewis-Jones, 2006). The impairment in quality of life resulting due to childhood eczema is thought to be greater than or equal to impairment due to diabetes, asthma and cystic fibrosis (Beattie and Lewis-Jones, 2006).

Itching, discomfort and soreness can limit activities during the day and can affect sleep. Poor sleep is common, with 60% of cases having disturbed sleep. Tiredness, behavioral difficulty (Daud et al., 1993) and impaired psychosocial functioning can result as a consequence (Dahl et al., 1995). Furthermore, school performance is occasionally affected due to bullying and embarrassment, contributing to low mood, depression and social isolation (Lewis-Jones and Finlay, 1995).

Symptoms and treatment regimens can restrict child and family life, with wider implications for owning a pet, holidays and taking part in sports. Parents and caregivers may find it difficult to cope with the chronic, relapsing and remitting nature of the condition as well as the taxing treatment regimens which often require multiple applications of topical therapies.

Additionally, the financial costs of managing atopic eczema can impact families and the health services (Emerson et al., 2001), with an estimated burden of £465 million/year in 1996 (Herd et al., 1996)and increasing to in excess of £1 billion/year in 2004 (Gupta et al., 2004) in the UK.

1.1.3 Pathogenesis

Skin barrier defects and immune dysregulation are implicated in the pathogenesis of atopic eczema. The stratum corneum and tight-junction proteins that aid in keratinocyte adhesion in the stratum granulosum are crucial for epidermal barrier structure and function. Correct keratinocyte differentiation is important in stratum corneum development. In atopic eczema, the stratum corneum demonstrates increased transepidermal water loss, reduced hydration (Flohr et al., 2010), raised pH (Rippke et al., 2004), decreased ceramides (Sator et al., 2003), overexpression of chymotryptic enzymes (chymase), reduced microbiome diversity with abundance of *Staphylococcus aureus* (Kong et al., 2012), and defects in filaggrin and other molecules in the epidermal differentiation complex. There are known genetically determined defects in the epidermis, such as inherited *FLG* loss-of-functions mutations which results in reduced expression of filaggrin; this is important for keratinocyte compaction in the development of the

stratum corneum as it aggregates keratin filaments (McGrath and Uitto, 2008). Consequently, this defect can result in poor water retention (Flohr et al., 2010). Abnormalities in the epidermal barrier function are not only related to structural proteins; exogenous environmental exposures such as proteases, soaps and detergents and repetitive scratching (Kezic et al., 2014) are also important. Defects in the skin barrier can allow penetration by allergens, bacteria and viruses (Proksch et al., 2006), contributing further to skin inflammation.

The inflammatory process in atopic eczema involves an imbalance in T cells, with a predominance of T-helper-2 (Th2) cells, increased Immunoglobulin E (IgE) levels and eosinophilia (Ong and Leung, 2006). In the acute phase there is excessive production of Th2 mediated cytokines (IL 4, 5, 13 and 31), but in chronic disease there is a shift to Th1/Th0 dominance (Grewe et al., 1998). Subclinical inflammation involving increased numbers of Th2 cells, Th22 cells, Th17 cells and a pro-inflammatory cytokines is also evident in non-affected areas of skin (Suarez-Farinas et al., 2011).

Atopy, the tendency for IgE antibodies and sensitisation to develop in response to environmental triggers (Johansson et al., 2004), underlies atopic eczema, asthma and allergic rhinitis (Spergel and Paller, 2003, Spergel, 2005). The “atopic march” describes the progression of atopic disorders from atopic dermatitis starting in infancy to allergic rhinitis and asthma in childhood (Spergel and Paller, 2003). Sensitisation against common food and aeroallergens is strongly linked to eczema and is thought to be largely a secondary epiphenomenon rather than as a causative factor (Williams and Flohr, 2006, Flohr et al., 2004, Elias and Steinhoff, 2008). Current research, however, is putting impaired barrier function at the forefront of the pathophysiology as it becomes apparent that dysfunction in the immune system is not the sole process causing atopic eczema.

It has been proposed that the risk of developing atopic disease represents the pleiotropic effects of genes transmitted from mother to child (Thomsen et al., 2006). However, maternally mediated environmental modulation of gene expression in offspring and gene-environment interactions appear to be more important than purely heritable genetic risk (Upham and Holt, 2005).

There is also growing evidence that epigenetic mechanisms are responsible for tissue-specific gene expression during growth and development and that these mechanisms underlie the processes of developmental plasticity. Epigenetics refers to the alterations in the expression of genes arising from deoxyribonucleic acid (DNA) methylation, histone modification and non-coding ribonucleic acid

(RNA), rather than from fixed changes in the genetic sequence. Emerging evidence shows that regulation of neonatal Th1/Th2 balance is under epigenetic control (Adcock et al., 2007). A Th2 profile, which favours the development of allergic disease (Hollingsworth et al., 2008), can be determined during the development of the fetal immune system (Adcock et al., 2007, Prescott and Clifton, 2009), as genes can be activated or inactivated by methylation of DNA CpG sites, determining when and where gene are expressed. In infants at high risk for allergy, DNA methylation in dendritic cells processing antigens is altered (Fedulov and Kobzik, 2011) and affects the Th1/Th2 balance. Increased DNA methylation also affects naive T-cell differentiation into Th2 cells. (Winders et al., 2004, Shin et al., 2005) Furthermore, decreased methylation of DNA has been reported to aid the switch of T-cells into a Th1 phenotype (Lee et al., 2006).

There is increasing experimental evidence that epigenetic and phenotypic changes induced, by an unbalanced maternal diet during pregnancy, for instance, can be influenced by endocrine or nutritional interventions during early postnatal life (Godfrey et al., 2013). Elucidation of these environmental influences and epigenetic processes may permit perinatal identification of individuals at risk of later atopy and facilitate a new generation of early intervention strategies to reduce such risk.

1.1.4 Management of atopic eczema

Identifying, avoiding and attempt at eradication of aggravating factors is important in preventing eczema flares. Common irritants include chemicals (soaps, detergents), clothing (nylon, wool) and atmospheric conditions (temperature, humidity). Food and inhalant allergens can also aggravate the symptoms of atopic eczema.

Treatment for atopic eczema is centred around two aims; improving barrier function and reducing inflammation. Hydration of the skin using emollients, improves barrier function, aids retention of water and can help reduce itching. Emollients can also reduce the need for topical steroids and reduce flare ups (Sher et al., 2012, Anderson and Dinulos, 2009). Advice from clinicians regarding the amount, frequency and type of emollient is important. Bathing in warm water can also hydrate the stratum corneum and has the advantage of being able to remove irritants and allergens, as well as scale, crust and sweat (Eichenfield et al., 2014a). Wet dressings can reduce transepidermal water loss, improve epidermal water content, enhance transepidermal penetration of topical glucocorticoids and

protect skin from scratching, and can help with the clinical resolution of the skin changes.

Topical glucocorticoids are the mainstay of treatment of atopic eczema as they have anti-inflammatory, anti-proliferative, immunosuppressive and vasoconstrictive effects. At the cellular level, they bind to the cytoplasmic glucocorticoid receptors, forming a glucocorticoid-receptor complex which has a direct regulatory effect on gene transcription. Indirectly, they also regulate transcription by blocking the effects of other transcription factors that promote pro-inflammatory molecules such as interleukins (IL), interferon gamma (IFN-γ) and tumour necrosis factor (TNF-α) (Norris, 2005). Topical steroid preparations vary in potency, and are prescribed depending on severity of skin and site affected. Potential adverse effects of topical glucocorticoids include skin atrophy and adrenal suppression, which are associated with inappropriate use of potent preparations.

Topical calcineurin inhibitors are immuno-modulators that work by binding to FK-binding protein and thus can inhibit the production of cytokines from activated T cells and inflammatory cells. Their use as a 'proactive' preventative is usually well tolerated, with some reporting transient burning sensation on application. Unlike topical glucocorticoids, they have not been associated with skin atrophy; however, their use in children under the age of 2 years is unlicensed.

If topical therapies fail, alternative and/or systemic therapies may be required. Alternative therapies such as phototherapy may be considered; ultraviolet A-1 (UVA-1) targets epidermal Langerhans cells and eosinophils and can be helpful in acute severe lesions, while narrow-band ultraviolet B (UVB) exerts immunosuppressive effects by blocking the function of antigen-presenting lymphocytes and by altering keratinocyte cytokine production (Majoie et al., 2009), and is used for chronic atopic eczema (Krutmann, 2000). Phototherapy needs to be carefully considered because of potential side effects including cutaneous malignancies as well as erythema, skin pain, pruritus, and pigmentation (Sidbury et al., 2014).

Systemic therapy with glucocorticoids, cyclosporine, antimetabolites (such as methotrexate, azathioprine, and mycophenolate mofetil), IFN-γ, allergen immunotherapy, and biologics that inhibit specific components in the inflammatory process may be indicated in severe uncontrolled disease. Impaired growth has been associated with systemic glucocorticoid use (Aylett et al., 1992),

amongst a number of other complications; thus use of systemic glucortoicoids is generally not recommended and the aforementioned therapies may be more appropriate. Reports of effectiveness of new biologics in atopic eczema, such as dupilumab, a monoclonal antibody, suggest that this targeted therapy may be promising in the future (Lee et al., 2017).

1.2 The Developmental Origins of Health and Disease

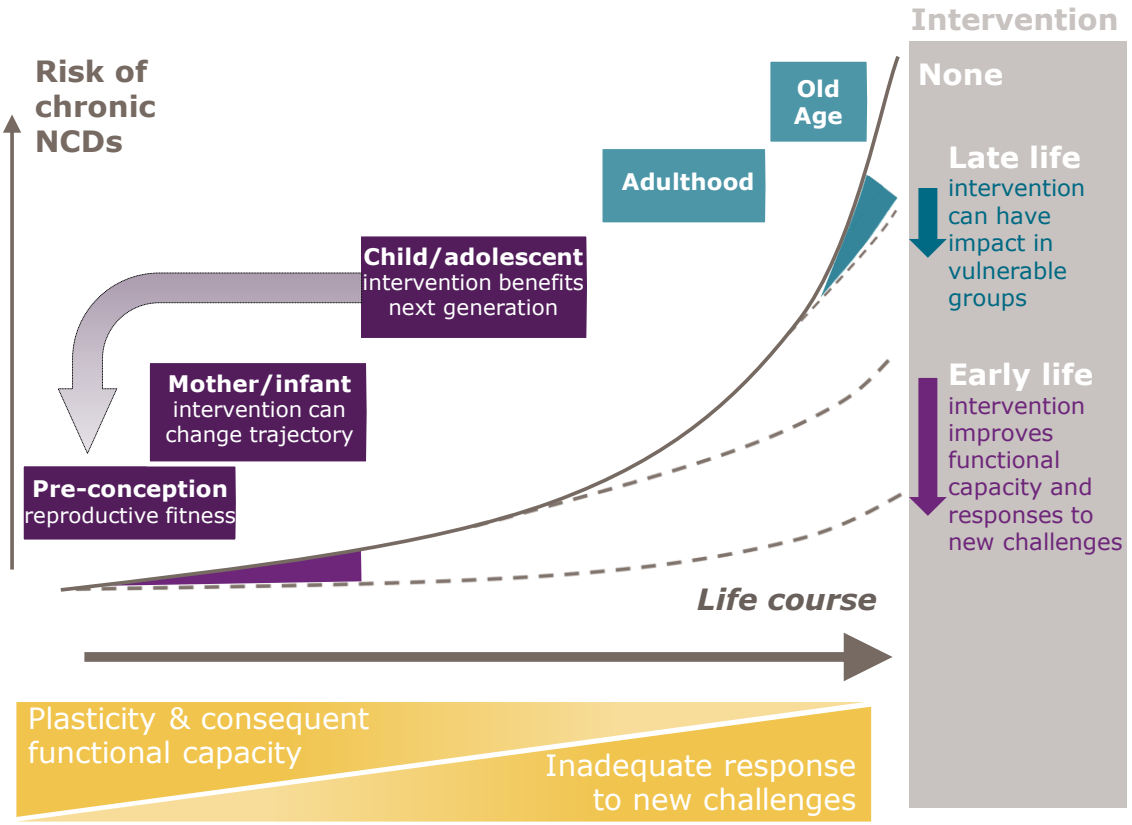
Patterns of health, illness and disease are influenced at different stages of the lifecourse by a combination of genetic, epigenetic and environmental factors. Substantial research has demonstrated that during early development responses to a range of stimuli are likely to 'programme' the risk of non-communicable disorders, as articulated by the 'developmental origins' or 'Developmental Origins of Health and Disease (DOHaD)' concept (Barker, 2003).

Subsequent environmental exposures during childhood and adult life may modify or condition this later risk of disease. These observations have led to the hypothesis that non-communicable disease (NCD) originate through developmental plastic responses made by the fetus and infant as part of a prediction of the subsequent environment to which it anticipates that it will be exposed. Critical periods in development result in irreversible changes; if the environment in childhood and adult life differs from that predicted during fetal life and infancy, the developmental responses may increase the risk of adult disease. Evolutionary considerations and experimental findings in animals strongly support the existence of major developmental effects on health and disease in adulthood. The preservation of this "programming" phenomenon across species and within the normal range of fetal growth suggests a physiological rather than a pathological basis to the DOHaD phenomenon.

Figure 2 shows a conceptual framework for ongoing research (Godfrey et al., 2010). The risk of NCDs increases across the lifecourse as a result of declining plasticity and accumulative effects of inadequate responses to new challenges (orange triangles). The greatest increase occurs in adult life, but the trajectory is set much earlier, being influenced by factors such as the mother's diet and body composition before and during pregnancy, and fetal, infant and childhood nutrition. In early life, timely interventions can have a large effect on the risk of disease later (purple area/arrow), while later intervention can remain impactful for vulnerable groups (blue area/arrow). Intervention in childhood and

adolescence increases biological capital, and may have an important impact on the next generation.

Figure 2. Schematic conceptual framework for the developmental origins of health and disease (Derived from Godfrey et al, 2010)



These concepts have led the UK Department of Health and other international agencies to now advocate a lifecourse approach to disease prevention from pre-conception through pregnancy, infancy, early years, childhood, adolescence and teenage years, and through to adulthood and preparing for older age (Hanson et al., 2015).

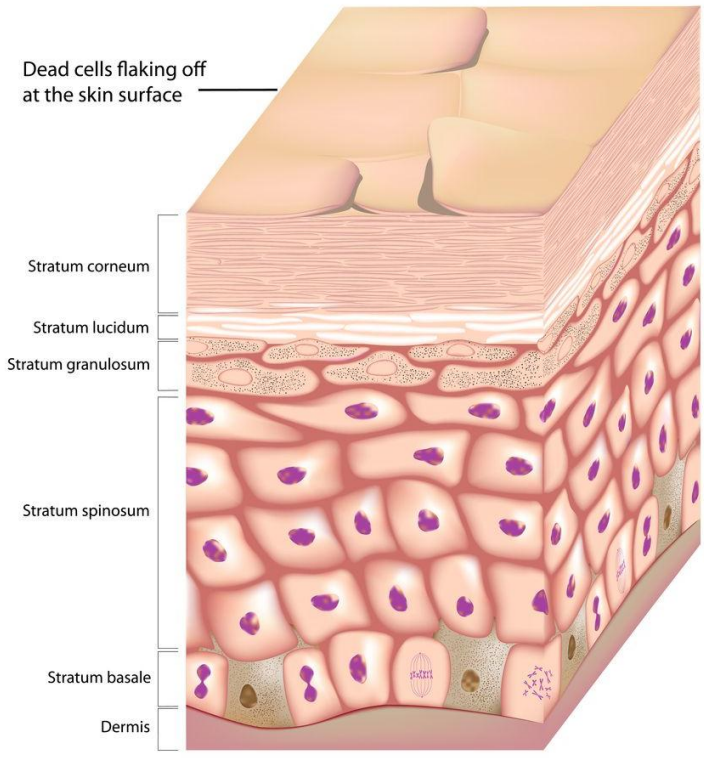
1.2.1 DOHaD and atopic eczema – early life development of the skin and immune system

Fetal skin at 4 weeks' gestation is made up of an inner basal layer and an outer periderm. Keratinisation begins at 9 weeks' and stratification into different layers is seen at 13 weeks (Ersch and Stallmach, 1999). At 14 weeks epidermal buds begin to form, and at 16 weeks, mesenchymal cells may be seen associated with

the bud. Sebaceous glands become apparent at 18 weeks. At 23 weeks, the basal layer bud begins to form primordial eccrine glands. Differentiation continues in utero, and at 34 weeks mature keratinocytes and adult like dermo-epidermal undulations form (Evans and Rutter, 1986).

The fetus is coated by the vernix caseosa, produced by the sebaceous glands and is made up water primarily as well as proteins and lipids; this coat protects the fetal epidermis from maceration, has antimicrobial properties and allows epidermal cornification and stratum corneum formation (Visscher et al., 2011). At birth, full term infants have mature- like skin structurally with tightly overlapping squamous keratinocytes making up the stratum corneum, the most superficial layer and columnar epidermal cells in the deeper stratum basale (Figure 3). Functionally, the stratum corneum in infants differs to that of adults (Nikolovski et al., 2008) in its ability act as a barrier and to hold and transport water. It has a higher water content (Nikolovski et al., 2008), higher trans-epidermal water loss at rest, absorbs more water and looses excess water faster than adults. The stratum corneum continues to develop and evolve in the first year of life, with neonatal skin adapting to the environment after birth by becoming more adult like in its thickness, pH and hydration (Chiou and Blume-Peytavi, 2004).

Figure 3. Anatomy of the adult epidermis



Embryonic macrophages can be seen in the yolk sac as early as 3 weeks' gestation and the first lymphocytes in the thymus by 9 weeks (Takashina, 1987). By 14 weeks, lymphocytes can be found in the lungs and gut, and circulating B cells with surface IgM are detectable from 12 weeks (Bofill et al., 1985). There is evidence that in utero exposure as early as 22 weeks' gestation can result in primary sensitisation to an allergen, leading to positive proliferative responses at birth (Jones et al., 1996).It is thought that the most probable route of antenatal sensitisation in the second trimester occurs through the fetal gut and that exposure to antigens through the fetal circulation occurs in the third trimester (Jones et al., 2001).

The developing uncornified fetal skin and mucosal surfaces may be more permissive to cytokines and allergens in the amniotic fluid (Jones et al., 2002), but the consequences of this are not fully understood. Antenatal events such as exposure to inflammatory processes (e.g. chorioamnionitis) can result in fetal gut inflammation (Wolfs et al., 2014), with mucosal injury and loss of the tight junctional proteins. These events have the potential to impact the integrity of skin and mucosa as they develop and thus result in very early mucosal immune dysregulation that is in turn associated with early postnatal clinical onset of eczema and food allergy (Palmer et al., 2013). Furthermore, normal gut colonization and microbial diversity can be affected by mucosal disturbances; however, this is not well understood.

Microbes (viruses, bacteria, archaea and fungi) that exist on and within the human body are known as the 'microbiome'. Microbiomes are unique, vary at different body sites, change with age and play a role in health and disease. It is recognised that the cutaneous microbiome differs in individuals with atopic eczema, increased *Staphylococcus aureus* colonisation being the most recognised difference. Animal studies have demonstrated an effect of the cutaneous microbiome on the development of skin immunity and disease (Naik et al., 2012, Scharschmidt et al., 2015, Kobayashi et al., 2015). In humans, early colonisation with commensal staphylococci at age 2 months, prior to the clinical onset of atopic eczema, has been associated with a lower risk of the condition at age 12 months (Kennedy et al., 2017).

Certain molecules such as short-chain fatty acids, produced by particular bacteria can affect epigenomic processes (Levy et al., 2016, Egert and Simmering, 2016). Toxins from *S. aureus* which colonises the skin of individuals with atopic eczema stimulate the inflammasomes and can contribute to inflammation and skin barrier

dysfunction (Park et al., 2016). Topical treatments, particularly topical corticosteroids, increase and normalise cutaneous microbiome diversity (Gonzalez et al., 2016, Kong et al., 2012).

Furthermore, changes in the intestinal microbiome can influence the risk of developing atopic eczema with evidence showing an increased risk of the condition at the age of 2 years in infants whose mothers' took antibiotics during pregnancy (Wohl et al., 2015). Early life changes to the cutaneous microbiome occurring in utero or early in the post-natal period are critical as skin barrier function and immune system have not fully matured. Probiotics and synbiotics have been shown to be beneficial in the prevention of atopic eczema in a number of meta-analyses (Chang et al., 2016b, Cao et al., 2015, Panduru et al., 2015, Pelucchi et al., 2012, Mansfield et al., 2014). However, despite this promising evidence, determining formulations, timing and dose requires further research.

1.3 Genetic influences and atopic eczema

Twin studies have consistently demonstrated a role for genetic influences in atopic eczema, with genomic studies identifying important genes on chromosome 1q21 making up the epidermal differentiation complex that functions in regulating epidermal homeostasis. A key gene in this complex is the *FLG* gene, encoding for filaggrin, a protein essential in terminal differentiation of the epidermis. *FLG* mutations have been strongly associated with atopic eczema (van den Oord and Sheikh, 2009, Paternoster et al., 2015) and supportive animal studies have shown have shown that *FLG* mutations play a role in eczema by causing a defect in the skin barrier function that allows enhanced penetration of allergens (Oyoshi et al., 2009, Scharschmidt et al., 2009). In individuals with atopic eczema, transepidermal water loss is increased in affected and non-affected skin (Cork et al., 2009, Seidenari and Giusti, 1995); this too has been linked with *FLG* mutations and can indicate a more severe atopic eczema (Flohr et al., 2010).

Furthermore, interactions between the most common *FLG* loss-of-function mutations (501x and 2282del4) and the presence of older siblings in relation to developing childhood eczema by the age of 6 years have been demonstrated and suggest that in children with inherent *FLG* loss-of-function mutations, the sibling effect acts as a strong risk factor (Cramer et al., 2010).

Recent genetic association studies have identified 31 genetic variants associated with atopic dermatitis risk predominantly in populations of European, African, Japanese and Latino ancestry (Paternoster et al., 2015). These loci include candidate genes involved in the regulation of innate host defences and T cell function, providing additional evidence for the important contribution of immune mechanisms to atopic eczema pathogenesis and show overlap with allergy, asthma and other inflammatory and autoimmune conditions (Paternoster et al., 2015).

1.4 Early life influences

Fetal development is influenced by the milieu in utero. Maternal health, diet, lifestyle and environment are therefore important in achieving optimal development.

Childhood eczema has been linked to higher parental socioeconomic status, higher educational achievement (Uphoff et al., 2015, Taylor-Robinson et al., 2016) and a more affluent neighbourhood environment (Ruokolainen et al., 2015, Astell-Burt et al., 2014). Reasons for social patterning have been proposed and include carpeting and heating that affect house dust mite populations, soap use, ultraviolet radiation and grime exposure, use of medications, contact with pets and other exposures linked with higher social class, including maternal diet and maternal age (Williams et al., 1994c). Many of these influences were postulated to act through mechanisms linked to “clean hygiene” (Strachan, 1989), and the recent interest in the role of the gut microbiome has strengthened the view that the above associations do not reflect confounding or reporting biases.

Smoking during pregnancy is recognised as a risk factor for atopic disorders; it has the potential to impact developing T-lymphocyte and can also affect fetal respiratory tract mucosa (Magnusson et al., 2005, Peters et al., 2013, Havstad et al., 2012). Environmental pollutants such as smoking can also impact immune regulation through effects on small non-coding RNA molecules, including microRNA (miRNA), which are important in posttranscriptional regulation of gene expression in immune regulation pathways. Maternal smoking in pregnancy is associated with miRNA-223 expression in maternal and cord blood, with reduced cord blood regulatory T cell (Treg) numbers and with a consequent increase in allergy risk in the offspring (Herberth et al., 2014, Hinz et al., 2012). Conversely, however, some studies have presented data showing protective effects of

smoking (Hjern et al., 2001, Magnusson et al., 2005, Taylor-Robinson et al., 2016).

The hygiene hypothesis proposes that atopic diseases are less prevalent in children who have a higher number older siblings, which may mean more exposure to allergens and microorganisms. The 'sibling effect' supports the hygiene-hypothesis, and a protective effect of having siblings on the risk of atopic disease has been observed in a number of studies (Golding J, 1986, Bodner et al., 1998, Christie et al., 1998, Butland et al., 1997, Olesen et al., 1996, Ponsonby et al., 1998, Lewis and Britton, 1998, Pekkanen et al., 1999).

Breastfeeding is recommended as an early strategy for allergy prevention, and there is supportive evidence of a protective relationship between breastfeeding and allergic disease (Dell and To, 2001). Some studies have reported that exclusive breastfeeding in the first months of life reduces the risk of asthma, with a greater protective effect in high risk children (Gdalevich et al., 2001) and further reports suggest this relationship is independent of maternal asthma (Oddy et al., 2002). The literature, however, is conflicting, with some recent studies reporting that breastfeeding is linked to an increased likelihood of developing allergic disease and sensitisation (Sears et al., 2002). Furthermore, published data have linked a longer duration of breastfeeding with an increased risk of atopic eczema (Bergmann et al., 2002) and reported that breast fed children of mothers with asthma are at an increased risk of asthma (Wright et al., 2001, Oberle et al., 2001). The inconsistency in the findings of these studies reflects the limitations in examining breastfeeding in observational studies; randomised controlled trials are few in number and incomplete control for confounding factors and potential recruitment biases are further constraints in observational studies (Zeiger, 2000).

As the infant immune system develops it can be influenced by immunomodulatory nutrients, cytokines and microbial factors that are sensitive to breast milk composition and maternal diet during lactation, which can alter the allergens that infants are exposed and sensitised to. Nevertheless, breastfeeding may delay exposure to other more allergenic proteins, such as cows' milk proteins and other antigenic formulations. Although no definitive conclusions can be made regarding the impact of breastfeeding on the offspring's risk of developing allergic immune responses, or the contribution of feeding practices to the increase in allergic disease in recent years, breastfeeding benefits both mother and child in many other ways and is endorsed by all health organisations.

1.4.1 Maternal diet and nutritional status

There is evidence pointing to the potential influences of maternal diet and nutritional status on offspring risk of allergic conditions (Netting et al., 2014, Miles and Calder, 2015). A recent systematic review of 21 cohort studies and 11 intervention studies that included over 40,000 children, however, found no consistent links between maternal diet and offspring atopic outcomes (Netting et al., 2014). Nevertheless, maternal diets rich in fruits, vegetables, fish and vitamin D and a Mediterranean dietary pattern were associated with a reduced risk of allergic disease, as opposed to diets rich in vegetable oils, margarine, nuts and fast food that were associated with an increased risk of allergic disease (Netting et al., 2014).

Vitamin D, omega-3 polyunsaturated fatty acids (PUFA), dietary fibre and antioxidants potentially have immunomodulatory properties and have been examined for their potential role in prevention of allergy and atopic conditions. The Princess Anne Hospital Study Group found that infants of mothers' whose late pregnancy serum vitamin D levels were greater than 75 nmol/L were more likely to have visible eczema at age 9 months, with a trend pointing to a higher likelihood of atopic eczema defined using modified UK Working Party Diagnostic Criteria (UKWPDC) at the same age, when compared to infants of mothers' whose serum vitamin D levels ware less than 30 nmol/L (Gale et al., 2008).

Dietary omega-3 PUFA also have immunomodulatory and anti-inflammatory effects (Prescott and Calder, 2004) and preliminary evidence shows that diets supplemented with these nutrients during pregnancy or the early postnatal period could have clinically evident immunomodulatory effects through inhibition of Th2 cell differentiation (Shek et al., 2012). A systematic review by Kremmyda et al. (Kremmyda et al., 2011) which included a number of randomised controlled trials found that infants of mothers whose intake of fish rich in omega-3 PUFA during pregnancy were less likely to develop atopic and allergic conditions. Omega-6 PUFA, present in vegetable oils rich in linoleic acid, on the other hand, have been associated with increased risk of atopy through promotion of Th2 cytokines (Palmer et al., 2012).

Higher maternal intakes during pregnancy of citrus fruit and green/yellow vegetables, which are rich in antioxidants and carotenoids, have been associated with a reduced risk of eczema in infants (Miyake et al., 2010). Excessive antioxidant intake, however, has been linked to a Th2 dominant profile through

immune regulatory mechanisms and suppression of Th1 differentiation (Allan et al., 2010).

Studies have shown no associations between maternal intakes of certain micronutrient and offspring eczema, specifically for maternal intakes of vitamin B9 (folic acid) (Nwaru et al., 2011, Miyake et al., 2011, Dunstan et al., 2012), vitamin B2 (Nwaru et al., 2011, Miyake et al., 2011), vitamin B12 (Miyake et al., 2011) or vitamin B6 (Miyake et al., 2011).

Acting through changes to the gut microbiome, modern high-fat, low-fibre diets are now thought to result in low-grade systemic inflammation and metabolic dysregulation, potentially contributing to chronic diseases (Walters et al., 2014). Few studies have as yet examined such influences in relation to infant atopic eczema. Advanced glycation end products (AGEs) produced as a result of modern food processing through non-enzymatic glycation and oxidation of proteins and lipids, have the ability to initiate and propagate inflammation through innate immune pathways and are involved in many inflammatory processes that can contribute to the development of atopic disease (Hilmenyuk et al., 2010, Akirav et al., 2014). Their role in utero and early infancy, however, is uncertain.

1.4.1.1 Nicotinamide and related tryptophan metabolites

Nicotinamide, the amide form of niacin (vitamin B3), is maintained by the dietary intake of vitamin B3 and tryptophan, an essential amino acid that is a constituent of most proteins. Nicotinamide is the precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) *in vivo*, and is an inhibitor of the nuclear enzyme poly-ADP-ribose polymerase 1 (PARP-1) (Jacobson et al., 1999). Through these functions, nicotinamide can enhance energy-dependent cellular processes such as DNA repair (Surjana et al., 2010), maintain genomic stability and regulation of some transcription factors, particularly in relation to the expression of inflammatory cytokines, chemokines, adhesion molecules and inflammatory mediators (Virag and Szabo, 2002). Nicotinamide inhibits cyclic adenosine monophosphate phosphodiesterase (cAMP PDE) and stabilises mast cells and leukocytes through inhibition of histamine and IgE release (Namazi, 2004). It has also been shown to increase the biosynthesis of ceramide and other stratum corneum lipids (Tanno et al., 2000) and prevents the upregulation of aquaporin, thereby decreasing water permeability and water loss (Song et al., 2008). Nicotinamide increases synthesis of collagen and proteins involved in formation of keratin, filaggrin, and involucrin in cultured cells, thus improving the overall structure, moisture, and elasticity of skin (Bissett et al.,

2003). Through these functions, nicotinamide has the potential to alter the disease processes associated with atopic eczema.

In a randomised control trial, topical 2% nicotinamide applied twice a day to atopic eczema for 4 and 8 weeks significantly reduced water loss and increased stratum corneum hydration when compared with white petrolatum (Soma et al., 2005). Orally, nicotinamide has been shown to reduce transepidermal water loss (Chen et al., 2016). It is not fully understood how oral nicotinamide administration may alter cellular inflammation *in vivo*; in a limited group of healthy human participants exposed to experimental endotoxaemia it had little effect on cytokines or exhaled nitric oxide (Soop et al., 2004).

1.4.2 Maternal stress and psychological distress

Pathways by which maternal stress can cause fetal immune dysregulation leading to a propensity to develop atopic eczema and other atopic disorders have been proposed. Transplacental passage of maternal stress hormones (Pincus-Knackstedt et al., 2006) and alteration of placental corticotrophin releasing hormone (CRH) regulation axis (Harris and Seckl, 2011) can affect the fetal hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids and catecholamines released as a result of stress can modulate differentiation of Th1 and Th2 cells, favouring a shift towards a Th2 humoral cell type reaction (Elenkov, 2004). This inflammatory reaction is seen in atopic eczema and other atopic conditions.

Andersson et al (Andersson et al., 2016) identified a number of studies linking prenatal maternal stress to an increased risk of offspring atopic eczema (Wen et al., 2011, Hartwig et al., 2014, de Marco et al., 2012, Sausenthaler et al., 2009, Larsen et al., 2014). de Marco et al. (de Marco et al., 2012) reported increased odds ratios for asthma, eczema and allergic rhinitis, supporting an immunomodulatory effect of stress and stress hormones on the development of atopy in humans.

It is possible that mothers experiencing stress may make poor health practice choices, including smoking, or differ in terms of their age, educational attainment, parity and history of eczema, which put their offspring at an increased risk of developing atopic eczema (Hoffman and Hatch, 1996). However, no published studies have explored the link between maternal preconception stress/ low mood and offspring risk of developing atopic eczema.

1.5 Early development and atopic eczema

Infant birthweight is often used as a crude measure of prenatal growth and studies examining birthweight in relation to atopic eczema have been inconsistent (Panduru et al., 2014, Olesen et al., 1997). The same birthweight can be achieved by different patterns of fetal growth, and studies examining outcomes other than atopic eczema have indicated that fetal growth patterns may show stronger associations with later outcomes than cross sectional assessments of size at single time points (Pike et al., 2010). A previous study using routinely collected fetal anthropometry data reported that fetuses with below average crown-rump length at 11 weeks' gestation and an above average bi-parietal diameter at 19 weeks' gestation were more likely to have eczema ascertained by postal questionnaire at age 10 years (Turner et al., 2011). However, no study to date has examined longitudinal measures of fetal and infant size in relation to infantile atopic eczema. More information has been published for other atopic outcomes; a large neonatal head circumference (Godfrey et al., 1994, Fergusson et al., 1997, Gregory et al., 1999, Shaheen et al., 1999) and higher abdominal circumference growth velocity between 11 and 19 weeks' gestation have been linked to an increased risk of atopy (Pike et al., 2010). The developmental origins of health and disease hypothesis describes fetal growth patterns showing late gestation growth faltering followed by subsequent rapid weight gain in infancy and childhood as strong predictors of non-communicable diseases in adult life (Barker, 2003).

In children with atopic eczema, possible reasons for poor growth in infancy and childhood have been proposed and include effects of the inflammatory disease (Wong et al., 2016), corticosteroid treatment (Aylett et al., 1992), poor nutrition as a result of an inappropriately restrictive diet (Keller et al., 2012), and eczema associated sleep disturbance (Silverberg and Paller, 2015). However, little attention has been paid to the possibility of pre-morbid changes in the growth trajectory of infants with atopic eczema. Any such changes in pre-morbid growth may help explain the growth impairment in children with atopic eczema, while also providing insights into etiology of the skin disorder.

1.6 Aims and objectives of this thesis

Better understanding of the developmental influences of atopic eczema is central to future preventive strategies of this highly prevalent chronic condition that has

substantial impact on the on the lives of the affected children and their families as well as the health care system.

This thesis examines particular aspects of the potential developmental influences on the etiology of atopic eczema, with a focus on three components acting in early life:

1. Examining the relationship between maternal serum nicotinamide and related tryptophan metabolite levels in pregnancy and risk of atopic eczema in infants.
2. Examining the influence of maternal stress and mood on the risk of atopic eczema in infants with particular interest in preconception stress and mood.
3. Examining fetal and infant size and growth patterns in relation to atopic eczema in infants.

Chapter 2: Methodology

As maternal influences on fetal development and subsequent health are becoming increasingly recognised, characterising women prior to conception has become imperative. Maternal factors can be influential from as early as before conception, preceding pregnancy related changes in diet, physical activity, body composition, endocrine profile, and psychological state that are known to impact the developing fetus (Gluckman and Hanson, 2005).

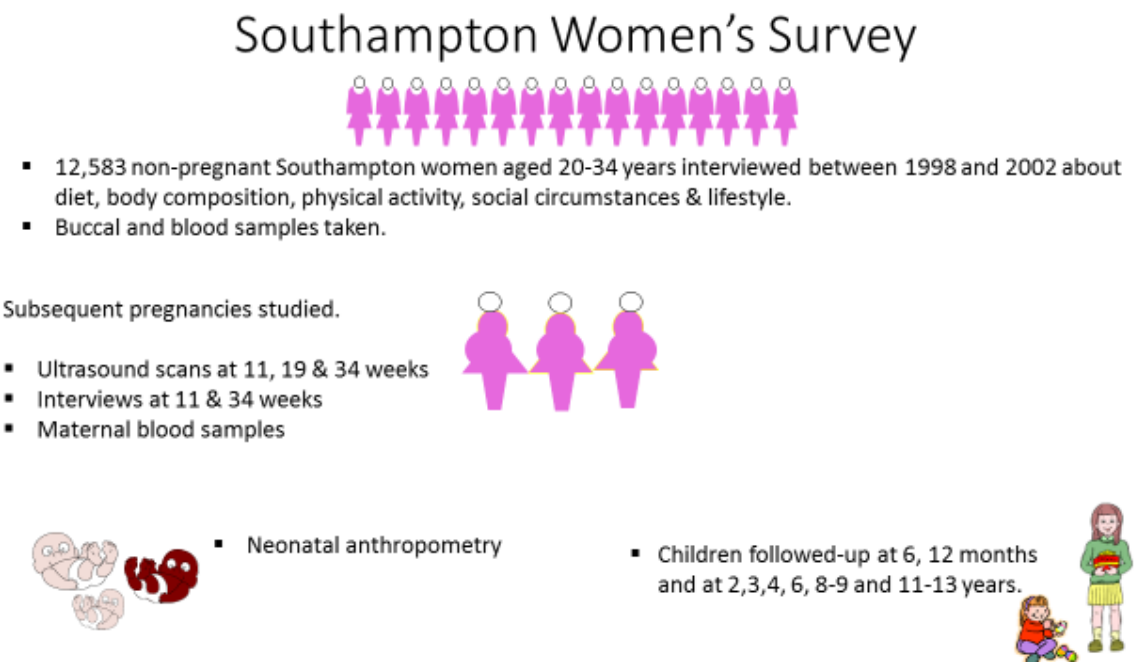
The focus of this thesis is on the developmental and early life influences on the risk of infantile atopic eczema at ages 6 and 12 months within the Southampton Women's Survey. Specifically, it examines maternal serum tryptophan metabolite concentrations, preconception and postpartum maternal stress and mood, and fetal and infant growth in relation to the risk of infantile atopic eczema. Briefly, these early life factors are also examined in relation to atopy with the aim of gaining insight to the potential various disease processes occurring in these conditions.

2.1 The Southampton Women's Survey

The Southampton Women's Survey (SWS), a prospective, longitudinal, mother-offspring cohort study, was established with the objective of exploring the effects of maternal factors on fetal and childhood development, growth and health and risk of adult NCDs including cardiovascular disease, type 2 diabetes, osteoporosis and atopic disease. The aim was to characterise women in detail prior to preconception, throughout the various stages of pregnancy and postnatally. The study examined maternal diet, physical activity, body composition, endocrine profile, and psychological state, as well as placental and fetal adaptive responses including fetal growth and body composition. (Inskip et al., 2006b)

Inskip et al (Inskip et al., 2006a) published a detailed description of the study cohort; Figure 4 and Appendix A show the various stages of the study. Between 1998 and 2002, 12583 women aged 20-34 years from the general population who were not pregnant were recruited through general practitioners in Southampton, UK, and the surrounding area.

Figure 4. The Southampton Women's Survey



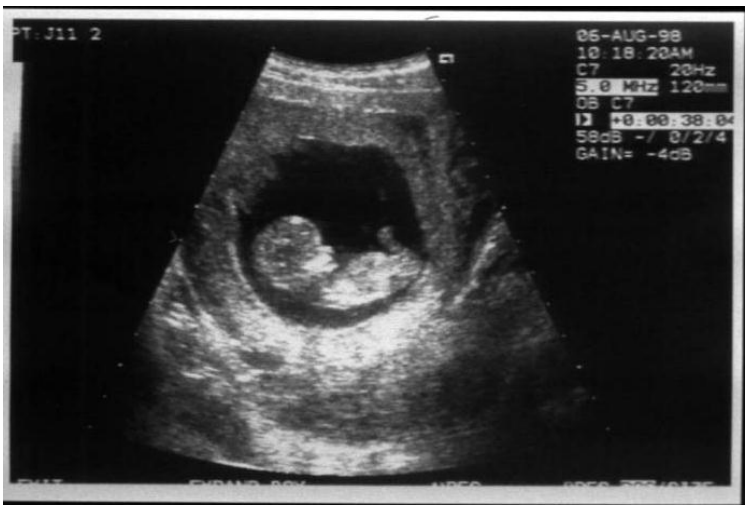
The preconception characteristics and lifestyles of these women were ascertained by a trained research nurse. This initial assessment included a nurse administered questionnaire capturing social circumstances, education, lifestyle, perceived stress, physical activity and diet (Figure 5 and Appendix B). The questions used to assess maternal stress are described in detail in Chapter 4.

Figure 5. Maternal circumstances and lifestyle

Women who became pregnant were interviewed by a research nurse at 11 and 34 weeks gestation assessing lifestyle and diet using a food frequency questionnaire that had previously been validated against 4-day food diaries. Maternal weight was measured, and questions about smoking and history of eczema were administered.

At 11, 19, and 34 weeks, ultrasound measurements of fetal and placental size and blood flows were performed (Figure 6). All ultrasound measurements were made by trained research ultrasonographers using detailed standardised operating protocols that followed the British Medical Ultrasound Society guidelines for the precise landmarks for fetal anthropometric measurements (Appendix C).

Figure 6. Fetal ultrasound at 11 weeks gestation



Bisoamples collected included venous blood and urine at the initial interview (at luteal phase of the menstrual cycle) and two further venous blood samples at 11 and 34 weeks gestation (Figure 7). Aliquots were stored at −80 degrees centigrade for later analysis, including the measurements of serum nicotinamide and related metabolites examined in this thesis. A venous blood sample was also collected from the woman's partner during her pregnancy. Buccal samples obtained from mouthwashes were collected from the women at initial interview and from the partner and parents of the women who became pregnant. DNA was extracted from blood and buccal samples.

Figure 7. Venous blood sampling

3158 infants were born to the study's participants. At birth, umbilical cord blood, placental and membrane samples were collected for functional studies with biopsies stored for later morphometry and molecular analyses. Anthropometric measurements of the neonates were made within 48 hours of birth.

The offspring were followed up at 6 and 12 months, 2 and 3 years, with subsequent follow up of sub-samples at 4, 6, 8-9 and 11-13 years of age. At the follow up visits, interviews were conducted to collect information on social circumstances, lifestyle, physical activity and diet as outlined in Appendix A.

Anthropometry including weight, height and skin fold thickness measurements (triceps, biceps, subscapular and suprailiac sites in the mothers, triceps and subscapular in the offspring) was performed providing data on body composition of both the women and their offspring (Figure 8).

Figure 8. Infant anthropometry: a) head circumference, b) skin fold thickness and c) upper arm circumference

A 100-item food frequency questionnaire was used to assess diets of women before and during pregnancy, where the average intake of the listed foods over a 3 month period preceding the interview was recorded. Further food frequency questionnaires assessed the diet of the offspring at 6 months, 1 year, and 3 years of age.

Assessment for atopy included a nurse administered questionnaire on allergies and parental atopy at infant age 1, 3 and 6 years. Skin prick testing was performed for the mothers and offspring at infant age 1 year, and again for their children at ages 3 and 6 years. Testing was undertaken to cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), grass pollens, egg and milk allergens (Hollister-Stier, Spokane, WA), together with positive and negative controls. Tree pollen was added (ALK Abelló Hørsholm, Denmark) at 6 years. Atopic eczema was ascertained by the trained research nurses at ages 6 and 12 months using a combination of questions and skin examination to collect the information required to applied modified UK working Party diagnostic criteria for the

definition of eczema. A detailed description of the information ascertained for assessment of this key outcome follows later in this Chapter.

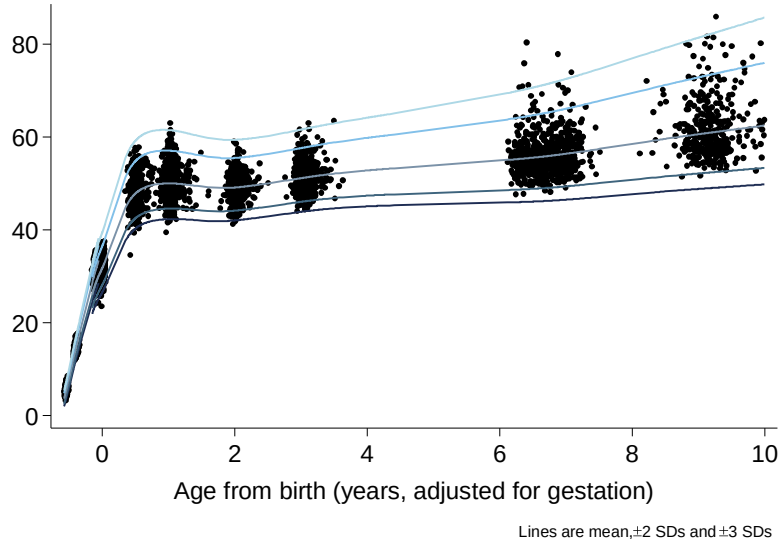
At infant age 6 months, mothers completed an Edinburgh Post-natal Depression Scale (EPDS) questionnaire. For a subset of the cohort, an assessment for psychological distress was incorporated part way through the initial preconception recruitment; 5513 women completed the 12-item General Health Questionnaire (GHQ-12) with an additional question about perceived financial strain. These women were also asked for their written consent to examine their medical records for diagnoses and treatment of depression in the 2 years after the interview.

The SWS is distinctive in collecting contemporaneous information from women before conception. Also, comprehensive longitudinal measurements of fetal and infant anthropometry enabled derivation of fetal growth rates in early, mid, and late pregnancy and later postnatal growth (Figure 9).

Figure 9. Abdominal circumference distribution in male and female offspring

Boys

Girls



The population of Southampton is broadly representative of the general population, although comparison of the Townsend Indices of Deprivation at recruitment between 1998–2002 for the enumeration districts of Southampton with those for England and Wales showed that at the time the recruitment was undertaken Southampton was slightly more deprived than average. For women aged 20–34 years in Southampton, approximately 94% were white, compared to 88% in England and Wales in 2001; 31% of SWS women were smokers at the time of the initial interview, compared with 33% of women of the same age in Britain; 21% had university degrees, comparable with 22% for women of working age in England as reported by the Labour Force Survey.

The interviews and assessments were standardised and conducted by trained nurses and inter-observer variation studies were performed to ensure reliability and validity of anthropometric measurements. Nevertheless, given the prospective longitudinal nature of the study, the recruitment of pregnancies occurred over a prolonged period of over 9 years, representing a limitation of the study.

2.1.1 Ethics statement

All phases of the Southampton Women's Survey were approved by the Southampton and South West Hampshire Local Research Ethics Committee and parents gave written informed consent. The relevant ethics approvals are shown in Table 1 below.

Table 1. SWS ethics approvals

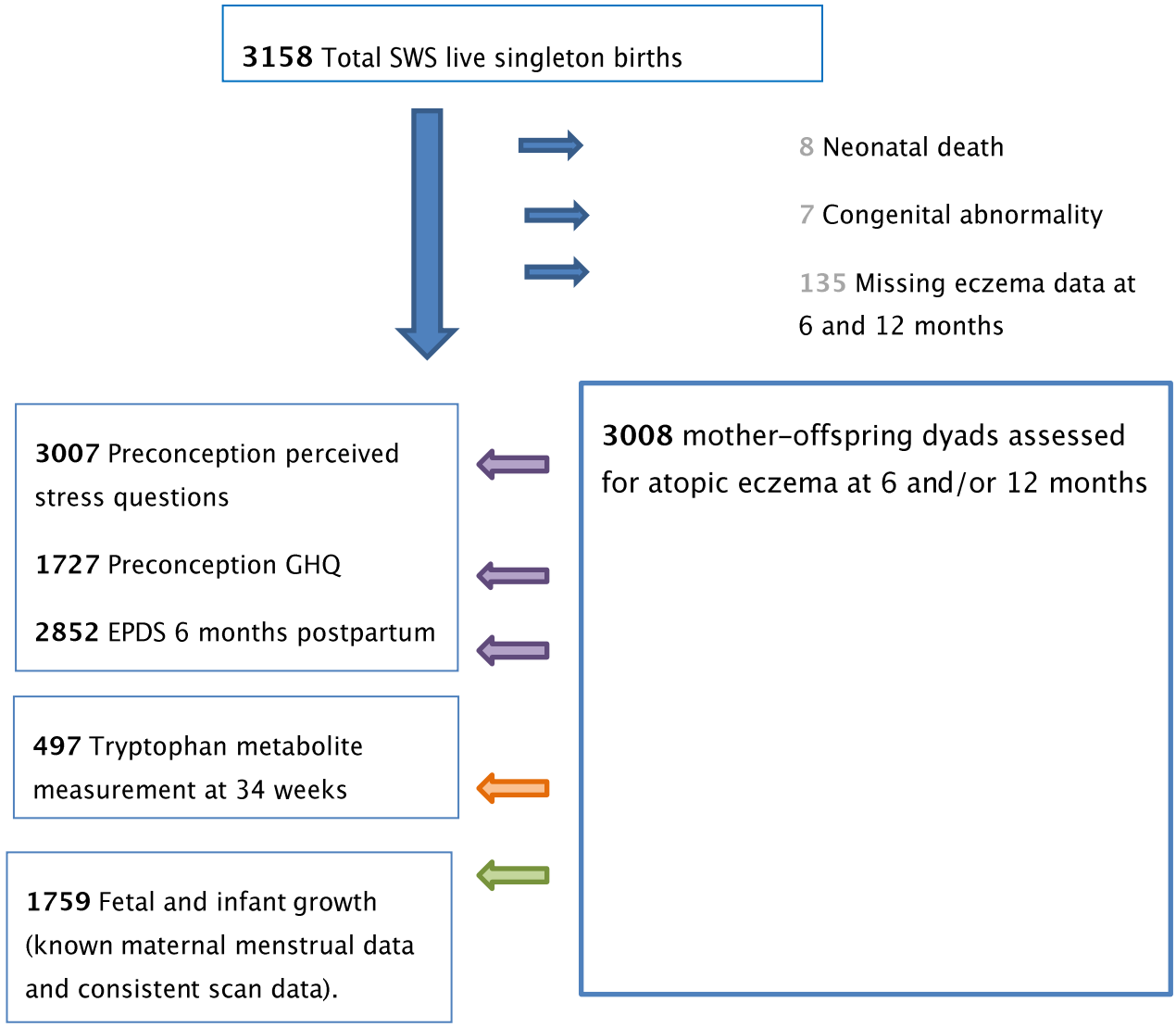
LREC no	SWS component	Title	Approval date
276/97	Recruitment	Survey of diet, body composition and hormone levels in young women in Southampton	28/11/1997
307/97	Pregnancy and birth	The effects of maternal nutrition, body composition and cardiovascular status on fetal development and metabolic programming	28/11/1997
018/99	Genetics: fetal, parental and (maternal) grandparental genetic studies	Genetic influences on foetal growth and adult disease	9/3/1999
089/99	6, 12, 24 and 36 months follow-up	Follow-up of infants born to women in the Southampton Women's Survey	30/4/1999
335/99	Depression (GHQ12) at recruitment, and follow-up through GP records for incident depression	Factors associated with depression among young women in the Southampton Women's Survey	3/11/1999
06/Q1702/104	6 year follow-up (1st phase), with focus on respiratory health *See 10/H0504/30 below for 2nd phase*	Developmental influences on childhood respiratory health. SWS cohort study at age 6 years	16/08/2006

LREC no	SWS component	Title	Approval date
10/H0504/30	6 year follow-up (2nd phase) focusing on cognitive function *See 06/Q1702/104 above for first phase*	Southampton Women's Survey cohort study at age 6 years: developmental influences on childhood respiratory health, body composition and thinking skills	28/6/2010

2.2 Sample size

12583 women were recruited to the Southampton Women's Survey. There were 3158 live singleton births. 8 neonatal deaths and 7 neonates with major congenital abnormalities were excluded from analyses. Eczema data at 6 and/or 12 months was missing for 135 infants and thus were excluded. 3008 mother-offspring dyads were assessed for atopic eczema at 6 and/or 12 months. Detailed flow diagrams showing the mother-offspring dyads contributing to each of the three Results chapters in this thesis are shown in each of the chapters. In brief, 3005 mothers completed preconception perceived stress questions, 1727 completed a preconception GHQ and 2852 completed an EPDS questionnaire at 6 months postpartum; making up the sample in which the effect of maternal stress and psychological distress preconception and postpartum low mood on the risk of offspring eczema was examined. Of the 3008 mother-offspring dyads assessed for atopic eczema at 6 and/or 12 months; 497 mothers had tryptophan metabolite measurements at 34 weeks gestation; the relation of these concentrations to offspring risk of atopic eczema was examined in this group. For the purpose of examining fetal and infant growth in relation to atopic eczema; 215 neonates with a gestational age of <37 weeks and 1055 participants with uncertain maternal menstrual data and/or discrepant or missing scan data were excluded, leaving 1759 infants in the examined group (Figure 10).

Figure 10. Selection of study group from the SWS cohort



2.3 Outcome assessment

2.3.1 Atopic eczema

Case definition of atopic eczema was based on a modified UKWPDC for the definition on atopic eczema (Williams et al., 1994a); as assessed by trained research nurses who administered a standardised questionnaire and ascertained other information required for the diagnostic criteria (a combination of history of itchy skin condition and two or more of the following: history of involvement of the skin creases such as folds of elbows, behind the knees, fronts of ankles, cheeks or around the neck; a history of a general dry skin in the last year; and visible flexural eczema or eczema involving the cheeks/forehead and outer

limbs). All infants were assessed for eczema before the age of 2 years, thus this criterion was met by all infants in the study cohort. However, as the infants were not old enough to have developed clearly defined atopic disorders, a personal history of atopy was omitted as a criterion. Atopic disease in a first degree relative was also omitted as a criterion as we were seeking to disentangle the apparent heterogeneous phenotypes that 'atopic eczema' is now thought to represent; excluding those with no family history of atopy would remove an important group of infants from such studies. Maternal history of atopic eczema in the past 12 month and maternal atopy, however, were considered potential confounding variables and were adjusted for in the analyses, where appropriate.

The UKWPDC were derived from the more extensive Hanifin and Rajka criteria (Hanifin and Rajka, 1980) that required an assessment of 4 major and 23 minor criteria, including patient history, clinical examination, laboratory and skin testing where meeting 3 major and 3 minor would indicate a diagnosis of AE (Appendix D). Extensive validation of the UKWPDC was undertaken by Williams et al as part of their derivation (Williams et al., 1994b).

Established in 1980, the highly sensitive Hanifin and Rajka criteria were for many years the most referred to criteria in in investigational studies, however, their use has not been considered valid in clinical trials and epidemiological studies and they may not be suitable for population based studies (Schultz Larsen and Hanifin, 1992).A number of studies found that some of the minor criteria had no clinical or diagnostic significance and were non-specific, such as, palmar hyperlinearlity and keratosis pillaris (Mevorah et al., 1985), nipple eczema, cheilitis, conjunctivitis, anterior subcapsular cataract, keratoconus, anterior neck fold and food intolerance (Kang and Tian, 1987, Kanwar et al., 1991, Nagaraja et al., 1996) Some criteria require invasive tests, for instance, ascertaining serum IgE and skin prick testing.

The UKWPDC, refined Hanifin and Rajka's criteria and consisted of 1 mandatory criterion (pruritus, i.e. itch), together with 3 or more of 5 other criteria; (i) onset under the age of 2 years; (ii) a history of flexural involvement; (iii) a history of asthma or hay fever (or a history of atopic disease in siblings and parents if the child is under 4 y); (iv) a history of generally dry skin in the last year; and (v) visible flexural dermatitis. Williams et al have also validated the use of this criteria in infants (Williams et al., 1994b).

Table 2. The UK Working Party Diagnostic Criteria for Atopic Eczema

UK working party diagnostic criteria for eczema	
Itchy skin condition (mandatory)	
In addition to three of the following:	▪ Onset of signs and symptoms under the age of 2 years (this criteria should not be used in children <4 years) ▪ History of eczema (flexural or of the cheeks and extensor surfaces if under 18 months) ▪ History of asthma or allergic rhinitis (or history of eczema in a first degree relative if <4 years old) ▪ History of dry skin in the last year ▪ Visible flexural eczema (or visible eczema of the cheeks and extensor surfaces if under 18 months)

Meeting the mandatory criterion of pruritus in addition to 3 other criteria indicates a diagnosis of atopic eczema. Studies have also validated the criteria when pruritus, the mandatory criterion, plus 2, 4 and 5 criteria were met (De et al., 2006, Williams et al., 1994a). Validation studies, including 7 UK independent studies with no conflict of interest, validated the criteria (Brenninkmeijer et al., 2008), and have shown uniform specificity but variation in sensitivity which may be attributable to differing and diverse cultural, lingual and socioeconomic population groups. (Hamada et al., 2005, Saeki et al., 2007, Chalmers et al., 2007).

Applying the UKWPDC is easy and quick and does not require invasive testing. Furthermore, the criteria has been the most comprehensively validated criteria, in both community and hospital settings, and have been shown to be replicable in various population groups (Brenninkmeijer et al., 2008, Weidinger and Novak, 2016, Gu et al., 2001).

A number of other diagnostic criteria for atopic eczema have been proposed. Some of these have not been sufficiently validated, such as The International Study of Asthma and Allergies in Childhood (ISAAC), Kang and Tian, Diepgen and Schulz-Larzen cirteria, while others have little or validation, such as the Danish Allergy Research Centre (DARC), Millennium, Japanese Dermatology Association and Lillehammer criteria (Brenninkmeijer et al., 2008).

2.3.2 Atopy

Assessment for atopy included nurse administered questionnaire on allergies and parental atopy at offspring age 1, 3 and 6 years. Skin prick testing were performed by trained research nurses for mothers at infant age 12 months and 3 years and for their children at age of 12 months, 3 and 6 years; testing to cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), grass pollens, egg and milk allergens (Hollister-Stier, Spokane, WA) were performed at age 12 months and 3 years. Tree pollen was added (ALK Abelló Hørsholm, Denmark) at 6 years. The protocol for this procedure is outlines in Appendix E. Atopy was defined as per skin prick test readings where a wheal response of ≥ 3 mm to valid readings with appropriate positive and negative control responses. The negative control, 50% glycerine, was considered valid if the response was 0 mm, and the histamine (10mg/ml^3) positive control ≥ 3 mm. The presence of at least one positive result was considered evidence of atopy.

Skin prick testing is commonly used in both the clinical and research settings to test for allergic sensitatisation. The testing kits are readily available and results are immediate and the risks of adverse reaction is small (Turkeltaub and Gergen, 1989). The allergen solutions are applied on marked standardised skin sites on the back of infants and forearm of adults. The skin is then pricked by a lancet at 90 degrees and responses read at 15 minutes. A dermal IgE response with rapid mast cell degranulation results in a wheal which is measured. Wheal size was determined by measuring the largest diameter and the diameter at right angles to this and then calculating the mean of the two measurements.

The skin prick testing procedure was standardised and followed the European Academy of Allergy and Clinical Immunology (EAACI) 1989 position paper in skin allergy testing. Specifically trained research nurses conducted the test to minimise operator variation. Reproducibility of their technique was monitored annually by performing 5 positive controls on the same subject on the same occasion.

All participants were asked to avoid taking antihistamines for 7 days prior to having the skin prick test. In the rare event of having received antihistamines, those who had taken antihistamines with appropriate responses to negative and positive control were not excluded from the study to prevent potential preferential exclusion of atopic children as supported by the EAACI.

Lancets with 1 mm point were used as there is evidence for their reliability (Brown et al., 1981) and achieve same penetration of skin on each occasion. A cut off of 3 mm can exclude those who develop a dermographism like response rather than a true wheal response to the allergen. In participants with more significant dermographism, the size of the negative control was subtracted from the allergen response.

Positive test is not necessarily indicate allergic disease. However, larger reaction are more likely to be clinically significant (Bernstein and Storms, 1995). Response to the test can depend on the age of the participant and a ratio of the wheal response to allergen to wheal response to histamine may be a more accurate indicator (Barbee et al., 1976). Infants examined, however, did not vary in age (all were aged 12 months (mean age in weeks 53.7 (IQR 52.6 – 55.0)).

2.4 Directed Acyclic Graphs

The selection of appropriate confounding factors in epidemiological studies such as the Southampton Women's Survey is important as studies are often observational and exposures are not randomised. Direct acyclic graphs (DAGs) provide a robust and objective means of selecting confounders.

Causal DAGs are a graphical representation of causal effects between variables. Based on prior knowledge and usual convention for the casual effects studied, these graphs identify potential confounding variables which are then adjusted for in multivariate analysis to minimise confounding bias. (Greenland et al., 1999) The potential confounders identified usually are those minimally sufficient for adjustment (Knuppel and Stang, 2010); other variables can be adjusted for to increase accuracy. DAGs are specified prior to data analysis and can be particularly helpful in observational studies where there is a large body of data and causal inferences are more difficult to determine.

The graph consists of an exposure, an outcome, and other variables represented by nodes and unidirectional arrows representing the causal effects. The software

programme used to graphically draw and analyse the DAGs for this thesis was DAGitty. (Textor et al., 2011)

This thesis mainly examined infant atopic eczema as the outcome with various early life exposures specifically;

- Maternal serum tryptophan metabolite concentrations
- Preconception and postpartum maternal mood and stress
- Fetal and infant growth.

Moreover, maternal serum tryptophan metabolites, preconception and postpartum maternal mood and stress and fetal and infant growth were examined as exposures influencing infant atopy as the outcome. DAGs were based on *a priori* hypotheses and knowledge, and determined the confounding variables to be taken into account in each setting.

2.5 Statistical analysis

Descriptive statistics were used to describe the characteristics of the studied populations, in comparison with the SWS participants included/not included in the individual analyses. Summary statistics are presented as mean (standard deviation (SD)) or median (Interquartile Range (IQR)) for continuous variables, and percentages for categorical variables. Histograms were used to check the distributions of outcome variables. Variables that were not normally distributed were log transformed to normality where possible. Transformed variables were standardised to allow comparison in SD units. For raw and transformed variables that were normally distributed, t tests and regression analyses were used; otherwise, non-parametric Wilcoxon-Mann-Whitney tests were used for analyses. Categorical variables were compared using Chi-square tests.

As atopic eczema is sufficiently rare, logistic regressions were an appropriate approach to interpret odds ratios as relative risk. Univariate and multivariate logistic regression analyses were performed (Stata version 13.0/14.1, Statacorp LP, TX) to relate the maternal, fetal and infant characteristics, and other early life exposures to infant atopic eczema at ages 6 and 12 months. Atopy, however, is a common outcome and Poisson regression analyses were therefore used. P values of <0.05 were considered statistically significant.

All statistical analyses were performed under the supervision and guidance of MRC LEU statisticians.

Chapter 3: Maternal serum nicotinamide and related tryptophan metabolites in relation to offspring risk of atopic eczema

3.1 Introduction

Evidence that atopic eczema partly originates in utero is increasing. The risk of developing atopic eczema has been linked with a variety of environmental factors in pregnancy, including mother's age, education and smoking, and some studies have proposed links with aspects of maternal diet and nutritional status during pregnancy (Beckhaus et al., 2015), with a number of studies examining a range of nutrients (Netting et al., 2014, Miles and Calder, 2015). Among B vitamins, maternal vitamin B9 (Nwaru et al., 2011, Miyake et al., 2011, Dunstan et al., 2012), vitamin B2 (Nwaru et al., 2011, Miyake et al., 2011), vitamin B12 and vitamin B6 (Miyake et al., 2011) have been examined in relation to the offspring's risk of atopic eczema, but no significant associations have been found. Vitamin B3 (niacin), however, has not been previously examined.

Nicotinamide is the amide form of niacin, also known as vitamin B3, an essential water-soluble vitamin. Both compounds are precursors of NAD and nicotinamide NADP *in vivo.* Nicotinamide is maintained by the intake of vitamin B3, found in foods including fish, meat, chicken, mushrooms, nuts, coffee and green leaves (Burtis et al., 2012, Rolfe, 2014) and by the intake of tryptophan, an essential amino acid that is a constituent of most proteins and constitutes approximately 1% of total dietary protein and is the precursor for serotonin and melatonin (Heine et al., 1995). In the liver, tryptophan can be converted to niacin via the kynurenine pathway, with quinolone acids as key intermediates, where approximately 60 mg of tryptophan is required to produce 1 mg of niacin, although the conversion ratio can be altered by numerous factors such as vitamin deficiency, food restriction, high protein intake, hormones, exercise and chemicals (Fukuwatari and Shibata, 2007). The recommended daily intake of vitamin B3 in niacin equivalents is 16 mg for men, 14 mg for women, 18 mg for pregnant women and 17 mg for lactating women (National Health Medical and Research Council, 2006) with reported accelerated degradation of tryptophan in pregnancy which may contribute to increased requirements (Wirleitner et al., 2003).

Deficiency of vitamin B3 leads to pellagra, a disease characterised by photosensitive dermatitis, gastrointestinal upset, widespread neurological deficit and cognitive decline (Wan et al., 2011). The skin is thought to be highly vulnerable to vitamin B3 deficiency though this vulnerability to vitamin B3 deficiency varies amongst the different tissues in the body (Jacobson et al., 1999). In developed countries, pellagra is now rare as a result of improved diet and the addition of vitamin B3 to food, and it is generally limited to individuals suffering from malabsorption syndromes, psychiatric illnesses or alcohol dependence. The deficiency of niacin is treated with nicotinamide supplementation because the latter causes less side effects, such as flushing, compared to treatment with niacin.

3.1.1 Tryptophan in pregnancy

During pregnancy, tryptophan demand is higher and the concentration of tryptophans been found to be increased in umbilical cord blood compared to maternal circulation (Badawy, 2015, Tricklebank et al., 1979). Tryptophan is required for protein synthesis for both the mother and the developing fetus. Its metabolites also have important functions in pregnancy; quinolinic acid (QA) for NAD synthesis, kynurenic acid (KA) for neuronal protection and other kynurenines for suppressing fetal rejection (Badawy, 2015). Tryptophan is also a precursor of serotonin, which is important in cellular signalling pathways (Badawy, 2015).

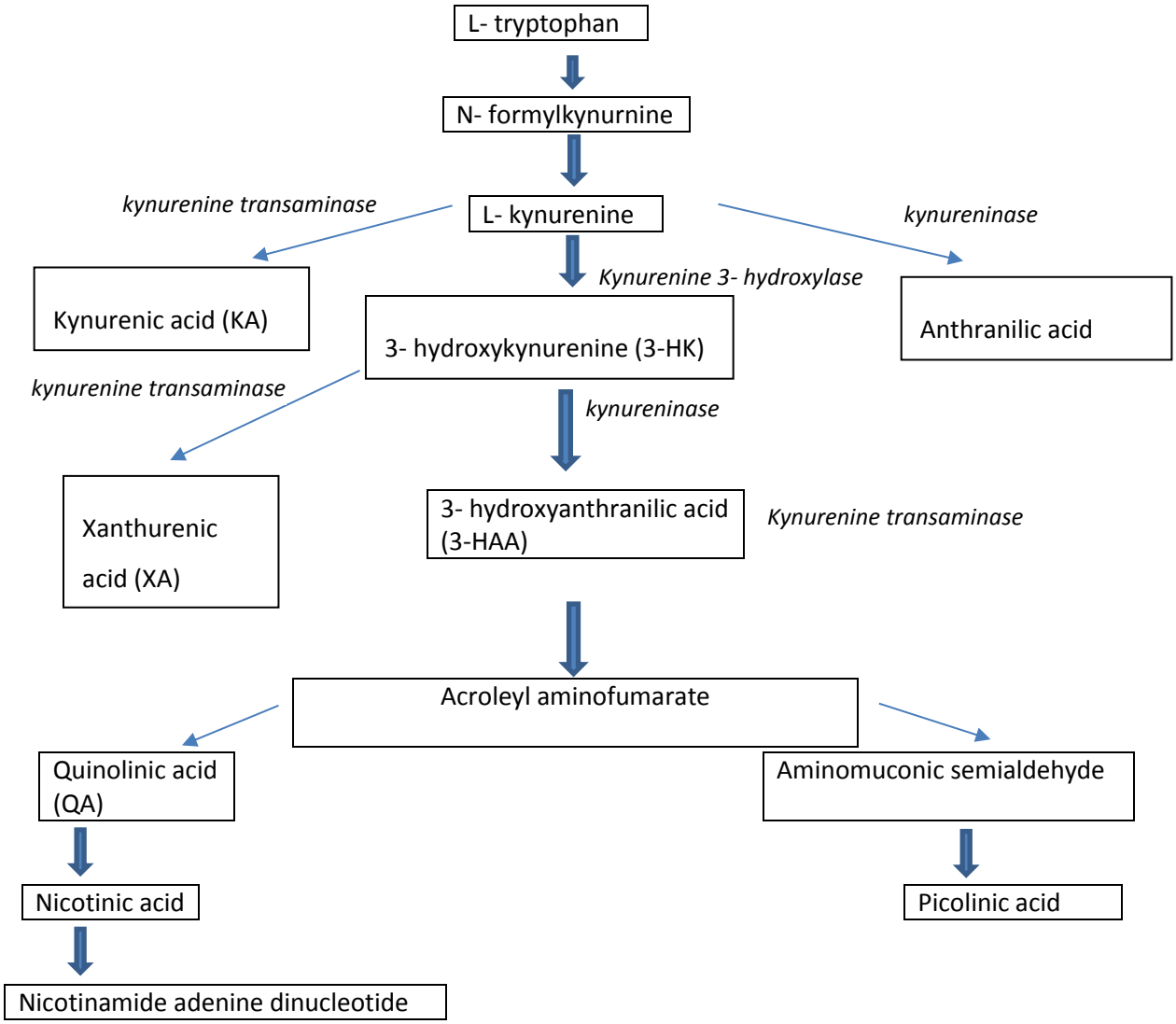
Total plasma tryptophan is reduced in pregnancy, but free tryptophan increases (De Antoni et al., 1980, Morita et al., 1992). In early pregnancy, progesterone and oestrogen inhibit maternal liver tryptophan 2,3-dioxygenase (TDO), thus increase tryptophan availability. Later in pregnancy, albumin is depleted and non-esterified fatty acid (NEFA) increase thus releasing albumin-bound tryptophan, which helps maintain tryptophan concentration and encourages metabolism through the Kynurenine pathway and subsequent increase in immunosuppressive kynurenines (Badawy, 2015, Badawy, 2014).

3.1.2 The kynurenine pathway

The kynurenine pathway (Figure 11) is the major route for tryptophan metabolism in mammals, and regulates several fundamental biological processes, including cell death. This pathway is controlled by hepatic TDO which is regulated by substrate concentration (i.e. tryptophan concentration), glucocorticoids and negative feedback from NADP (Bender, 1983) and inhibited by oestrogens and

progesterones. Extrahepatic activation of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), induced by inflammatory stimuli (most importantly IFN-γ) (Werner et al., 1987), leads to the formation of kynurenine and other metabolites that counter-regulate immune activation; in chronic immune activation continued immunosuppressive feedback mechanisms lead to elevated kynurenine concentrations (Mandi and Vecsei, 2012). Kynurenine has also been reported to enhance IgE-mediated responses (Kawasaki et al., 2014). Tryptophan is metabolised by kynureninase and kynurenine transaminase. Kynurenine is converted to kynurenic acid (KA), 3-hydroxykynurenine (3-HK) and anthranilic acid by kynurenine transaminase, kynurenine 3-hydroxylase and kynureninase, respectively. 3-HK is then to xanthurenic acid (XA) by kynurenine transaminase and to hydroxyanthranilic acid (3-HAA) by kynureninase. 3-HAA is further converted to acroleyl aminofumarate, which in turn is converted to quinolinic acid (QA) through non enzymatic cyclization, before it is converted to nicotinic acid (niacin), the precursor for NAD. 3-HAA and QA can alter Th1 cells (Fallarino et al., 2002), and tend to increase Th2 reactivity. N1-Methylnicotinamide is a metabolite of nicotinamide; produced primarily in the liver, it has anti-inflammatory properties and may also influence thrombosis through activation of prostacyclin activation (Gebicki et al., 2003).

Figure 11. The Kynurenine pathway



3.1.3 Rationale for examining nicotinamide in relation to infant atopic eczema

In a randomised control trial involving children and adults, topical 2% nicotinamide applied twice a day to atopic eczema for 4 and 8 weeks significantly reduced water loss and increased stratum corneum hydration when compared with white petrolatum (Soma et al., 2005). Orally, nicotinamide has been shown to reduce transepidermal water loss in a group of adult patients recruited to a study examining the effects of oral nicotinamide supplementation in non-melanoma skin cancers (Chen et al., 2016). It is not fully understood how oral nicotinamide administration may alter cellular inflammation and/or the skin

barrier *in vivo*; in a limited group of healthy human participants exposed to experimental endotoxaemia, nicotinamide was thought to have little effect on the endotoxin-induced inflammatory response as it did not influence cytokines (Tumour necrosis factor- α (TNF-α), IL-6, IL-8, and IL-10) or exhaled nitric oxide (Soop et al., 2004).

The above observations led us to examine the hypothesis that higher maternal serum concentrations of nicotinamide and related tryptophan metabolites in late pregnancy may be associated with a decreased risk of atopic eczema in the offspring. Furthermore, as similar mechanisms and processes of immune dysregulation occur in atopy and atopic eczema, maternal serum nicotinamide and related tryptophan metabolites were examined in relation to infant atopy.

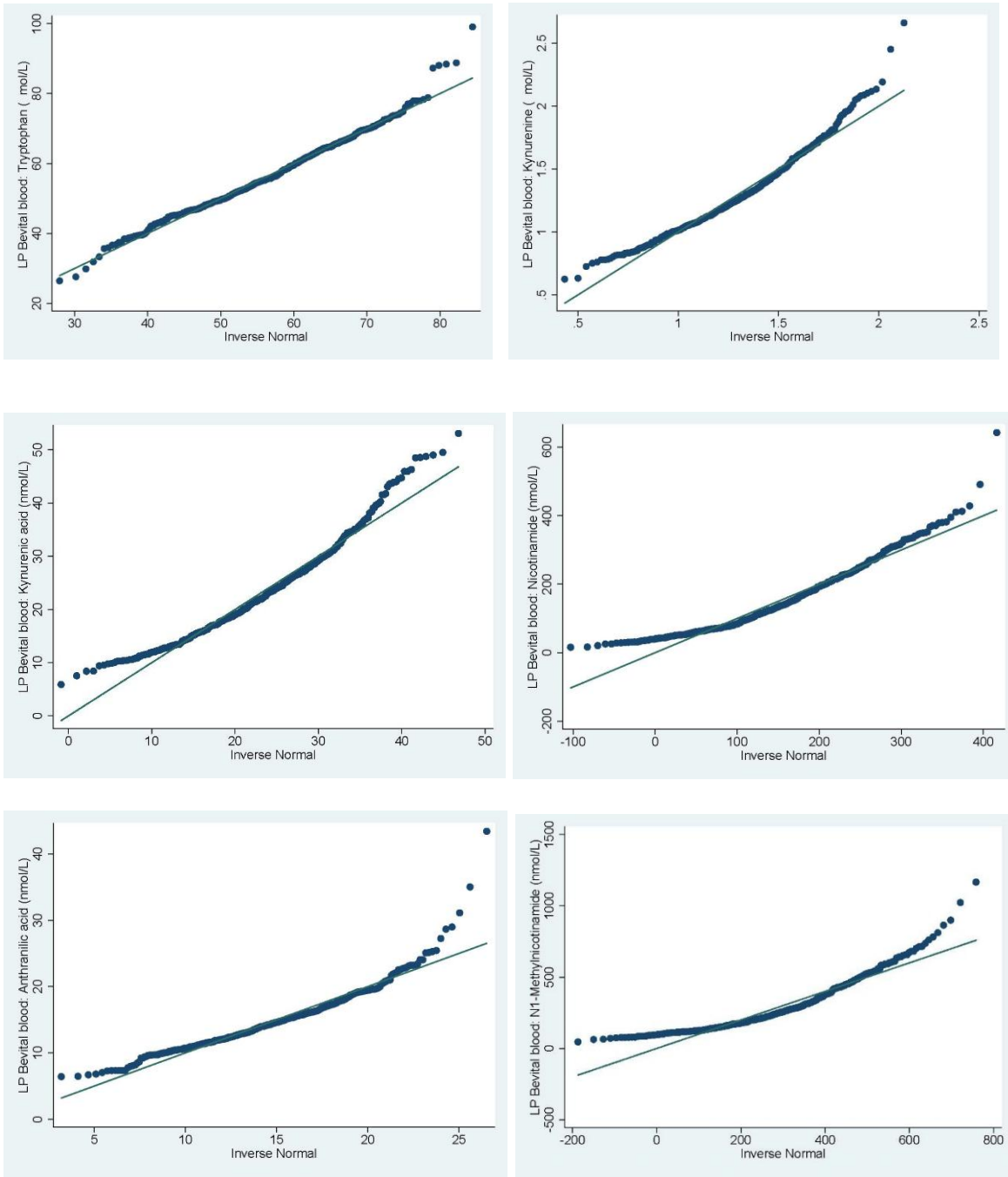
3.2 Methods

Within the SWS, 3008 live born infants with no major congenital abnormalities and no neonatal deaths were assessed for atopic eczema at 6 or 12 months. An aliquot of serum was available from 497 women for measurement of serum tryptophan, kynurenine, kynurenic acid, anthranilic acid, nicotinamide and N1-Methylnicotinamide concentrations in late pregnancy (Figure 12). Maternal serum samples were collected at 34 weeks' gestation and spun down within 24 hours. Serum was separated and stored at -80 degrees centigrade. Measurements of the above tryptophan metabolites were made by BEVITAL AS, Norway using liquid chromatography–mass spectrometry/mass spectrometry (Midttun et al., 2009); coefficients of variation were <12%.

Liquid chromatography- tandem mass spectrometry (LC-MS/MS) combines liquid chromatography (or high performance liquid chromatography HPLC) for physical separation of components and tandem mass spectrometry (MS/MS) for mass analyses to identify the components with a high molecular specificity and detection sensitivity. LC-MS a superior analytical specificity when compared to conventional high performance/pressure liquid chromatography (HPLC) or immunoassays for low molecular weight analytes (Grebe and Singh, 2011). It also has a higher throughput than gas chromatography-mass spectrometry (GC-MS) (Grebe and Singh, 2011). The use of high throughput LC-MS/MS produces the separation and sensitivity needed to quantify endogenous plasma levels of a number of compounds that are chemically varied but biologically similar, spanning a wide concentration range. The LC-MS/MS method used included 16 B

vitamin and inflammatory analytes, and is thought to be suitable for characterisation in epidemiological studies (Midttun et al., 2009).

Figure 12 Distribution of serum tryptophan metabolite concentrations in late pregnancy (LP)



3.2.1 Outcome definition

Case definition of atopic eczema and atopy was based on the UK Working Party diagnostic criteria for the definition of atopic eczema (Williams et al., 1994a, Williams et al., 1994b) and skin prick testing, respectively. Outcome definitions are described in chapter 2.

3.2.2 Statistical analysis

Metabolite concentrations were transformed using Fisher-Yates transformation (Armitage and Berry, 2002) to allow analysis of relations per standard deviation (SD) change in concentration. Univariate and multivariate logistic regression analyses were performed (Stata version 13.0, Statacorp LP, TX) to relate maternal demographic and lifestyle characteristics, maternal serum metabolite levels and early life factors to infant eczema at ages 6 and 12 months and atopy at 12 months.

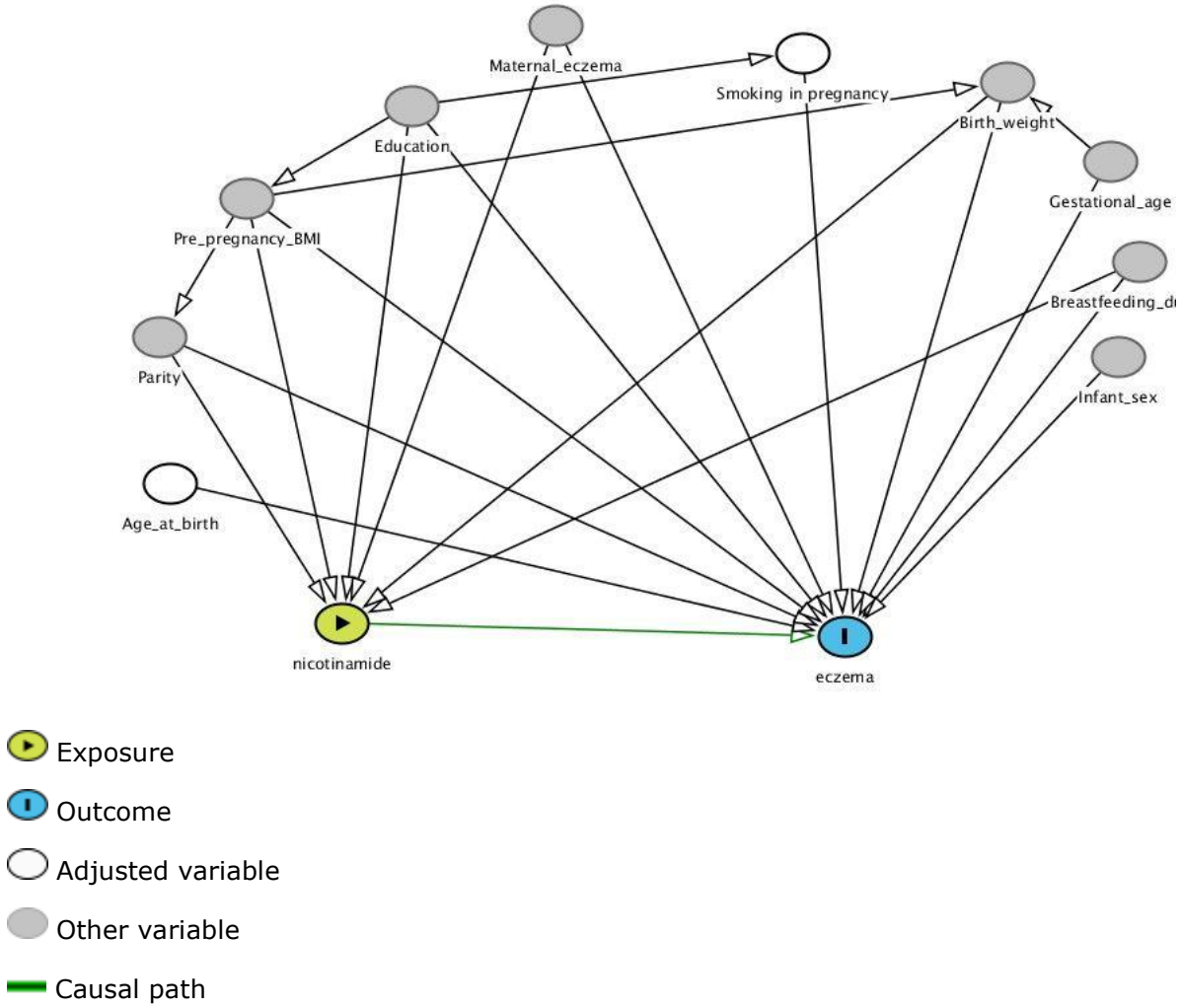
Maternal factors that were considered as potential confounders as identified on the DAG (Figure 13) were:

- Pre-pregnancy body mass index (BMI)
- Education
- Parity
- Maternal history of eczema in creases of elbows or knees in the 12 months prior to recruitment
- Smoking during pregnancy and age at child birth were considered competing exposures and were adjusted for to improve the accuracy of the model.

Potential infant confounders were:

- Sex
- Gestational age
- Birth weight
- Breastfeeding duration

Figure 13. Maternal nicotinamide and related tryptophan metabolites in relation to offspring atopic eczema DAG



Additional analysis with the inclusion of filaggrin single-nucleotide polymorphism rs7512552 as a confounder was performed. This single-nucleotide polymorphism (SNP) has been as related to atopic eczema in the SWS cohort as part of the replication studies following a recent multi-ancestry genome-wide association (Paternoster et al., 2015) study, and was available for 414 infants in this subsample

Univariate (unadjusted) and multivariate (adjusted) Poisson regression analyses were used to relate maternal tryptophan metabolite levels to infant atopy at 12 months. The same confounders were considered when examining infant atopy and atopic eczema to allow for comparison of the two outcomes, with the exception of accounting for maternal atopy instead of maternal eczema as this is more relevant to this analysis.

3.3 Results

3.3.1 Associations between maternal tryptophan metabolites and infant atopic eczema

Maternal and infant characteristics are summarised in Table 3. Among the 497 participants, the mother's average age at child's birth was 31.2 years (Standard Deviation (SD) = 3.5); 48.9% were primiparous and 12.5% smoked during pregnancy. 50.7% of infants were male; mean birthweight of infants was 3.51 kg (SD 0.47) and gestational age 40.1 weeks (IQR 39.1 – 41.0). 30.6% of the infants had the rs 7512552 SNP. 10.7% and 13.7% had atopic eczema at ages 6 and 12 months, respectively.

Distributions of the measured tryptophan metabolites are in keeping with reference ranges from the analysing laboratory but are rarely reported in population samples. Values are reported as medians and inter-quartile ranges as there was some element of positive skew, and were transformed as described in the Methods to enable statistical analysis.

Table 3. Characteristics of the study population

	n	%, Mean (SD), Median (IQR)
Maternal		
Age at child's birth (years)	497	31.2 (3.5)
Pre-pregnancy BMI (kg/m^2)	493	24.2 (22.2- 27.2)
A level or higher education	311	62.8%
Smoking in pregnancy	497	62 (12.5%)
Primiparous	243	48.9%
Eczema in the last 12 months	270	9.4%
Duration of gestation at late pregnancy blood sample (weeks)	492	34.5 (0.6)
Maternal serum metabolite concentrations in late pregnancy (n = 497)		
Tryptophan (µmol/L)	497	55.5 (49.1-62.8)
Kynurenine (µmol/L)	497	1.2 (1.1-1.4)
Kynurenic acid (nmol/L)	497	21.9 (17.0-27.6)
Anthranilic acid (nmol/L)	497	14.5 (12.1- 16.9)
Nicotinamide (nmol/L)	497	140.2 (81.1-213.6)
N1- Methylnicotinamide (nmol/L)	497	246.2 (164.4-368.1)
Infant		
Male	252	50.7%
Gestational age (weeks)	497	40.1 (39.1- 41.0)
Birthweight (kg)	494	3.51 (0.47)
SNP (rs7512552)	414	30.6%
Breast feeding (completed months)		
Never breast fed	74	15.4%
<1	95	19.8%
1 to 3	86	17.9%
4 to 6	94	19.6%
7 to 11	86	17.9%
12 or more	45	9.4%

	n	%, mean (SD), median (IQR)
6 month assessment		
Age (weeks)	495	26.7 (25.9 – 27.6)
Weight (kg)	494	7.99 (0.94)
Atopic eczema as per UK Working Party criteria	53	10.7%
12 month assessment		
Age (weeks)	467	53.6 (52.4 – 54.6)
Weight (kg)	465	10.12(1.1)
Atopic eczema as per UK Working Party criteria	64	13.7%

SD: standard deviation

Table 4 represents SWS infants assessed for eczema at ages 6 and/or 12 months and shows number of infants with atopic eczema at these ages. 248 infants had eczema at age of 6 months, this increased to 265 infants having eczema at both 6 and 12 months of age.

Table 4. The number of infants with atopic eczema at age 6 months and those at 12 months

	Atopic eczema at age 12 months		
Atopic eczema at age 6 months	No	Yes	Total
No	2441	131	2572
Yes	114	134	248
Total	2555	265	2820

Table 5 shows that the characteristics of the 497 participants in the study group with maternal metabolite measurements in late pregnancy were similar to the remaining SWS participants who were assessed for atopic eczema. However, the study group mothers were older at child's birth, smoking was less prevalent and infants' birthweight was higher.

Table 5. Comparison of the study population with the remainder of the SWS participants who were assessed for atopic eczema

	Study population (n = 497) Median (IQR), Mean (SD) or %	Other SWS participants (n = 2511) Median (IQR), Mean (SD) or %	P value for difference between the two groups
Maternal			
Age at child's birth (years)	31.2 (3.5)	30.6 (3.9)	0.001
Pre-pregnancy BMI (kg/m^2)	24.2 (22.2- 27.2)	24.1 (21.8-27.4)	0.33
% A level or higher degree	62.8%	58.3%	0.07
% Smoking in pregnancy	12.5%	16.4%	0.029
% Primiparous	48.9 %	52.0%	0.21
Infant			
% Male	50.7%	52.2%	0.55
Gestational age (weeks)	40.1 (39.1- 41.6)	40.0(39.0-41.0)	0.40
Birth weight (kg)	3.51 (0.47)	3.43 (0.56)	0.003

Table 6 shows univariate analyses of maternal and infant characteristics in relation to infant atopic eczema at age 6 months. Girls had a lower odds ratio of atopic eczema at age 6 months (p=0.002). None of the maternal serum metabolite concentrations were associated with offspring atopic eczema at age 6 months. However, univariate analyses of maternal and infant characteristics showed that higher maternal serum concentrations of nicotinamide and anthranilic acid were associated with lower odds ratios of atopic eczema at age 12 months (Table 7; nicotinamide OR 0.70, 95%CI 0.53-0.90 per SD change, p= 0.007, Figure 14a; anthranilic acid OR 0.63, 95%CI 0.48-0.83 per SD change, p= 0.001, Figure 14b). No significant associations were found with late pregnancy serum levels of tryptophan, kynurenine, kynurenic acid or N1-methylnicotinamide. Higher maternal pre-pregnancy BMI (p= 0.001) and male sex (p= 0.009) were also associated with higher odds ratios of atopic eczema at age 12 months.

Table 6. Univariate analyses of maternal and infant characteristics as predictors of atopic eczema at age 6 months

	n	OR	95%CI	p-value
Maternal characteristics				
Age (years)	495	1.01	0.93 – 1.10	0.76
Pre-pregnancy BMI (SD)	491	1.29	0.99 – 1.68	0.06
Education (6 levels)	493	0.99	0.80 – 1.23	0.95
Smoking in pregnancy (no/yes)	495	0.70	0.27 – 1.84	0.47
Parity (0/ 1+)	495	0.65	0.36 – 1.15	0.14
Eczema in last 12 months (no/yes)	492	1.98	0.83–4.75	0.13
Maternal serum metabolite concentrations in late pregnancy				
Tryptophan (SD)	495	1.05	0.79 – 1.40	0.73
Kynurenine (SD)	495	1.04	0.78 –1.38	0.80
Kynurenic acid (SD)	495	1.25	0.93 – 1.67	0.14
Anthranilic acid (SD)	495	0.85	0.63 – 1.13	0.26
Nicotinamide (SD)	495	0.94	0.71 – 1.26	0.70
N1-Methylnicotinamide (SD)	495	0.92	0.69 – 1.23	0.57
Infant characteristics				
Sex (male=1/ female=2)	495	0.37	0.20 – 0.69	0.002
Gestational age (weeks)	495	0.99	0.80 – 1.22	0.93
Birth weight (kg)	492	1.30	0.71–2.36	0.40
Breast feeding (6 groups)	480	1.02	0.85 – 1.22	0.86
SNP rs7512552 (no/yes)	413	0.70	0.46–1.09	0.11

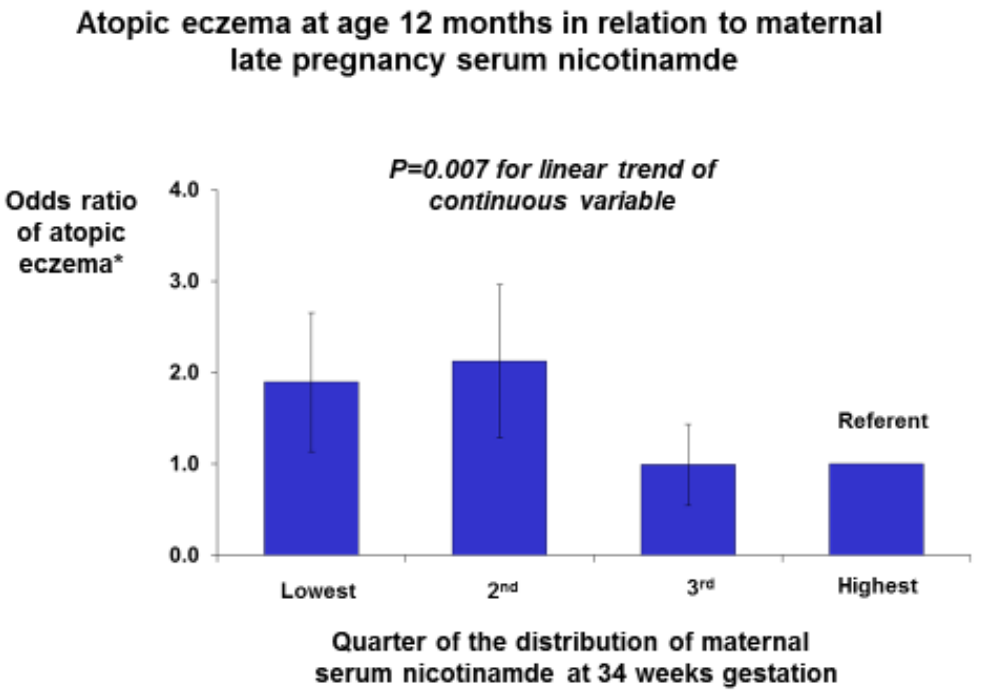
SD: standard deviation

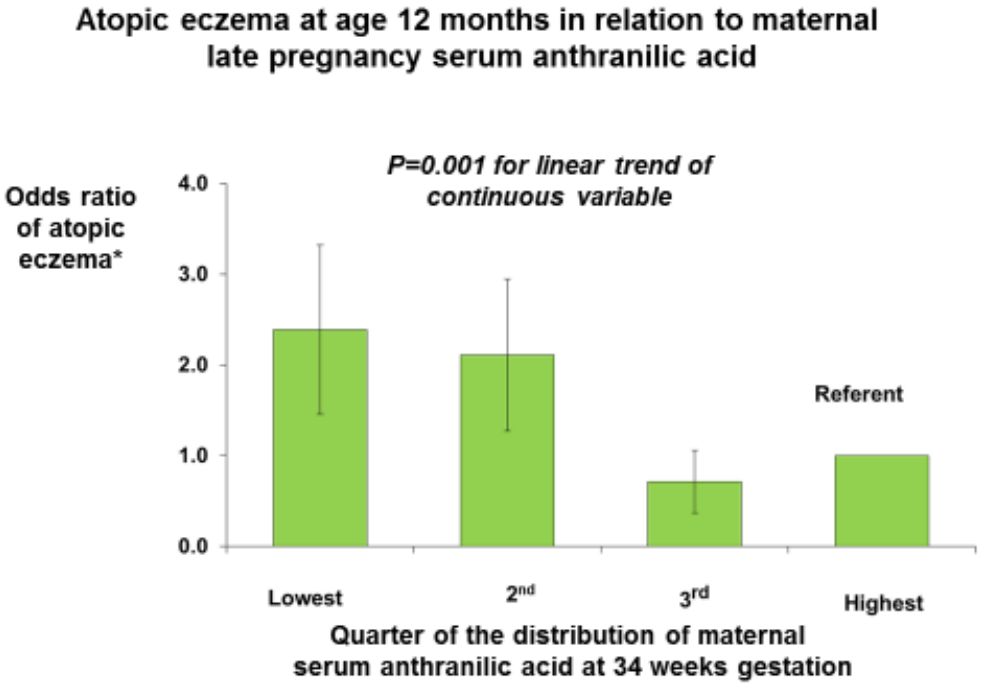
Table 7. Univariate analyses of maternal and infant characteristics as predictors of atopic eczema at age 12 months

	n	OR	95% CI	p-value
Maternal characteristics				
Age (years)	467	0.96	0.89 -1.03	0.27
Pre-pregnancy BMI (SD)	463	1.49	1.17-1.92	0.001
Education (6 levels)	465	0.85	0.69-1.04	0.12
Smoking (no/yes)	467	1.67	0.83-3.34	0.15
Parity (0/ 1+)	467	0.71	0.42-1.20	0.20
Eczema in the last 12 months (no/yes)	464	1.28	0.51-3.20	0.60
Maternal serum metabolite concentrations in late pregnancy				
Tryptophan (SD)	467	1.03	0.79 - 1.34	0.84
Kynurenine (SD)	467	0.90	0.69 - 1.18	0.47
Kynurenic acid (SD)	467	1.03	0.79 - 1.35	0.80
Anthranilic acid (SD)	467	0.63	0.48 - 0.83	0.001
Nicotinamide (SD)	467	0.70	0.53 - 0.90	0.007
N1-Methylnicotinamide (SD)	467	0.78	0.59 - 1.01	0.06
Infant characteristics				
Sex (male=1/ female=2)	467	0.48	0.28 -0.84	0.009
Gestational age (weeks)	467	0.95	0.79-1.16	0.64
Birth weight (kg)	464	0.78	0.44-1.37	0.38
Breast feeding (6 groups)	457	0.92	0.78- 1.09	0.36
SNP rs7512552 (no/yes)	387	0.80	0.54-1.18	0.26

SD: Standard deviation

Figure 14. Atopic eczema at age 12 months in relation to maternal late pregnancy serum concentrations of a) nicotinamide and b) anthranilic acid





*Values are OR (95%CI)

As concentrations of nicotinamide and anthranilic acid showed a significant correlation ($r=0.218, p<0.001$), we undertook separate multivariate analyses of each metabolite in relation to atopic eczema, taking account of maternal characteristics and other potential confounding variables (Table 8). Taking account of these other variables, higher maternal serum nicotinamide ($p= 0.013$) and anthranilic acid ($p= 0.003$) concentrations remained significantly associated with lower risks of atopic eczema at age 12 months.

Table 8. Multivariate analysis of maternal and infant characteristics as predictors of atopic eczema at age 12 months, including a) serum nicotinamide, b) serum anthranilic acid levels in late pregnancy (n = 467)

	OR	CI	P-value
(a)			
Maternal			
Serum nicotinamide (SD)	0.70	0.52–0.93	0.013
Maternal age (years)	1.01	0.93 – 1.09	0.86
Pre-pregnancy BMI(kg/m^2)	1.07	1.01 – 1.13	0.02
Maternal education (6 groups)	0.83	0.65 – 1.06	0.14
Smoking in pregnancy (no/yes)	1.39	0.64 – 3.04	0.41
Parity (0/ 1+)	0.62	0.35 – 1.11	0.14
Eczema (no/yes)	1.28	0.49–3.34	0.62
Infant			
Sex (male=1/ female=2)	0.48	0.27 – 0.86	0.014
Gestational age (weeks)	0.97	0.79 – 1.19	0.74
Breast feeding duration (6 groups)	0.99	0.81 – 1.22	0.95
(b)			
Maternal			
Serum anthranilic acid (SD)	0.64	0.48 – 0.86	0.003
Maternal age (years)	1.00	0.92 – 1.09	0.98
Pre-pregnancy BMI(kg/m^2)	1.07	1.02 – 1.14	0.013
Maternal education (6 groups)	0.86	0.68 – 1.10	0.24
Smoking in pregnancy (no/ yes)	1.12	0.51 – 2.46	0.79
Parity (0/ 1+)	0.62	0.35 – 1.10	0.11
Eczema (no/yes)	1.09	0.41 – 2.92	0.86
Infant			
Sex (male=1/ female=2)	0.47	0.26 – 0.84	0.011
Gestational age (weeks)	0.96	0.78 – 1.18	0.70
Breast feeding duration (6 groups)	0.96	0.78 – 1.17	0.67

Maternal education groups (1= none, 2= GCSE, 3= O level (Ordinary Level General Certificate of Education), 4= A level (General Certificate of Education Advanced Level), 5= HND (Higher National Diploma), 6= Degree).

Breast feeding duration groups (completed months (1= Never tried, 2= <1, 3= 1–3, 4= 4–6, 5= 7–11, 6= 12 or more)).

The odds ratios and P values changed little after additionally taking account of rs 7512552 SNP, available for a subsample of the group (Table 9).

Table 9 . Multivariate analysis of maternal and infant characteristics as predictors of atopic eczema at age 12 months, including a) serum nicotinamide (n=372), b) serum anthranilic acid levels in late pregnancy (n = 372) taking into account rs 7512552 SNP

	OR	CI	P-value
(a)			
Maternal			
Serum nicotinamide (SD)	0.69	0.51-0.93	0.016
Maternal age (years)	1.02	0.94 - 1.12	0.62
Pre-pregnancy BMI(kg/m^2)	1.28	0.97 - 1.70	0.09
Maternal education (6 groups)	0.81	0.63 - 1.06	0.13
Smoking in pregnancy (no/yes)	1.46	0.64 - 3.31	0.37
Parity (0/ 1+)	0.63	0.34 - 1.16	0.14
Eczema (no/yes)	1.63	0.60- 4.37	0.34
Infant			
Sex (male=1/ female=2)	0.54	0.30 - 1.00	0.051
Gestational age (weeks)	0.96	0.76 - 1.21	0.76
Breast feeding duration (6 groups)	0.96	0.77 - 1.20	0.72
SNP rs7512552(no/yes)	0.78	0.51 - 1.19	0.25
(b)			
Maternal			
Serum anthranilic acid (SD)	0.61	0.45 - 0.83	0.002
Maternal age (years)	1.02	0.93 - 1.12	0.64
Pre-pregnancy BMI(kg/m^2)	1.30	0.98 - 1.72	0.07
Maternal education (6 groups)	0.86	0.66 - 1.12	0.28
Smoking in pregnancy (no/ yes)	1.05	0.45 - 2.44	0.91
Parity (0/ 1+)	0.61	0.33 - 1.13	0.12
Eczema (no/yes)	1.56	0.57 - 4.25	0.39
Infant			
Sex (male=1/ female=2)	0.54	0.29 - 1.00	0.048
Gestational age (weeks)	0.93	0.73 - 1.17	0.53
Breast feeding duration (6 groups)	0.90	0.72 - 1.13	0.39
SNP rs7512552(no/yes)	0.77	0.51 - 1.18	0.24

Maternal education groups (1= none, 2= GCSE, 3= O level (Ordinary Level General Certificate of Education), 4= A level (General Certificate of Education Advanced Level), 5= HND (Higher National Diploma), 6= Degree).

Breast feeding duration groups (completed months (1= Never tried, 2= <1, 3= 1-3, 4= 4-6, 5= 7-11, 6= 12 or more)).

Further examination of the data was undertaken specifically to analyse the potential influence of 52 infants who had questionnaire data but missing data on visible eczema that could potentially have contributed to the case definition of atopic eczema (52 infants at age 6 months, 5 infants at age 12 months). However, in the cohort of mothers who had tryptophan metabolite measured, this only affected one mother-offspring dyad in the group analysed for eczema at 6 months (n= 494) with no change in group characteristics (Table 3). Analysis showed no change in relation to the findings for either eczema at 6 month (Table 10) or eczema at 12 month.

Table 10 . Univariate analyses of maternal and infant characteristics as predictors of atopic eczema at age 6 months excluding one mother-offspring dyad with missing eczema data.

	N	OR	95%CI	P value
Maternal characteristics				
Age (years)	494	1.01	0.93 – 1.10	0.75
Pre-pregnancy BMI (SD)	490	1.29	0.99 – 1.68	0.06
Education (6 levels)	492	0.99	0.80 – 1.24	0.95
Smoking in pregnancy (no/yes)	494	0.70	0.27 – 1.84	0.47
Parity (0/ 1+)	494	0.65	0.37 – 1.16	0.15
Eczema in last 12 months (no/yes)	491	1.98	0.83-4.74	0.13
Maternal serum metabolite concentrations in late pregnancy				
Tryptophan (SD)	494	1.06	0.79 – 1.41	0.70
Kynurenine (SD)	494	1.04	0.78 -1.39	0.79
Kynurenic acid (SD)	494	1.25	0.94 – 1.67	0.13
Anthranilic acid (SD)	494	0.85	0.64 – 1.14	0.27
Nicotinamide (SD)	494	0.95	0.71 – 1.26	0.71
N1-Methylnicotinamide (SD)	494	0.92	0.69 – 1.23	0.57
Infant characteristics				
Sex (male=1/ female=2)	494	0.37	0.20 – 0.68	0.002
Gestational age (weeks)	494	0.99	0.80 – 1.22	0.92
Birth weight (kg)	491	1.29	0.71-2.35	0.41
Breast feeding (6 groups)	479	1.02	0.85 – 1.22	0.86
SNP rs7512552 (no/yes)	412	0.70	0.45-1.08	0.11

SD: Standard deviation

3.3.2 Associations between maternal tryptophan metabolites and infant atopy

81/2244 infants had both atopic eczema at age 6 months and atopy at 12 months; 75/2310 had atopic eczema and atopy at age 12 months. 81/198 infants who had eczema at age 6 months also had atopy at 12 months; 75/225 infants who had atopic eczema at age 12 months also have atopy at this age (Table 11). Maternal serum tryptophan metabolites concentrations did not relate to infant atopy at 12 months (Tables 12 and 13).

Table 11. Infant atopy at 12 months in relation to atopic eczema at age a) 6 months and b) 12 months.

a)

	Atopic eczema at 6 months	
Atopy at 12 months	No	Yes
No	1882	117
Yes	164	81

b)

	Atopic eczema at 12 months	
Atopy at 12 months	No	Yes
No	1907	150
Yes	178	75

Table 12 – Univariate analyses of maternal serum tryptophan metabolites as predictors of infant atopy at age 12 months

	n	RR	95% CI	P value
Maternal serum metabolite concentrations in late pregnancy				
Tryptophan (SD)	426	1.14	0.86–1.51	0.36
Kynurenine (SD)	426	1.12	0.83–1.52	0.46
Kynurenic acid (SD)	426	1.07	0.81–1.40	0.63
Anthranilic acid (SD)	426	0.94	0.70–126	0.67
Nicotinamide (SD)	426	0.89	0.68–1.17	0.39
N1–Methylnicotinamide (SD)	426	1.08	0.84–1.39	0.57

Table 13 – Multivariate analyses of maternal serum tryptophan metabolites as predictors of infant atopy at age 12 months

	n	RR	95% CI	P value
Maternal serum metabolite concentrations in late pregnancy				
Tryptophan (SD)	362	1.07	0.81–1.41	0.62
Kynurenine (SD)	362	1.04	0.79–1.37	0.79
Kynurenic acid (SD)	362	1.08	0.81–1.44	0.59
Anthranilic acid (SD)	362	0.92	0.71–1.20	0.53
Nicotinamide (SD)	362	1.00	0.76–1.33	0.98
N1–Methylnicotinamide (SD)	362	1.11	0.83–1.49	0.47

SD: Standard deviation

Adjusted for maternal age at childbirth, BMI, education, parity, smoking during pregnancy and atopy, and infant sex, gestational age and breastfeeding duration.

3.4 Discussion

The results show that maternal nicotinamide and related tryptophan metabolite concentrations were not associated with offspring atopic eczema at 6 months. However, higher late pregnancy maternal concentrations of nicotinamide and anthranilic acid were associated with a lower prevalence of eczema at age 12 months. These associations were robust to adjustment for potential confounding variables.

Nicotinamide is the precursor of NAD and is the sole substrate and an inhibitor of the nuclear enzyme PARP-1 (Jacobson et al., 1999). Through these functions, nicotinamide can enhance energy-dependent cellular processes such as DNA repair (Surjana et al., 2010), and maintain genomic stability and regulation of some transcription factors, particularly in relation to the expression of inflammatory cytokines, chemokines, adhesion molecules and inflammatory mediators (e.g. TNF- α, IL-6, IL-10, inducible nitric-oxide synthase (iNO)) (Virag and Szabo, 2002). Nicotinamide inhibits cAMP PDE and stabilises mast cells and leukocytes through inhibition of histamine and IgE release (Namazi, 2004). It has also been shown to increase the biosynthesis of ceramide and other stratum corneum lipids (Tanno et al., 2000) and prevents the upregulation of aquaporin, thereby decreasing water permeability and water loss (Song et al., 2008). Nicotinamide can improve the overall structure, moisture, and elasticity of skin as it increases the synthesis of collagen and may alter protein transcription and translocation in ways that alter the formation of keratin, filaggrin, and involucrin that are crucial for the barrier function of the stratum corneum (Bissett et al., 2003). Through these functions, nicotinamide has the potential to alter the disease processes associated with atopic eczema.

There is only limited evidence of an effect of dietary factors on serum tryptophan metabolite concentrations. Dietary niacin is readily converted to nicotinamide in the body and challenge studies have shown a robust rise in anthranilic acid concentrations after administration of sodium benzoate, a widely used preservative found in many foods and soft drinks (Lennerz et al., 2015) .These observations suggest that influences other than the intake of niacin and tryptophan may underlie the associations we found between tryptophan metabolites and infant atopic eczema.

Although maternal serum samples provide an objective measure of substances, serum levels may not be representative of stores or concentration of substances

in relevant tissue. Maternal serum concentrations levels may also not compare with fetal exposure and uptake.

As can be inferred from Figure 1, lower levels of anthranilic acid and nicotinamide do not point to a specific enzymatic block in the tryptophan pathway either genetically or environmentally induced, and lower levels of these two metabolites may be a general reflection of perturbation of the pathway or low substrate availability. Additionally, the findings (Figure 3) show a graded association between lower maternal nicotinamide and anthranilic acid levels and the offspring's risk of atopic eczema, pointing away from a simple genetically determined enzymatic block. However, the pathway has not been intensively investigated and we hope our data will stimulate more intensive investigation including analysis of genetic polymorphisms.

Although total tryptophan decreases in the third trimester of pregnancy (Luan et al., 2014), free tryptophan is increased due to decreased binding to albumin, as albumin decreases and non-esterified fatty acids increase in late pregnancy (Badawy, 2015). Nicotinamide is able to cross the human placenta, and fetal blood levels of nicotinamide are greater than corresponding maternal blood levels (Stockton and Paller, 1990). There are no reports of adverse effects due to nicotinamide in human fetuses, but the effect of high doses is unknown (Stockton and Paller, 1990). There are no human data on maternal use of B vitamins and tryptophan in relation to offspring atopic eczema. However, animal studies on long-term high dose nicotinamide supplementation in pregnancy have been associated with nicotinamide-induced oxidative tissue injury, insulin resistance and disturbed methyl metabolism that can lead to epigenetic changes (Li et al., 2013).

Maternal late pregnancy concentrations of nicotinamide and anthranilic acid were associated with infant eczema at age 12 months but not at age 6 months; this could also reflect heterogeneity in the etiology and pathogenesis of atopic eczema in early childhood (Loo et al., 2015). Maternal tryptophan metabolites in late pregnancy were not significantly related to the offspring risk of atopy at age 12 months. This is in contrast to the relationship between nicotinamide and anthranilic acid to offspring risk of atopic eczema at that age, suggesting that in addition to the immunomodulatory changes seen in these conditions, there may be other pathophysiological changes and genetic predispositions determining the risk of atopic eczema.

The data highlights a link between lower maternal nicotinamide levels and related tryptophan metabolites and an increased risk of atopic eczema in the offspring. Strengths of the data are its prospective nature, the standardised assessment of eczema by trained staff and control for confounding factors. Limitations of the data were the relatively small sample size of 497 and the lack of measurements of metabolite levels in early pregnancy. Replicating the findings in other populations would be a valuable next step. Modified UK Working Party Diagnostic Criteria for Atopic Dermatitis were used, where atopic disease in a first degree relative was omitted , aiming todisentangle the apparent heterogeneous phenotypes that ‘atopic eczema’ is now thought to represent; excluding those with no family history of atopy would remove an important group of infants from such studies. This modification has, however, not previously been validated. Although the association was independent of rs7512552 filaggrin status, no information was available on genetic variants that may influence tryptophan metabolism.

The data in this chapter are the first evidence of a link between maternal serum levels of nicotinamide and related metabolites to the risk of atopic eczema in the offspring. The findings point to potentially modifiable maternal influences on this complex, multifactorial condition and support the evidence that atopic eczema partly originates in utero.

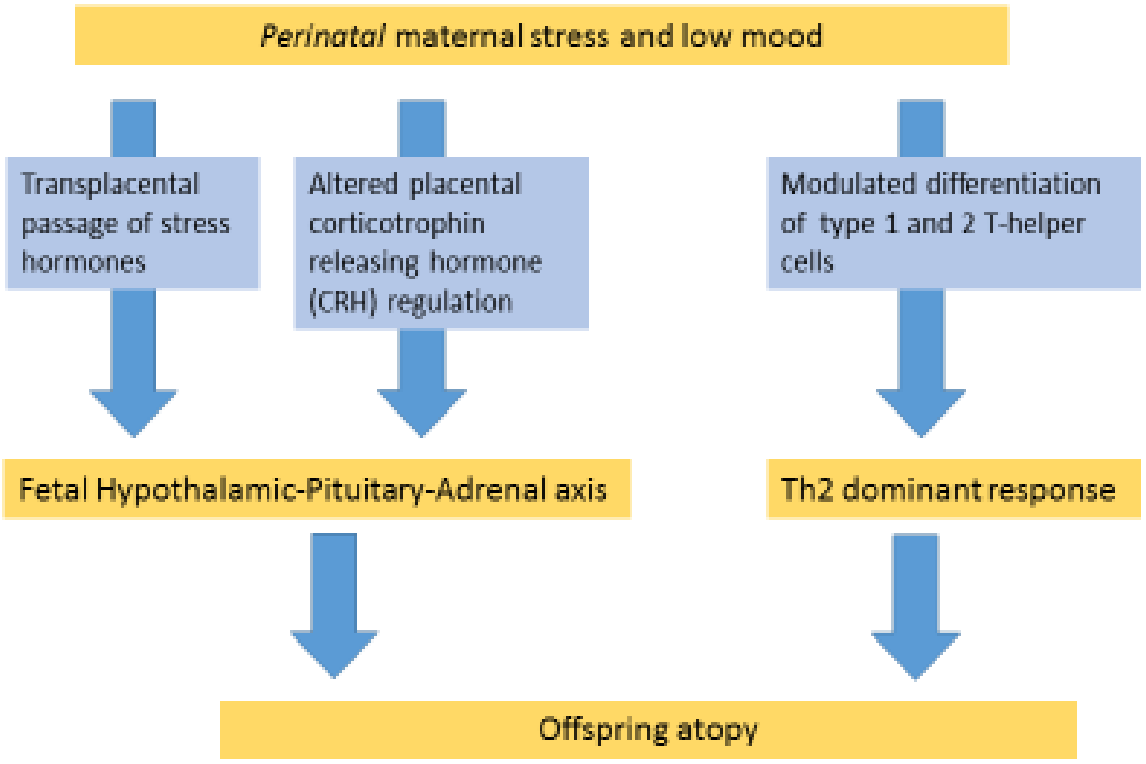
Chapter 4: Maternal stress and low mood in relation to offspring risk of atopic eczema

4.1 Introduction

There is increasing evidence that atopic eczema partly originates in utero, where genetic susceptibility and environmental exposures can result in immune dysregulation (Segerstrom and Miller, 2004), influencing the risk of developing the skin condition. Better understanding of such early life environmental exposures could help identification of preventative strategies.

Pathways by which maternal stress can cause fetal immune dysregulation leading to a propensity to develop atopic eczema and other atopic disorders have been proposed (Figure 15). In mice, transplacental passage of maternal stress hormones affects fetal HPA axis development, with prenatally stressed offspring demonstrating airway inflammation and hyper-responsiveness (Pincus-Knackstedt et al., 2006). Maternal stress also alters placental corticotrophin releasing hormone (pCRH) regulation, with the potential to influence the fetal HPA axis (Harris and Seckl, 2011). In addition, glucocorticoids and catecholamines released as a result of stress can modulate differentiation of type 1 and 2 T-helper (Th1 and Th2) cells, favouring a shift towards a Th2 humoral cell type reaction with overproduction of IL-4 and IL-10 and suppression of interleukin 12 (Elenkov, 2004). This inflammatory reaction is seen in atopic eczema and other atopic conditions.

Figure 15 Pathways by which perinatal maternal stress and low mood can lead to offspring atopy (evidence from animal studies)



Experimental studies in rodents and sheep have shown that maternal glucocorticoid exposure during the period around conception can have important effects on a range of organs, including the HPA axis, with implications for immune disorders such as atopic eczema (Langley-Evans et al., 1996, Bloomfield et al., 2004). In humans the consequences of stress around conception remain underexplored. In a recent systematic review, Andersson et al (Andersson et al., 2016) identified a number of studies linking prenatal maternal stress to an increased risk of offspring atopic eczema (Wen et al., 2011, Hartwig et al., 2014, de Marco et al., 2012, Sausenthaler et al., 2009, Larsen et al., 2014). Sausenthaler et al. (Sausenthaler et al., 2009) observed a link between maternal stress during pregnancy and an increased risk of atopic eczema in the first 2 years of life but not beyond that age, suggesting that the impact of prenatal stress weakens in the presence of other influencing factors. When examining other atopic disorders in relation to prenatal maternal stress, de Marco et al. (de Marco et al., 2012) reported higher odds ratios for asthma, eczema and allergic rhinitis, supporting an immunomodulatory effect of stress and stress hormones on the development of atopic conditions in humans. Furthermore, postpartum depression during the first 6 months of life has been linked with childhood atopic eczema at age 3 years, independently of prenatal maternal stress (Wang et al., 2016). Early life

persistent and increasing maternal postnatal depressive symptoms (Giallo et al., 2015, Kozyrskyj et al., 2008), greater caregiver perceived stress (Wright et al., 2002), and prenatal and postnatal negative life events (Chiu et al., 2012) have also been associated with an increased risk of offspring asthma.

It is possible that mothers experiencing stress may make poor health practice choices, including smoking, or differ in terms of their age, educational attainment, parity and history of eczema, which put their offspring at an increased risk of developing atopic eczema (Hoffman and Hatch, 1996), and thus previous studies may not have controlled for all potential confounding variables. Moreover, no previous studies have explored the link between maternal preconception stress/ low mood and offspring risk of developing atopic eczema.

The objective in this study was to determine whether the risk of developing infantile atopic eczema at ages 6 and 12 months was influenced by maternal stress and low mood, while controlling for a range of potential confounding factors and with particular focus on the effects of stress prior to conception. Furthermore, as similar mechanisms and processes of immune dysregulation occur in atopy and atopic eczema, infant atopy was examined in relation to maternal stress and low mood.

4.2 Methods

4.2.1 Southampton Women's Survey

In the UK Southampton Women's Survey (SWS), information on maternal diet, lifestyle, socioeconomic status, stress and psychological distress was collected from women at recruitment as described in Chapter 2 (Inskip et al., 2006a). Women who became pregnant were followed up through their pregnancies; 3158 live born infants were delivered. The findings reported in this chapter are based on the 3008 mother-offspring dyads assessed for atopic eczema at 6 and/or 12 months (Figure 16).

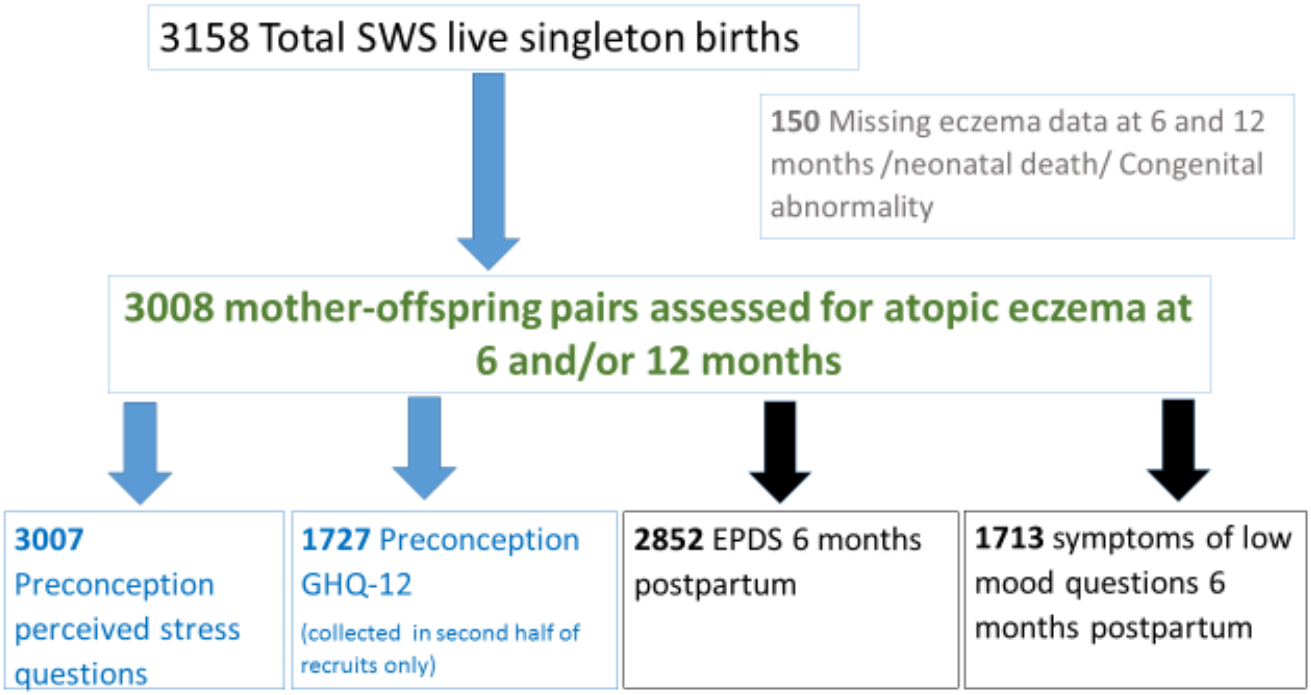
4.2.2 Stress and mood assessments

At recruitment (preconception) women were asked to report perceived stress using two questions ('To what extent do you feel that the stress or pressure you have experienced in your life has affected your health?' (n=3002), and 'In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?'(n=3007)), grading the impact of stress into one of five groups (none,

slightly/just a bit, moderately/a good bit, quite a lot, extremely/a great deal). These were adapted from the Short Form (36) Health Survey (SF-36) (Ware and Sherbourne, 1992). A subgroup of participants also completed the 12 item General Health Questionnaire (GHQ-12) (n= 1729) (Appendix F) assessing mental wellbeing, in which a score of ≥3 is considered indicative of psychological distress (Goldberg and Williams, 1988).

At 6 months postpartum, mothers completed the Edinburgh Post-natal Depression Scale (EPDS) (Appendix G), where higher scores indicate lower mood and a score of 13 or more is considered indicative of probable major depression (Matthey et al., 2006). A subgroup of mothers were also asked if they had experienced symptoms of low mood between delivery and infant age 6 months (episodes of feeling sad, depressed or gloomy for most of day, unable to find pleasure in things normally enjoyed, lost interest in things normally enjoyed, feeling tired or worn out and episodes of having less energy than usual); these questions were adapted from the Patient Health Questionnaire- 9 (PHQ-9) (Kroenke et al., 2001)(Appendix H).

Figure 16. Study sample



4.2.3 Outcome assessment

4.2.3.1 Atopic eczema

Case definition of atopic eczema and atopy was based on the UKWPDC Working for the definition on atopic eczema (Williams et al., 1994a) and skin prick testing, respectively, as described in Chapter 2.

4.2.4 Statistical analysis

Confounding variables were determined prior to the analysis using a DAG (Figure 17) (Greenland et al., 1999). Maternal factors that were identified as confounding variables were:

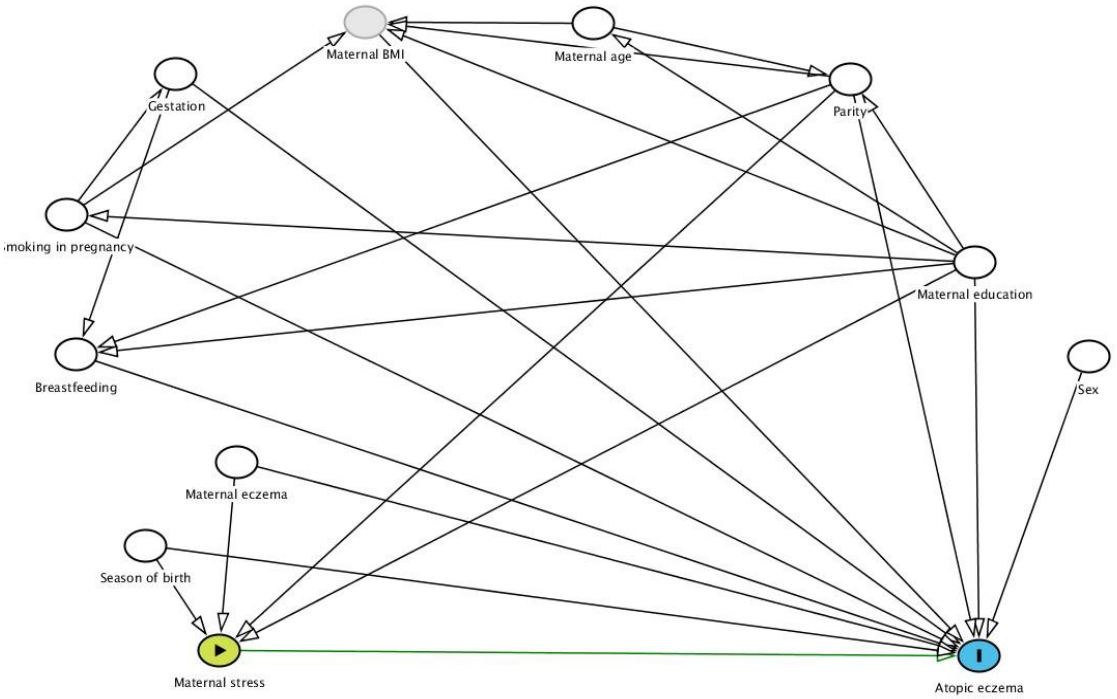
- Age at child's birth
- Education
- Smoking
- Parity
- Eczema in the 12 months preceding recruitment.

Infant confounding variables were:

- Gestational age
- Season of birth
- Breastfeeding

Infant sex, was identified as a competing exposure. This was adjusted for to improve the accuracy of the model.

Figure 17. Maternal stress/mood and infantile atopic eczema DAG



Logistic regression analyses were performed (Stata version 14.1, Statacorp LP, TX) to relate maternal stress/mood to infant atopic eczema at ages 6 and 12 months. P values < 0.05 were considered statistically significant. The five-category variables describing stress preconception were analysed as continuous variables.

Sensitivity analysis was used to compare the risk of atopic eczema in infants conceived less than 1 year after the mothers reported perceived stress at the initial preconception assessments to the risk of atopic eczema in infants in the whole study cohort.

Further logistic regression models were used to analyse the relations between preconception stress (as determined by both stress affecting health and stress in daily living jointly) and postnatal low mood (as determined by EPDS score) and

infant atopic eczema at ages 6 and 12 months. GHQ-12 scores and postnatal symptoms of low mood were not included in this analysis as these were only assessed in subsets of the study population.

Similarly, univariate (unadjusted) and multivariate (adjusted) Poisson regression analyses were used to relate maternal stress and low mood to infant atopy at 12 months. The same confounders were considered when examining infant atopy and atopic eczema to allow for comparison of the two outcomes, with the exception of accounting for maternal atopy instead of maternal eczema as this is more relevant to this analysis.

4.3 Results

4.3.1 Cohort characteristics

Maternal and infant characteristics are summarised in Table 14. Among the study group, the mothers' average age at their children's birth was 30.7 years (standard deviation (SD) = 3.8); 51.4% were primiparous, 15.7% smoked during pregnancy and 7.0% of the mothers had eczema in the past 12 months. The mean duration between the initial questionnaire and conception was 2.2 years. 51.9% of infants were male; mean birthweight of infants was 3.44 kg (SD 0.55) and gestational age 40.1 weeks (IQR 39.0-41.0). 2956 infants were assessed for eczema at age 6 months, 8.9% of which had atopic eczema. At age 12 months 2872 infants were assessed and 9.4% had atopic eczema.

Table 14. Characteristics of the study population

	Total n	%, Median (IQR) or Mean (SD)
Maternal		
Age at child's birth (y)	3008	30.7 (3.8)
% A level or higher	2999	59.1%
% Smoking in pregnancy	2870	15.7%
% Primiparous	3005	51.4%
% Eczema in last 12 months	2815	7.0%
Infant		
% Male	3008	51.9%
Gestational age (weeks)	3008	40.1 (39.1-41.0)
Birthweight (kg)	2981	3.44 (0.55)
Born in winter	673	22.4%
Born in spring	726	24.1%
Born in summer	807	26.8%
Born in autumn	802	26.7%
Breast feeding (completed months)		
Never breast fed	527	18.4%
<1	581	20.2%
1 to 3	615	21.4%
4 to 6	484	16.9%
7 to 11	422	14.7%
12 or more	241	8.4%
6 month assessment		
Age (wks)	2956	27.4 (26.1-33.7)
% Atopic eczema as per UK working party criteria	2956	262 (8.9%)
12 month assessment		
Age (wks)	2872	53.7 (52.6-55.0)
% Atopic eczema as per UK working party criteria	2872	270 (9.4%)

Table 15 provides descriptive data on maternal mood. Preconception, 28.2% had a GHQ-12 score of ≥3, suggestive of psychological distress; 17.7% reported that stress affected their health 'quite a lot' or 'extremely' preconception and 24.6% reported 'quite a lot' or 'a great deal' of stress experienced in daily living. At 6 months after delivery, 46.8% reported episodes of feeling sad, depressed or gloomy for most of the day; the median EPDS score was 10 (IQR 6-15) and 36.7% had a score of 13 or more.

Table 15. Descriptive data of maternal stress and mood

	Total n	n (%) or Median (IQR)
PRECONCEPTION		
Maternal stress preconception		
Stress in life affecting health	3007	
None		723 (24.0%)
Slightly		1203 (40.0%)
Moderately		548 (18.2%)
Quite a lot		448 (14.9%)
Extremely		85 (2.8%)
Stress in daily living in last 4 weeks	3002	
None		248 (8.3%)
Just a bit		1373 (45.7%)
A good bit		644 (21.5%)
Quite a lot		572 (19.1%)
A great deal		165 (5.5%)
Maternal mental wellbeing preconception		
Psychological distress ascertained by General Health Questionnaire	1729	
No (score <3)		1241 (71.8%)
Yes (score ≥3)		488 (28.2%)
POSTNATAL		
Maternal mood in worst 2 week period between birth and infant age 6 months		
Edinburgh Postnatal Depression Score	2853	10 (6-15)
Edinburgh Postnatal Depression Score ≥13		1046 (36.7%)
Postnatal maternal mood between delivery and infant age 6 months		
Between delivery and infant age 6 months experienced episodes of:		
Feeling sad, depressed or gloomy for most of day	1713	802 (46.8%)
Unable to find pleasure in things normally enjoy	1660	502 (30.2%)
Lost interest in things normally enjoy	1660	490 (29.5%)
Feeling tired of worn out	1660	1319 (79.7%)
Less energy than usual	1660	1294 (78.0%)

4.3.2 Association between maternal stress and mood and infant atopic eczema at age 6 months

Table 16 shows univariate (unadjusted) and multivariate (adjusted) analyses of maternal and infant characteristics in relation to infant atopic eczema at age 6 months. Stress in daily living preconception was associated with increased risk of offspring atopic eczema (OR 1.13, 95% CI 1.01-1.28, p= 0.039); this association weakened slightly after adjusting for potential confounders (adjusted OR 1.12, 95% CI 0.99-1.28, p= 0.072). Episodes of feeling sad, depressed or gloomy for most of the day experienced between delivery and infant age 6 months were associated with an increased risk of atopic eczema after taking confounding variables into account (OR 1.40, 95%CI 1.00-1.96, p= 0.048). Other measures of maternal postnatal mood and postnatal EPDS score were not associated with offspring atopic eczema at age 6 months. As family history of atopy was omitted as a criterion in the case definition of atopic eczema, maternal and paternal eczema in childhood was additionally controlled for in the analyses; this had little effect on the 6 month eczema analyses shown in Table 16 (Table 17).

Table 16. Maternal stress/mood preconception and postnatally in relation to infant atopic eczema at age 6 months

	Unadjusted				Adjusted *			
Outcome	OR	95% CI	P value	n	OR	95% CI	P value	N
Preconception maternal mood								
Stress in life affected health (5 levels)	1.10	0.98–1.24	0.093	2956	1.08	0.96–1.23	0.22	2548
Stress in daily living in the past 4 weeks (5 levels)	1.13	1.01–1.28	0.039	2951	1.12	0.99–1.28	0.072	2546
Psychological distress ascertained by GHQ–12 (no/yes)	1.21	0.85–1.73	0.30	1694	1.24	0.84–1.83	0.28	1419
Postnatal maternal mood between delivery and age 6 months								
Edinburgh Postnatal Depression Score	1.01	0.99–1.03	0.28	2816	1.01	0.99–1.04	0.26	2431
Edinburgh Postnatal Depression Score ≥13	1.13	0.87–1.48	0.35	2816	1.12	0.84–1.49	0.44	2431
Between delivery and infant age 6 months experienced episodes of:								
Feeling sad, depressed or gloomy for most of day (yes/no)	1.33	0.97–1.83	0.08	1713	1.40	1.00–1.96	0.048	1646
Unable to find pleasure in things normally enjoy (yes/no)	1.34	0.95–1.87	0.09	1660	1.26	0.88–1.80	0.20	1596
Lost interest in things normally enjoy (yes/no)	1.27	0.90–1.79	0.17	1660	1.26	0.88–1.79	0.22	1596
Feeling tired or worn out (yes/no)	1.07	0.72–1.61	0.73	1660	1.01	0.67–1.54	0.95	1596
Less energy than usual (yes/no)	1.35	0.89–2.06	0.16	1660	1.26	0.81–1.94	0.30	1596

*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, and infant sex, gestational age at birth, season of birth and breastfeeding duration

Table 17 . Maternal stress/mood preconception and postnatally in relation to infant atopic eczema at age 6 months, with additional adjustment for maternal and paternal history of atopic eczema

Outcome	OR	95% CI	P value	n
Preconception maternal mood				
Stress in life affected health (5 levels)	1.05	0.93–1.20	0.44	2504
Stress in daily living in the past 4 weeks (5 levels)	1.10	0.97–1.25	0.14	2502
Psychological distress ascertained by GHQ-12 (no/yes)	1.12	0.75–1.67	0.58	1397
Postnatal maternal mood between delivery and age 6 months				
Edinburgh Postnatal Depression Score	1.01	0.99–1.03	0.36	2388
Edinburgh Postnatal Depression Score ≥13	1.07	0.80–1.44	0.63	2388
Between delivery and infant age 6 months experienced episodes of:				
Feeling sad, depressed or gloomy for most of day (yes/no)	1.36	0.97–1.91	0.071	1616
Unable to find pleasure in things normally enjoy (yes/no)	1.20	0.84–1.73	0.32	1566
Lost interest in things normally enjoy (yes/no)	1.19	0.83–1.73	0.34	1566
Feeling tired or worn out (yes/no)	0.99	0.65–1.51	0.96	1566
Less energy than usual (yes/no)	1.20	0.78–1.87	0.40	1566

* Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, infant sex, gestational age at birth, season of birth, breastfeeding duration and paternal eczema.

4.3.3 Association between maternal stress and mood and infant atopic eczema at age 12 months

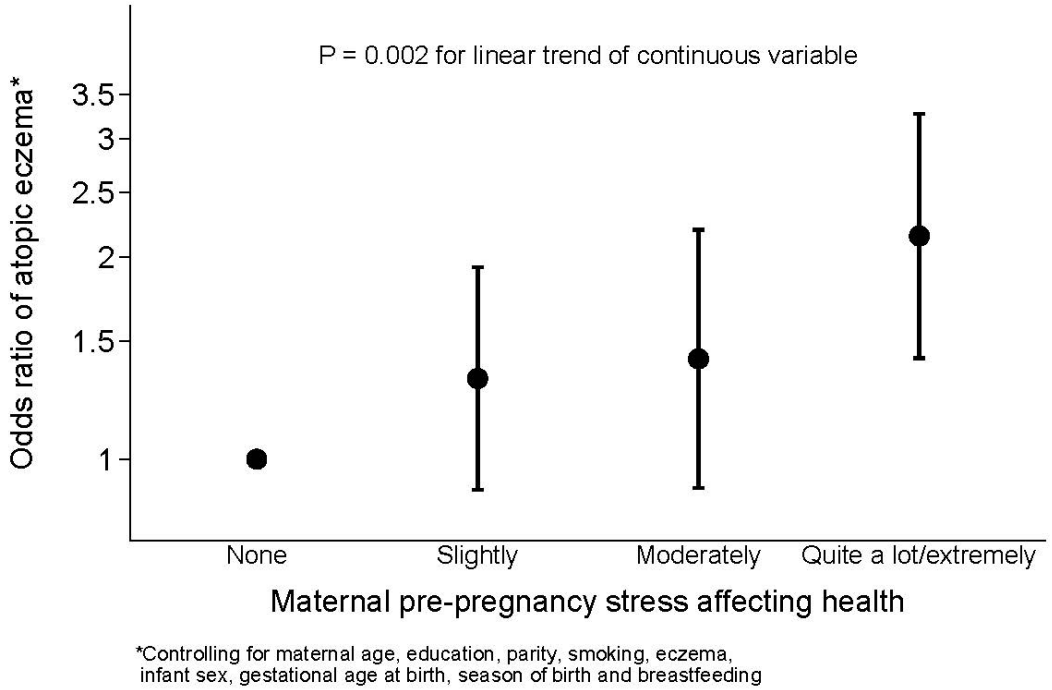
Table 18 shows similar analyses in relation to infant atopic eczema at age 12 months. Psychological distress ascertained by the GHQ-12 preconception was associated with increased risk of atopic eczema (OR 1.43, 95% CI 1.00-2.04, p= 0.044), but the association weakened slightly after adjusting for confounding variables (OR 1.37, 95% CI 0.93-2.00, p= 0.11). Increased stress in life affecting health (OR 1.21, 95% CI 1.08-1.35, p= 0.001, Figure 18) and increased stress in daily living (OR 1.16, 95%CI 1.03-1.30, p= 0.014, Figure 19) preconception were associated with an increased risk of offspring atopic eczema at age 12 months; these associations remaining significant after adjusting for confounding variables (p= 0.014 and p= 0.046, respectively).

Table 18. Maternal stress/mood preconception and postnatally in relation to infant atopic eczema at age 12 months

	Unadjusted				Adjusted *			
Outcome	OR	95% CI	P value	n	OR	95% CI	P value	n
Preconception maternal mood								
Stress in life affected health (5 levels)	1.21	1.08–1.35	0.001	2871	1.21	1.07–1.37	0.002	2448
Stress in daily living in the past 4 weeks (5 levels)	1.16	1.03–1.30	0.014	2867	1.14	1.00–1.29	0.046	2447
Psychological distress ascertained by GHQ-12 (no/yes)	1.43	1.00–2.04	0.044	1629	1.37	0.93–2.01	0.11	1340
Postnatal maternal mood between delivery and age 6 months								
Edinburgh Postnatal Depression Score	1.02	1.00–1.04	0.045	2721	1.02	1.00–1.05	0.041	2330
Edinburgh Postnatal Depression Score >13	1.09	0.84–1.42	0.52	2721	1.08	0.82–1.44	0.58	2330
Between delivery and infant age 6 months experienced episodes of:								
Feeling sad, depressed or gloomy for most of day (yes/no)	1.47	1.06–2.04	0.022	1621	1.52	1.08–2.13	0.016	1574
Unable to find pleasure in things normally enjoy (yes/no)	1.54	1.09–2.18	0.013	1569	1.55	1.08–2.20	0.016	1524
Lost interest in things normally enjoy (yes/no)	1.56	1.11–2.21	0.011	1569	1.57	1.10–2.24	0.013	1524
Feeling tired or worn out (yes/no)	1.51	0.95–2.40	0.08	1569	1.52	0.95–2.42	0.08	1524
Less energy than usual (yes/no)	1.75	1.09–2.79	0.020	1569	1.68	1.05–2.70	0.032	1524

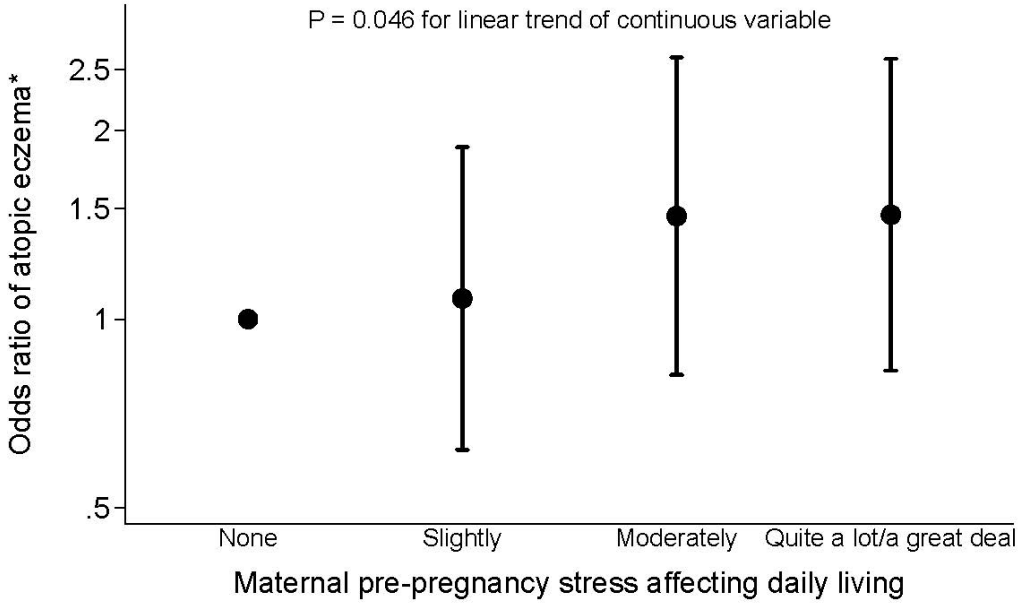
*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, and infant sex, gestational age at birth, season of birth and breastfeeding duration

Figure 18 Maternal preconception perceived stress affecting health in relation to offspring atopic eczema at age 12 months



*Controlling for maternal age, education, parity, smoking, eczema, infant sex, gestational age at birth, season of birth and breastfeeding

Figure 19. Maternal preconception perceived stress affecting daily living in relation to offspring atopic eczema at age 12 months



*Controlling for maternal age, education, parity, smoking, eczema, infant sex, gestational age at birth, season of birth and breastfeeding

Values are OR (95% CI)

Examining postnatal maternal mood, all five measures (experiencing episodes of feeling sad, depressed or gloomy for most of the day unable to find pleasure in things normally enjoyed, loss of interest in things normally enjoyed, having less energy than usual) ascertained 6 months after delivery showed associations or trends towards lower maternal mood being associated with a higher odds of offspring atopic eczema at age 12 months in both unadjusted and adjusted analyses (p= 0.08 to p= 0.013 (Table 18)); adjusting for potential confounders generally increased the odds ratios of atopic eczema. A higher EPDS score examined as a continuous variable was also significantly associated with increased risk of atopic eczema at 12 months (OR 1.02, 95%CI 1.00-1.04, p= 0.045), but there was no significant association with an EPDS score of ≥13.

Additionally controlling for maternal and paternal eczema in childhood somewhat attenuated the association of maternal preconception stress in daily living in the past 4 weeks with atopic eczema at 12 months (adjusted OR 1.10, 95%CI 0.97-1.25, p= 0.14 (Table 19)), but had little effect on the associations of preconception stress in life affecting health and postnatal stress variables with atopic eczema at age 12 months (Table 19).

Table 19. Maternal stress/mood preconception and postnatally in relation to infant atopic eczema at age 12 months, with additional adjustment for maternal and paternal history of atopic eczema

	Adjusted *			
Outcome	OR	95% CI	P value	n
Preconception maternal mood				
Stress in life affected health (5 levels)	1.17	1.03–1.33	0.014	2404
Stress in daily living in the past 4 weeks (5 levels)	1.10	0.97–1.25	0.14	2403
Psychological distress ascertained by GHQ-12 (no/yes)	1.27	0.86–1.89	0.23	1318
Postnatal maternal mood between delivery and age 6 months				
Edinburgh Postnatal Depression Score	1.02	1.00–1.05	0.066	2287
Edinburgh Postnatal Depression Score >13	1.05	0.78–1.40	0.80	2287
Between delivery and infant age 6 months experienced episodes of:				
Feeling sad, depressed or gloomy for most of day (yes/no)	1.51	1.07–2.14	0.020	1544
Unable to find pleasure in things normally enjoy (yes/no)	1.52	1.06–2.19	0.024	1494
Lost interest in things normally enjoy (yes/no)	1.51	1.05–2.18	0.028	1494
Feeling tired or worn out (yes/no)	1.56	0.96–2.53	0.07	1494
Less energy than usual (yes/no)	1.67	1.03–2.72	0.039	1494

*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, infant sex, gestational age at birth, season of birth, breastfeeding duration and paternal eczema.

Sensitivity analysis indicated that infants of women who reported greater perceived stress affecting health and stress in daily living and were conceived less than 1 year after the mother completed the initial preconception assessment had higher odds of developing atopic eczema at age 12 months compared with the offspring whose mothers did not report stress; this effect was greater than that seen among women in the whole study cohort (ORs of 1.25 versus 1.21 for stress affecting health, 1.22 versus 1.14 for stress in daily living for adjusted analyses) (Table 20).

Table 20 . Maternal preconception perceived stress affecting heath and stress in daily living in relation to offspring atopic eczema at age 12 months in infants conceived in less than a year from initial preconception maternal assessment

	Unadjusted				Adjusted*			
	OR	95% CI	P value	n	OR	95% CI	P value	n
Stress in life affected health (5 levels)	1.21	1.01–1.45	0.034	918	1.25	1.04–1.51	0.019	879
Stress in daily living in the past 4 weeks (5 levels)	1.22	1.01–1.46	0.039	917	1.22	1.01–1.48	0.042	879

*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, and infant sex, gestational age at birth, season of birth and breastfeeding duration.

We undertook further multivariate analyses to examine the relation of offspring atopic eczema with preconception stress as determined by perceived stress affecting health and stress in daily living, in combination with postnatal low mood as determined by EPDS (Table 21). For atopic eczema at age 6 months, we found no association with stress/low mood either preconception or postnatally. However, examining atopic eczema at age 12 months; stress affecting health at preconception (p= 0.015) was associated with an increased risk of atopic eczema but no significant association was seen between postnatal mood and eczema after taking account of preconception stress/mood variables.

Table 21. Maternal preconception stress and postnatal mood in relation to infant atopic eczema at ages 6 and 12 months

	OR	95% CI	P value	n
Infant atopic eczema age 6 months				2429
Preconception				
Stress in life affected health (5 levels)	1.04	0.91–1.20	0.57	
Stress in daily living in the past 4 weeks (5 levels)	1.09	0.94–1.25	0.24	
Postnatal				
EPDS	1.01	0.98–1.03	0.48	
Infant atopic eczema age 12 months				2329
Preconception				
Stress in life affected health (5 levels)	1.80	1.03–1.35	0.015	
Stress in daily living in the past 4 weeks (5 levels)	1.07	0.93–1.22	0.34	
Postnatal				
EPDS	1.01	0.99–1.04	0.24	

*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and eczema, and infant sex, gestational age at birth, season of birth and breastfeeding duration

A sensitivity analysis omitting 52 infants who had questionnaire data but missing data on visible eczema that could potentially have contributed to the case definition of atopic eczema (52 infants at age 6 months, 5 infants at age 12 months) showed little change in relation to the findings for either 6 month eczema and perceived stress in life affecting health (OR 1.11, 95% CI 0.99–1.25), perceived stress affecting daily living in the past 4 weeks (OR 1.13, 95% CI 1.01–1.28) and EPDS (OR 1.01, 95% CI 0.99–1.03) or 12 month eczema and the same variables OR 1.21 (95% CI 1.08–1.36), 1.16 (95% CI 1.03–1.30) and 1.02 (95% CI 1.00–1.04), respectively.

4.3.4 Association between maternal stress and mood and infant atopy at age 12 months

No significant associations were seen between prenatal and postnatal maternal stress and mood variables and infant atopy at age 12 months (Table 22). Infants of mothers reporting episodes of less energy than usual between delivery and

infant age 6 months had a higher risk of atopy at age 12 months (RR 1.61, 95%CI 1.04–2.50, p= 0.034). This isolated variable, however, may not indicate low mood in the absence of other indicators to suggest low mood.

Table 22. Maternal stress/mood preconception and postnatally in relation to infant atopy at age 12 months

	Unadjusted				Adjusted *			
Outcome	RR	95% CI	P value	n	RR	95% CI	P value	n
Preconception maternal mood								
Stress in life affected health (5 levels)	1.03	0.93–1.14	0.61	2312	1.11	0.99–1.24	0.077	1719
Stress in daily living in the past 4 weeks (5 levels)	0.92	0.82–1.03	0.14	2309	0.95	0.84–1.08	0.46	1718
Psychological distress ascertained by GHQ-12 (no/yes)	0.97	0.69–1.37	0.85	1375	1.05	0.71–1.55	0.82	990
Postnatal maternal mood between delivery and age 6 months								
Edinburgh Postnatal Depression Score	1.00	0.98–1.02	0.73	2261	1.01	0.99–1.03	0.43	1686
Edinburgh Postnatal Depression Score ≥13	0.91	0.71–1.17	0.47	2261	0.93	0.70–1.24	0.63	1686
Between delivery and infant age 6 months experienced episodes of:								
Feeling sad, depressed or gloomy for most of day (yes/no)	0.94	0.71–1.26	0.69	1416	0.99	0.73–1.35	0.95	1228
Unable to find pleasure in things normally enjoy (yes/no)	1.10	0.81–1.50	0.54	1405	1.03	0.74–1.45	0.85	1218
Lost interest in things normally enjoy (yes/no)	0.98	0.71–1.35	0.91	1405	0.98	0.70–1.39	0.93	1218
Feeling tired or worn out (yes/no)	1.17	0.80–1.70	0.41	1405	1.00	0.68–1.48	0.99	1218
Less energy than usual (yes/no)	1.62	1.07–2.45	0.022	1405	1.61	1.04–2.50	0.034	1218

*Adjusted for maternal age at birth, education, smoking in pregnancy, parity and atopy, and infant sex, gestational age at birth, season of birth and breastfeeding duration

4.4 Discussion

We found that maternal preconception stress was associated with an increased risk of offspring atopic eczema at age 12 months but not at age 6 months. These associations were robust to adjustment for potentially confounding variables including maternal history of eczema in the past 12 months, maternal smoking during pregnancy, infant gestational age, sex and breastfeeding duration. Findings were similar for maternal psychological distress preconception. Low maternal mood between delivery and 6 months postpartum assessed using the EPDS as a continuous variable (but not as a dichotomous variable) was also associated with an increased risk of infantile atopic eczema at age 12 months, but no significant association between postnatal mood and eczema was seen after taking account of preconception stress/mood variables.

Maternal stress in the prenatal (Anderson and Dinulos, 2009) and postnatal (Wang et al., 2016) periods have previously been shown to be linked to offspring atopic eczema. The data described in this chapter, however, are the first to show a link between maternal stress preconception and the risk of this skin disorder.

Previous studies have described potential mechanisms by which prenatal maternal stress can impact the developing fetus, some of which are thought to lead to atopy in the offspring. For example, stress is associated with raised cortisol levels and although the majority of the maternal cortisol is metabolised as it crosses the placenta, 10–20% can pass through the placenta un-metabolised; this amount can double the much lower fetal cortisol concentration, and a linear relationship between fetal cortisol concentration and maternal cortisol concentration has been observed (Gitau et al., 1998). pCRH which is also released as a result of maternal stress, creates a positive feedback loop and a subsequent increase in pCRH, adrenocorticotropic hormone (ACTH) and cortisol. pCRH affects both mother and fetus and has been shown to impact fetal programming and influencing health outcomes (Sandman, 2015). Furthermore, mothers experiencing psychosocial stress or nervousness during pregnancy have been found to have raised maternal serum pro-inflammatory cytokines (Coussons-Read et al., 2007) and higher umbilical cord IgE concentrations (Lin et al., 2004), respectively. Despite maternal neuroendocrine changes occurring as part of pregnancy, maternal psychosocial status has also been linked with circulating plasma levels of neuroendocrine parameters including ACTH, β-endorphin and cortisol (Wadhwa et al., 1996). Chang et al. (Chang et al., 2016a) proposed a further potential mechanism linking maternal stress with increased

offspring susceptibility to atopic eczema where the placenta fails to maintain its detoxifying ability when reactive oxygen species are high during stress, exposing the developing foetus to oxidative stress.

The stress model is complex and has evolved in recent years with some studies exploring the link between atopic eczema and certain aspects of this model, including exposure to stressors, such as significant life events, perceived stress and stress related disorders including anxiety and depression prenatally. Wen et al. (Wen et al., 2011) noted that self-reported psychological distress in the third trimester, cord blood IgE and lymphotoxin (LT-α) and high-affinity receptor for IgE (FcεRI-β) genotypes were associated with offspring atopic eczema at 2 years of age. Children of mothers who experienced stressful life events (divorce, mourning, loss of job) during pregnancy were found to have a moderately increased risk of atopic eczema, wheezing, asthma and allergic rhinitis (de Marco et al., 2012). Hartwig et al (Hartwig et al., 2014) observed similar associations where a higher likelihood of atopic asthma and eczema in 14 year olds was seen in those whose mothers experienced negative life events in the second half of pregnancy.

Physiological responses to acute and chronic stress differ. The release of stress hormones in adaptation to acute stress in order to maintain homeostasis is referred to as allostasis (Sterling and Eyer, 1988). Increased allostatic load develops as a result of recurring exposure to stress, protracted response to stress and incapability to adapt (McEwen, 2000). Although the nature, severity, significance and persistence of stress are important (von Hertzen, 2002), personality traits, coping ability and physical state effect the way humans respond to stress (Conti, 2000). As individuals respond to stress differently, quantifying stress exposures and an individual's resistance to stress can be difficult to determine (Kopin, 1995).

The data shows that infants conceived within 1 year of their mothers reporting increased stress affecting health and stress in daily living had higher odds of developing atopic eczema when compared with the remainder of the study cohort, suggesting that stress/low mood experienced closer to the time of conception may have a greater impact on the risk offspring atopic eczema. The stages of preconception and pregnancy that are most sensitive to immunomodulatory effects of maternal stress have not been identified. Evidence from animal studies support the notion that maternal exposure to stress such as undernutrition around conception (Langley-Evans et al., 1996, Bloomfield et al.,

2004, Dodic et al., 1998, De Blasio et al., 2007) and stress such as restraint, noise and light stress in early pregnancy can affect the development of a number of systems, including the immune system and the HPA axis (Henry et al., 1994, Kay et al., 1998). However, evidence is scarce on the impact of periconceptional stress on HPA axis development in humans. It is unclear whether neuroendocrine changes related to maternal stress occurring around the time of conception influence the risk of atopic eczema in the same mechanistic ways if they occurred later in pregnancy. Furthermore, evidence that exposure to stress during sensitive stages of development in early life can affect the programming of the HPA axis through epigenetic changes and may result in a Th2 dominant immune response has been reported (Cheng et al., 2015). Similarly, methylation of the gene encoding the glucocorticoid receptor (NR3C1) has been shown to be associated with exposure to prenatal psychological distress (Braithwaite et al., 2016).

The current study found that maternal stress was associated with infant eczema at age 12 months but not at age 6 months; this could reflect heterogeneity in the aetiology and pathogenesis of atopic eczema in early childhood (Loo et al., 2015). These data are consistent with the observations previously described, where atopic eczema occurring in late infancy was associated with environmental factors and atopic eczema with an early onset in the first 6 months of life was mainly associated with familial factors (Loo et al., 2015). However, taking maternal and paternal history of childhood eczema into account in this study had little effect on the associations between maternal stress and mood infant atopic eczema at age 6 months, nonetheless it attenuated the effect of maternal stress of daily living on infant atopic eczema at age 12 months.

Furthermore, the data shows no links between preconception and postpartum maternal stress and mood and infant atopy at age 12 months. This contrasts to the findings in relation to offspring risk of atopic eczema at age 12 months. Allergic disease has been linked to maternal stress and a number of mechanisms have been proposed as described above. However, the discrepancy between the associations of preconception and postpartum stress and mood with infant atopic eczema but not with infant atopy suggests that other mechanisms and pathways may be involved in the various allergic conditions within atopy.

These data highlight a link between maternal stress at preconception and an increased risk of atopic eczema in the offspring at 12 months. Strengths of the data are the large sample size, the prospective nature of the Southampton Women's Survey, collection of true preconception data, the standardised

assessment of eczema by trained staff and control for confounding factors. The questions on perceived stress in daily living and stress affecting health preconception were directly adapted from the SF-36 Health Survey, which is an established research tool for ascertaining perceived stress (Ware and Sherbourne, 1992). The GHQ-12 which was introduced half way through the study, is likewise, a widely utilised standard questionnaire assessing mental wellbeing, which has been validated as measuring psychological distress (Goldberg and Williams, 1988). There are many research papers utilizing EPDS as a continuous scale in relation to different outcomes (Venkatesh et al., 2014, Chaudron et al., 2010). Using the EPDS as a continuous outcome better captures the multiple dimensions ascertained by the questionnaire, including anxiety, low mood and a sense of helplessness (Reichenheim et al., 2011), which may not be captured by dichotomising the score (as used in clinical practice to screen for depression).

Nevertheless, limitations were the lack of evaluation of stress during pregnancy and the use of questionnaire-based assessments of stress where there is potential for reporting bias. A further limitation is that 52 infants at age 6 months and 5 infants at age 12 months had missing information on visible eczema that could potentially have contributed to the case definition of atopic eczema; however, a sensitivity analysis omitting these infants with a missing skin examination information showed little change in the findings. Bioindicators relating to stress were not collected; however, indicators such as salivary cortisol may be difficult to interpret as concentrations fluctuate, particularly during pregnancy.

In summary, the data in this study demonstrates a novel link between preconception maternal stress / low mood and the risk of atopic eczema in the offspring. The findings point to potentially modifiable maternal influences on this complex, multifactorial skin condition and add to the evidence that atopic eczema partly originates during development before birth.

Chapter 5: Fetal and infant growth in relation to infant atopic eczema

5.1 Introduction

Linear growth impairment in children with atopic eczema is a clinical concern (Kristmundsdottir and David, 1987, Massarano et al., 1993, Park et al., 2013). National recommendations in the US (Eichenfield et al., 2014b), UK (Royal College of Paediatrics and Child Heath, 2011, The National Institute for Health and Care Excellence and National Collaborating Centre for Women's and Children's Health (UK), 2007) and other settings are that growth is monitored as part of clinical care for children with atopic eczema. Possible reasons for growth faltering have been proposed and include effects of the inflammatory skin disease (Wong et al., 2016), corticosteroid treatment (Aylett et al., 1992), poor nutrition as a result of an inappropriately restrictive diet (Keller et al., 2012), and eczema associated sleep disturbance (Silverberg and Paller, 2015). Hitherto, little attention has been paid to the possibility of pre-morbid changes in growth trajectory in infants with atopic eczema. Any such changes in pre-morbid growth may help explain the growth impairment in children with atopic eczema while also providing insights into the etiology of the skin disorder. The development of inflammatory diseases such as atopic eczema is influenced by both genetic determinants and environmental exposures in early life, including poor nutrition (Beach et al., 1982, Varg et al., 2011), maternal stress (Andersson et al., 2016, El-Heis et al., 2017), smoking (Wang et al., 2013) and microbiome-related exposures (West et al., 2015). With increasing evidence that atopic disease is partly determined by the fetal environment, better understanding of early life environment becomes crucial for identifying potential preventative strategies.

Studies examining infant birthweight in relation to atopic eczema have been inconsistent (Panduru et al., 2014, Olesen et al., 1997), and it is now recognised that infant birthweight is only a crude proxy for patterns of fetal growth, which may show stronger associations with later outcomes than cross sectional assessments of size at single time points (Pike et al., 2010). A previous study reported that fetuses with below average crown-rump length at 11 weeks' gestation and an above average bi-parietal diameter at 19 weeks' gestation were more likely to have eczema ascertained by postal questionnaire at age 10 years (Turner et al., 2011), but no previous study has examined longitudinal measures

of fetal and infant size in relation to infantile atopic eczema. More information is available for other atopic outcomes, and a large neonatal head circumference (Godfrey et al., 1994, Fergusson et al., 1997, Gregory et al., 1999, Shaheen et al., 1999) and higher abdominal circumference growth velocity between 11 and 19 weeks' gestation have been linked to an increased risk of atopy.(Pike et al., 2010)

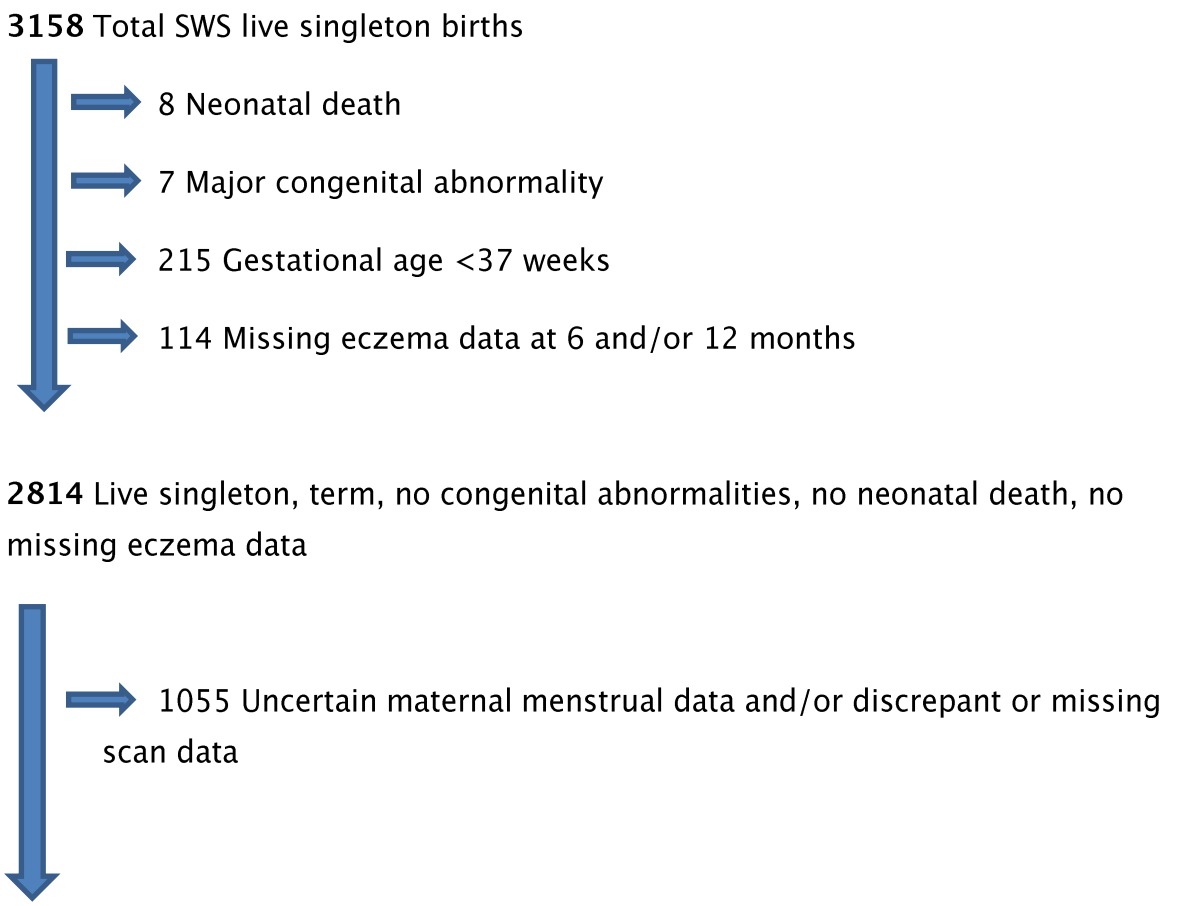
In this chapter, fetal and infant anthropometric measurements and growth velocities were examined in relation to the risk of atopic eczema at ages 6 and 12 months, to look for evidence of altered growth prior to the clinical onset of atopic eczema which might support a prenatal developmental influence on the disorder. Similarly, the relation of fetal and infant growth velocities were examined in relation to infant atopy at age 12 months. Furthermore, evidence supports the role of maternal nutrition and psychological state in impacting fetal and infant growth, therefore growth velocity outcomes were examined in relation to maternal serum nicotinamide and anthranilic acid, and preconception and postnatal maternal stress and low mood exposures.

5.2 Methods

5.2.1 Southampton Women's Survey

In the SWS, information on maternal diet, lifestyle, socioeconomic status, and body composition was collected at the initial visit prior to women becoming pregnant (Inskip et al., 2006b). Women who became pregnant were followed up through their pregnancies; ultrasound measurements of fetal size were performed at 11, 19, and 34 weeks. 3158 live born singleton infants were delivered. Further anthropometry was performed at birth, and at ages 6 and 12 months. The findings reported here are based on 1759 term, live singleton births with no congenital abnormalities, who were assessed for atopic eczema at 6 and/or 12 months and had fetal and infant anthropometric measurements and known maternal menstrual data (Figure 20).

Figure 20. Selection of study group sample from the SWS cohort



*Non assisted conception, regular menstrual cycle, sure/certain of last menstrual period (LMP), not on oral contraceptive pill prior to LMP, dating range scan data available, LMP consistent with each of date of conception where known, first positive pregnancy test, scan data and gestation at birth)

5.2.2 Fetal and infant anthropometric measurements

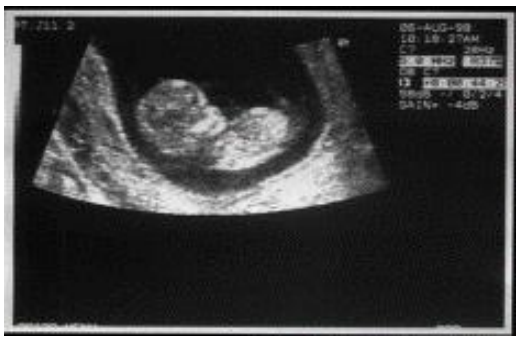
At 11, 19 and 34 weeks' gestation, women underwent high-resolution ultrasound scanning by experienced research staff using Kretz Voluson® 730 (GE Kretz Ultrasound, Tiefenbach, Austria) or Acuson Sequoia® 512 (Siemens, Erlangen, Germany) systems, which were cross-calibrated. Measurements of fetal linear size (crown-rump length (CRL) at 11 weeks), femur length (FL) at 19 and 34 weeks), head circumference (at 11, 19 and 34 weeks) and abdominal circumference (at 11, 19 and 34 weeks) were made according to internationally accepted and validated methodology (Chitty et al., 1994). As measurements of length (linear size) were not directly available from prenatal ultrasound scan measurements, fetal length was estimated from CRL and FL; assuming that they are proportional

to fetal length. An appropriate multiplier was determined by comparing the summary statistics for total length from fetal autopsies provided by Guihard-Costa et al. (Guihard-Costa et al., 2002) with those for CRL and FL in the SWS dataset. These multipliers of 1.71, 7.66 and 6.91 were used to predict fetal length from CRL at 11 weeks and FL at 19 weeks and 34 weeks, respectively.

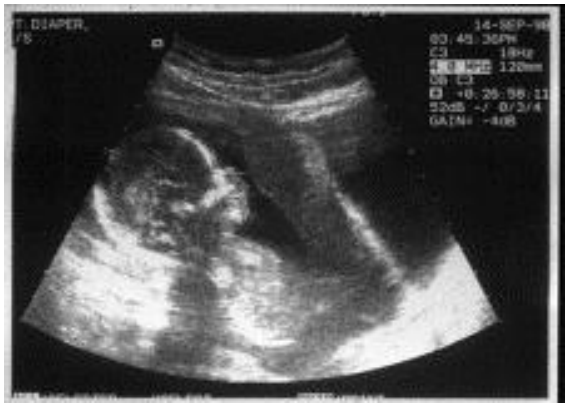
Fetal ultrasound scans (Figure 21) were performed by any of three particular ultrasonographers who were trained on specific standard operating procedures. Fetal size measurements were taken at a number of time points during pregnancy, allowing growth patterns to be calculated. The detailed measurements made by research staff according to specific protocols allow the examination of growth patterns prior to birth and prior to the clinical onset of conditions such as atopic eczema.

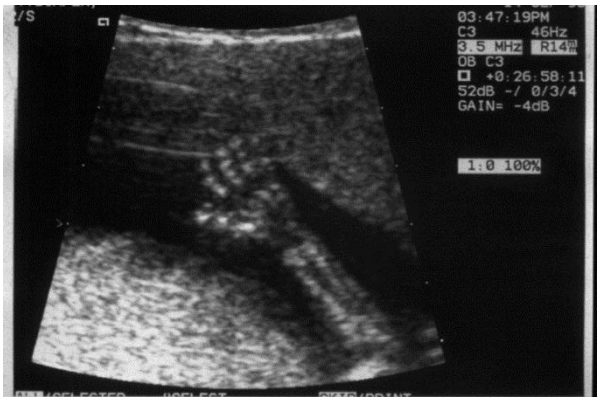
Figure 21. Fetal ultrasound scans at 11, 19 and 34 weeks gestation

11 weeks

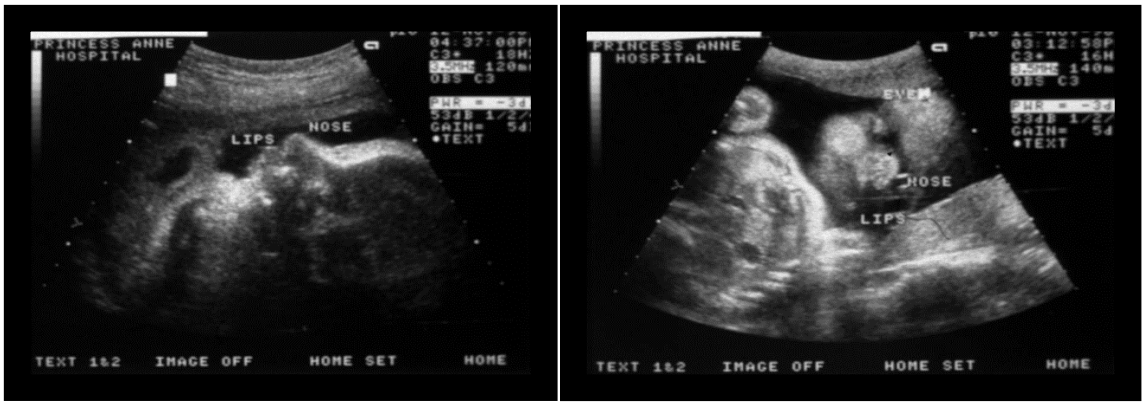


19 weeks

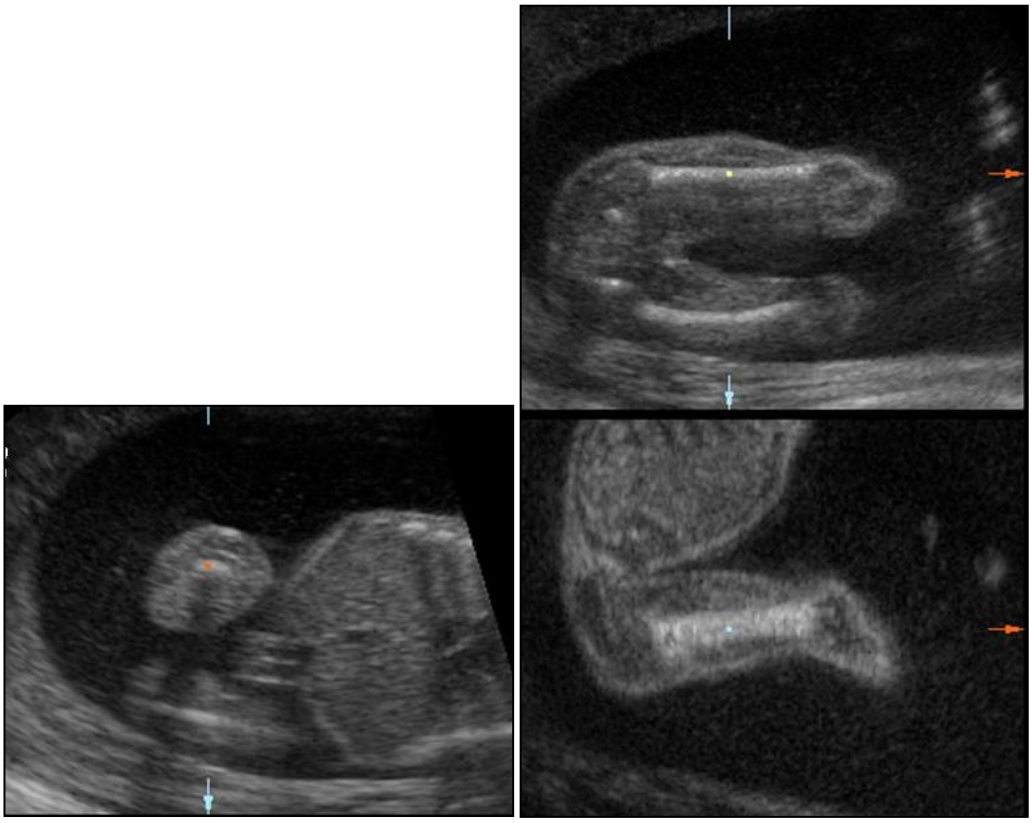




34 weeks



Femur length measurements



Postnatal anthropometry was performed by trained research nurses according to standardised procedures (Appendices I and J), with each measurement repeated three times and the mean value used for analysis. Crown-heel length at birth was measured using a neonatometer (Harpenden, Wrexham, UK) and at ages 6 and 12 months using an infantometer (Seca Ltd, Birmingham, UK); head and abdominal circumferences were measured using unmarked tapes read off against a metal ruler at birth, 6 and 12 months.

The fetal and infant anthropometric measures of interest examined were:

- Linear size
- Head circumference
- Abdominal circumference
- Head: abdominal circumference ratio

5.2.3 Outcome assessment

Case definition of atopic eczema and atopy was based on the UKWPDC for the definition of atopic eczema (Williams et al., 1994a, Williams et al., 1994b) and skin prick testing, respectively. Outcome definitions are described in Chapter 2.

Assessment of maternal serum nicotinamide and anthranilic acid and maternal stress and mood exposures are described in Chapters 3 and 4, respectively.

5.2.4 Statistical analysis

As measurements were taken close to but not at the exact ages specified, the associations between anthropometric measures and age were modelled using Cole's LMS (Cole and Green, 1992) in LMSchartmaker (Pan and Cole, 2011), to create sex-specific-size-for-age z-scores. Cole's LMS method smoothens growth curves and concurrently allows calculation of the z-score. The parameters of the method involve; the Box-Cox power calculation (L) which represent skewness, the median (M) and the generalised coefficient variation (S). These parameters are all used together to create the preferred percentiles. The LMS has been previously used and adopted in a number of studies and are thought of as reliable method of smoothing growth curves (Inokuchi et al., 2006, Roelants et al., 2009, Silva et al., 2012, Stanojevic et al., 2009).

Conditional models of change were built using linear regression analysis: thus size z-score at 11 weeks was the starting point. Conditional change in z-score from 11 to 19 weeks was defined as the standardised residuals resulting from the linear regression model of z-score at 19 weeks on z-score at 11 weeks. The conditional change in z-score from 19 to 34 weeks was obtained from the standardised residuals resulting from regressing z-score at 34 weeks on both z-score at 19 weeks and z-score at 11 weeks simultaneously. This process was continued for each subsequent time point, resulting in independent measures of conditional growth. Using the measures of linear size at 11 weeks, birth and 6 months, additional conditional growth measures using this subset of time points were created; linear size at 11 weeks, linear growth from 11 weeks to birth, from

birth to 6 months and from 6 to 12 months. This approach would allow for comparison of linear growth patterns prenatally and postnatally and highlight growth patterns prior to the clinical observation of atopic eczema at 6 or 12 months. Measures of conditional growth were mutually uncorrelated and yielded standard deviation scores, enabling comparison of relationships between growth in different time intervals and risk of eczema at ages 6 and 12 months.

Confounding variables were determined prior to the analysis using a DAG (Greenland et al., 1999) (Figure 22). The resulting maternal factors that were included as confounding variables in the analyses were:

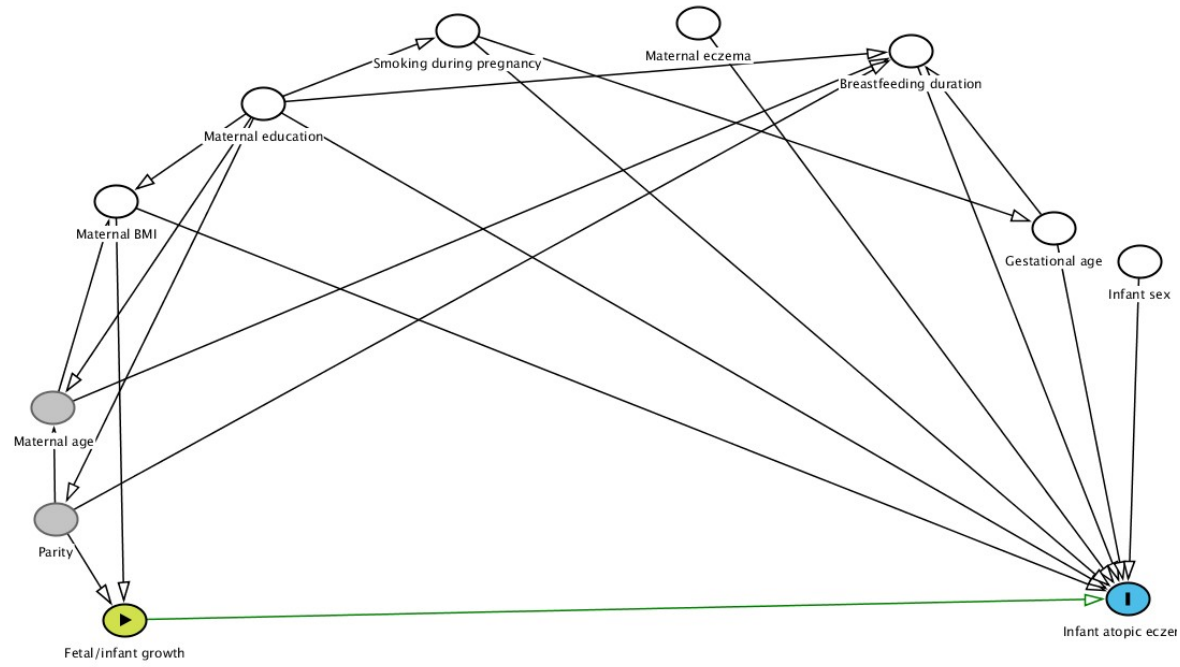
- BMI at initial assessment
- Educational attainment
- Smoking

The infant confounding variables were:

- Gestational age
- Breastfeeding

Maternal eczema in the 12 months prior to recruitment and infant sex were considered competing exposures and were adjusted for in the analyses to improve the accuracy of the model.

Figure 22. Fetal and infant growth and atopic eczema DAG.



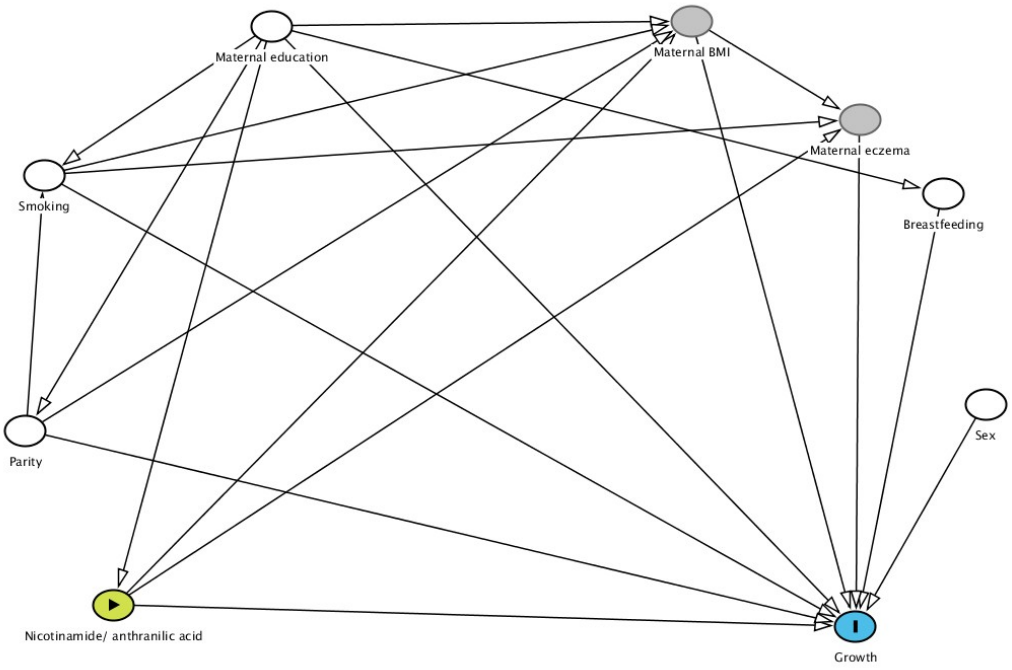
Logistic regression analyses were performed (Stata version 14.1, Statacorp LP, TX) to relate fetal and infant anthropometric measures (in SD) and growth velocities (in SD) to infant atopic eczema at ages 6 and 12 months, with results presented as eczema OR per SD increase (OR/SD). $P<0.05$ was considered statistically significant.

Univariate (unadjusted) and multivariate (adjusted) Poisson regression analysis was used to relate maternal tryptophan metabolite levels to infant atopy at 12 months. The same confounders were considered when examining infant atopy and atopic eczema to allow for comparison of the two outcomes, with the exception of accounting for maternal atopy instead of maternal eczema as this is more relevant to this analysis.

Further multivariate (adjusted) regression analyses examining associations of late pregnancy maternal serum nicotinamide and anthraniliac acid and maternal

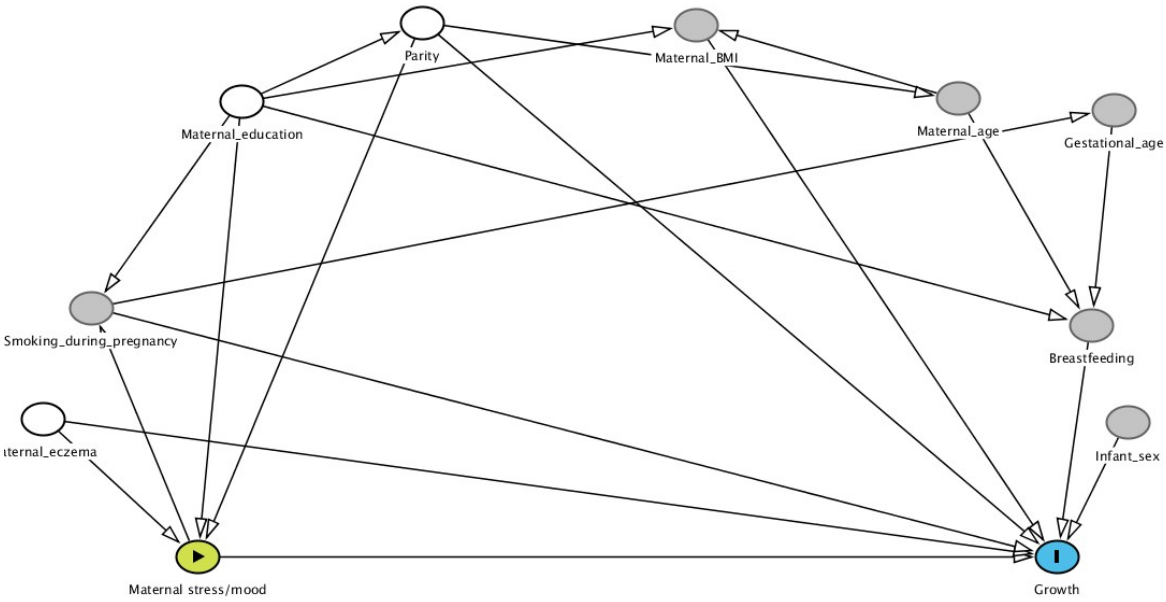
preconception and postnatal stress and low mood exposures with fetal and infant growth velocities were undertaken. The confounding variables for these were determined using DAGs (Figure 23, Figure 24, respectively). Maternal education, smoking during pregnancy, parity, infant sex and duration of breastfeeding were considered confounders when examining maternal nicotinamide and anthranilic acid. Maternal eczema, education and parity were considered confounders when examining maternal stress and mood.

Figure 23. Maternal serum nicotinamide/anthranilic acid and fetal and infant growth



Confounding variables: maternal education
Variables to improve precision of model: smoking during pregnancy, parity, infant sex and duration of breastfeeding.

Figure 24. Maternal stress and mood and fetal and infant growth DAG.



Confounding variables: maternal eczema, education and parity

5.3 Results

5.3.1 Cohort characteristics

Table 23 summarises maternal, fetal and infant characteristics. Among the study group, the mothers' mean (SD) age at their child's birth was 31.0 (3.7) years; 52.2% were primiparous, 11.7% smoked during pregnancy and 6.7% of mothers had eczema in the 12 months prior to recruitment. 51.1% of infants were male; mean (SD) infant birthweight was 3.52 (0.47) kg and median gestational age at birth 40.1 weeks (IQR 39.3-41.0). 1698 infants were assessed for eczema at age 6 months, 9.5% of whom had atopic eczema. At age 12 months, 1684 infants were assessed and 10.0% had atopic eczema. Table 24 shows the characteristics of the 1759 participants in the study group in comparison with the overall SWS pregnancy cohort; the study group mothers were slightly older at child's birth, higher proportions were primiparous,had attained A level or higher education, and were non-smokers.

Table 23. Characteristics of the study population.

	Total n	%, Median (IQR) or Mean (SD)
Infant		
n (%) Male	1759	899 (51.1%)
Gestational age at birth (weeks)	1759	40.1 (39.3-41.0)
Birthweight (kg)	1750	3.52 (0.47)
n (%) Breast feeding (completed months)		
Never breast fed	1677	270 (16.1%)
<1		330 (19.7%)
1 to 3		341 (20.3%)
4 to 6		325 (19.4%)
7 to 11		266 (15.9%)
12 or more		145 (8.7%)
6 month assessment		
Age (weeks)	1731	27.4 (26.1-32.9)
n (%)Atopic eczema	1698	162 (9.5%)
12 month assessment		
Age (weeks)	1684	53.7 (52.6-55.0)
n (%) Atopic eczema	1683	168 (10.0%)
Maternal		
Age at child's birth (y)	1759	31.0 (3.7)
n (%) A level or higher	1755	1111 (63.3%)
n (%) Smoking in pregnancy	1698	199 (11.7%)
n (%) Primiparous	1758	917 (52.2%)
n (%) Eczema in last 12 months	1585	106 (6.7%)
Pre-pregnancy BMI	1742	24.1 (21.9-27.4)

	Total	%, Median (IQR) or Mean (SD)
Fetal/infant measurements		
Linear size (mm)		
11 weeks (CRL)	1489	53.0 (8.9)
19 weeks (FL)	1728	30.7 (2.1)
34 weeks (FL)	1747	64.9 (2.7)
Birth (CHL)	1667	500.7 (18.7)
6 months (CHL)	1326	675.1 (25.2)
12 months (CHL)	1592	759.7 (28.7)
Head circumference (mm)		
11 weeks	1123	69.8 (9.1)
19 weeks	1726	168.4 (8.6)
34 weeks	1688	317.8 (10.8)
Birth	1682	351.0 (12.6)
6 months	1337	440.7 (14.1)
12 months	1645	468.6 (14.4)
Abdominal circumference		
11 weeks	1044	55.7 (7.5)
19 weeks	1718	146.3 (9.0)
34 weeks	1748	307.7 (15.0)
Birth	1680	317.9 (20.2)
6 months	1342	477.3 (32.6)
12 months	1636	497.2 (33.2)

CRL: Crown-Rump Length, FL: Femur Length, CHL: Crown-Heel Length
IQR: Interquartile Range, SD: Standard Deviation

Table 24. Comparison of the study population with the remainder of the SWS participants.

	Study population (n = 1759) Median (IQR), Mean (SD) or %	Other SWS participants* (n = 1169) Median (IQR), Mean (SD) or %	P value for difference between the two groups
Maternal			
Age at child's birth(y)	31.0 (3.7)	30.2 (4.0)	<0.001
% A level or higher degree	63.3%	52.6%	<0.001
% Smoking in pregnancy	11.7%	22.3%	<0.001
% Primiparous	52.2 %	47.8%	0.021
% Eczema in the last 12 months	6.7%	7.3%	0.56
Pre-pregnancy BMI (kg/m^2)	24.1 (21.9- 27.4)	24.2 (21.9-27.3)	0.86
Infant			
% Male	51.1%	52.0%	0.65
Gestational age (weeks)	40.1 (39.3- 41.0)	40.2 (39.2-41.0)	0.71
Birth weight (kg)	3.52 (0.47)	3.49 (0.47)	0.08

*Includes live singleton infants born at term, with no congenital abnormalities and who survived the neonatal period.

5.3.2 Associations of fetal size and growth velocities with infant atopic eczema at age 6 months

Univariate and multivariate analyses of fetal size and growth velocities in relation to atopic eczema at age 6 months are shown in Tables 25 and 26, respectively. At 34 weeks' gestation a shorter femur length, smaller abdominal circumference and higher head to abdominal circumference ratio were associated with increased risks of eczema at age 6 months. Expressed per SD increase, higher femur length and abdominal circumference were associated with decreased risks of atopic eczema (eczema OR/SD increase 0.81, 95%CI 0.69–0.96, p= 0.017; 0.78, 95%CI 0.65–0.93, p= 0.006, respectively), while, every SD increase in head to abdominal circumference ratio (indicating disproportionate growth) was associated with an increase in risk of atopic eczema (1.37, 95%CI 1.15–1.63, p= 0.001) (Table 25, Figure 25). Fetal head circumference was not related to infant atopic eczema at age 6 months (Table 25, Figure 25). Postnatal anthropometry showed that infants with atopic eczema at 6 months were shorter at age 6 months (eczema OR/SD increase 0.78, 95%CI 0.65–0.93, p= 0.006) (Table 25, Figure 25).

Figures 26,27 and 28 show a graphical comparison of fetal and infant size measurements of infants who have atopic eczema at age 6 months and those who do not have eczema at this time point. Mean fetal and infant linear size, head circumference and abdominal circumference of infants who have atopic eczema at the age of 6 months appeared persistently lower prenatally and postnatally when compared to infants who do not have atopic eczema at the stage. Also, infants who have atopic eczema at this age appeared to have a higher head: abdominal circumference ratio indicating disproportionate growth both prenatally and at age 12 months but not 6 months.

Lower velocities of linear growth from 11 weeks' gestation to birth, and birth to age 6 months were associated with eczema at age 6 months (eczema OR/SD increase 0.80, 95% CI 0.65–0.98, p= 0.034; 0.81, 95%CI 0.66–1.00), p= 0.051, respectively) (Figure 30a); this particularly reflected lower linear growth velocity from 11–19 weeks' gestation (Table 26). A lower abdominal circumference growth velocity from 11–19 weeks' gestation was associated with an increased risk of eczema at age 6 months (eczema OR /SD increase 0.71, 95%CI 0.55–0.92, p= 0.009, Table 26), but there were no associations of head circumference growth velocities with eczema at age 6 months (Table 26).

Table 25 Static size measurements in relation to eczema at age 6 months

	Univariate				Multivariate*			
	n	OR	95% CI	P value	n	OR	95% CI	P value
Linear size (SD)								
11 weeks (CRL)	1434	1.00	0.84–1.18	0.96	1254	1.01	0.83–1.23	0.90
19 weeks (FL)	1667	0.89	0.75–1.05	0.18	1445	0.90	0.75–1.09	0.30
34 weeks (FL)	1686	0.86	0.74–1.01	0.07	1457	0.81	0.69–0.96	0.017
Birth (CHL)	1620	0.91	0.77–1.09	0.32	1425	0.85	0.71–1.03	0.09
6 months (CHL)	1324	0.79	0.66–0.94	0.007	1263	0.78	0.65–0.93	0.006
12 months (CHL)	1538	0.88	0.74–1.05	0.16	1350	0.86	0.72–1.03	0.11
Head circumference (SD)								
11 weeks	1078	1.04	0.85–1.27	0.69	942	1.07	0.85–1.34	0.56
19 weeks	1665	0.96	0.82–1.14	0.68	1443	0.98	0.82–1.19	0.86
34 weeks	1628	1.02	0.86–1.21	0.80	1404	1.03	0.85–1.24	0.78
Birth	1635	0.89	0.75–1.05	0.15	1439	0.87	0.73–1.04	0.13
6 months	1335	0.88	0.74–1.05	0.16	1273	0.88	0.73–1.06	0.18
12 months	1588	0.89	0.75–1.05	0.16	1385	0.89	0.75–1.06	0.19
Abdominal circumference (SD)								
11 weeks	999	0.94	0.77–1.16	0.59	875	0.96	0.76–1.21	0.72
19 weeks	1657	0.97	0.82–1.16	0.76	1436	0.94	0.78–1.14	0.53
34 weeks	1687	0.81	0.69–0.96	0.014	1458	0.78	0.65–0.93	0.006
Birth	1633	0.86	0.73–1.02	0.08	1437	0.83	0.70–1.00	0.045
6 months	1340	0.99	0.84–1.17	0.93	1278	1.01	0.85–1.20	0.90
12 months	1580	0.90	0.76–1.05	0.19	1380	0.87	0.73–1.03	0.11

	Univariate				Multivariate*			
	n	OR	95% CI	P value	n	OR	95% CI	P value
Head: abdominal circumference ratio Z scores (SD)								
11 weeks	945	1.09	0.88–1.35	0.44	823	1.04	0.83–1.30	0.74
19 weeks	1654	1.04	0.88–1.22	0.67	1433	1.09	0.92–1.29	0.34
34 weeks	1628	1.32	1.12–1.56	0.001	1404	1.37	1.15–1.63	0.001
Birth	1633	1.13	0.96–1.33	0.15	1437	1.13	0.95–1.35	0.16
6 months	1334	0.96	0.81–1.14	0.66	1272	0.94	0.79–1.13	0.52
12 months	1570	1.07	0.90–1.26	0.45	1371	1.09	0.92–1.30	0.33

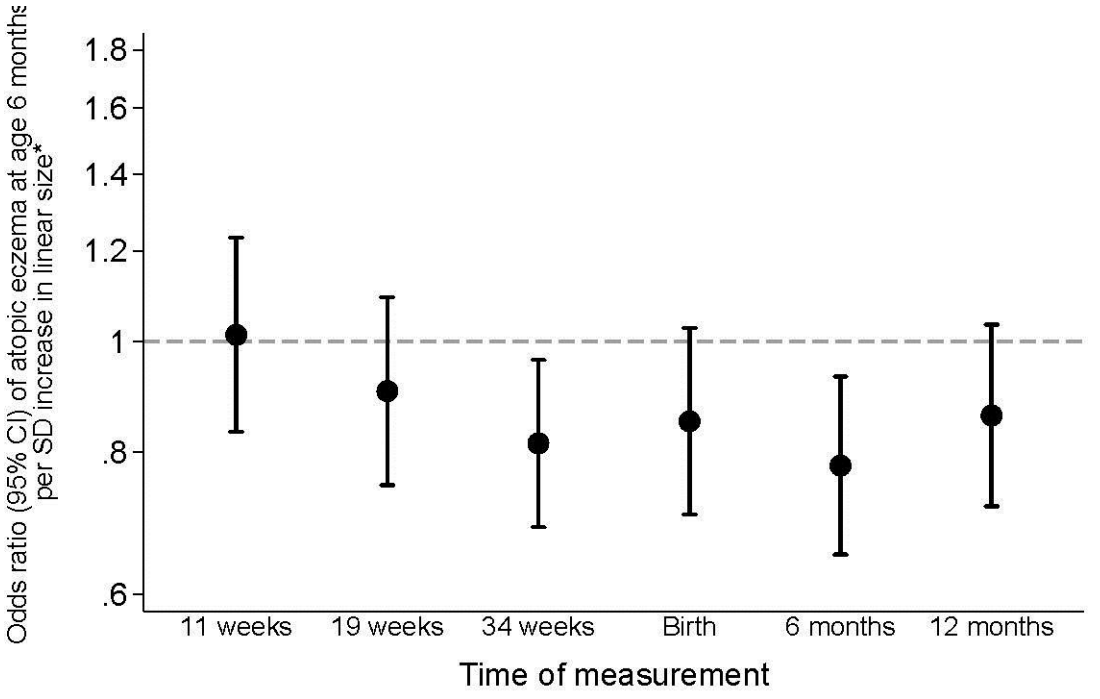
*Adjusted for gestational age at birth, infant sex, maternal BMI, education and smoking in pregnancy. SD: standard deviation.

Table 26. Growth velocities in relation to eczema at age 6 months

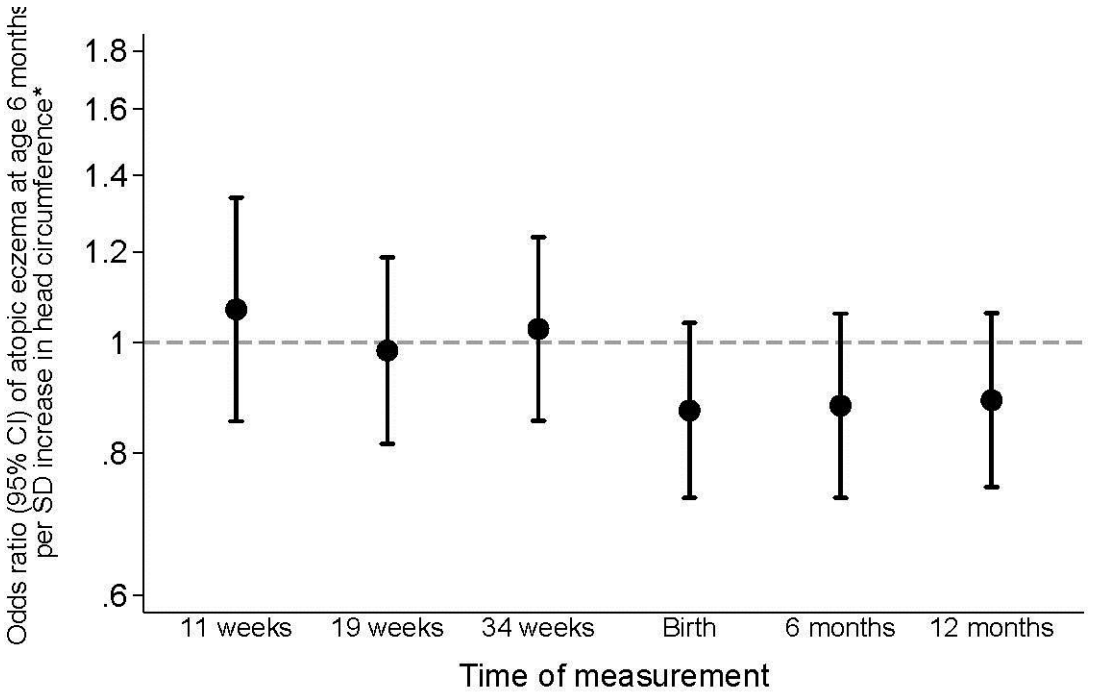
	Univariate				Multivariate*			
	n	OR	95% CI	P value	n	OR	95% CI	P value
Linear growth (SD)								
11 weeks	1003	1.02	0.84–1.24	0.87	973	1.01	0.82–1.25	0.91
11 – 19 weeks	1003	0.75	0.61–0.93	0.008	973	0.75	0.61–0.93	0.010
19 – 34 weeks	1003	0.85	0.69–1.04	0.12	973	0.83	0.67–1.02	0.07
34 weeks – Birth	1003	0.95	0.78–1.16	0.61	973	0.93	0.76–1.15	0.51
Birth – 6 months	1003	0.81	0.66–0.99	0.040	973	0.82	0.67–1.01	0.07
6 – 12 months	1003	1.11	0.91–1.36	0.31	973	1.12	0.91–1.38	0.29
Head circumference (SD)								
11 weeks	748	1.09	0.86–1.38	0.49	726	1.08	0.84–1.40	0.54
11 – 19 weeks	748	0.85	0.66–1.08	0.19	726	0.82	0.64–1.06	0.14
19 – 34 weeks	748	1.10	0.87–1.40	0.44	726	1.06	0.83–1.36	0.64
34 weeks – Birth	748	0.84	0.66–1.06	0.15	726	0.85	0.66–1.08	0.19
Birth – 6 months	748	1.03	0.81–1.31	0.79	726	1.05	0.82–1.34	0.68
6 – 12 months	748	0.91	0.72–1.16	0.47	726	0.89	0.69–1.15	0.37
Abdominal circumference (SD)								
11 weeks	723	0.97	0.76–1.23	0.78	703	0.94	0.73–1.22	0.66
11 – 19 weeks	723	0.98	0.78–1.25	0.90	703	0.96	0.76–1.22	0.75
19 – 34 weeks	723	0.79	0.62–1.00	0.051	703	0.71	0.55–0.92	0.009
34 weeks – Birth	723	1.09	0.86–1.38	0.47	703	1.14	0.89–1.46	0.29
Birth – 6 months	723	1.08	0.85–1.37	0.53	703	1.09	0.86–1.40	0.47
6 – 12 months	723	0.85	0.68–1.08	0.18	703	0.83	0.66–1.05	0.13

*Adjusted for gestational age at birth, infant sex, maternal BMI, education and smoking in pregnancy. SD: standard deviation.

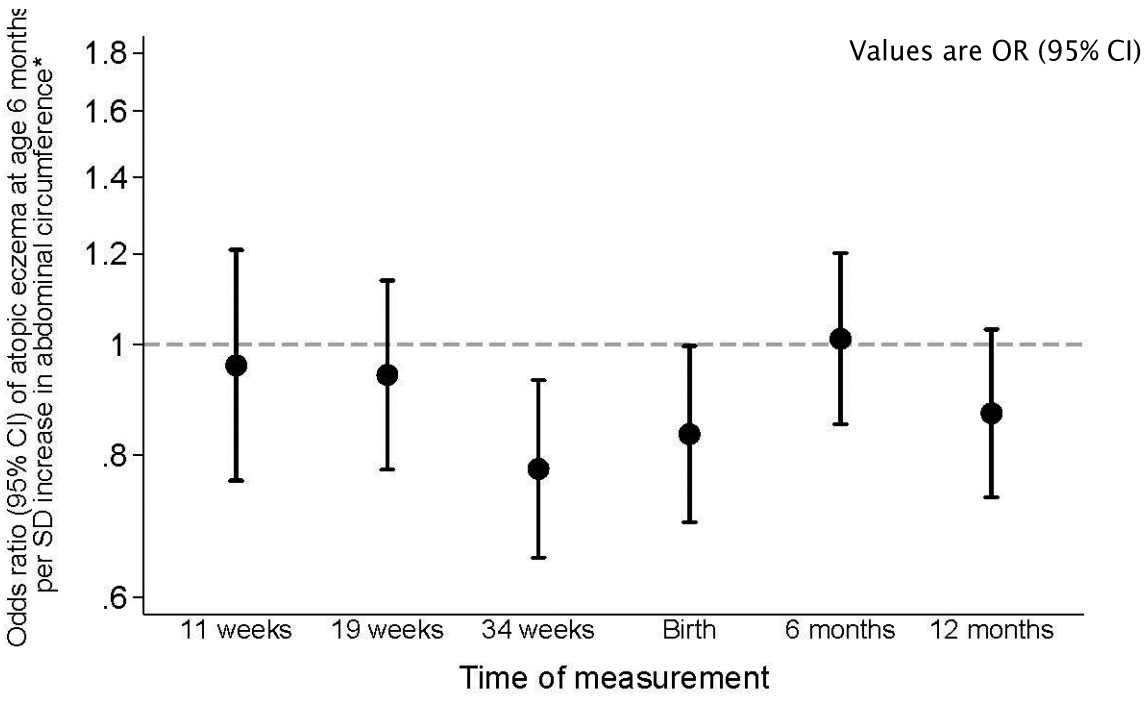
Figure 25. Size measurements in relation to atopic eczema at age 6 months



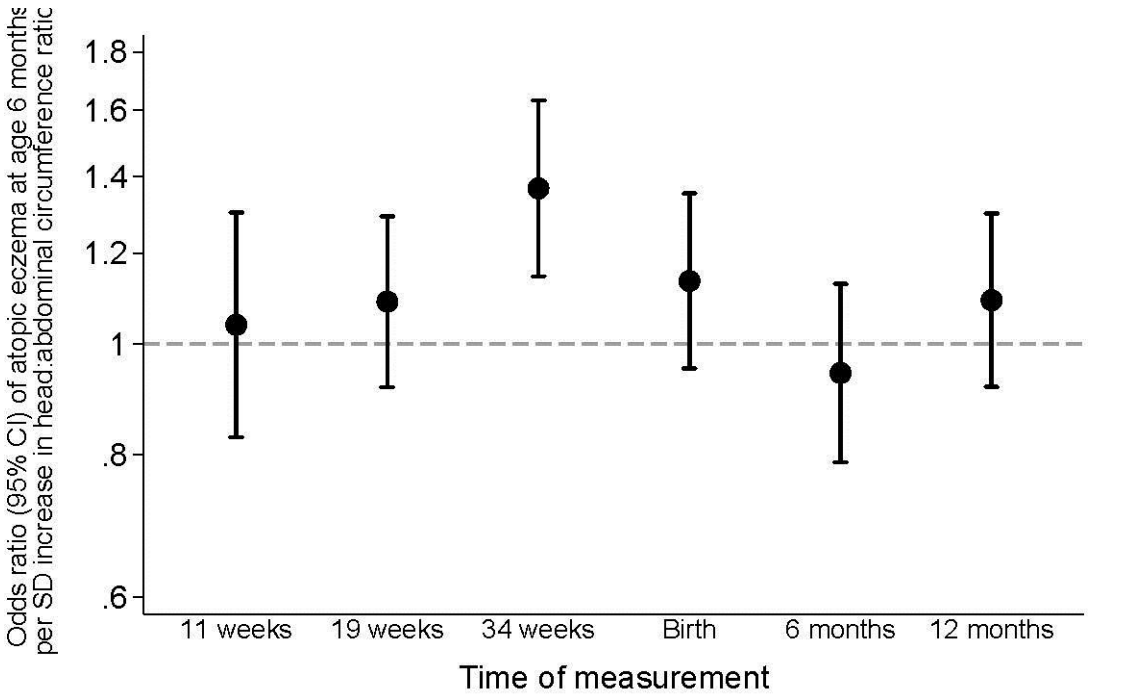
*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy



*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy



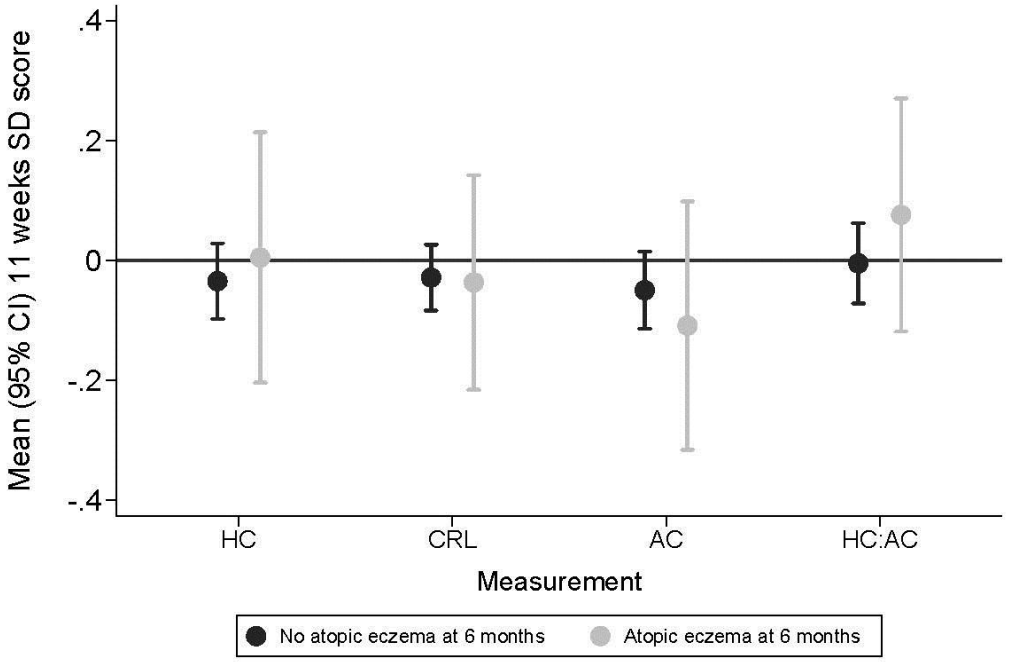
*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy

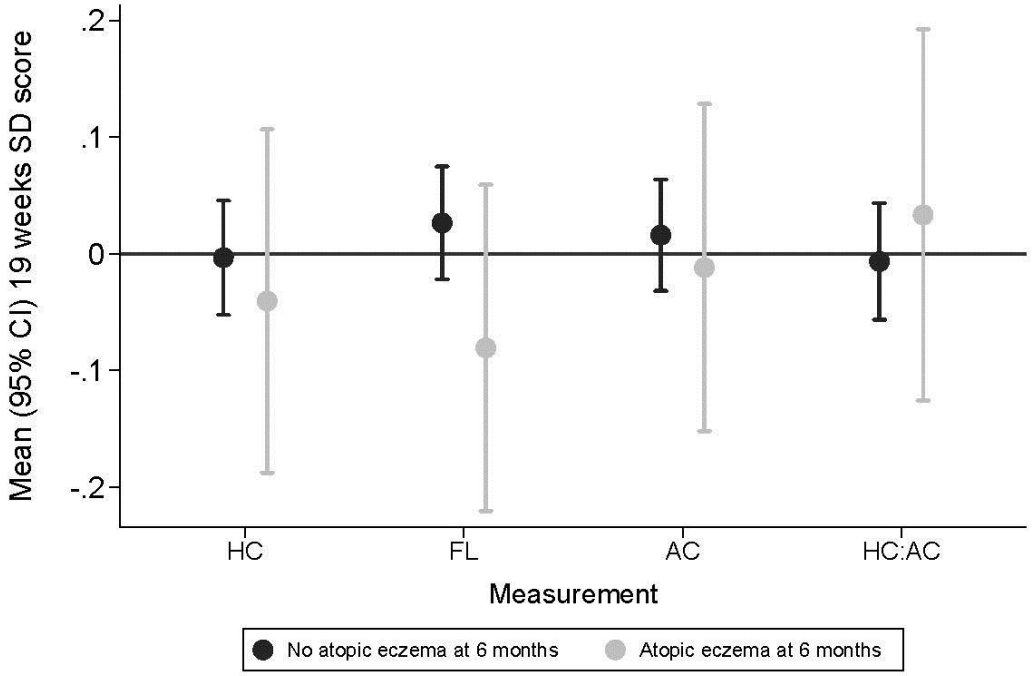


*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy

Figure 26. Mean fetal size of infants with and without atopic eczema at age 6 months

Values are OR (95% CI)





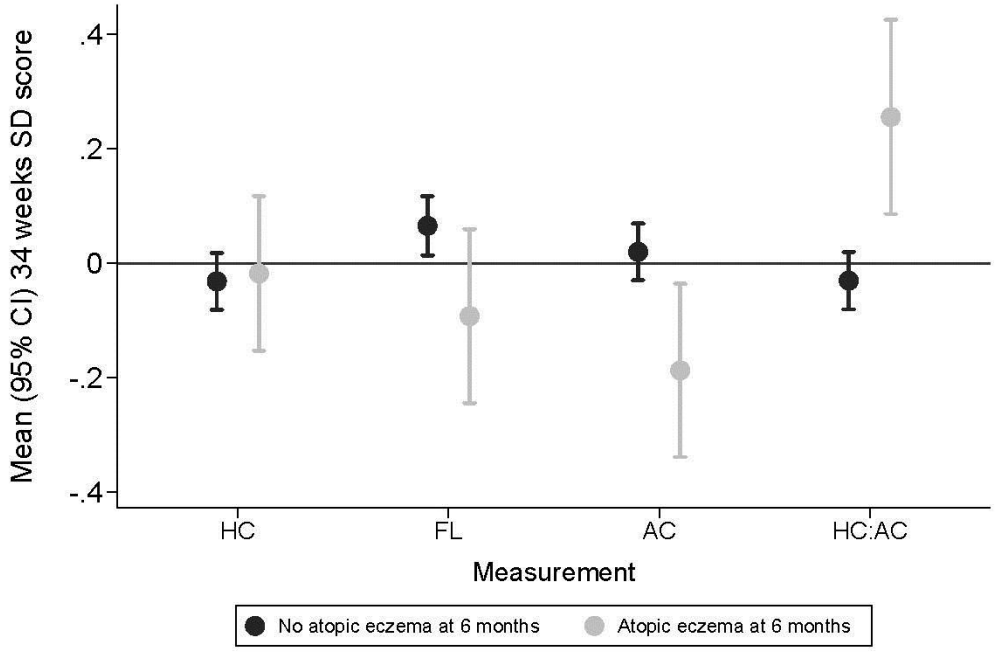
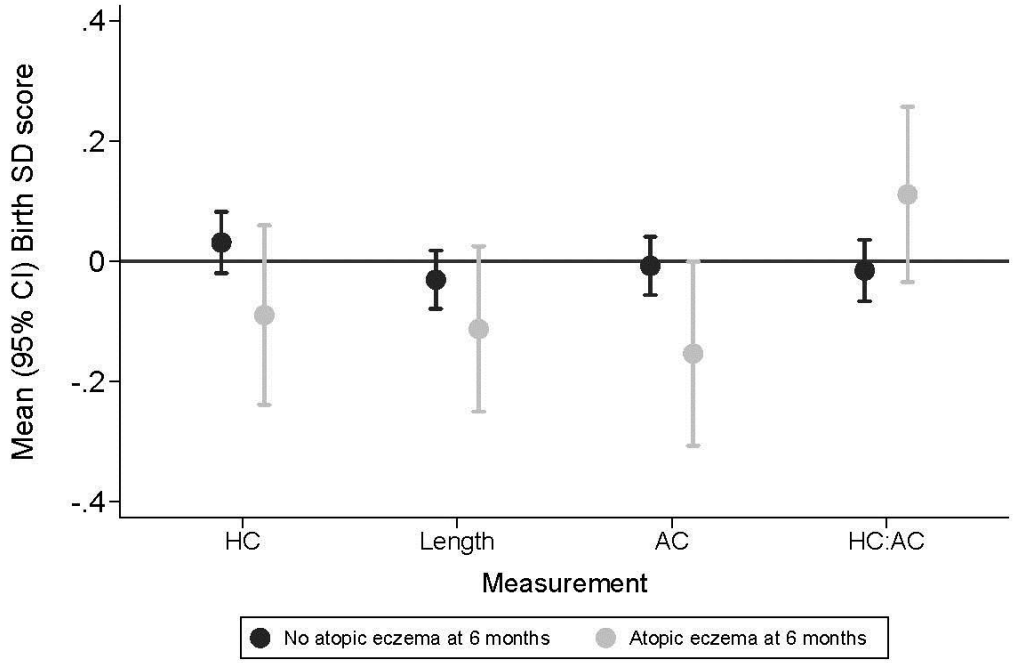
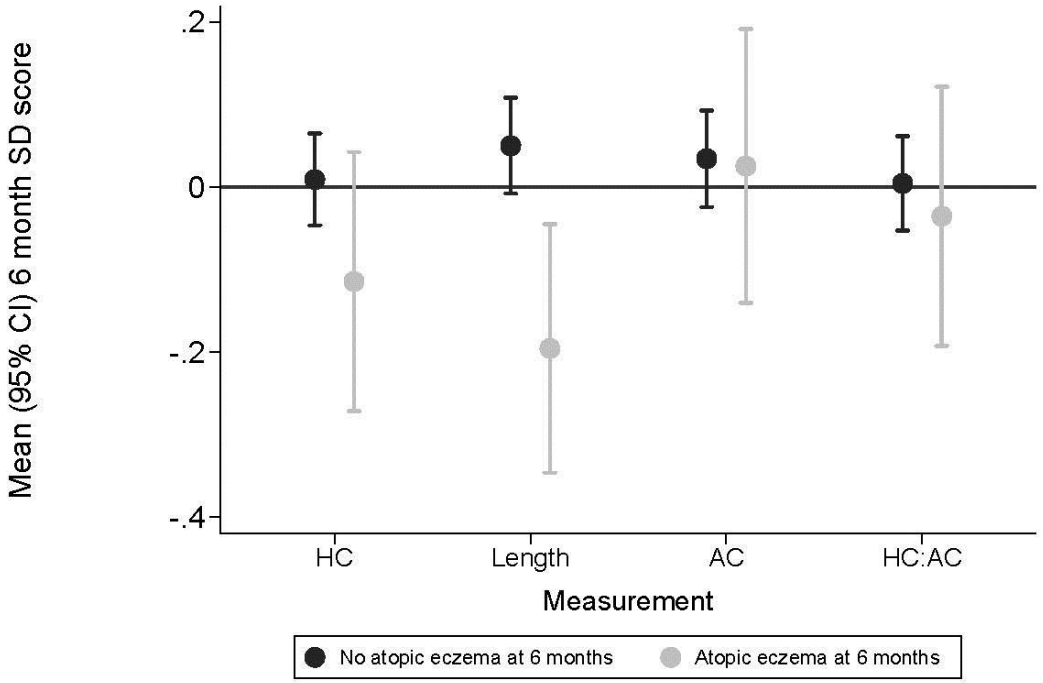


Figure 27. Mean postnatal size of infants with and without atopic eczema at age 6 months




Mean (95% CI) 6 month SD score
.2
0
-.2
-.4
HC
Length
AC
HC:AC
Measurement
No atopic eczema at 6 months
Atopic eczema at 6 months

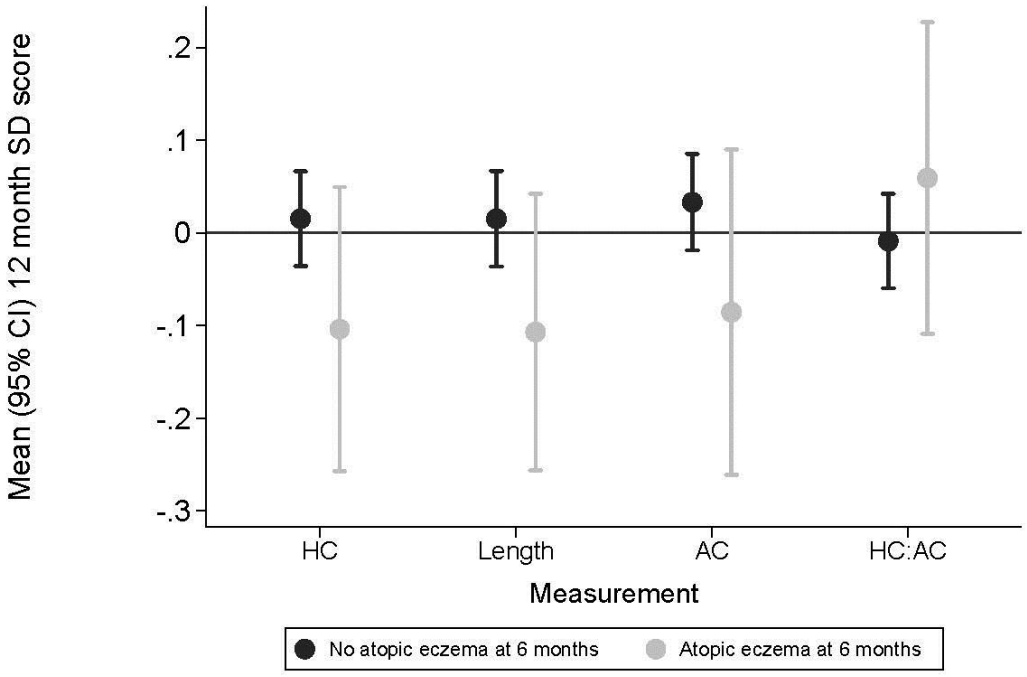
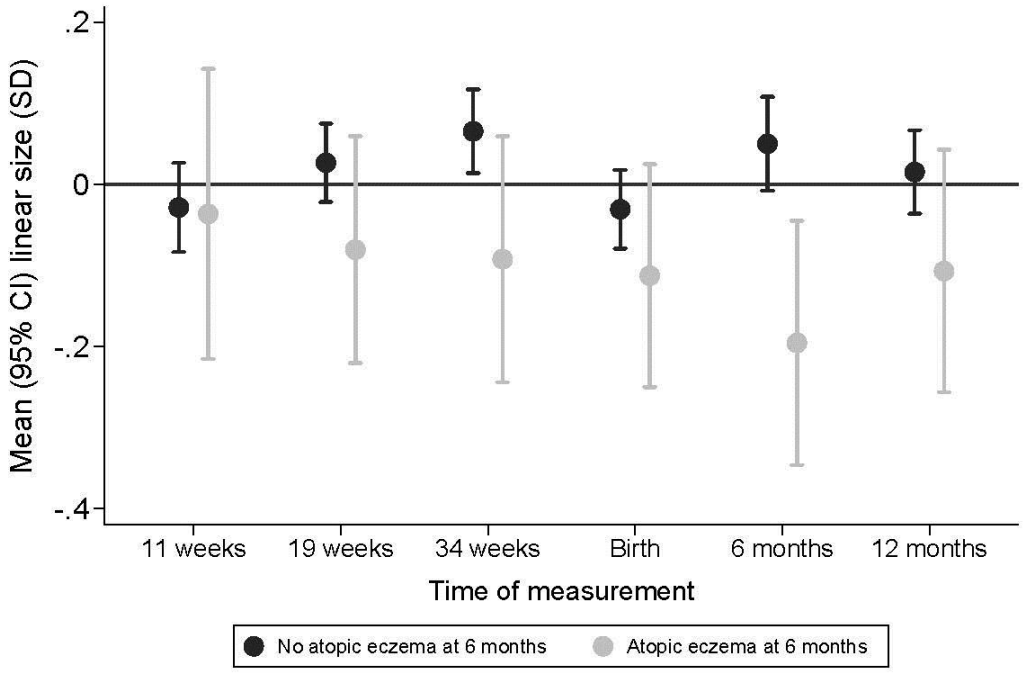
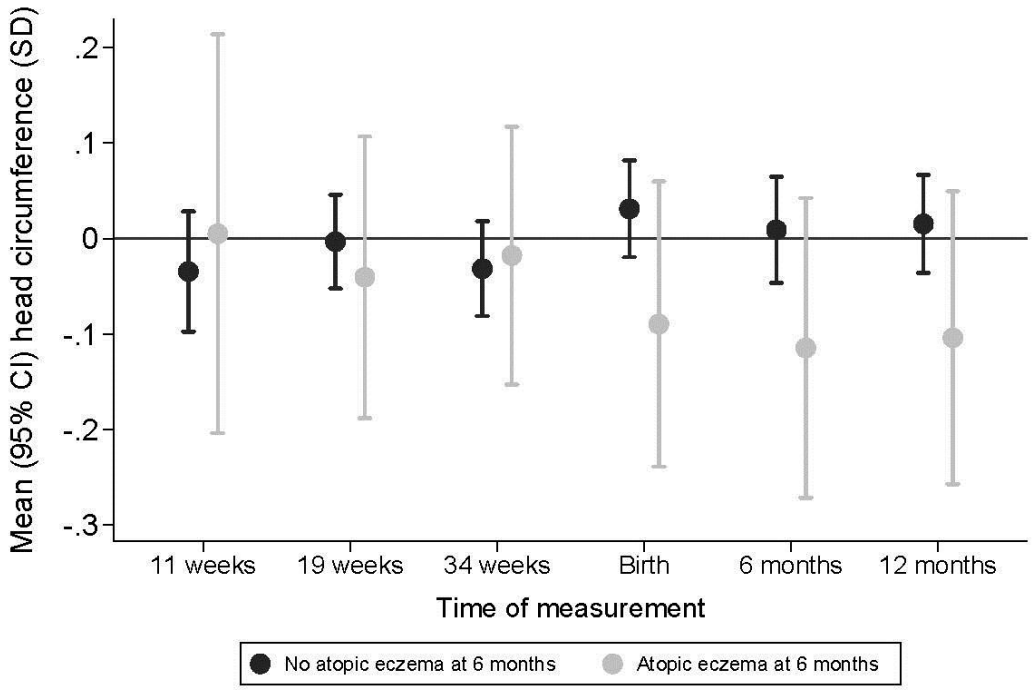
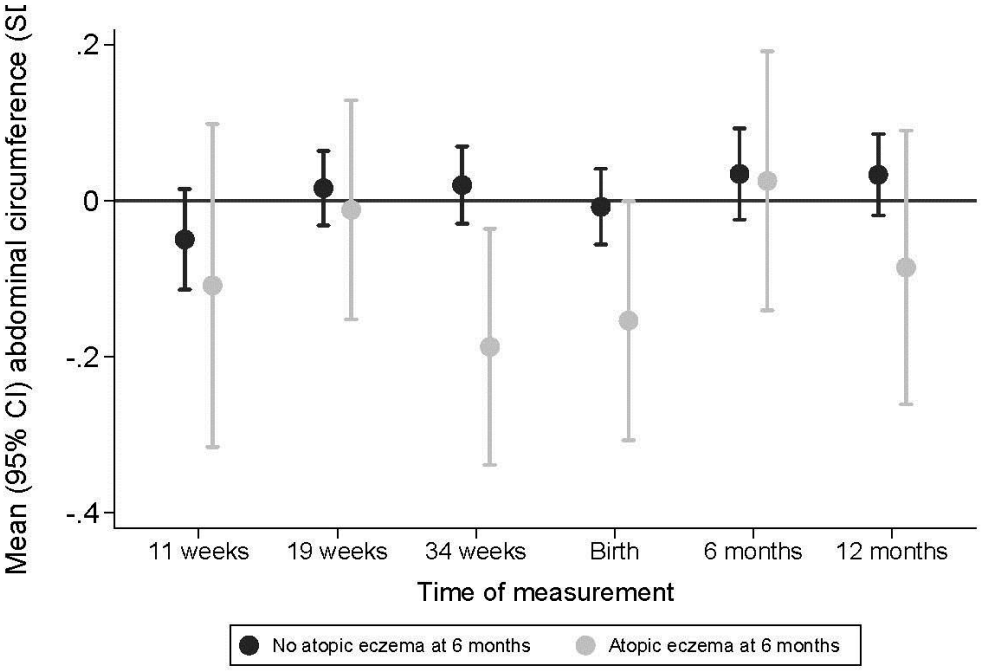
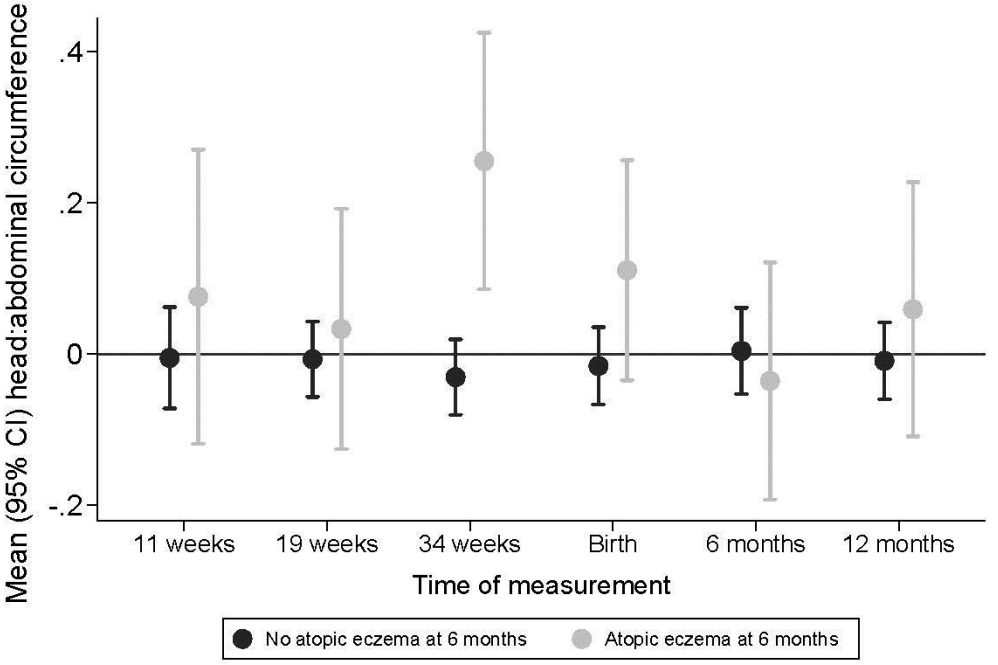

Mean (95% CI) 12 month SD score
.2
.1
0
-.1
-.2
-.3
HC
Length
AC
HC:AC
Measurement
No atopic eczema at 6 months
Atopic eczema at 6 months

Figure 28. Mean fetal and postnatal size of infants with and without atopic eczema at age 6 months






Mean (95% CI) abdominal circumference (SI
.2
0
-.2
-.4
11 weeks
19 weeks
34 weeks
Birth
6 months
12 months
Time of measurement
No atopic eczema at 6 months
Atopic eczema at 6 months

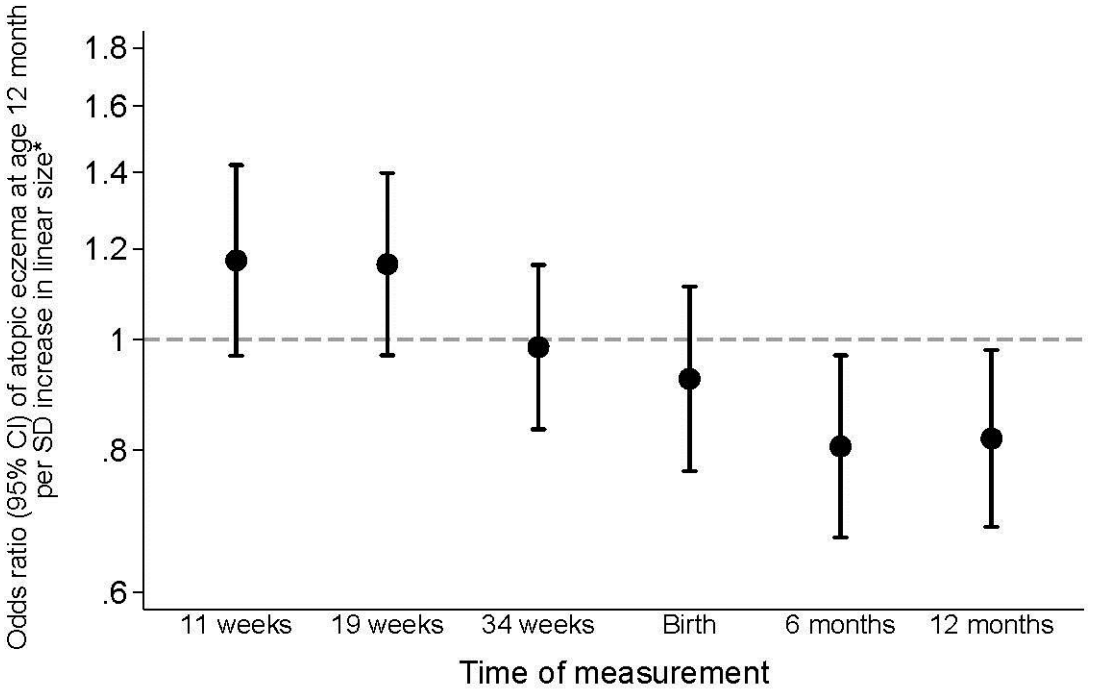

Mean (95% CI) head:abdominal circumference
.4
.2
0
-.2
11 weeks
19 weeks
34 weeks
Birth
6 months
12 months
Time of measurement
No atopic eczema at 6 months
Atopic eczema at 6 months

5.3.3 Associations of fetal size and growth velocities with infant atopic eczema at age 12 months

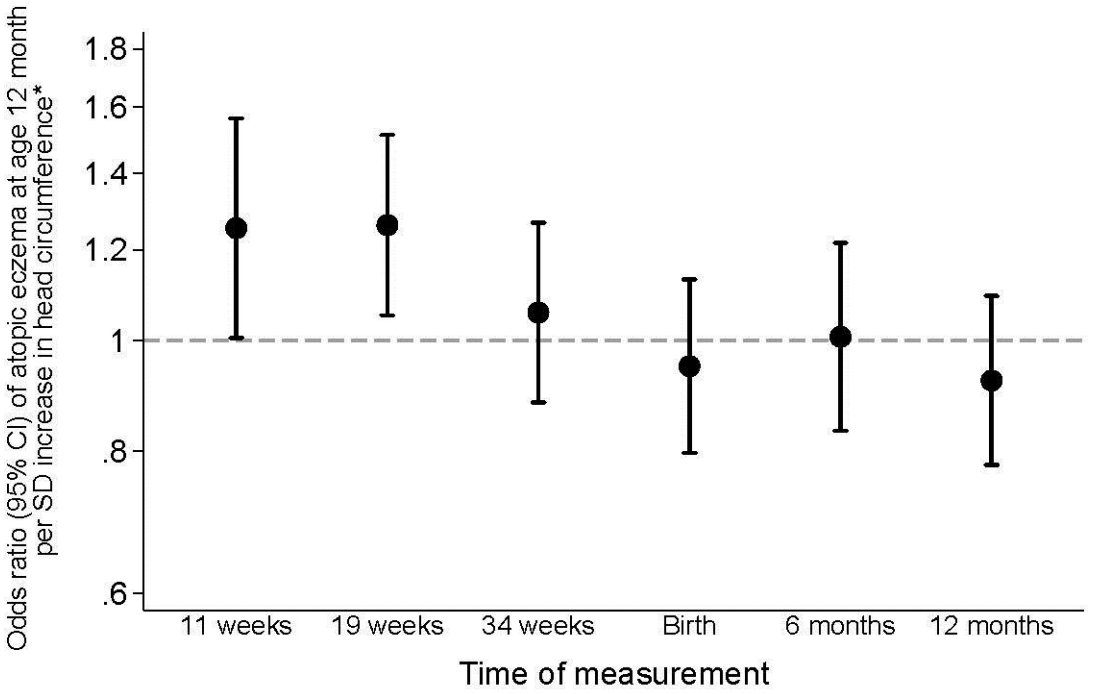
Univariate and multivariate analyses of fetal size and growth velocities in relation to atopic eczema at age 12 months are shown in Tables 27 and 28, respectively. Infants with atopic eczema age 12 months had a larger head circumference in early pregnancy (at 11 and 19 weeks' gestation eczema OR /SD increase 1.25, 95%CI 1.00–1.56, p=0.045 and 1.26, 95%CI 1.05–1.51, p=0.012, respectively), higher head to abdominal circumference ratio at 34 weeks (eczema OR /SD increase 1.22, 95%CI 1.03–1.46, p=0.025) (Table 27 and Figure 29). Postnatally, infants with atopic eczema at 12 months were shorter at ages 6 and 12 months (eczema OR /SD increase 0.81, 95%CI 0.67–0.97, p=0.021, OR 0.82, 95% CI 0.69–0.98, p=0.028, respectively) (Table 17).

Patterns of faltering of abdominal circumference growth velocity from 19–34 weeks gestation were seen (eczema OR /SD increase 0.67 (0.51–0.88), p= 0.003, Table 28), with trends towards faltering of linear growth velocity from 11 weeks to birth and birth to age 6 months (eczema OR /SD increase 0.81 (0.66–1.00), p= 0.051; 0.83 (0.68–1.03), p=0.087, respectively) (Figure 30b).

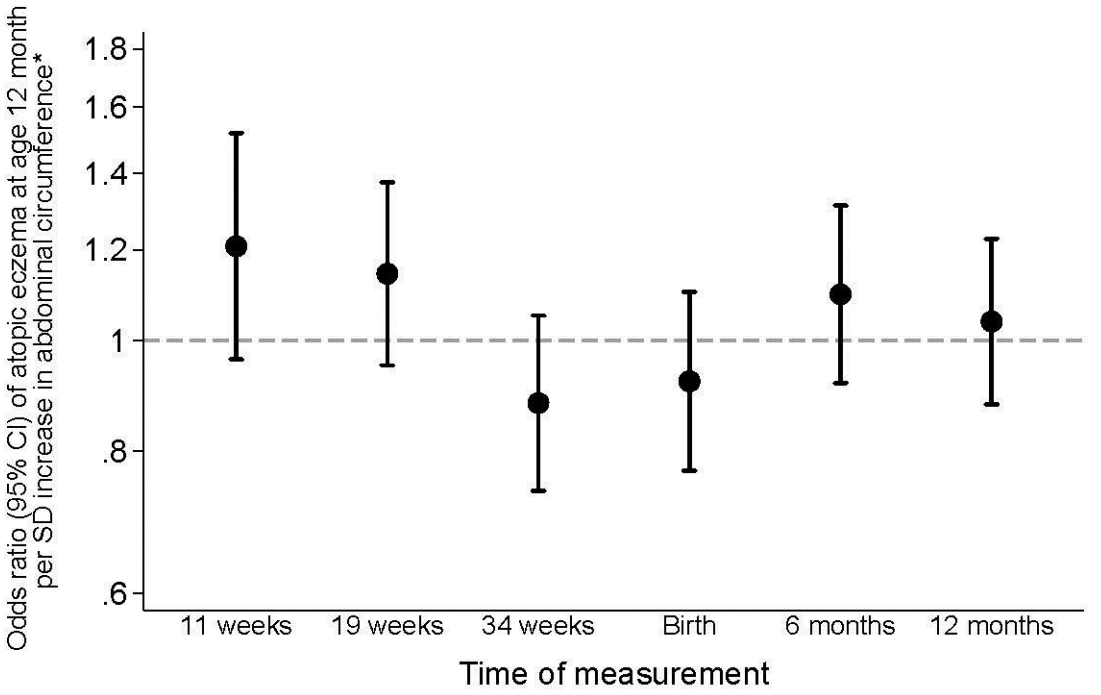
Figure 29. Size measurements in relation to atopic eczema at age 12 months



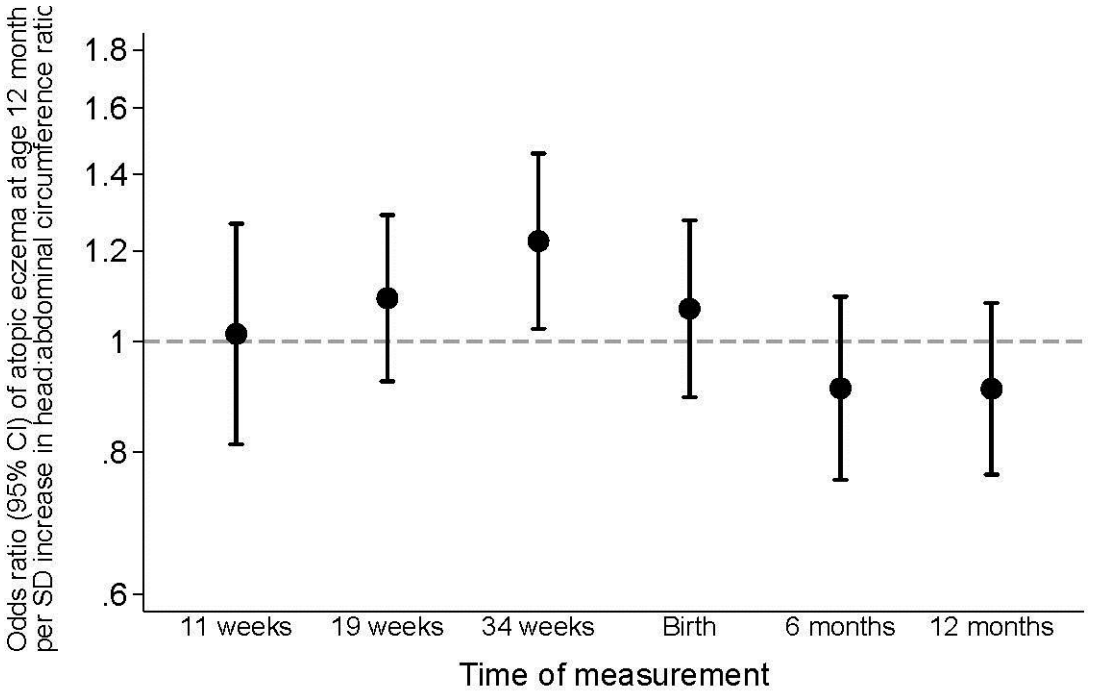
*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy



*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy



*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy



*Controlling for gestation, sex, breastfeeding, maternal BMI, qualifications and smoking in pregnancy

Table 27 Static size measurements in relation to eczema at age 12 months.

	Univariate				Multivariate*			
	N	OR	95% CI	P value	n	OR	95% CI	P value
Linear size (SD)								
11 weeks (CRL)	1426	1.08	0.91–1.28	0.41	1232	1.17	0.97–1.42	0.10
19 weeks (FL)	1655	1.09	0.92–1.29	0.30	1418	1.16	0.97–1.40	0.11
34 weeks (FL)	1671	0.96	0.82–1.12	0.57	1427	0.98	0.83–1.16	0.85
Birth (CHL)	1595	0.92	0.78–1.09	0.34	1396	0.92	0.77–1.11	0.40
6 months(CHL)	1252	0.80	0.67–0.96	0.017	1212	0.81	0.67–0.97	0.021
12 months (CHL)	1591	0.79	0.67–0.94	0.007	1372	0.82	0.69–0.98	0.028
Head circumference (SD)								
11 weeks	1076	1.17	0.96–1.42	0.12	927	1.25	1.00–1.56	0.045
19 weeks	1653	1.16	0.98–1.37	0.08	1416	1.26	1.05–1.51	0.012
34 weeks	1614	1.00	0.84–1.18	0.97	1375	1.06	0.88–1.27	0.55
Birth	1609	0.94	0.80–1.11	0.45	1409	0.95	0.80–1.13	0.56
6 months	1263	1.00	0.83–1.20	0.96	1222	1.01	0.83–1.22	0.94
12 months	1644	0.90	0.77–1.06	0.20	1407	0.92	0.78–1.09	0.35
Abdominal circumference (SD)								
11 weeks	1002	1.16	0.94–1.43	0.16	863	1.21	0.96–1.52	0.10
19 weeks	1647	1.10	0.93–1.30	0.28	1411	1.14	0.95–1.38	0.15
34 weeks	1672	0.87	0.74–1.03	0.11	1428	0.88	0.74–1.05	0.16
Birth	1607	0.96	0.81–1.13	0.62	1407	0.92	0.77–1.10	0.37
6 months	1268	1.07	0.90–1.28	0.43	1227	1.10	0.92–1.31	0.31
12 months	1635	1.06	0.90–1.24	0.48	1402	1.04	0.88–1.23	0.66

	Univariate				Multivariate*			
	n	OR	95% CI	P value	n	OR	95% CI	P value
Head: abdominal circumference ratio Z scores (SD)								
11 weeks	949	1.06	0.86–1.30	0.61	813	1.02	0.81–1.27	0.90
19 weeks	1644	1.07	0.91–1.26	0.40	1408	1.09	0.92–1.29	0.31
34 weeks	1614	1.20	1.02–1.41	0.030	1375	1.22	1.03–1.46	0.025
Birth	1607	1.02	0.87–1.20	0.82	1407	1.07	0.89–1.28	0.47
6 months	1262	0.93	0.77–1.11	0.41	1221	0.91	0.76–1.09	0.32
12 months	1625	0.89	0.76–1.05	0.17	1393	0.91	0.76–1.08	0.28

*Adjusted for gestational age at birth, infant sex, maternal BMI, education and smoking in pregnancy. SD: standard deviation.

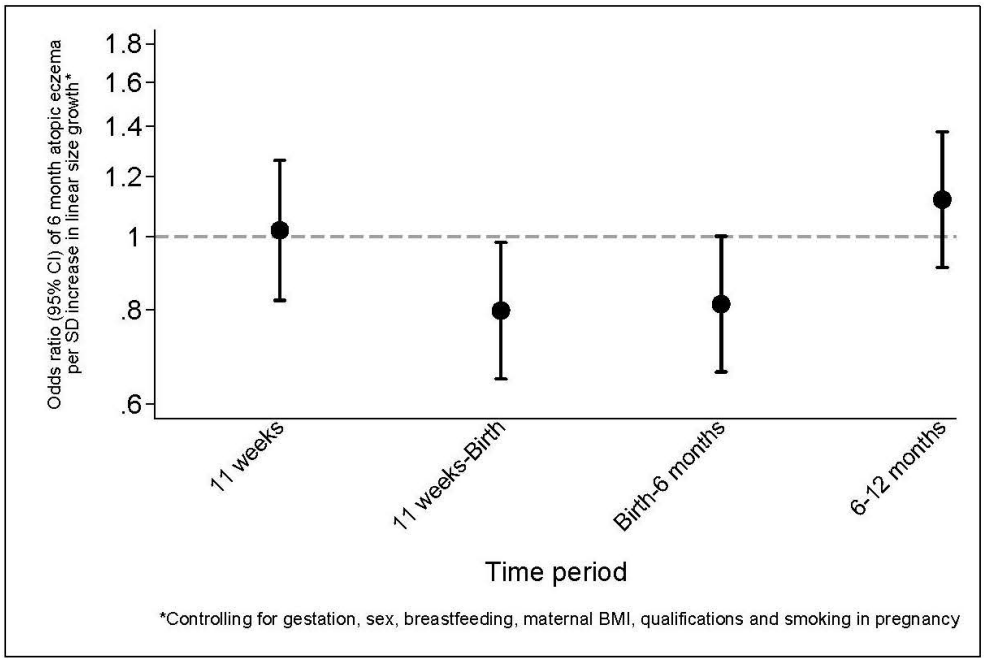
Table 28. Growth velocities in relation to eczema at age 12 months

	Univariate				Multivariate*			
	n	OR	95% CI	P value	n	OR	95% CI	P value
Linear growth (SD								
11 weeks	1005	1.15	0.94–1.40	0.18	975	1.22	0.98–1.52	0.07
11 – 19 weeks	1005	0.94	0.77–1.16	0.58	975	0.94	0.76–1.16	0.55
19 – 34 weeks	1005	0.99	0.81–1.22	0.96	975	0.98	0.79–1.20	0.81
34 weeks – Birth	1005	0.84	0.69–1.04	0.11	975	0.83	0.68–1.02	0.08
Birth – 6 months	1005	0.81	0.66–0.99	0.039	975	0.81	0.66–1.00	0.052
6 – 12 months	1005	0.91	0.74–1.12	0.36	975	0.90	0.73–1.11	0.34
Head circumference (SD)								
11 weeks	749	1.30	1.02–1.66	0.033	727	1.31	1.01–1.70	0.039
11 – 19 weeks	749	0.95	0.74–1.21	0.66	727	0.94	0.73–1.20	0.63
19 – 34 weeks	749	0.95	0.75–1.21	0.70	727	0.93	0.72–1.19	0.55
34 weeks – Birth	749	0.88	0.69–1.12	0.29	727	0.88	0.69–1.12	0.30
Birth – 6 months	749	1.06	0.83–1.35	0.64	727	1.07	0.84–1.37	0.56
6 – 12 months	749	0.90	0.71–1.15	0.41	727	0.91	0.70–1.17	0.45
Abdominal circumference (SD)								
11 weeks	724	1.24	0.97–1.60	0.09	704	1.24	0.95–1.62	0.11
11 – 19 weeks	724	0.97	0.76–1.24	0.82	704	0.97	0.76–1.24	0.82
19 – 34 weeks	724	0.73	0.57–0.93	0.012	704	0.67	0.51–0.88	0.003
34 weeks – Birth	724	1.13	0.88–1.44	0.35	704	1.17	0.91–1.51	0.23
Birth – 6 months	724	1.12	0.87–1.44	0.37	704	1.11	0.86–1.43	0.42
6 – 12 months	724	1.00	0.78–1.27	0.98	704	0.99	0.77–1.26	0.91

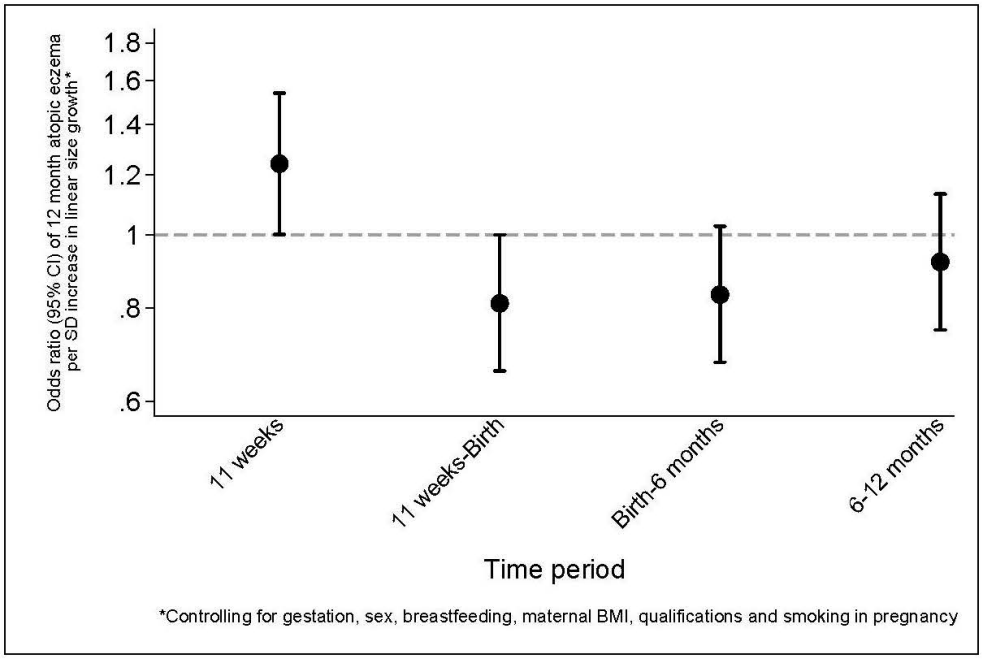
*Adjusted for gestational age at birth, infant sex, maternal BMI, education and smoking in pregnancy. SD: standard deviation.

Figure 30. Linear growth velocities in relation to atopic eczema at age a) 6 and b)12 months

a. Linear growth velocities in relation to atopic eczema at 6 months



b. Linear growth velocities in relation to atopic eczema at 12 months



5.3.4 Associations of fetal size and growth velocities with infant atopy at age 12 months

Rapid infant head growth between birth and 6 months was associated with an increased risk of infant atopy at age 12 months, this relationship remained significant after taking into account of confounding variables (RR 1.62, 95% CI 1.21–2.17, p 0.001). Infant and fetal linear and abdominal growth at various stages did not relate to infant atopy at age 12 months (Table 29).

5.3.5 Associations of maternal serum nicotinamide/anthranilic acid with fetal and infant growth velocities

Maternal serum nicotinamide concentrations were not significantly related to fetal and infant growth velocities (Table 30). Anthranilic acid, however, was related to fetal head circumference growth at 19–34 weeks gestation (β = −0.15 (95% CI −0.27to −0.03) SD/SD, p = 0.017) where a higher maternal serum anthranilic acid concentration was associated with faltering of fetal head growth, with no other significant associations with the other growth parameters (Table 31).

5.3.6 Associations of maternal stress and mood with fetal and infant growth velocities

Maternal perceived stress affecting health was linked with fetal linear growth velocity between 11–19 weeks (β = 0.05 (95%CI 0.01 to 0.09) SD/category, p = 0.019), where greater stress affecting health was associated with a higher 11–19 week linear growth velocity (Table 32). There were no links with other growth parameters. Maternal perceived stress in daily living was linked with infant abdominal circumference growth velocity between birth and age 6 months (β = 0.07 (95%CI 0.00 to 0.14) SD/category, p = 0.045) where greater stress in daily living was associated with higher early infancy abdominal circumference growth (Table 33). There were no links with other growth parameters. Maternal distress as ascertained by GHQ was not significantly related to fetal and infant growth velocities (Table 34).

Table 29. Fetal and infant growth velocities in relation to atopy at age 12 months

	Univariate				Multivariate*			
	n	RR	95% CI	P value	n	RR	95% CI	P value
Linear growth (SD)								
11 weeks	825	1.00	0.84–1.20	0.98	720	0.98	0.79–1.20	0.82
11 – 19 weeks	825	1.20	0.92–1.56	0.19	720	1.11	0.84–1.47	0.46
19 – 34 weeks	825	1.03	0.82–1.30	0.78	720	1.07	0.84–1.36	0.56
34 weeks – Birth	825	1.15	0.92–1.44	0.23	720	1.12	0.89–1.41	0.35
Birth – 6 months	825	0.92	0.72–1.17	0.50	720	0.90	0.70–1.17	0.44
6 – 12 months	825	0.74	0.53–1.03	0.076	720	0.75	0.51–1.11	0.15
Head circumference (SD)								
11 weeks	598	1.09	0.87–1.37	0.47	519	1.11	0.87–1.41	0.42
11 – 19 weeks	598	0.96	0.72–1.30	0.81	519	0.92	0.66–1.28	0.62
19 – 34 weeks	598	1.07	0.83–1.36	0.61	519	1.11	0.85–1.45	0.44
34 weeks – Birth	598	0.84	0.58–1.21	0.34	519	0.89	0.61–1.30	0.56
Birth – 6 months	598	1.47	1.08–2.00	0.014	519	1.62	1.21–2.17	0.001
6 – 12 months	598	1.01	0.60–1.71	0.96	519	1.07	0.62–1.84	0.82
Abdominal circumference (SD)								
11 weeks	577	0.95	0.75–1.22	0.71	505	0.96	0.72–1.26	0.75
11 – 19 weeks	577	1.20	0.81–1.79	0.37	505	1.26	0.84–1.89	0.27
19 – 34 weeks	577	0.97	0.72–1.30	0.83	505	0.88	0.63–1.23	0.45
34 weeks – Birth	577	1.26	0.91–1.76	0.16	505	1.36	0.96–1.94	0.087
Birth – 6 months	577	1.16	0.89–1.50	0.27	505	1.22	0.90–1.65	0.19
6 – 12 months	577	0.85	0.62–1.16	0.31	505	0.81	0.61–1.07	0.13

*Adjusted for gestational age, infant sex, maternal BMI, education, smoking in pregnancy and maternal atopy. SD: standard deviation.

Table 30. Multivariate analysis of fetal and infant growth velocities in relation to maternal serum nicotinamide.

	n	β	95% CI	P value
Linear growth (SD)				
11 weeks	229	0.03	-0.10 to 0.17	0.61
11 – 19 weeks	229	0.03	-0.06 to 0.12	0.47
19 – 34 weeks	229	0.11	-0.01 to 0.23	0.063
34 weeks – Birth	229	0.00	-0.10 to 0.11	0.97
Birth – 6 months	229	-0.07	-0.17 to 0.04	0.22
6 – 12 months	229	-0.01	-0.08 to 0.07	0.85
Head circumference (SD)				
11 weeks	179	0.01	-0.15 to 0.16	0.94
11 – 19 weeks	179	0.05	-0.03 to 0.14	0.23
19 – 34 weeks	179	0.09	-0.04 to 0.21	0.16
34 weeks – Birth	179	0.03	-0.07 to 0.13	0.60
Birth – 6 months	179	-0.03	-1.14 to 0.08	0.63
6 – 12 months	179	0.02	-0.04 to 0.09	0.48
Abdominal circumference (SD)				
11 weeks	172	0.02	-0.14 to 0.19	0.77
11 – 19 weeks	172	0.01	-0.09 to 0.12	0.84
19 – 34 weeks	172	0.09	-0.04 to 0.22	0.18
34 weeks – Birth	172	-0.00	-0.11 to 0.10	0.95
Birth – 6 months	172	-0.03	-0.18 to 0.12	0.67
6 – 12 months	172	-0.05	-0.17 to 0.08	0.48

Adjusted for maternal education, smoking during pregnant, parity, infant sex and breastfeeding duration.

Table 31. Multivariate analysis of fetal and infant growth velocities in relation to maternal serum anthranilic acid.

	n	β	95% CI	P value
Linear growth (SD)				
11 weeks	229	0.01	−0.12 to 0.14	0.85
11 – 19 weeks	229	0.08	−0.00 to 0.17	0.06
19 – 34 weeks	229	0.02	−0.10 to 0.14	0.76
34 weeks – Birth	229	−0.03	−0.14 to 0.07	0.52
Birth – 6 months	229	0.07	−0.04 to 0.17	0.21
6 – 12 months	229	−0.04	−0.11 to 0.04	0.32
Head circumference (SD)				
11 weeks	179	0.10	−0.05 to 0.25	0.20
11 – 19 weeks	179	−0.05	−0.13 to 0.04	0.29
19 – 34 weeks	179	−0.15	−0.27 to −0.03	0.017
34 weeks – Birth	179	−0.04	−0.14 to 0.06	0.45
Birth – 6 months	179	0.06	−0.06 to 0.17	0.33
6 – 12 months	179	0.05	−0.01 to 0.12	0.11
Abdominal circumference (SD)				
11 weeks	172	0.06	−0.10 to 0.21	0.48
11 – 19 weeks	172	0.01	−0.09 to 0.12	0.79
19 – 34 weeks	172	0.09	−0.04 to 0.22	0.18
34 weeks – Birth	172	−0.04	−0.15 to 0.06	0.41
Birth – 6 months	172	0.06	−0.08 to 0.20	0.41
6 – 12 months	172	0.01	−0.11 to 0.13	0.93

Adjusted for maternal education, smoking during pregnant, parity, infant sex and breastfeeding duration

Table 32. Multivariate analysis of fetal and infant growth velocities in relation to maternal stress affecting health; β coefficients are SD per category of maternal stress affecting health (5 categories–none to extremely).

	n	β	95% CI	P value
Linear growth (SD)				
11 weeks	997	-0.04	-0.10 to -0.02	0.17
11 – 19 weeks	985	0.05	0.01 to 0.09	0.019
19 – 34 weeks	997	-0.02	-0.07 to 0.04	0.57
34 weeks – Birth	997	0.04	-0.00 to 0.09	0.07
Birth – 6 months	997	-0.00	-0.05 to 0.04	0.94
6 – 12 months	997	0.01	-0.02 to 0.05	0.44
Head circumference (SD)				
11 weeks	743	0.01	-0.06 to 0.08	0.74
11 – 19 weeks	743	-0.00	-0.05 to 0.04	0.86
19 – 34 weeks	743	0.02	-0.04 to 0.07	0.57
34 weeks – Birth	743	0.00	-0.04 to 0.05	0.89
Birth – 6 months	743	0.01	-0.04 to 0.06	0.83
6 – 12 months	743	-0.00	-0.03 to 0.02	0.79
Abdominal circumference (SD)				
11 weeks	718	0.02	-0.05 to 0.09	0.52
11 – 19 weeks	718	0.02	-0.03 to 0.07	0.36
19 – 34 weeks	718	0.04	-0.02 to 0.09	0.24
34 weeks – Birth	718	0.02	-0.03 to 0.08	0.43
Birth – 6 months	718	0.01	-0.05 to 0.08	0.68
6 – 12 months	718	-0.00	-0.06 to 0.05	0.91

Adjusted for maternal eczema, maternal education and parity.

Table 33. Multivariate analysis of fetal and infant growth velocities in relation to maternal stress in daily living; β coefficients are SD per category of maternal stress in daily living (5 categories – none to a great deal).

	n	β	95% CI	P value
Linear growth (SD)				
11 weeks	996	-0.04	-0.10 to 0.03	0.25
11 – 19 weeks	984	-0.00	-0.04 to 0.04	0.90
19 – 34 weeks	996	-0.03	-0.08 to 0.02	0.29
34 weeks – Birth	996	0.01	-0.04 to 0.05	0.76
Birth – 6 months	996	-0.01	-0.06 to 0.03	0.62
6 – 12 months	996	-0.00	-0.04 to 0.03	0.94
Head circumference (SD)				
11 weeks	742	-0.03	-0.10 to 0.03	0.40
11 – 19 weeks	742	-0.02	-0.06 to 0.03	0.46
19 – 34 weeks	742	0.02	-0.04 to 0.07	0.58
34 weeks – Birth	742	-0.03	-0.07 to 0.02	0.29
Birth – 6 months	742	0.01	-0.04 to 0.07	0.57
6 – 12 months	742	-0.01	-0.04 to 0.02	0.69
Abdominal circumference (SD)				
11 weeks	717	-0.05	-0.12 to 0.01	0.12
11 – 19 weeks	717	0.01	-0.04 to 0.06	0.73
19 – 34 weeks	717	0.02	-0.04 to 0.07	0.58
34 weeks – Birth	717	-0.04	-0.09 to 0.01	0.14
Birth – 6 months	717	0.07	0.00 to 0.14	0.045
6 – 12 months	717	-0.03	-0.08 to 0.03	0.32

Adjusted for maternal eczema, maternal education and parity.

Table 34. Multivariate analysis of fetal and infant growth velocities in relation to maternal psychological distress (GHQ 3 or more as a binary outcome).

	n	β	95% CI	P value
Linear growth (SD)				
11 weeks	533	0.03	-0.16 to -0.23	0.75
11 – 19 weeks	528	0.04	-0.10 to 0.18	0.54
19 – 34 weeks	533	-0.04	-0.20 to 0.13	0.68
34 weeks – Birth	533	0.10	-0.04 to 0.25	0.17
Birth – 6 months	533	0.08	-0.07 to 0.22	0.29
6 – 12 months	533	0.02	-0.10 to 0.13	0.77
Head circumference (SD)				
11 weeks	399	-0.11	-0.34 to 0.12	0.34
11 – 19 weeks	399	0.14	-0.01 to 0.30	0.07
19 – 34 weeks	399	-0.06	-0.23 to 0.12	0.54
34 weeks – Birth	399	-0.01	-0.16 to 0.13	0.89
Birth – 6 months	399	-0.05	-0.22 to 0.12	0.60
6 – 12 months	399	0.03	-0.07 to 0.12	0.56
Abdominal circumference (SD)				
11 weeks	380	-0.18	-0.39 to 0.04	0.11
11 – 19 weeks	380	-0.01	-0.16 to 0.14	0.91
19 – 34 weeks	380	0.08	-0.11 to 0.26	0.41
34 weeks – Birth	380	-0.04	-0.21 to 0.13	0.63
Birth – 6 months	380	0.19	-0.02 to 0.40	0.07
6 – 12 months	380	-0.01	-0.20 to 0.17	0.88

Adjusted for maternal eczema, maternal education and parity.

5.4 Discussion

We found that infants with atopic eczema at age 6 months have faltering of linear growth beginning after 11 weeks' gestation, with a higher head to abdominal circumference ratio at 34 weeks' gestation. Infants with atopic eczema at age 12 months had a larger head circumference in early pregnancy and faltering of abdominal growth in the second half of pregnancy. Postnatally, the infants with eczema at ages 6 and 12 months were shorter than infants without eczema, but the longitudinal measurements of fetal size suggest that this growth faltering commenced prior to birth. These associations were robust to adjustment for potentially confounding variables, notably maternal age, BMI, education, smoking in pregnancy and eczema in past 12 months and infant sex and duration of breastfeeding.

The findings provide the first longitudinal data examining fetal and infant growth velocities to infant atopic eczema at ages 6 and 12 months. Previous studies have generally focused on anthropometric measurements at birth or during infancy, finding that these were not related to the prevalences of reported eczema or hay fever by the age of 13 years (Leadbitter et al., 1999), or to eczema at age 7 years (Carrington and Langley-Evans, 2006). The same infant size at birth can be achieved through different patterns of fetal growth and few previous studies have examined patterns of fetal growth in relation to atopic outcomes. An increase in size between first trimester crown-rump length and second trimester bi-parietal diameter has been associated with higher risks of eczema and asthma at age 10 years (Turner et al., 2011). Pike and colleagues (Pike et al., 2010) reported that rapid early gestation fetal abdominal growth followed by late gestation faltering of abdominal circumference growth was associated with atopy at age 3 years, and late gestation abdominal growth faltering was associated with atopic wheeze.

Mechanistically, it is known that intrauterine growth restriction leads to disproportionate fetal growth and a high head to abdominal circumference ratio as a result of "brain sparing" responses, which direct nutrient-rich blood to maintain brain growth away from truncal organs including the thymus, with potential impact on immune development. Animal and human studies have linked fetal and birth anthropometric parameters indicative of undernutrition during pregnancy with smaller thymic size and impaired thymic development (Varg et al., 2011, Lang et al., 2000, Fulford et al., 2013). We found that a larger fetal head circumference at 11 and 19 weeks' gestation was linked with a higher risk of eczema, with evidence of disproportionate head to abdominal circumference at

34 weeks. Therefore, we suspect that these growth alterations might influence thymus development, resulting in a diminished population of Th 1 lymphocytes, favouring Th2 populations and consequently raised serum IgE (Godfrey et al., 1994), an immune reaction that is seen in atopic eczema and other atopic conditions (Prescott et al., 1999). Whereas the exact mechanisms responsible for the association of altered fetal growth with development of atopic eczema are unknown, the observation points to involvement of periconception or early pregnancy factors in the etiology of infantile atopic eczema. The patterns of association between anthropometric measurements and infant eczema at ages 6 and 12 months differed; this could reflect a chance finding, or heterogeneity in the etiology and pathogenesis of atopic eczema in early childhood (Loo et al., 2015).

Hitherto, it has been thought that the chronic inflammatory process associated with atopic eczema may result in growth impairment because proinflammatory cytokines such as IL-6, which promotes Th2 differentiation and simultaneously inhibit Th1, can act at the level of the growth plate or may alter the Growth Hormone – Insulin-Like Growth Factor 1 (IGF-1) axis (Wong et al., 2016) Animal studies show growth impairment in juvenile chronic arthritis and chronic inflammatory bowel disease independent of nutritional intake as a result of an IL-6-mediated decrease in IGF-1 (Ballinger et al., 2000, De Benedetti et al., 1997). Alternatively, it has been proposed that infants with eczema may be susceptible to postnatal growth impairment due to treatment of the condition with topical or systemic corticosteroids (Aylett et al., 1992), poor nutrition as a result of an inappropriately restrictive diet (Keller et al., 2012), and associated disturbance in sleep (Silverberg and Paller, 2015). However, the findings in this current chapter suggest that intrinsic and intrauterine factors that influence growth may modify the risk of developing atopic eczema as opposed to growth faltering developing postnatally as a result of the inflammatory skin condition or its treatment. These findings have important clinical implications, and suggest that improved control of the inflammatory process in infantile atopic eczema or avoidance of topical corticosteroids may not necessarily resolve the growth impairment seen in many infants with eczema.

This data also shows a link between rapid fetal head circumference growth between delivery and age 6 months with increased risk of infant atopy at age 12 months. It is notable that the links seen with infant atopic eczema are not seen in relation to infant atopy although the two conditions are believed to share common mechanisms in their pathogenesis.

Additionally, the data demonstrate an association between maternal serum anthranilic acid and fetal head circumference growth in late pregnancy, although this association was not demonstrated for nicotinamide. Little is known about the role of anthranilic acid in growth, although it is recognised that tryptophan (its precursor) is involved in protein synthesis and nicotinamide (a related tryptophan metabolite) is involved in energy production and therefore tryptophan and nicotinamide may have a role in growth. It is possible that the link between maternal anthranilic acid and head circumference growth may represent downstream effects of another signalling event or molecule.

Moreover, greater maternal preconception perceived stress affecting health was associated with increased linear growth between 11 and 19 week's gestation, and maternal perceived stress experienced in daily living with increased abdominal circumference growth between birth and 6 months. These findings appear counterintuitive and can be chance findings. However, it does parallel experimental data from a rodent study, which reported that prenatal maternal restraint stress was associated with subsequently higher bone area and a potentially higher bone formation rate in the offspring (Amugongo and Hlusko, 2014). This faster growth rate could reflect a faster lifecourse trajectory in response to early life stress, which has been described in birds (Farrell et al., 2015) and humans (Entringer et al., 2011).

The strengths of this study are its large sample size, its prospective nature, the standardised assessment of fetal/infant size and eczema by trained staff and control for confounding factors. Nevertheless, limitations were the use of questionnaire-based assessments for part of the assessment for the diagnosis of atopic eczema, which may introduce bias. A modified UKWPDC for diagnosis of atopic dermatitis was used. Atopic disease in a first degree relative was omitted from the case definition to avoid too narrow a focus on familial cases of atopic eczema. Exploratory and hypothesis-generating methods were used to determine the described growth patterns, this reduced total participants included in the growth pattern analyses as it excluded those with missing data and introduced multiple statistical testing which is a potential limitation of this study, however, only three parameters (linear size, abdominal circumference and head circumference) were examined to lessen the inherent risks.

In conclusion, the data in this chapter demonstrates links between fetal and infant anthropometric measurements and growth patterns with the risk of atopic eczema at ages 6 and 12 months. The findings suggest that growth falters prior

to the onset of the inflammatory process associated with atopic eczema or its treatment, and provide additional support for important prenatal influences on this skin condition.

Chapter 6: Discussion

The case for a developmental contribution to the origin of atopic eczema is becoming stronger, with the combination of genetic susceptibility and early life environmental exposures influencing the risk of developing the condition. As research into new treatments continues with little in the way of simple, safe and effective new therapeutics currently under evaluation, developing preventative strategies becomes crucial as atopic eczema is a common condition that has a major impact on the individuals affected, their families and the healthcare system as a whole.

6.1 Summary of findings

The objectives for this thesis are outlined in Chapter One. Exploratory analyses were carried out to examine *a priori* hypotheses formed based on previous literature and clinical observations. This thesis reports the research conducted to examine specific developmental influences on infant atopic eczema at ages 6 and 12 months. Work in the thesis examined the influences of maternal serum nicotinamide and related tryptophan metabolite concentrations and of maternal stress and low mood on infant atopic eczema risk, and the associations of fetal and infant growth with infantile atopic eczema. The principal findings were:

- Higher maternal serum concentrations of nicotinamide and anthranilic acid were associated with a lower risk of eczema at age 12 months.
- Preconception perceived stress affecting health and stress in daily living were associated with an increased risk of offspring atopic eczema at age 12 months. Findings were similar for maternal psychological distress preconception. Low maternal mood between delivery and 6 months postpartum was associated with an increased risk of infantile atopic eczema at age 12 months, but no significant association between postnatal mood and atopic eczema was seen after taking account of preconception stress.
- Infants with atopic eczema at age 6 months demonstrated premorbid altered patterns of growth, including lower velocities of linear growth from 11 weeks' gestation to birth, and from birth to age 6 months. Infants with atopic eczema at age 12 months had a larger head circumference in early gestation and faltering of abdominal growth velocity from 19–34 weeks gestation.

6.2 Mechanistic considerations in relation to the findings

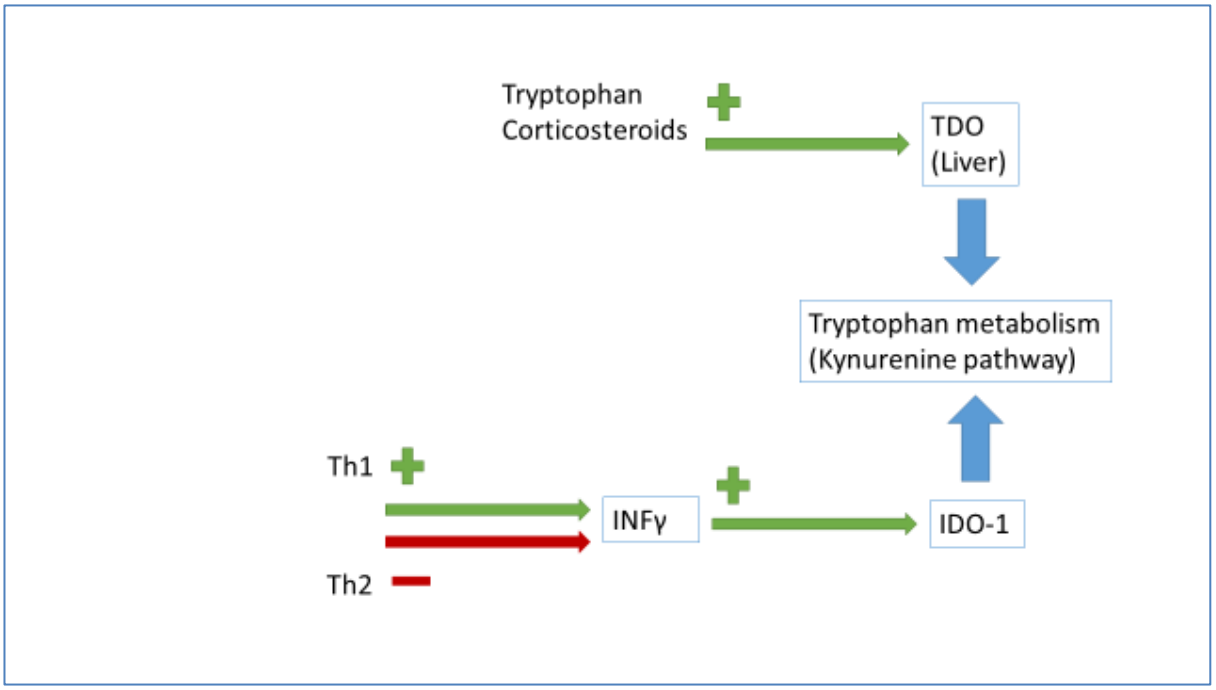
6.2.1 Maternal nicotinamide and related tryptophan metabolites

Nicotinamide has been used topically in the treatment of atopic eczema (Soma et al., 2005). Its biological functions in immune-regulation and in modifying the structure and function of skin are described in Chapter 3. Serum nicotinamide concentrations have not previously been examined in relation to skin disorders, although deficiency of niacin, a precursor of nicotinamide, is linked to dermatitis as part of the clinical picture of pellagra. Maternal serum concentrations of nicotinamide and related tryptophan metabolites have not hitherto been examined in relation to the offspring's risk of atopic eczema.

The results described in Chapter 3 show that higher maternal nicotinamide and anthranilic acid, a related tryptophan metabolite concentrations in pregnancy were associated with a lower risk of offspring atopic eczema at age 12 months. There is an abundant literature on the metabolism of tryptophan, the precursor of nicotinamide but less so for nictoinamide.

Through the kynurenine pathway, its main route of metabolism, tryptophan generates kynurenine derivatives. In this pathway, there are two key enzymes; TDO that is chiefly found in the liver and is induced by tryptophan concentrations and corticosteroids, and IDO-1 that is found in various cells such as macrophages and is induced by cytokines, preferentially by INF-γ (Gostner et al., 2016) . Th1 associated cytokines such as INF- γ are downregulated in a Th2 dominant inflammatory response. These cytokines have the capability of strongly inducing IDO which is important in tryptophan metabolism and the resultant regulation of the immune system (Gostner et al., 2016) (Figure 31). Through depletion of tryptophan and the production of bioactive catabolites, IDO has the potential to inhibit the proliferation of immune cells (Gostner et al., 2016).

Figure 31. Inducers of tryptophan metabolism



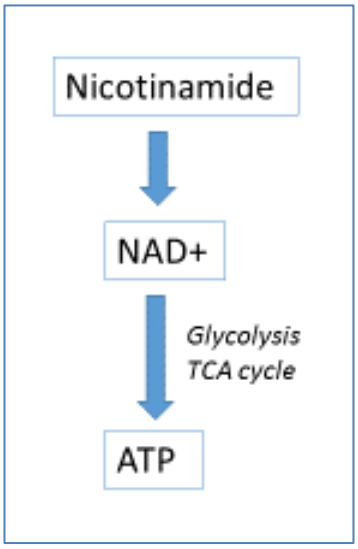
Moreover, antioxidants in food preservatives, additives and colourants can inhibit the Th1-type immune responses in vitro, mediated by IDO activity and neopterin production (Gostner et al., 2014, Maier et al., 2010) and thus increase risks of allergy (Fuchs, 2012, Zaknun et al., 2012). Tryptophan has a role in immunomodulation, with animal studies demonstrating a role for IDO in the immunological tolerance of pregnancy preventing rejection of the fetus (Munn et al., 1998). An increased kynurenine to tryptophan ratio results from activation of IDO and is associated with neopterin, a marker of immune activation (Murr et al., 2002); further supporting the immunomodulating function of these substances.

Although no links between maternal tryptophan and tryptophan metabolite concentrations with infant atopy at age 12 months were found in the analyses described in Chapter 3, previous studies have demonstrated that in individuals predisposed to atopic conditions (with an imbalance of Tregs), oxidative stress and T cell imbalances related to allergen NADPH oxidases can cause allergic disease. High serum concentrations of tryptophan and low IDO activity have been associated with allergic disease (Gostner et al., 2016, Buyuktiryaki et al., 2016). Raised tryptophan concentrations in individuals with allergy to pollen (Kositz et al., 2008) that only occurs outside of the pollen season has been reported (Ciprandi et al., 2010). There is, however, only slight change to the kynurenine to tryptophan ratio, an index of tryptophan breakdown (von Bubnoff et al., 2004).

Reduced tryptophan concentrations in pregnancy were recognised in 1996 (Schrocksnadel et al., 1996) and the active transport mechanisms of tryptophan across the placenta have been previously described (Cleal and Lewis, 2008). Indirect effects of increased tryptophan on the fetus as a result of maternal stress have also been suggested (Bonnin and Levitt, 2011). As demonstrated in Chapter 3, lower levels of anthranilic acid and nicotinamide do not point to a specific enzymatic block in the tryptophan pathway, and it is possible that lower levels of these two metabolites are a general reflection of perturbation of the pathway or low substrate availability. Also, a simple genetically determined deficiency is not likely as the association between lower maternal nicotinamide and anthranilic acid levels and the offspring's risk of atopic eczema is graded across the range of concentrations. There is evidence that the concentrations of the main kynurenine metabolites found in umbilical cord venous blood are in higher than in maternal venous blood, indicating the presence and activity of IDO, TDO and other pathway enzymes in the placenta, alongside fetal liver and splenic production of kynurenine metabolites (Munoz-Hoyos et al., 1998). Furthermore, during the onset of labour and associated activation of inflammatory pathways in spontaneous vaginal deliveries; maternal kynurenine and 3-hydroxyanthranilic acid concentrations have been found to be higher when compared to those delivered by caesarean section before labour had been fully established (Murthi et al., 2017, Kazda et al., 1998). Concentrations were also higher in cold blood (Murthi et al., 2017), supporting tryptophan consumption during labour.

In the SWS cohort studied, maternal late gestation serum nicotinamide was not related to infant and fetal growth parameters. Interestingly, higher anthranilic acid concentration, however, was associated with faltering head circumference growth velocity between 19-34 weeks. Tryptophan is important in protein synthesis, and can therefore impact fetal growth. Specifically, nicotinamide is thought to the primary source of NAD in mammalian cells even in the absence of deliberate dietary supplementation (Rongvaux et al., 2003). In a mouse model, nicotinamide administered to mothers resulted in an increase in fetal brain nicotinamide and brain NAD content, with nicotinamide preventing the decrease in adenosine triphosphate (ATP), a crucial 'energy' molecule, in fetal brains and thus averting fetal growth restriction (FGR) (Figure 32).

Figure 32. Nicotinamide metabolism and ATP production



Metabolomics studies suggest that in mothers receiving nicotinamide, fetal growth is maintained through sustained ATP production and occurs due to enhanced glycolysis and tricarboxylic acid (TCA) cycling as a result of improved NAD pools (Li et al., 2016). It is uncertain whether the changes seen in hypoxia can impact the developing fetus in the same way as suboptimal maternal serum nicotinamide concentrations, but the mechanisms proposed nonetheless highlight the importance of nicotinamide in maintaining ATP and fetal growth. In the third trimester, placental expression of genes relating to the tryptophan–kynurenine pathway enzymes, such as IDO, is significantly decreased with fetal growth restriction compared with gestation-matched placentas of uncomplicated pregnancies, suggesting a role for IDO in increased placental inflammatory status in fetal growth restriction (Murthi et al., 2017). Murthi et al (Murthi et al., 2017) also proposed that lower IDO expression would increase oxidative stress in placentas.

Although no links between fetal growth and maternal nicotinamide were identified in the SWS cohort studied, anthranilic acid was related to fetal head circumference growth in late gestation suggesting that the kynurenine pathway in the setting of pregnancy is indeed complex and poorly understood.

6.2.2 Maternal stress and low mood and infantile atopic eczema

The data described in Chapter 4 show that maternal preconception stress was associated with an increased risk of offspring atopic eczema at age 12 months but not at age 6 months. Findings were similar for maternal psychological distress preconception. Low maternal mood between delivery and 6 months

postpartum was also associated with an increased risk of infantile atopic eczema at age 12 months, but no significant association between postnatal mood and eczema was seen after taking account of preconception stress/mood variables. The data, are the first to show a link between maternal stress preconception and the risk of infantile atopic eczema.

Mechanisms for maternal prenatal stress resulting in effects in the fetus have been proposed and are described in Chapter 4. The stages at which the fetus is most sensitive to maternal stress are unknown, but the data presented in Chapter 4 point to the importance of maternal psychological wellbeing in the periconceptional period.

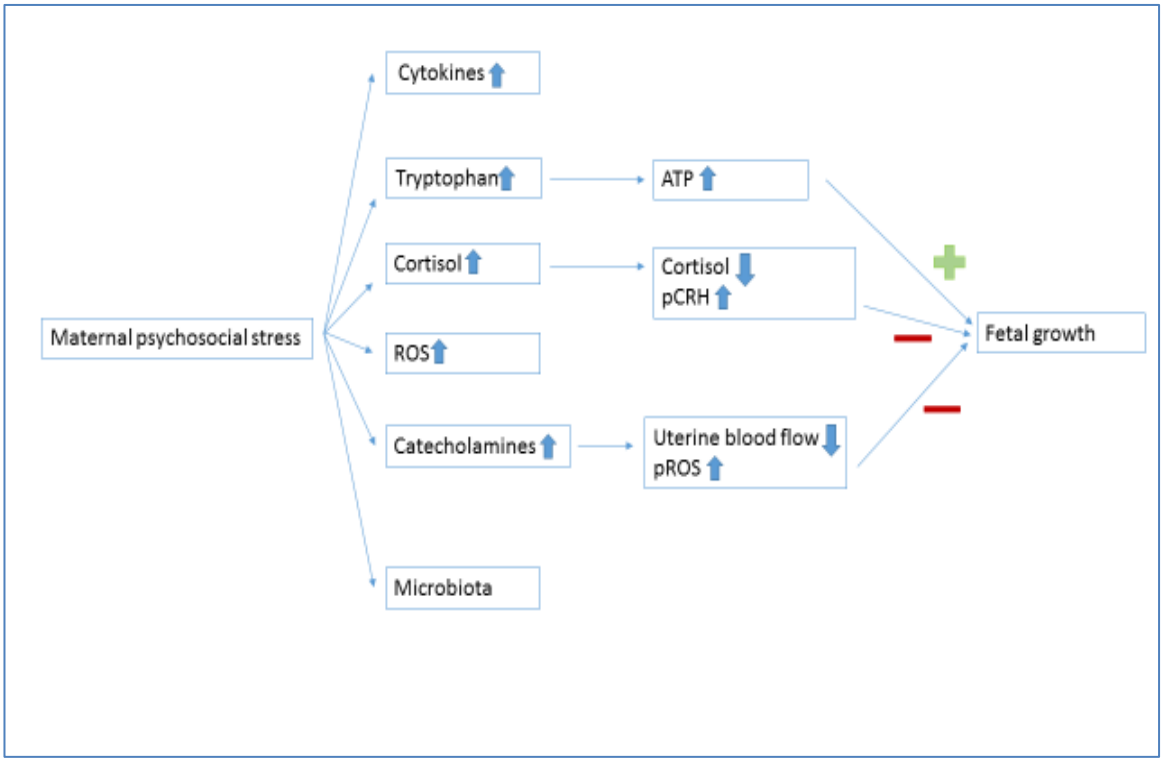
In pregnant rats, psychosocial stress has been linked with an increase in maternal tryptophan plasma concentrations and an associated increase in tryptophan and serotonin (5-HT) levels in the fetal brain (Peters, 1990). Other studies report various types of stressors leading to an increase in plasma tryptophan and 5-HT concentrations, this increase can be transient is some scenarios (Malyszko et al., 1994). Stress can impact serotonin metabolism, but this depends on the type, duration and intensity of the stressor. Whether stressors in humans can result in the same effects described above is unknown. In adult male rats an episode of restraint stress has been reported to increase corticosterone, a glucocorticoid, and stimulate hepatic TDO expression and activity. Repeated stress resulted in depression-related behaviour and changes similar to those seen with a single episode of stress. As TDO activity increased, kynurenine concentrations also increased and tryptophan concentrations fell indicating activation of the kynurenine pathway (Gibney et al., 2014). Placental TDO function is not well understood. Liver TDO is induced by glucocorticoids (Gibney et al., 2014, Liao et al., 2007) and it is therefore possible that placental TDO may be impacted by the developing fetal HPA axis. It is unclear how 5-HT and tryptophan act when fetal HPA axis is not yet fully mature and glucocorticoid receptors are not widely expressed. The placenta itself is a source of 5-HT in early pregnancy which is important to the developing fetal brain.

Furthermore, a high tryptophan diet administered to pregnant rats caused hyperserotonaemia (Castrogiovanni et al., 2014, Musumeci et al., 2014), low growth hormone concentrations (Musumeci et al., 2014) and delayed maturation of the developing offspring's central serotonergic system (Huether et al., 1992). Tryptophan concentrations in various fetal tissue increase in a dose-dependent

manner and there is evidence that the placenta is unable to prevent the influx of tryptophan to the fetus (Arevalo et al., 1991).

Mouse studies have suggested a number of plausible mechanisms by which maternal stress can influence fetal growth (Figure 33), but it is unknown if stress induced molecular changes occurring in a murine pregnancy have the same influences in the developing human fetus.

Figure 33. Potential pathway by which maternal psychosocial stress can impact fetal growth



6.2.3 Fetal and infant growth patterns

In the SWS cohort studied, infants with atopic eczema at age 6 months had faltering of linear growth beginning after 11 weeks' gestation, with a higher head to abdominal circumference ratio at 34 weeks' gestation. Infants with atopic eczema at age 12 months had a larger head circumference in early pregnancy and faltering of abdominal growth in the second half of pregnancy. Postnatally, the infants with eczema at ages 6 and 12 months were shorter than infants without eczema, but the longitudinal measurements of fetal size suggested that this growth faltering commenced prior to birth. Furthermore, the data presented suggest a link between rapid head circumference growth between delivery and 6 months and a higher risk of infant atopy at 12 months.

The findings described in this thesis provide the first longitudinal data examining fetal and infant growth velocities to infant atopic eczema at ages 6 and 12 months. Short stature in children with atopic eczema suggesting linear growth impairment has been previously reported (Kristmundsdottir and David, 1987, Massarano et al., 1993, Park et al., 2013). Patel et al (Patel et al., 1997) suggested this impairment is temporary as within their cohort, participants with a history of atopic eczema starting before the age of 5 years with a severity requiring specialist care were not short as adults. These studies however did not include fetal growth measurements or control for several important confounding variables. Anthropometric measurements at birth or during infancy have been used a proxy for fetal growth in some studies; these have found that these measurements not related to the prevalences of reported eczema or hay fever by the age of 13 years (Leadbitter et al., 1999), or to eczema at age 7 years (Carrington and Langley-Evans, 2006). Furthermore, head circumference at birth has not found to be associated with atopic eczema, asthma, rhinitis or sensitisation to common allergens (Leadbitter et al., 1999, Gregory et al., 1999, Lopuhaa et al., 2000). One study has, however, associated a large head circumference at birth with childhood asthma (Fergusson et al., 1997).

The inconsistencies in the evidence may reflect the variation in study methodology, outcome definition and confounding factors taken in account. However, it is clear that in- utero influences are important in determining later risk of atopic disease as demonstrated by twin studies showing that birth weight is influential on the risk of atopic eczema with similar risk in monozygotic and dizygotic twin pairs (Lundholm et al., 2010). Other studies have shown that birth weight had more influence on the risk of asthma in monozygotic twins than in dizygotic twin pairs, suggesting fetal growth and childhood atopic conditions may be associated independently of shared genetic factors (Ortqvist et al., 2009, Kindlund et al., 2010).

Godfrey et al reported that individuals with raised serum IgE had a larger head circumference and weighed more at birth but had similar crown heel length at birth representing disproportionate growth of head in relation to trunk. (Godfrey et al., 1994) Similar associations have been reported by others (Leadbitter et al., 1999, Gregory et al., 1999, Oryszczyn et al., 1999, Lopuhaa et al., 2000). Disproportionate fetal growth has been related to rapid fetal growth and catch up, putting the fetus at risk of undernutrition in the later stages of pregnancy. Brain growth is then preserved and favoured over other body parts and organs including the thymus, representing an adaptive response to late gestation

undernutrition; such mechanisms may explain the association between fetal growth and later infant risk of eczema (Gluckman et al., 2005). Benn et al. (Benn et al., 2001), however, debated that thymus size and head circumference are positively related and that thymus size was not associated to allergic disease at age 5 years, counter to the brain sparing hypothesis.

The data showed that a larger fetal head circumference at 11 and 19 weeks' gestation was linked with a higher risk of eczema, with further evidence of a disproportionate head to abdominal circumference ratio at 34 weeks gestation. One possibility is that these growth alterations might influence fetal thymus development, possibly resulting in a diminished population of Th1 lymphocytes, favouring Th2 populations. We also found that postnatally, rapid head circumference growth in the first 6 months of life is linked to increased infant atopy at 12 months. Pike et al. (Pike et al., 2010) who have previously examined atopy at age 3 years in the SWS cohort, found no association with postnatal growth velocities (linear growth, weight, skin fold thickness), prenatal head circumference growth or birth weight but an increased risk of atopy with greater crown-heel length at birth.

It has been proposed that infants with eczema may be susceptible to postnatal growth impairment due to treatment of the condition with topical or systemic corticosteroids (Aylett et al., 1992), poor nutrition as a result of an inappropriately restrictive diet (Keller et al., 2012), or associated disturbances in sleep (Silverberg and Paller, 2015). It has also been proposed that the chronic inflammatory process associated with atopic eczema might result in growth impairment as proinflammatory cytokines such as IL-6, which promotes Th2 differentiation and simultaneously inhibits Th1, could act at the level of the growth plate or might alter the Growth Hormone-IGF-1 axis (Wong et al., 2016). The data shown in Chapter 5 suggest, however, that changes in growth are already apparent before birth, pointing to the possibility that postnatal growth changes may at least in part reflect alterations in growth trajectory that have a prenatal origin.

Epigenetic influences also play a role in the development of the immune system with nutrition during the time around conception and during pregnancy being linked with DNA methylation that can potentially modify the risk of immune disease later in life (Barton et al., 2017) . For instance, animal studies have found that periconceptional restriction of co-factors for the methionine cycle resulted in

altered DNA methylation of fetal liver tissue and influencing immune responses, body composition and insulin-sensitivity (Sinclair et al., 2007).

The results presented in this thesis suggest periconception and early pregnancy are important periods in the etiology of atopic eczema. However, the exact mechanisms for the associations of altered fetal growth with the development of atopic eczema remain unknown and the most sensitive periods remain to be identified. The findings suggest that intrinsic and intrauterine factors that influence growth may modify the risk of developing atopic eczema, as opposed to growth faltering developing postnatally as a result of the inflammatory skin condition or its treatment.

6.3 Implications

The evidence presented in this thesis supports the concept that, in an adverse intrauterine environment resulting from suboptimal maternal nutrition or maternal psychological stress, the fetus adapts in a number of ways which can impact its growth and the development of organs and systems (Barker et al., 1991).

The data support a significant developmental contribution to the etiology of atopic eczema and highlight the importance of early life environmental exposures that have the potential to influence the development of the fetal skin and immune system. The data present evidence that ‘exposures’ are interlinked, with biologically plausible associations between maternal serum nicotinamide concentrations and maternal stress and between both maternal serum nicotinamide and maternal stress with fetal growth, acting together in impacting fetal health outcomes . The results described can help form future public health strategies for prevention of atopic eczema. The findings relating to maternal serum nicotinamide concentrations and risk of infant atopic eczema at 12 months suggest that maternal dietary or microbiome manipulation may have a role in reducing the risk of atopic eczema in infants; however, this may not be simple but certainly women planning to conceive should be advised to optimise their nutrition in preparation for pregnancy and lactation.

6.3.1 Directions for potential preventative strategies

Associations between dysbiosis, where there is a decrease in the diversity in the microflora, and inflammatory and allergic disease have been previously described

(Hill and Artis, 2010). A host's diet can be the source of substrate for intestinal bacteria metabolism and some metabolites produced can impact immune system development and regulation (Brestoff and Artis, 2013); for example, commensal bacteria can metabolise essential amino acids such as tryptophan that can be acquired through diet. Amino acids are in turn important to the microbiome; L-amino acids make up proteins and D-amino acids make up some bacterial cell walls (Cava et al., 2011). Bacterial derived amino acids are also thought to play a role in the development, function and homeostasis of immune cells. For example, melatonin, an L-tryptophan metabolite, can prevent some cytokines from being produced (Konturek et al., 2008, Kim et al., 2010). Tryptophan is produced by probiotic bacteria such as *Lactobacillus rhamnosus GG* and *Lactobacillus casei W56*, which have been reported to increase Tregs and decrease Th2 inflammatory responses in the lungs (Kepert et al., 2017). It is important to bear in mind, however, that commensal bacterial in the gut can also produce pro-inflammatory metabolites. The equilibrium between these functions may be genetically determined and influenced by external factors such as diet (Hirata and Kunisawa, 2017). Nevertheless, probiotic strains, *Bifidobacterium lactis Bb-12* and Lactobacillus strain GG, have been shown to clinically modify signs of inflammation in individuals with atopic eczema, suggesting that their favourable effects are not limited to the intestines, and may be beneficial in infants when they are risk of sensitisation to new allergens during weaning (Isolauri et al., 2000).

Early life environmental exposure, including diet, may alter disease risk through epigenetic processes, such as DNA methylation, which induce heritable changes in gene expression without a change in gene sequence (Prescott and Saffery, 2011). Methylation of key Th1/Th2 genes modulated by early life environment can affect infant and childhood risk of asthma, atopic dermatitis and atopy. Severe blunting of Th2 effects, both in the lungs and systemically are seen in transgenic mice with a dominant negative mutant of GATA3 (Zhang et al., 1999) and a number of SNPs in the GATA3 promoter and exons of GATA3 have been linked with high IgE atopic eczema and asthma phenotypes (Arshad et al., 2008, Pykalainen et al., 2005). GATA3 mRNA levels have been found to be elevated in peripheral blood mononuclear cells in individuals with atopic eczema, with levels returning to normal following treatment (Arakawa et al., 2004). Within the SWS, higher methylation of GATA3 CpGs -2145/-2143bp, IL-4R CpG +28239, IL-4R CpG +28269 bp and STAT4 CpG -229 were reported to be associated with a higher incidence of atopic eczema at the age of 12 months, although some

associations were attenuated after taking into account potential confounding variables (Barton et al., 2017). Methylation of IL-4R CpG +28180 has been reported to be positively associated with asthma incidence in the peripheral blood of 18 year old adolescents (Soto-Ramirez et al., 2013), while methylation of the neighbouring CpG site IL-4R CpG +28269 from umbilical cord samples has been positively associated with atopic eczema at 12 months (Barton et al., 2017). This suggests that tissue specific differences in DNA methylation and could explain why the findings reported in this thesis for atopic eczema differed to those for atopy.

Appropriate intervention studies and randomised controlled trials are needed to better evaluate the merit in nutrient and micronutrient supplementation; these trials may however be challenging to carry out. Moreover, low circulating levels of nutrients in maternal serum may not simply indicate dietary insufficiency and low maternal serum levels should be interpreted with caution as many nutrient are albumin bound and as part of the physiological process, partitioning of nutrients during pregnancy may favour the fetus. Nonetheless, the feasibility of undertaking randomised trials has however been demonstrated by interventions such as the preconception NiPPeR (Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health) nutritional and probiotic supplementation study currently underway in Southampton (Godfrey et al, 2017).

The thesis presents evidence for effects of stress acting in the preconception period on offspring eczema risk and interventions for reducing maternal stress preconception may be helpful to mothers planning a pregnancy. It remains unknown, however, when the most sensitive times of fetal development are. The findings also highlight the importance of optimising maternal nutrition and psychological health from as early as preconception.

The implications of the findings described are important clinically. They suggest that improved control of the inflammatory process in infantile atopic eczema or avoidance of topical corticosteroids may not necessarily resolve the growth impairment seen in many infants with eczema. Monitoring of growth parameters should be continued and encouraged, but recognising that they may be a more fundamental alteration in the trajectory of growth which commenced before birth.

6.3.2 Phenotypic variation

The evidence presented also supports that although atopic eczema and atopy share pathological mechanisms in terms of immunomodulation, there may be other important mechanisms acting in these particular manifestations of "allergy". For instance, atopy, determined by positive skin prick testing, indicates sensitisation where mast cells activate an immediate hypersensitivity response through their high affinity IgE receptor. Mast cells have a role in driving inflammation in asthma, but their role and that of IgE is less clear in atopic eczema (Kawakami et al., 2009).

The data presented shows a difference in the associations of maternal tryptophan metabolites, maternal stress and mood and fetal and infant growth patterns to offspring eczema at age 6 and 12 months. This suggests that atopic eczema occurring at age 6 months may be a different phenotype to the condition occurring at 12 months. This also raises the question whether infants with atopic eczema at age 12 months have different disease progression, severity and prognosis to infants with the condition at age 6 months. It is possible that phenotypes of atopic eczema and other atopic conditions may differ in terms of dominant determining factors. For instance, in GUSTO (Growing Up in Singapore Towards healthy Outcomes), an Asian mother-offspring cohort, early onset atopic dermatitis developing at age 6-12 months was linked with familial factors, while late onset atopic dermatitis developing after the age of 12 months was linked with consumption of antibiotics or probiotics (Loo et al., 2015).

6.4 Strengths and limitations of the research

The Southampton Women's Survey is a prospective study where women were characterised when they were not pregnant; this is a notable feature the study. The population is broadly representative of the UK population (Inskip et al., 2006b). Primary health care providers invited women to take part in the study. Women who participated may have already been thinking about becoming pregnant or may have been more 'health aware' and interested in their health than those who chose not to participate, thus may not have been a 'true' reflection of the general population. Some women may have chosen not take part due to inability to dedicate time to the study due to work or family commitments and this may also have influenced participant retention with the study. Participants who were likely to make good health choices, such as attending antenatal care and not smoking, were more likely to have complete fetal

ultrasound data for growth pattern analyses. Comparison of the study groups to the SWS cohort were carried about and are shown in Chapters 3, 4 and 5.

Participants' behaviours may be altered due to the Hawthorne effect, whereby modified aspects of behaviours result due to participant awareness of being observed. However, within the SWS, participants were part of a discovery platform and were not aware of the precise hypotheses being studied, so altering diet and lifestyle depending on their perceptions of what is 'healthy' is unlikely to have had a major effect on outcomes that were assessed by the research staff (i.e. atopic eczema). Similarly, participant reporting bias is less likely to have affected the results; for instance, participants would have not been aware that preconception stress questions would be studied in relation to offspring risk of atopic eczema. The SWS is a long term longitudinal study with follow up at various time points with internal checks on consistency, therefore further minimising this effect.

Numerous potentially confounding factors were accounted for and DAGs were used to help identify these; providing a robust method for identifying confounding factors that should be included in the statistical analyses. This objective method provides a robust means of assessing the causal relationship between exposure and outcome. Residual confounding cannot however be excluded.

Maternal educational attainment has been used a proxy for social class in this cohort. Educational attainment has been found to predict maternal and child diet and is thought to better reflect the status of women than occupation or household income. It appears to be an important confounding factor as health, nutritional and behavioural choices are socially patterned with some exposures being linked to one another occurring or not together. Satisfactory correction for social class is difficult to determine, but in the SWS maternal education was used as a categorical variable which allows clearer classification.

The primary outcome measure of atopic eczema has been thoroughly validated. The UKWPDC combines both a standardised questionnaire and physical examination. In this study, the assessments were undertaken by a suitably trained health care professional. The criteria is appropriate for use in Southampton, UK as it has been found to have high sensitivity and specificity particularly if applied to developed countries (Williams et al., 1994b, Popescu et al., 1998, Girolomoni et al., 2003, Saeki et al., 2007, Fleming et al., 2001, Gu et al., 2001),but perhaps less sensitive in less developed countries (Firooz et al., 1999). Nevertheless, the

criteria is easy and quick to use and does not involve invasive testing, making it an ideal tool for use in large epidemiological studies such as the Southampton Women's Survey.

Likewise, serum tryptophan metabolite measurements, stress and mood assessments and growth measurements have been previously extensively validated; these are detailed in Chapters 3, 4 and 5 respectively.

Multiple statistical testing is a potential limitation. Formal adjustments for multiple testing were not undertaken as methods used to determine the associations described are considered exploratory and hypothesis-generating.

6.5 Future work

6.5.1 Extending and replicating the analyses

Extending the analyses presented in this thesis on childhood atopic eczema and atopy data at ages 3 and 6 years would be valuable in understanding the development and progression of these conditions in the lifecourse. Participant retention within the SWS was sufficient, and would allow for comparison between the findings at age 12 months and those beyond. Replication in other mother-offspring cohorts would be a valuable further step.

Other atopic conditions such as allergic rhinitis and wheezing/asthma were not examined as the main outcome of interest was atopic eczema and airway disorders were outside the remit of this thesis. Examining these related conditions would provide further understanding of the findings described for atopy and provide support for the role of other pathological mechanisms acting in various allergic conditions.

6.5.2 Building on from the findings

Analysis of maternal tryptophan metabolites in relation to maternal stress and low mood was beyond the remit of this thesis. This would ideally require measurement of tryptophan metabolites in maternal serum taken preconception and in early pregnancy, rather than simply using the existing measurements from samples taken in late pregnancy. Examining metabolites from all tryptophan metabolism pathways not limited to the kynurenine pathway should also be considered. Links discovered could point to the interaction between diet and psychological state in influencing offspring health.

Investigating the relation of maternal dietary intake, particularly of niacin, nicotinamide and tryptophan, to serum concentrations of tryptophan metabolites would be of interest. Metabolism of these nutrients in pregnancy is altered and measurement of cord blood concentrations could be informative. Data on the expression of tryptophan transporters in the placenta is available for 32 participants who had serum tryptophan measurements and assessments for atopic eczema; additional analysis of this data may help improve understanding of changes in tryptophan metabolism and transport in pregnancy and their influence of infantile eczema, albeit this is a small sample size.

Exploring the genomic influences on maternal nicotinamide and anthranilic acid levels linked with infant eczema and atopy and epigenetic networks in DNA extracted from relevant maternal pregnancy blood would be of great interest. Within the PARP-1 gene, for instance, common polymorphisms (such as rs1136410, V762A) can be studied. The prevalence of the minor PARP-1 allele (C encoding alanine) varies across ethnic groups, being present in ~11%-47% of the population, and the protein encoded by the minor allele has approximately 57% the enzymatic activity of the major (valine encoding) allele (Wang et al., 2007). It is plausible that such polymorphism, along with others on the biosynthetic pathways could affect maternal levels of nicotinamide and/or be predictive of infant eczema, but their associations with such phenotypes have not been investigated.

Genetic polymorphisms can influence nearby DNA methylation levels (Teh et al., 2014) and genetic effects of disease susceptibility can be mediated via DNA methylation (Liu et al., 2013, Kato et al., 2015); these might be considered as modifiable aspects of genetic predisposition. Environmental influences (van Dongen et al., 2016) such as nutritional interventions (Hoile et al., 2014) can also modify DNA methylation levels. Delineation of the genetic and epigenetic (DNA methylation) marks associated with maternal nicotinamide status and infant atopic eczema may suggest targets for intervention. For example, it would be possible to examine whether proteins activated by low levels of nicotinamide and associated with development of atopic eczema are inhibited by naturally occurring compounds, or whether certain compounds affect and change the epigenetic marks associated with atopic eczema. Additionally, maternal blood RNA could be examined for transcriptomic (RNA-seq) analysis, potentially providing mechanistic explanations for the associations between tryptophan metabolites and atopic eczema. Given the findings described above, Epigenome Wide Association Studies (EWAS) exploring epigenetic variations and the various

phenotypes in relation to tryptophan metabolites, stress and low mood and fetal and infant growth patterns in association with infant eczema would also be of interest.

Likewise, examining genes associated with stress and mood in the study cohort could add more insight to the role of stress in influencing the risk of atopic eczema. One gene that would be of interest is *brain-derived neurotrophic factor* (*BDNF*), a gene on chromosome 11p13, which is thought to be involved in stress-induced hippocampal adaptation and pathogenesis of depression (Duman et al., 1997). BDNF is also thought to be involved in the pathogenesis of atopic eczema, where levels in eosinophils, eosinophil supernatant, serum and plasma are higher in individuals with atopic eczema (Ma et al., 2009) and can be offer a measure of disease activity (Namura et al., 2007). Eosinophils and Th2 cells are capable of producing serum BDNF (Nockher and Renz, 2006, Namura et al., 2007), which suppresses production of Th1-type cytokines and skews the response toward Th2-type pattern although it has no effect on production of TH2 cytokine (Kimata, 2005).

In conclusion, this thesis describes associations of maternal serum tryptophan metabolite (nicotinamide and anthranilic acid) concentrations in late pregnancy and maternal stress and low mood preconception with the infant's risk of atopic eczema at age 12 months. Altered trajectories of growth have been identified in infants with atopic eczema, with evidence of faltering linear growth prior to the clinical onset of the condition at age 6 months. The findings have highlighted phenotypic heterogeneity in infantile atopic eczema, with infant atopic eczema at age 6 months showing no associations with maternal tryptophan metabolite concentrations or maternal stress and low mood. Furthermore, the exposures examined did not relate to infant atopy, suggesting that different pathological processes are involved in these two often co-occurring outcomes.

Mechanistic links between tryptophan metabolites in relation to maternal stress and potential pathways by which both or either of these exposures can influence fetal growth are discussed. The complex interactions between prenatal exposures in influencing offspring outcomes and the value of further studies examining genomic and microbiome influences are also highlighted.

The findings add to the evidence that atopic eczema partly originates during development before birth and point to potentially modifiable maternal influences on this multifactorial skin condition.

Appendices

Appendix A

SECTION	Pre-pregnant	11 w	19 w	34 w	Birth	6m	1 y	2 y	3 y	4 y	6 y	8 y
WOMAN:												
Occupation/ employment	✓			✓	✓		✓	✓				
Activity & exercise	✓	✓		✓						✓	✓	
Food frequency questionnaire	✓	✓		✓								
Prospective diet record	✓	✓		✓								
Food supplements	✓	✓		✓	✓	✓						
General diet questions	✓											
Dietary changes		✓										
Alcohol consumption	✓	✓		✓								
Smoking	✓	✓		✓		✓		✓			✓	✓
General health	✓					✓		✓				
Menstrual cycle & LMP	✓	✓										
Medications		✓		✓								
Pregnancies & illnesses		✓										
Appetite & nausea		✓		✓								
Mental health	GHQ12					EPDS						
EXAMINATION:												
Body measurements	✓	✓		✓		✓					✓	
Grip strength			✓									

Appendix A

SECTION	Pre-pregnant	11 w	19 w	34 w	Birth	6m	1 y	2 y	3 y	4 y	6 y	8 y
BIOLOGICAL SAMPLES:												
Mouthwash (DNA)	✓											
Bloods	✓	✓		✓								
DEMOGRAPHICS /SOCIAL:												
Family background	✓											
Education	✓											
Ethnic group	✓											
Marital status	✓											
Housing	✓							✓				
Household heating						✓		✓				
Household composition	✓					✓		✓				
Childcare arrangements	✓							✓				
Benefits	✓							✓				
Income/ Household/ Shopping								✓				
OBSTETRICS:												
Delivery/ labour					✓							
Obstetric history					✓							
Family medical history					✓							

SECTION	Pre-pregnant	11 w	19 w	34 w	Birth	6m	1 y	2 y	3 y	4 y	6 y	8 y
BP/weights/ urine analyses					✓							
Pregnancy complications					✓							
PARTNER:												
Asthma/eczema		✓										
Height/weight/ Date of birth		✓										
Occupation/employment		✓						✓				
Grip strength			✓									
DXA scan					✓							✓
BIOLOGICAL SAMPLES:												
Bloods			✓									
Mouthwash (DNA)			✓									
INFANT/ CHILD:												
Supplement use						✓	✓		✓	✓		
Milk or formula feeding					✓	✓	✓		✓			
Food frequency questionnaire						✓	✓		✓	✓	✓	✓
24 hour dietary recall						✓						

Appendix A

SECTION	Pre-pregnant	11 w	19 w	34 w	Birth	6m	1 y	2 y	3 y	4 y	6 y	8 y
Prospective food diary									✓			
Introduction of foods						✓	✓					
(Dummy &) bottle use						✓	✓					
Eating behaviour/dietary restraint/ food allergies									✓		✓	
Illnesses						✓	✓	✓	✓	✓	✓	✓
Antibiotics							✓				✓	
Skin conditions						✓	✓		✓		✓	
Allergies						✓	✓				✓	
Sleeping arrangements						✓	✓	✓				
Sleep activity									✓			
TV watching								✓	✓			
Physical activity									✓	✓	✓	✓
Immunisations									✓			
Parenting/Strengths & Difficulties									✓			
Exposure to animals						✓	✓				✓	
EXAMINATION:												
Gender					✓							

SECTION	Pre-pregnant	11 w	19 w	34 w	Birth	6m	1 y	2 y	3 y	4 y	6 y	8 y
Fetal anthropometry		✓	✓	✓								
Body measurements					✓	✓	✓	✓	✓	✓	✓	✓
Placental weight/appearance					✓							
DXA scan					✓					✓	✓	✓
Skin examination						✓	✓					
Skin prick testing							✓		✓		✓	
Dental eruption							✓	✓				
Blood pressure									✓			✓
Bio-electrical impedance									✓			
Grip strength										✓	✓	✓
Cardiovascular assessment												✓
Clinical respiratory assessment											✓	
BIOLOGICAL SAMPLES:												
Umbilical cord blood					✓							
Umbilical cord					✓							

Placenta & membranes					✓							
Buccal DNA											✓	✓

Appendix B

Relevant sections from the SWS initial questionnaire

SWS serial number

QUESTIONNAIRE

Appendix B

SMOKING

8.1 Have you ever smoked regularly (at least once a day for a year or more)?

0. No

1. Yes

8.2 How old were you when you first smoked regularly?

8.3 Are you currently smoking?

0. No

1. Yes

8.4 How many per day? Record maximum stated

EDUCATION

10.1 How old were you when you left full-time education?

(don't round up; enter current age if still studying)

(count a year or less out as continuous education)

10.2 Have you passed any exams or do you have any formal qualifications?

1. None

2. CSE/ School cert/ GCSE grade D or lower/ NVQ1/ Foundation GNVQ

3. O levels/ Matric/ GCSE grade A,B,C/ RSA secretarial/ NVQ2/ Intermediate GNVQ

4. A levels/ City & Guilds/ EN(G)/ ONC/ NNEB/ BTech (day release)/ NVQ3/ Advanced GNVQ/ OND / HNC

5. HND/ RGN/ Teaching Cert/ NVQ4

6. Degree/ NVQ5

7. Other (specify)

GENERAL HEALTH

20.4 To what extent do you feel that the stress or pressure you have experienced in your life has affected your health?

* 1. None
2. Slightly
3. Moderately
4. Quite a lot
5. Extremely

20.5 In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?

* 1. None
2. Just a little
3. A good bit
4. Quite a lot
5. A great deal

Appendix B

Appendix C

Relevant protocol sections for fetal anthropometric measurements

PROTOCOL FOR ANTHROPOMETRIC MEASUREMENTS

All women participating in the Southampton Women's Survey who become pregnant will be offered ultrasound scans at 11, 19 and 34 weeks of gestation. The following measurements will be taken:

1) Crown-rump Length (CRL)

2) Bi-parietal Diameter (BPD)

3) Head Circumference (HC)

4) Femur Length (FL)

5) Abdominal Circumference (AC)

The following Ultrasound machines, calibrated to 1540 m/s will be used:

a) ACUSON 128 XP using the following multi hertz transducer's:

C7 curvi-linear with 7 and 5 MHz frequencies,

C3 curvi -linear with 3.5 and 2.5 Mhz frequencies.

b) ACUSON ASPEN using the following transducer's;

C7 curvi-linear with 7, 5 and 4 MHz frequencies,

C3 curvi-linear with 4, 3.5 and 2.5 MHz frequencies.

CRITERIA FOR BIOMETRY:

1) Crown Rump Length (11 wks)

The embryo can be visualised from 6 weeks postmenstrual age transabdominally. Correctly performed measurements of the CRL are the most accurate at estimating gestational age, as the fetus grows rapidly at this stage. However, the CRL depends on the operator's ability to obtain a true, unflexed, longitudinal section of the fetus with the end points clearly defined. Owing to fetal movement there can be no standardised technique. A longitudinal section of the uterus and gestation sac should be identified. Once the fetus has been identified, the transducer should be rotated until the long axis of the fetus is obtained. A measurement is then taken from the frozen image, from the top of the head (crown) to the end of the trunk (rump), using the callipers. The Ultrasound machine then calculates the distance between the two callipers. The yolk sac should not be included in the measurement, as this will artefactually increase the gestational age. From 9 weeks the fetal spine can be identified and therefore any degree of flexion can be assessed. Any degree of flexion of the fetal spine will underestimate the CRL. With increasing gestational age the fetus is likely to be in a flexed position, and therefore it is likely to be inaccurate after 12 weeks postmenstrual age. (Chudleigh & Pearce 1992a, Dewbury, Meire & Cosgrove 1993)

2) Bi-parietal Diameter (11, 19 & 34 wks)

The BPD measurement is a linear one, with well-defined landmarks for reproducibility. To measure the BPD a longitudinal section of the fetus with the spine or aorta is obtained. The transducer is then moved cranially so that the head and neck are visualised, and then rotated through 90 degrees keeping the same angle of asynclitism. The transducer is then moved up and down until the correct transverse section is obtained. The correct section is at the level of the fetal head where the cavum septum pellucidum (csp) breaks the midline echo, approximately one third of the way from the anterior border of the skull (Campbell et al, 1977). The BPD is the maximum diameter of the transverse section of the fetal skull, at the level of the csp, with the callipers placed on the outer aspect of the proximal skull surface and the inner aspect of the distal skull surface (outer-inner), at 90 degrees to the midline.

3) Head Circumference (11, 19 & 34 wks)

The same section as the B.P.D. is used.. The first calliper is placed on the outer aspect of the skull at the occiput and the second calliper on the outer aspect of the skull at the sinciput. Then using the ellipse key on the ultrasound machine the Head Circumference is measured and the machine calculates the circumference.

4) Femur Length (19 & 34 wks)

The femur length is also an accurate linear measurement. It is easily located. A cross section of the fetal abdomen is obtained and then the transducer is moved caudally, so that the iliac bones are seen. The transducer is than rotated until the full length of the femur has come into view and both ends are clearly seen. The measurement of the femur is then taken from the centre of the 'U' shape at each end of the bone, which represents the length of the diaphysis (Chudleigh & Pearce 1992b).

5) Abdominal circumference (11, 19 & 34 wks)

The section used for measuring the above should have the following features;

The outline is circular. There should be a short length of umbilical vein. This should be imaged so that it is centrally placed between the lateral abdominal walls and is a third of the way along an imaginary line drawn from the anterior abdominal wall to the fetal spine. The stomach is usually visualised as a transonic area in the left side of the abdomen (Chudleigh & Pearce 1992c). A longitudinal length of the fetus is obtained .The transducer should then be moved until the fetal aorta is visualised from the fetal chest and through the abdomen. The transducer should then be rotated through 90 degrees at the level of the fetal stomach to obtain a cross-section. The transducer is then moved until the correct section is obtained as described above. The ellipse method will then be used to measure the circumference.

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Appendix D

Hanifin and Rajka Criteria for Atopic Dermatitis

Hanifin and Rajka Criteria for Atopic Dermatitis	
Major criteria (must have 3)	Pruritus Dermatitis affecting flexural surfaces in adults or face and extensor surfaces in infants Chronic or relapsing dermatitis Personal or family history of cutaneous or respiratory allergy
Minor criteria (must have 3)	Facial features Facial pallor, erythema, hypopigmented patches, infraorbital darkening, cheilitis, infraorbital folds, recurrent conjunctivitis, anterior neck folds Triggers Emotional factors, environmental factors, food, skin irritants Complications Susceptibility to skin infections, impaired cell-mediated immunity, predisposition to keratoconus and anterior subcapsular cataracts, immediate skin reactivity Other Early age of onset, dry skin, ichthyosis, hyperlinear palms, keratosis pilaris, hand and foot dermatitis, nipple eczema, white dermatographism, perifollicularaccentuation

Appendix D

Appendix E

Southampton Women's Survey - 12 MONTH INFANCY & 3 YEAR HOME VISITS

Skin Scratch Testing

1) Explain to the mother that we are trying to find out if a baby's development in the womb determines the later development of allergies and that we would like to do a very simple skin test on her and her baby. Say that this simply involves putting a drop of liquid containing small amounts of each allergy substance on the skin, very lightly scratching the surface and looking at the skin 15 minutes later to see if there is a slight bump at the site. Explain that we are testing for reactions to cat, dog, egg, grass pollen, house dust mite and milk proteins. If the mother is concerned, explain that it doesn't hurt and it doesn't draw blood or anything like that & illustrate on yourself if necessary! A history of allergic reactions is not a contraindication to the tests unless these have been anaphylactic in nature.

Ask "Is he/she currently taking steroid tablets/syrup, or have they been admitted for a bad flare of asthma or eczema in the past 14 days, or ever for a severe allergic reaction?" If the answer to any of these elements is "Yes" then skin prick testing should not be performed in the community.

2) Scratch test the mother first -

a) with a pen lightly mark 2 rows (3 cm apart) of 4 dots (again 3 cm apart) on the volar aspect of the (left) forearm between the elbow & the wrist

b) open 8 prick lancets & apply a small drop from each bottle near the dots in the standard order. Don't touch the dropper itself on the skin.

- inner row: (elbow) Cat, Dog, Egg, saline neg control (wrist)

3 cm gap then

- outer row: (elbow) Grass pollen, House dust mite, Milk, histamine pos control (wrist)

c) using a new prick lancet for each drop scratch the solution into the top-most layer of the skin, holding the lancet at 30□ to the skin - this should not draw blood. Dispose of the lancets into the sputum pot.

d) start the timer (15 minutes) and lay an absorbent tissue on the skin to remove surplus fluid.

3) Scratch test the infant

a) undress the infant and, with he/she sitting on the mother's lap, lightly mark 2 rows (3 cm to the left & right of the midline) of 4 dots (again 3 cm apart) on the central back

b) open 8 prick lancets & apply a small drop from each bottle near the dots in the standard order. Don't touch the dropper itself on the skin.

- left row: (top) Cat, Dog, Egg, saline neg control (bottom)

3 cm gap then

- right row: (top) Grass pollen, House dust mite, Milk, histamine pos control (bottom)

c) using a new prick lancet for each drop scratch the solution into the top-most layer of the skin as above.

d) lay an absorbent tissue on the skin to remove surplus fluid.

4) Perform the infant anthropometry while the tests are "cooking"

5) Read the mother's tests when the timer "bleeps" by looking & feeling for a wheal reaction (do not measure the "flare"). The “resistance to a ball point pen test” may help in difficult cases. Read off the average (not maximum) diameter using the circles and record. Take care that it is entered under the correct allergen on the data entry form.

6) Read the infant's scratch tests in the same way.

7) If some of the tests are "positive" explain to the mother that usually it is only the strongest positives that may be of any relevance & that most infant's get minor reactions like this that they soon grow out of and don't cause any problems as such. If, for example, the child has eczema and is positive to cow's milk & the mother presses you about the possible benefit from a milk free diet explain that avoiding milk can sometimes do more harm than good to the baby, and that if the mother wishes to pursue this it would be best to do so only in consultation with a qualified dietician.

Adverse reactions

It is questionable whether skin scratch testing (as opposed to intra-dermal testing) has ever been associated with an anaphylactic reaction. It is however a sensible precaution to always have adrenaline available, and to be aware of the correct management. This is –

Administer adrenaline and basic ABC life support

Infants & 3 year olds: 1 in 1000 Adrenaline, 12 Units in insulin syringe, i/m to thigh (0.12 ml) (120 ìg)

Adults: 1 in 1000 Adrenaline, 0.5 ml in 1ml syringe/green needle, i/m to thigh (500 ìg)

Dial 999 for emergency assistance

Repeat adrenaline in 5 minutes if no clinical improvement

(2002 Resuscitation Council guidelines for use in the community, updated 2005)

Dr Keith Godfrey BM FRCP

MRC Clinical Scientist & Consultant Dermatologist

Appendix E

Appendix F

General Health Questionnaire

SWS serial no:

Date:

Please read this carefully:

We should like to know if you have had any medical complaints, and how your health has been in general, *over the past few weeks.* Please answer ALL the questions on the following pages simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about present and recent complaints, not those that you had in the past.

Appendix F

It is important that you try to answer ALL the questions.

Thank you very much for your help.

Have you recently:

1. been able to concentrate on whatever you're doing? Better than usual Same as usual Less than usual Much less than usual

2. *lost much sleep over worry?* Not at all No more than usual Rather more than usual Much more than usual

3. *felt that you are playing a useful part in things?* More so than usual Same as usual Less useful than usual Much less useful

4. felt capable of making decisions about things? More so than usual Same as usual Less so than usual Much less capable

5. *felt constantly under strain?* Not at all No more than usual Rather more than usual Much more than usual

6. *felt you couldn't overcome your difficulties?* Not at all No more than usual Rather more than usual Much more than usual

7. been able to enjoy your normal day-to-day activities? More so than usual Same as usual Less so than usual Much less than usual

8. been able to face up to your problems? More so than usual Same as usual Less able than usual Much less able

9. *been feeling unhappy and depressed?* Not at all No more than usual Rather more than usual Much more than usual

10. *been losing confidence in yourself?* Not at all No more than usual Rather more than usual Much more than usual

11. *been thinking of yourself as a worthless person?* Not at all No more than usual Rather more than usual Much more than usual

12. *been feeling reasonably happy, all things considered?* More so than usual Same as usual Less so than usual Much less than usual

13. *How well would you say you are managing financially these days?* Living comfortably or doing alright Just about getting by Finding it difficult or very difficult

Any previous treatment for mental health problems?

14. Have you ever received treatment for depression, anxiety, or other mental health problem in the past? (Please tick one)

☐ Yes ☐ No

(Treatment might be tablets, or counselling, or seeing a psychiatrist or other mental health professional)

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Appendix G

Edinburgh Postnatal Depression Scale

Date _____ SWS serial no:

We would just like to ask you a few further questions about how you have felt over the last 6 months since the baby was born.

Please **UNDERLINE** the answer which comes closest to how you have felt ***during the worst 2 week period*** since the baby was born, not just how you feel today.

Here is an example, already completed:

1. I felt happy

Yes, all the time

Yes, most of the time

No, not very often

No, not at all

This would mean that even during the worst 2 week period since the baby was born "I felt happy most of the time".

Please complete the other questions in the same way.

During the worst 2 week period since the baby was born

1. I was able to laugh and see the funny side of things

As much as I always could

Not quite so much

Definitely not so much

Not at all

2. I looked forward with enjoyment to things

As much as I ever did

Rather less than I used to

Definitely less than I used to

Hardly at all

3. I blamed myself unnecessarily when things went wrong

Yes, most of the time

Yes, some of the time

Not very often

No, never

4. I was anxious or worried for no good reason

No, not at all

Hardly ever

Yes, sometimes

Yes, very often

During the worst 2 week period since the baby was born

5. I felt scared or panicky for no very good reason

Yes, quite a lot

Yes, sometimes

No, not much

No, not at all

6. Things were getting on top of me

Yes, most of the time I wasn't able to cope at all

Yes, sometimes I wasn't coping as well as usual

No, most of the time I coped quite well

No, I coped as well as ever

7. I was so unhappy that I had difficulty sleeping

Yes, most of the time

Yes, sometimes

Not very often

No, not at all

8. I felt sad or miserable

Yes, most of the time

Yes, quite often

Not very often

No, not at all

9. I was so unhappy that I cried

Yes, most of the time

Yes, quite often

Only occasionally

No, never

10. The thought of harming myself occurred to me

Yes, quite often

Sometimes

Hardly ever

Never

Many thanks for your help with this questionnaire.

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Committee No: 335/99

Appendix H

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✓" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

Appendix I

Relevant section of SWS protocol for neonatal anthropometry

PROTOCOL FOR NEONATAL ANTHROPOMETRY

Measure the baby's head circumference - maximum occipitofrontal circumference - using the 3 plastic unmarked tapes. You do not need to disturb the baby's position to do this. Place the tape on the most anterior protuberance of the forehead, pass the tape around the head, placing the tape on the most posterior protuberance and mark the circumference with a water soluble marker. Do the 3 measurements then read them off against the fixed rule on the measurement trolley, placing the mark against the zero and reading off the other end.

Fully remove the baby's clothes to expose the mid & lower chest. Feel for the xiphisternum where the ribs meet the sternum and mark the **base** of the xiphisternum using a water soluble marker. Pass the tape around the lower chest so that the mark is at the **upper** border of the tape. Do the 3 chest circumference measurements **at end of expiration** then read the tapes off against the fixed rule on the measurement trolley.

To measure the lower abdominal circumference pass the tape around the baby at the level of the umbilicus. If the umbilicus protrudes too much and it is not possible to measure at this level, then the reading should be taken immediately above the umbilicus. Take 3 measurements **at end of expiration** then read the tapes off against the fixed rule on the measurement trolley.

Use a steret to clean off the water soluble marks.

Remove the nappy and check hip stability if not already done by the paediatricians. Ensure that an assistant is at hand.

Place the baby inside the neonatometer and get the assistant to fix the head against the head plate by raising their fingers under the baby's axillae. Extend both legs by flexing at the hips, tickling the back of the legs and then extending until they are **flat on the inco pad**. Bring the foot plate up to the heels, **ensure that the foot and knee are flat**, lock the foot plate and read off the length. Relax the legs, unlock the foot plate and repeat X 2, checking each time that the head remains against the head plate. In a few breech deliveries it may not be possible to extend the hips - code these as 99.9.

Measure crown-rump length in the same way, bringing the foot plate up the baby's rump with the hips fully flexed. **Ensure that the baby's bottom is flat on the trolley (i.e. rotate the anus / sacrum downwards** with firm pressure) & the knees together. Bring the foot plate up until you encounter **firm resistance.**

Appendix J

Relevant section of the SWS infant anthropometry protocol

SWS INFANT ANTHROPOMETRY

A protocol for fieldworkers at the MRC Epidemiology Resource Centre, University of Southampton

Length/ Crown-foot (left foot next to bare skin) at 6 months & 1 yr.

(measurer and assistant required)

Place the baby/infant in a supine position lying on the infantometer. Get the assistant to hold the infant's head in the Frankfort Plane against the head plate.

Extend the left leg by flexing at the hips, (tickling the back of the leg may help) and then extend until flat.

Ensure that the baby is lying straight with their back flat on the infantometer.

Bring the foot plate up to the heel of the left foot, ensure that the foot and knee are flat, lock the foot plate and read off the length.

Relax the leg, unlock the foot plate and repeat x 2

Take three readings. Check each time that the head remains against the head plate.

Abdominal circumference – Sitting (1, and 2yr) blank tapes over bare skin

Pass the tape around the baby at the level of the umbilicus. Ensure the nappy is clear and not constricting the area; undo nappy if necessary.

Take the reading at the end of expiration.(when the abdomen is relaxed)

Do the 3 measurements then read them off against the fixed ruler.

Appendix J

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