The Influence of Diet Prenatally and during the First Year of Life on Sour Taste Development – A Longitudinal Investigation within an Irish Setting.

Aileen Kennedy BA (Sci), MSc (Med Sci), PG Dip

School of Nursing & Human Sciences

Dublin City University

Submitted for the award of PhD

Supervisors:

Dr Tracey Harrington, Dublin City University

Dr Sandra O'Neill, Dublin City University

November 2013

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: ______________________ ID No. : _____________ Date: __________

TABLE OF CONTENTS

ABSTRACT

The Influence of Early Learning Environments on Sour Taste Development in the First Year of Life

Aileen Kennedy

A mother's diet during pregnancy and lactation influences the infant's taste development as early exposure to flavour in amniotic fluid and breast milk can modify innate taste preferences in infants. Infants are born with an innate preference for sweet flavours while rejecting bitter and sour tastes. Sour taste preference in infants is linked to high fruit intake and given the increase in childhood obesity it is important we maximise the chances that children developing healthy food preferences. This study examined the relationship between maternal fruit intake during pregnancy and lactation and the development of sour taste preference during the first year of life. Mothers completed a 7-day food diary during the 3rd trimester of pregnancy and at 12 weeks post-partum. Infant feeding practices were recorded at birth, 3, 6 and 12 months and infant food intake was assessed at 12 months using a 3-day food dairy.

At 6 and 12 months sour taste acceptance in infants was examined by offering a base drink with increasing molar concentration (M) of citric acid (0.00M, 0.013M, 0.029M and 0.065M). The infant was allowed to consume these solutions *ad libitum* over 60 seconds. Sour taste acceptance was measured using three methods, amount ingested by the infant, the mother's perception of the infants acceptance and the frequency of the negative responses by the infant to the solutions as measured by video analysis.

In general, infants rejected extreme sour tastes at 6 and 12 months. However, a large variability within the group was observed, with some accepting these tastes. Fruit consumption by mothers during pregnancy, gender and the length of exclusive breastfeeding were positively associated with acceptance of sour tastes at 6 months ($p<0.05$). At 12 months, only an infant's own fruit consumption was positively associated with sour taste acceptance ($p<0.05$). This study also provided insights into mothers' diet during pregnancy and lactation. While in general their diet was adequate, participants had higher than recommended fat, saturated fat and salt intakes and lower than recommended intakes of folate vitamin D. Infants' diet at 12 months also had poor intakes of vitamin D. This study sheds light on the relationship between early exposure to fruit and sour taste acceptance infants, which could be exploited to improve fruit intake from infancy.

ACKNOWLEDGEMENTS

It would not have been possible to complete this research without the support of many kind people along the way, only some of whom it is possible to give particular mention here.

Firstly, I would like to thank my supervisors, Dr. Tracey Harrington and Dr. Sandra O'Neill for the time and effort they devoted to supervising this work. I appreciate their expertise, guidance, help and encouragement throughout this process.

I would like to thank the School of Nursing and Human Sciences, whose backup support has made this work possible. I am grateful to my fellow PhD students who helped and encouraged me throughout this journey. I'm also very grateful to Dr. Mary Rose Sweeney for her generosity of time and advice and to Dr. Michael Parkinson for his statistical advice and help throughout the study. Many thanks to Dr Pamela O'Connor and the staff of the CWIUH for their assistance with the project. I am also grateful to Ms. Caroline King, Imperial College London, who advised me on aspects of my methodology and research design.

I would also like to thanks Dr. Donal O'Gorman and my colleagues on the DEXLIFE project, for their constant support and encouragement over the past year as I learned to balance my new role in the project whilst finishing this thesis.

I would like to express my sincerest gratitude to all of the parents and the babies who very generously gave up several hours of their time to take part in my study. I am especially indebted to those parents who willingly agreed to have their infants consume something they often did not like and for allowing me to visit their homes, a person they barely knew.

I'd also like to thank my family and friends for their on-going support during the ups and downs of the past few years. I owe everything to my parents, Margaret and Séamus Kennedy, who have always given me continuous support throughout my education and life. I would also like thank my sister, Sinéad for her advice, support and encouragement over the years. To Maeve, Laura and Siobhán for all your support. When times got tough, the laughs, dinners and tea, help me put it all in perspective!

Finally and most importantly a special word of gratitude and appreciation to my new husband Colm Ó Riain for his invaluable editing skills but most importantly for his patience,

understanding, unwavering support and love. I could not have done any of this without you and it is to you that I dedicate this thesis.

LIST OF TABLES

TABLE OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS USED

ADA	American Diabetes Association
AICR	American Institute for Cancer Research
ALSPAC	Avon Longitudinal Study of Pregnancy and Childhood
ANOVA	ANalysis Of VAriance
AR	Average Requirement
BMI	Body Mass Index
BMR	Basal Metabolic Rate
$Ca2^{+}$	Calcium
CHD	Coronary Heart Disease
cm	centimetres
COMA	Committee on Medical Aspects of Food and Nutrition Policy
CoV	Coefficients of Variation
CWIUH	Coombe Women and Infants University Hospital
DAG	Diacylglycerol
DCU	Dublin City University

DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DNSIYC	Diet and Nutrition Survey of Infants and Young Children (UK)
DRV	Dietary Reference Values
EAR	Estimated Average Requirements
EBF	Exclusively Breastfeeding
EFSA	European Food Safety Authority
EI	Energy Intake
ENaC	Epithelial Sodium Channel
ERSI	Economic and Social Research Institute
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology & Nutrition
EU	European Union
FAO	Food and Agriculture Organisation
FFQ	Food Frequency Questionnaire
FSA	Food Standards Agency

FSAI	Food Safety Authority of Ireland
g	Grams
GDM	Gestational Diabetes
GP	General Practitioner
H^+	Hydrogen
HbA1C	Haemoglobin A1C
HBSC	Health Behaviour in School-aged Children Study (WHO)
HCl	Hydrochloric Acid
HCP	Health Care Professional
HSE	Health Service Executive
IOM	Institute of Medicine
IR	Ingestion Ratio
IU	International Unit
IUNA	Irish Universities Nutrition Alliance
K^+	Potassium
kcal	kilocalorie

kg	kilograms
LTI	lowest threshold intake
m	meters
M	Molar concentration
mins	minutes
M- LR	Mother's Liking Ratio
max	Maximum
min	Minimum
ml	Millilitres
mmHg	One millimetre of mercury (the unit of barometric pressure)
mo	months
MCY	milk, cheese and yoghurt shelf of Food Pyramid
MFPA	meat, fish, poultry and alternatives shelf of Food Pyramid
Mg	Magnesium
MJ	Mega Joule
MSG	Monosodium Glutamate

Na^+	Sodium
NaCl	Sodium Chloride
NANS	National Adult Nutrition Survey
NDNS	National Diet and Nutrition Survey (UK)
NPNS	National Pre-School Nutrition Survey (Ireland)
NHS	National Health Service (UK)
NTD	Neural Tube Defect
OCED	Organisation for Economic Co-operation and Development
PAL	Physical Activity Level
PIP2	Phosphatidylinositol-4,5-bisphosphate
PKD2L1	Polycystic kidney disease-like ion channel
PLC	Phospholipase C
PROP	6-n-Propylthiouracil
PTC	Phenylthiocarbamide
RCPCH	The Royal College of Paediatrics and Child Health (UK)
RDA	Recommended Dietary Allowance

R-LR	Rater's Liking Ratio
SACN	Scientific Advisory Committee on Nutrition
SD	Standard Deviation
SE	Standard Error
SLÁN	Survey of Lifestyle, Attitudes and Nutrition
SPSS	Statistical Package for the Social Sciences
SR	self-reported
TRP	Transient Receptor Potential
UK	United Kingdom
UNICEF	United Nations International Children's Emergency Fund
US	United Sates
WCRF	World Cancer Research Fund
WHO	World Health Organisation
wks	weeks
yrs	Years

"*I will add that formerly it looked to me as if the sense of taste, at least with my own children when they were still very young, was different from the adult sense of taste; this shows itself by the fact that they did not refuse rhubarb with some sugar and milk which is for us an abominable disgusting mixture and by the fact that they strongly preferred the most sour and tart fruits, as for instance unripe gooseberries and Holz apples.*"

Charles Darwin, 1877

THESIS SUMMARY

Everyone wants to do the best for their child, to maximise their health, happiness and success in life. David Barker's 'Foetal Origins Hypothesis' (Barker, 1997) hypothesises that the origins of many of the common diseases in today's society can be tracked back to the responses of the baby or toddler to under or over-nutrition during the first 1000 days of life (from conception through to a child's 2^{nd} birthday). In fact, right up until the child's 2^{nd} birthday, nutrition can have a long term impact on health, predicting a child's risk of developing coronary heart disease, hypertension, type 2 diabetes, certain cancers and even obesity. Therefore nutrition, from early conception right through until the second year of life, is vitally important for any infants' long term health. Infants have an innate preference for sweet, salty and fatty tastes and an innate rejection of bitter and sour tastes. However, these innate rejections can be overcome and infants can develop a liking for these flavours. Darwin reported this liking for sourness, as early as 1877, in his own children. The aim of this thesis to examine the relationship between diet during pregnancy and lactation and the development of sour taste preference during the first year of life.

This thesis is comprised of eight chapters.

Chapter 1 comprises of a detailed review of the existing literature and gives an overview of the many factors that may shape infants' food acceptance patterns during the first year of life. The review is divided into 9 subsections covering the ontogeny of flavour perception and its function in infants, exposure to volatiles in milk sources, a discussion of potential

determinants of transfer of compounds into mothers' milk, a review of early infant feeding practices including weaning and finally a review of preference learning and a discussion of the possible links to later obesity. Finally, it outlines the purpose of the present study, as well as its specific aims, objectives and hypotheses.

Chapter 2 provides an in-depth description of the methodologies used in this thesis. It includes a description of the research design, participants, measures, procedures, and statistical analyses employed in the present study.

Chapter 3 focuses on the diets of women during the third trimester of pregnancy. It reports on macro- and micro- nutrient intakes and compares these intakes for the group with the government recommendations and the national surveys.

Chapter 4 evaluates the diets of women at approximately 12 weeks post-pregnancy. It reports on macro- and micro- nutrient intakes and compares these intakes for the group with the government recommendations and the national surveys.

Chapter 5 focuses on early infant feeding practices of the infants in this study. Detailed information on breastfeeding initiation and prevalence rates, using specific well-defined breastfeeding definitions were collected and reported in this chapter. Detailed information regarding the introduction of complementary foods to the infants' diets is also discussed. Data are also presented on the weaning practices of mothers specifically pertaining to the timing of weaning.

Chapter 6 evaluates the diets of 12 month old infants. It reports on macro- and micro-nutrient intakes and compares these intakes for the group with the government recommendations and the latest national infant surveys.

The results of the acceptance of sour taste of infants at 6 months and 12 months are reported in **Chapter 7**. A subsequent analysis was undertaken to identify predictors of sour tastes acceptance at both ages.

Finally, **Chapter 8** provides a general discussion of the present study, exploring its strengths and limitations, the practical and theoretical implications of its findings, and suggestions for potential future research.

.

CHAPTER 1. LITERATURE REVIEW

1.1 *Introduction*

The 1,000 days from conception until the infant's 2nd birthday offer a unique window of opportunity to shape a healthier future for the infant. The nutritional requirements of infants are very high and change rapidly, making optimal nutrition during this time not only critical for a child's ability to grow, learn, and develop, but also in influencing the long term health of the infant into adulthood (Barker, 2000). During the first year of life, infants triple their birth weight and increase their length by 50%, a growth that is unmatched at any other time during their lives (Thomas & Bishop, 2007). During this time, patterns of food intake also change dramatically. By the time infants are half-way through their first year of life, they can no longer obtain sufficient nutritional requirements from milk alone and require a transition to a variety of solid foods for their continued growth and development. This transition occurs over a period of several months, during which infants are exposed to a variety of foods and combinations of tastes and flavours (Birch, 1998).

Research has demonstrated that children's food consumption patterns are predominantly determined by their food preferences (Baxter, Thompson, & Davis, 2000; Pérez-Rodrigo, Ribas, Serra-Majem, & Aranceta, 2003). These preferences can be strongly tracked over time, with regards to fat, carbohydrates and protein (Stein, Shea, Basch, Contento, & Zybert, 1991; Wang, Bentley, Zhai, & Popkin, 2002), higher fruit and vegetable consumption (Resnicow *et al.,* 1997) and overall food preferences (Skinner, Carruth, Bounds, & Ziegler, 2002a). Evidence suggests that dietary experience and patterns have their origins before the child is born, as foetuses in the third trimester can taste the flavours from their mother's food in utero and this can influence later food preferences (Skinner, Ziegler, Pac, & Devaney, 2004). Given the early development of dietary patterns, it is of key public health importance to understand how food preference develops from the third trimester into early infancy.

To better understand food preference, a basic knowledge of the five primary tastes which are perceived by humans viz. bitter, sweet, salty, sour and umami, is important. Evidence has linked exposure to these tastes in utero or in early infancy to the development of particular food preferences in later life (Mennella & Beauchamp, 2009). This thesis examines sour taste preference at an early age and its link to the development of healthy food consumption patterns among children. This focus on sour taste is due to its association with low energy density foods such as fruit (Liem, Bogers, Dagnelie, & de Graaf, 2006). Few studies have examined the link between sour taste acceptance and fruit consumption and it has been shown that the acceptance of fruit by children is important as high fruit consumption is linked to decreased weight gain (Buijsse *et al.,* 2009). Given the growth in childhood obesity and associated co-morbidities seen in the past few decades, a better understanding of food preference and patterns may play a role in promoting future health.

There exists a continuum of opportunities for early exposure to flavour of fruits during infancy. Infants learn about flavours prior to their first taste of solid food via amniotic fluid and breast milk. Maternal and family diet plays a critical role in the timing and variety of flavours and types of complementary foods proffered to the child. This study will examine sour taste preference development in young infants, exploring whether early learning environments, viz. uterine and through breast milk; and the introduction of solid food influences the acceptance of sour tastes. This study will also afford us an opportunity to examine the mothers' diet during the third trimester of pregnancy and post-pregnancy. Furthermore, it will also provide an opportunity to examine weaning practices in Ireland.

1.2 *Nutritional Requirements of Infants from Conception to 1 year*

It is important that mothers have adequate nutrition to meet the demands of their developing foetus, thus allowing the foetus to grow and develop physically and mentally to its full potential (Van Teijlingen *et al.,* 1998). Sufficient nutrition helps to protect against premature birth, congenital malformations and low birth-weight (Ortega, 2001). Once born, the infants receive all of their nutrition from milk initially - either from breast or formula milk. As infants grow, solid food is introduced into the diet (known as weaning) to make up the increasing shortfall between their growing nutritional requirements and what they are getting from breast or formula milk. Initially the nutrient content from solid food is small but as weaning progresses the infant becomes more reliant on solid food to meet their nutritional requirements. The following sections will discuss the nutritional requirements from pregnancy through the first year of life for the infant and its mother. It will try to elucidate on some of the issues that occur during this period.

1.2.1 Nutritional Requirements during Pregnancy

Energy intake during pregnancy is of particular concern, as food consumption must meet the increased demands of the growing foetus, the increasing maternal tissues and fat stores as well as the increase in maternal Basal Metabolic Rate (BMR), which is a result of being pregnant. While there are wide variations between individuals' energy requirements during pregnancy, the total energy cost of pregnancy has been estimated as approximately 76,500 kilocalories (kcal) (FAO/WHO/UNU, 1985). Many physiological adaptations occur to help meet this increased requirement; better absorption, less energy expenditure and some energy sparing adaptations allow the mother to meet increased energy demands. For example, pregnant women make greater use of lipids as a source of energy for their own needs, thus conserving their glucose stores for the needs of their foetus (Herrera, 2000).

The quality of maternal diet, both at conception and during gestation, is of great importance due to its association with pregnancy outcomes. For example, a low folate status peri-conceptionally can increase the risk of neural tube defects (MCR Vitamin Study Research Group, 1991; Scholl & Johnson, 2000) or intrauterine exposure to low concentrations of vitamin D is associated with less muscle mass and higher insulin resistance in children (Krishnaveni *et al.,* 2011). Adaptations to the muscular activity of the intestine also occur, which lengthen the transit time of food in the gut. This increases the efficiency of nutrient absorption from ingested food, especially with regard to nutrients like calcium and iron (Barsai, 2003). Increasing evidence now suggests that maternal nutrition might not only affect immediate pregnancy outcomes but may also have effects on infant health in later life (Godfrey *et al.,* 1996; Godfrey & Barker, 2001; Krishnaveni *et al.,* 2011).

Often maternal increases in energy intake will involve an increase in many of the required vitamins and minerals. The UK Dietary Reference Values (DRVs) for pregnancy recommend small increases in energy intakes (Department of Health, 1991). None of their recommendations include mineral intake increases, while the only increased requirements for vitamins are for thiamin, riboflavin, folate and vitamins C, D & A. In Ireland, there are similar changes to nutrient requirements along with added increases in several minerals - particularly calcium, iron, vitamin B12 (Table 1.1).

Table 1-1 Recommended Dietary Allowances for non-pregnant women aged 18-64 in Ireland and aged 19-50 in the UK as well as recommended increments during pregnancy for both Ireland and the UK (Department of Health, 1991; Food Safety Authority of Ireland 1999)

Nutrient (units)	*Irish Females 18-64*	*Irish Pregnant women**	*UK Females 19-50*	*UK Pregnant women*
Energy (kcal/day)	1900	+300***	1900	+200***
Protein (g/kg/day)	0.75	+10g/d	45g/day	+10g/d
Thiamin (mg/d)	1.0	0.9	0.8	0.9
Riboflavin (mg/d)	1.3	1.6	1.1	1.4
Folate (µg/d)	300	500	200	300
Vitamin C (mg/d)	60	80***	40	50***
Vitamin D (µg/d)	0-10	10	-	10
Vitamin A (µg/d)	600	700	600	700
Calcium mg/d	800	1200	700	700
Iron mg/d	14	15	14.8	14.8
Vitamin B12 (µg/d)	1.4	1.6	1.5	1.5

*** For last trimester only

Pregnant women are advised to avoid certain foods in order to decrease potential risks for both mother and child (Brundage, 2002; Gilbert, 2002), though these recommendations change from country to country. In Ireland, women are recommended to avoid fish like shark, marlin and swordfish as they may contain high levels of mercury (Food Safety Authority of Ireland, 2004). In New Zealand, guidelines suggest that pregnant women can consume these fish up to once a fortnight (Ministry of Health, 2008). Pregnant women face the risk of infection viz. listeriosis, toxoplasmosis and salmonella through eating certain foods such as raw or lightly cooked eggs, meat, fish and poultry, unpasteurised milk and dairy products, pate as well as unwashed fresh produce. Infection of toxoplasmosis in early pregnancy can lead to miscarriage or stillbirth and to hydrocephalus and retinochoroditis in late pregnancy (Health Service Executive, 2010; Center for Disease Control and Prevention 2013). These dangers can lead to avoidance of certain foods during pregnancy (Health Service Executive, 2010; Center for Disease Control and Prevention 2013).

Research has shown that the most significant change in women's behaviour during pregnancy occurs with regard to eating habits and food choices (Lewallen, 2004). Some changes in food choices occur during pregnancy due to specific food cravings or aversions experienced by the prospective mother (Bayley, Dye, Jones, DeBono, & Hill, 2002). One study showed that pregnant women's greatest aversions were to high protein foods such as meats, fish, poultry and eggs (Flaxman & Sherman, 2000). It has been speculated that this may be due to the effects of morning sickness, as recent research shows that during the first trimester, prospective mothers' intake of meat products were lower in those who suffered from nausea and vomiting (Latva-Pukkila, Isolauri, & Laitinen, 2010). Fruit juice, fruit and sweet foods were found to be the most commonly craved foods (Bowen, 1992; Verbeke & De Bourdeaudhuij, 2007), with one study showing that women in their second trimester of pregnancy had the highest cravings for sweet foods (Bowen, 1992).

1.2.2 Nutritional Requirements of Neonates and Infants to 1 year

Neonates and infants have a much higher requirement, relative to their size, for both energy and nutrients in comparison to adults because of their high growth velocity and their small gastric capacity, which cannot process large quantities of food. A nutritionally dense diet is therefore essential for healthy infant growth (Thomas & Bishop, 2007). Table 1.2 details the macro- and micro- nutrient recommended allowances for infants from birth to 12 months of age (Department of Health, 1991; Food Safety Authority of Ireland, 1999).

Table 1-2 Recommended Dietary Allowances for infants aged 0-12 months in Ireland (DoH 1991, FSAI 1999)

Nutrient (units)	*0-3 months*	*4-6 months*	*7-9 months*	*10-12 months*
Energy (MJ/day)				
Males	2.28	2.89	3.44	3.85
Females	2.16	2.69	3.20	3.61
Protein (g/day)	12.5	12.7	13.7	14.9
Vitamin A (μg/d)	350	350	350	350
Riboflavin (mg/d)	0.4	0.4	0.4	0.4
Vitamin C (mg/d)	25	25	25	25
Folate (μg/d)	50	50	50	50
Vitamin B12 (μg/d)	0.3	0.3	0.4	0.4
Vitamin D (μg/d)	8.5	8.5	7	7
Calcium mg/d	525	525	525	525
Iron mg/d	1.7	4.3	7.8	7.8
Zinc mg/d	4	4	5	5
Sodium	210	280	320	350
Mg	55	60	75	80
Vitamin B6 (mg/d)	0.2	0.2	0.3	0.4
Thiamin	0.2	0.2	0.2	0.3

1.2.2.1 Nutritional composition of breast milk

Breast milk meets an infant's requirements for the first few months of life.

Table 1-3 Nutritional Composition of Mature Human Milk, unmodified cow's milk and a cow's milk whey dominant infant formula.

Nutrient (units)per L	*Human[a,b] Milk*	*Cow Milk[a,b]*	*Formula[c]*
Energy (MJ	3.0	2.8	2.8
Total Carbohydrate (g)	74	49	73
Total Lipid (g)	42	38	36
Total Protein (g)	9	31	13
Casein (g)	2-3	27	4.7
Whey Proteins (g)	6.4	5.8	8.7
Non-protein Nitrogen (% total N)	18-30	5	10
Sodium (mmol)	7	25	7
Potassium (mmol)	15	37	17
Calcium (mmol)	9	30	10.5
Magnesium (mmol)	1	5	3
Phosphate (mmol)	5	31	7.7
Chloride(mmol)	12	29	12
Iron (μmol)	13	11	143
Zinc(μmol)	48	63	92
Iodine(μmol)	0.6	1.2	0.8
Vitamin A (RE μg)	600	350	660
Vitamin E (mg)	3.5	1.4	7.4
Vitamin D (μg)	0.4	0.8	12
Vitamin C (mg)	41	18	90
Thiamin (mg)	0.2	0.4	1.0
Riboflavin (mg)	0.3	1.7	1.1
Niacin (NE mg)	7.2	8.2	9.0
Folic Acid (μg)	52	55	80
Vitamin B12 (μg)	0.1	4.5	1.8

[a](McCance & Widdowson, 2002); [b](Golden, 2000); [c](SMA, 2012)

The approximate concentrations of the constituents of breast milk are shown in Table 1.3, but ranges are wide as its composition varies from mother to mother, with time of day, the time into the feed and with the length of time post-partum. The composition of fat and the amount of fat soluble vitamins in mature milk are particularly variable (Jensen, 1999; Donovan, 2009).

Table 1-4 Overview of the influence of Maternal Diet on milk composition (Donovan, 2009)

Level of effect of Maternal Diet	*Breast Milk Component*
Little or no effect	Lactose concentration Marco-mineral concentration (e.g. Ca, P, Mg) Some trace elements (e.g. Zn, Cu) Electrolytes (Na, K, Cl)
Minimal effect except in severe under-nutrition	Protein concentration & composition Non protein nitrogen composition and concentration
Influence	Fatty acid content and composition -PUFA; Trans Fatty acids Manganese, Iodine and Selenium concentration Water soluble vitamin concentration (e.g. vitamin C & B12; folate) Fat soluble vitamin concentration (vit D, A, E & K)

Ca- Calcium; P- Phosphorous; Mg- Magnesium; Zn- Zinc; Cu –Copper; Na- Sodium; K – Potassium Cl –Chloride; PUFA- Polyunsaturated fatty acids

As breastfed infants are dependent on breast milk for their entire nutrient intake, the nutritional composition of the milk is very important. This is dependent on the nutritional status of the mother in some aspects (Table 1.4). For example, infants born to mothers with low vitamin D status are at high risk of deficiency as they will have low stores and their mothers' breast milk will be low in vitamin D (Mølgaard & Fleischer Michaelsen, 2003). The recommended nutrient intakes are set at higher levels than non-pregnant women due to the increased nutritional demands on the mother during lactation. Table 1.5 shows the Irish and UK Recommended Dietary Allowances for non-lactating and lactating women (Department of Health, 1991; Food Safety Authority of Ireland, 1999).

1.2.2.2 Nutritional Composition of Formula

Unmodified cow's milk is very different from human milk (Table 1.3) and entirely unsuitable for infants' requirements (Table 1.2). The major reasons for this are that cow's milk contains high concentration of protein, sodium, calcium, phosphate and chloride. Moreover, the concentrations of iron and copper in cow's milk are too low. To address these issues, the food industry started to develop modified milk, now known as formula in the mid-19th century. The early 20th century saw the growth of modern day formula milks and by the 1940s, formula milk was considered a safe substitute for breast milk (Stevens, Patrick, & Pickler, 2009). In the US, the Infant Formula Act (1980) authorized the Food and Drug Administration to assure quality control of infant formulas (Fomon, 2001) and requires the following nutrients be present in all infant formulas: protein; fat; vitamins C, A, D, E, K, B1, B2, B6, and B12; niacin; folic acid; pantothenic acid; calcium; phosphorous; magnesium; iron; zinc; manganese; copper; iodine; sodium; potassium; and chloride. It was not until 2005 that global consensus was reached by an International Expert Group on the composition of infant formula (Koletzko *et al.,* 2005). Although the nutrients in synthetic formulas appear almost identical to the nutrients in breast milk on labels, the bioavailability of these nutrients in formula varies significantly when compared to breast milk (Stevens *et al.,* 2009). Moreover, infant formula does not change in composition as the infant ages. Thus, formula is not responsive to a growing infant's nutritional needs, which makes the digestive process more difficult (Lawrence, 1994).

1.2.2.3 Nutritional Requirements of the Mother while Breastfeeding

As exclusively breastfed infants are dependent upon breast milk for their entire nutrient intake, the nutritional composition of the milk is very important. This is dependent on the nutritional status of the mother. The major determinant of extra energy required during this time are the volume and energy content of the milk produced. Well-nourished women produce approximately 750 ml/day for the first 4-6 months of lactation. After the introduction of solid food, this tends to fall to approximately 600 ml/day (Garza & Rasmussen, 2000). The recommended nutrient intakes for energy as well as for many vitamin and minerals are set at higher levels than non-pregnant/lactating women due to the increased nutritional demands on the mother during lactation. Table 1.3 summarises the Irish and UK Recommended Dietary Allowances for non-lactating and lactating women (Department of Health, 1991; Food Safety Authority of Ireland, 1999).

Table 1-5 Recommended Dietary Allowances for women aged 18-64 in Ireland and aged 19-50 in the UK as well as recommended increments during lactation for both Ireland and the UK (DoH 1991, FSAI 1999).

Nutrient (units)	*Irish Females 18-64*	*Irish Lactating women*	*UK Females 19-50*	*UK Lactating women*
Energy (kcal/day)	1900	+430	1900	+325-425
Protein (g/kg/day)	0.75	0.75 +10 g/day	45 g/day	65 g/day
Vitamin A (μg/d)	600	950	600	1300
Riboflavin (mg/d)	1.3	1.7	1.1	1.8
Vitamin C (mg/d)	60	80	40	95
Folate (μg/d)	300	400	200	280
Vitamin B12 (μg/d)	1.4	1.9	1.5	2.6
Vitamin D (μg/d)	0-10	10	-	10
Calcium mg/d	800	1200	700	1200
Iron mg/d	14	15	14.8	15
Zinc mg/d	7	12	7	19

1.2.2.4 Nutritional Requirements of Infant during Weaning

The significant change in dietary experience occurs with the introduction of solid food. The introduction to solid feeding and the gradual replacement of milk by solid foods is known as weaning (Foote & Marriott, 2003). The Committee on Medical Aspects of Food and Nutrition Policy (COMA, 1994) defines weaning as "*the process of expanding the diet to include foods and drinks other than breast milk or formula milk*". The WHO (2001) describes weaning in very narrow terms, viz. "*the complete cessation of breastfeeding*". Instead the WHO (1998) uses the term Complementary Feeding to describe the introduction of solid foods and has defined the term as *"a process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk"* (WHO, 1998; pp128-167). In Ireland and the UK, the terms complementary feeding and weaning are used synonymously to describe the introduction of solid foods to infants. For the purpose of this thesis, the term weaning will be used to describe the process of introducing solid foods to infants in tandem with either formula or breast milk.

The weaning period is associated with significant changes in both the macronutrient and micronutrient composition of the infant's diet. Table 1.2 shows how the increases in nutritional requirements of the infants for many nutrients (e.g. energy, protein, iron, zinc, magnesium) coincides with the age that solid foods are introduced - at 6 months approximately. Yet compared to the vast amount of literature on breast and formula feeding, little attention has been given to this period and its significance in later infant health and development.

Weaning is a very important event during an infant's development. The total energy requirements of breastfed infants have been estimated to be 615 kcal/day at 6-8 months, 686 kcal/day at 9-11 months and 894 kcal/day at 12-23 months of age (Dewey & Brown, 2003). The values were slightly higher when based on a combined group of breastfed and formula-fed infants (634, 701 and 999 kcal/d at 6-8 months, 9-11 months and 12-23 months respectively; Butte *et al.,* 2000). This was explained by the higher resting metabolic rate of formula-fed infants compared to breastfed infants. Butte et al. (1990) previously suggested that energy digestibility and the composition of the newly synthesized tissues may differ between formula-fed and breast-fed infants, accounting for this higher resting metabolic rate among formula-fed infants.

By the time infants are 4 months old, their neonatal iron stores have been reduced by 50%, while by the age of 6 months these stores have been further reduced. Sufficient iron is required for development and low-iron status in infants may result in delayed psycho-motor development and defects in cellular immunity. Therefore, it is very important that, between 4-12 months of age, adequate dietary sources of iron are provided to maintain haemoglobin levels (Thomas & Bishop, 2007). During this time, the UK and Irish recommended reference intake values increase from 1.7 mg/day at 0-3 months to 7.8 mg/day at 7-12 months. Studies on iron status in infants are limited but research suggests that in Europe (including Irish data) 7.2% of infants at 12 months old are iron deficient with 2.3% having iron deficiency anaemia (Male, Persson, Freeman, Guerra, & Hof, 2001). The National Preschool Nutrition Survey 2012 (Walton, 2012) found that 23% of one-year-olds, 10% of two-year-olds and 11% of three year- olds were estimated to have inadequate iron intakes.

1.3 *Current Infant Feeding Recommendations and Practice*

There is a great deal of research which indicates the benefits of breastfeeding to both the mother (Heinig, 1997) and the infant (Beaudry, Dufour, & Marcoux, 1995; Fewtrell, 2004; Golding, Emmett, & Rogers, 1997a; Golding, Emmett, & Rogers, 1997b; Hamosh, 2001; Kramer *et al.,* 2009; Oddy *et al.,* 2003). Further research has indicated that maternal breastfeeding has implications for later infant food acceptance (Birch, 1998; Sullivan, 1994). A growing body of literature also suggests that breastfeeding affords a small, yet consistent, protective effect against infant obesity (Owen *et al.,* 2005; Owen, Martin, Whincup, Smith, & Cook, 2005). However, contradictory findings have emerged in research by Kramer (2009), who, in a large cluster-randomised trial of breastfeeding, found no relationship between breastfeeding and adiposity at 6.5 years. Further research is needed to better understand this relationship.

Despite widely reported benefits, worldwide breastfeeding rates are low, with less than 40% of infants exclusively breastfed during the first six months of life (WHO, 2013b). Some countries have excellent initiation rates, notably Canada 90.3% (Chalmers *et al.,* 2009); Sweden 98%; Iceland 98% and Norway 99% (Cattaneo, Yngve, Koletzko, & Guzman, 2005). In these countries, mothers also breastfed for longer. In Norway, 80% of mothers still breastfed infants at 6 months, while in Sweden 72% and Iceland 65% of mothers' breastfed at six months (Cattaneo *et al.,* 2005). In Canada, there was a higher decline in breastfeeding rates at six months with 53.9% still breastfeeding (Chalmers *et al.,* 2009).

Ireland has one of the lowest breastfeeding rates in Europe and the world. The latest national data available indicates that 46% of mothers were exclusively breastfeeding on discharge from hospital in 2011, compared to 44.3% in 2008, 42.5% in 2004 and 36% in 1999 (The Economic and Social Research Institute - Health Research and Information Division, 2012). Studies in Ireland and the UK showed that women who initiated breastfeeding often stopped after a few weeks (Williams *et al.,* 2010; Tarrant, Younger, Sheridan-Pereira, & Kearney, 2011; Williams, Murray, McCrory & McNally, 2013). The recent Growing-Up in Ireland study showed that the mean cessation time point for breastfeeding was at 11 weeks for Irish born mothers. Women who had been educated to Leaving Certificate (final school examination at ~18 years) stopped breast-feeding after 10 weeks on average, in comparison to those who had completed third level education, who ceased breastfeeding at 14 weeks (Williams *et al.,* 2010).

In 2001, the WHO recommended that all infants should be exclusively breastfed until 6 months and that breastfeeding should continue until the baby is at least 2 years old (WHO, 2001). Earlier WHO recommendations (1995) recommended that solid foods should be introduced after the 4th but before the 6th month of life. This new recommendation was based on the findings of a systematic review, which concluded that there was no significant difference in growth between infants exclusively breastfed for 3-4 months compared to those breastfed exclusively for 6 months (Kramer & Kakuma, 2002). Moreover, research had shown that early introduction of solid food before 4 months was associated with an increased risk of obesity, gastrointestinal infection and risk of wheeze in developed countries (Forsyth *et al.,* 1993; Wilson *et al.,* 1998; Duijts, Jaddoe, Hofman, & Moll, 2010; Huh *et al.,* 2011;). The rationale for the WHO policy change was to help reduce morbidity in the developing world (WHO, 1995; Prescott et al., 2008).

Irish national recommendations have concurred with this new WHO recommendation that all infants should be breastfed for the first six months of life (Department of Health and Children, 2003). However, the evidence base supporting a major, population-wide change in public health policy underwent surprisingly little scrutiny (Fewtrell, Wilson, Booth, & Lucas, 2011). Recently, after a detailed review, commissioned by the European Commission, the European Food Safety Authority's panel on dietetic products, nutrition, and allergies concluded that, for infants across the EU, complementary foods might be introduced safely between four to six months, and six months of exclusive breast feeding might not always provide sufficient nutrition for optimal growth and development (European Food Safety Agency, Panel on Dietetic Products, Nutrition and Allergies, 2009). This is similar to recent guidance issued by the British Dietetic Association Paediatric Group (British Dietetic Association, 2013). Furthermore, a recent Swedish study has shown the potential protective effect of introducing gluten-containing foods, gradually and in small amounts whilst still breastfeeding, to increase the opportunity for the child to build up an oral tolerance to coeliac disease (Ivarsson *et al.,* 2013).

It is, therefore, clear that there is much debate over the timing of the introduction of solid foods. Moreover, despite the current recommendations of health authorities worldwide, several countries show marked departures from official recommendations (Cattaneo *et al.,* 2009). In Italy, 34% of infants were reported to have received complementary foods before 4 months (Giovannini *et al.,* 2004), while in the UK the figure stood at 51% (McAndrew *et al.,* 2012). The Euro-Growth Study found that the median age for solid-food introduction was 19 weeks. About 7% of children were introduced to solid food before the end of the third month of life and 77% of all infants were introduced to solid food by 21 weeks (Grote *et al.,* 2011). In Perth in Australia, 44% of infants were introduced to solid food, before 17 weeks and 93% of infants had been introduced to solid foods before 26 weeks (Scott, Binns, Graham, & Oddy, 2009). Similarly, in Ireland, a recent study (n=401) showed that 70.5% of infants received solid foods before 16 weeks and that 99.7% had been introduced to solid foods by 20 weeks (Tarrant *et al.,* 2010). The finding that 5.1% of Irish infants (Tarrant *et al.,* 2010) had been introduced to solid foods by the age of 1 month is of great concern.

Notwithstanding the timing of weaning, which may have negative effects on infant health, the process itself is of importance for the development of the infant. Weaning, as well as providing additional calories to meet the infant's growing nutritional needs, provides an opportunity for the infant to learn about food through exposure to novel tastes. There are four key stages in this process, from smooth puree to soft finger foods, which are age-specific and designed to meet the nutritional and developmental needs of the infant, which are described in detail in Appendix A. The timing of the introduction of solid foods to infants is conditional on several developmental conditions being met. Infants must have matured to the extent that they have sufficient neuromuscular coordination so that they are able to lift their head, sit without support and take food from a spoon. The timing of this development varies among infants but tends to occur between 4 and 6 months of age. Developmental readiness is an important factor to consider with regard to the introduction of complementary foods to infants. As an infant grows, it starts to develop the ability to chew and starts to show an interest in food. Renal and gastrointestinal function need to be mature in order to metabolise non-milk foods and this usually occurs by the age of 4 months (Ziegler *et al.,* 1990).

Oral motor development is also important in the transition to solid foods. At birth, an infant has 5 oral reflexes present; reflex swallowing; reflex sucking; gag reflex; phasic bite reflex and rooting. These reflexes fade or disappear over the first year of life and it is these changes which allow the transition from liquid to semi-solid to solid foods to occur (Naylor & Morrow, 2001). The ability to manipulate a bolus of food in the mouth and swallow it, to coordinate a utensil and bring food to the mouth as well as drink from a cup, all help in the development of muscles in the face. This muscle development is important in the acquisition of speech, while eating finger food helps develop hand eye coordination (Dunne, Farrell, & Kelly, 2011). These milestones are reached at different ages by infants.

However, it has been noted that there are critical windows for introducing different textures of foods. Studies show that those infants introduced to lumps in their food at a relatively late stage (9 months or older) are more difficult to feed, have more definite likes and dislikes, have long-term feeding problems and have reduced consumption of important food groups such as fruit and vegetables (Northstone, Emmett, Nethersole & the ALSPAC Study Team, 2001; Coulthard, Harris, & Emmett, 2009).

The WHO recommendation for 6 months of exclusive breastfeeding has caused much professional debate because of the lack of scientific evidence to support these recommendations. While the evidence is strong for the initiation of exclusive breastfeeding, there is probably no definitive age at which all infants should be introduced to solid foods but rather a range, somewhere between 4-6 months, depending on the individual development of the infant. Further research is needed to provide evidence based guidelines for weaning. The Scientific Advisory Committee (SACN) Subgroup on Maternal and Child Nutrition (2011) is currently carrying out a review of the scientific evidence underpinning the UK recommendations and will consider the need for updating these, though this will not be completed until 2015.

1.4 *Link between Early Nutrition and Disease*

There is an increasing body of evidence which indicates that there are critical or sensitive periods in life during which nutrition and growth may permanently influence dietary behaviour or "*programme*" long-term development and disease in later life (Godfrey, Lillycrop, Burdge, Gluckman, & Hanson, 2007; Gluckman, Hanson, Cooper, & Thornburg, 2008; Gluckman, Hanson, Buklijas, Low, & Beedle, 2009; Hanson, Godfrey, Lillycrop, Burdge, & Gluckman, 2011). The health and nutritional status of the mother before conception, as well as the growth, energy and nutritional demands placed on her during her pregnancy affect foetal growth. In recent decades, an emphasis has been put on foetal nutrition and the potential impact it may have on an individual's health during childhood and adulthood. The Barker hypothesis (Barker, 1997) suggests a link between prenatal, early life exposures and the development of a range of chronic diseases in later life such as coronary heart disease (CHD), lung cancer and obesity through foetal programming (Oken, Taveras, Kleinman, Rich-Edwards, & Gillman, 2007). This hypothesis is based on the fact that under-nutrition during pregnancy can result in foetal adaptive changes; including metabolic, circulatory and endocrine changes which last into adulthood (De Boo & Harding, 2006).

The Dutch famine was a period of extreme food shortage in the west of the Netherlands that occurred during the last 5–6 months of World War II. It is considered an important historical cohort, which has been used to further investigate the Barker Hypothesis. Studies have shown that those exposed to the famine in early gestation had a higher prevalence of CHD (Roseboom *et al.,* 2000); were twice as likely to consume a high-fat diet (>39% of energy from fat) and had a tendency to be less physically active (Lussana *et al.,* 2008). Stillbirths increased sharply during first trimester exposure to the famine, as did death in the first week of life (Susser & Stein, 1994). Prenatal exposure to the Dutch famine during late gestation was found to be linked to impaired glucose tolerance in later adult life (Ravelli *et al.,* 1998).

This growth restriction during gestation may be linked to programming later food preferences and dietary patterns. Studies have shown that infants exposed to intrauterine growth restriction tended to eat more foods rich in carbohydrates and fat upon reaching adulthood than those who had not experienced such growth restriction (Lussana *et al.,* 2008; Barbieri *et al.,* 2009; Stein *et al.,* 2009). Recently, Perälä *et al.* (2012) showed that small body size at birth was associated with lower consumption of fruits and berries in adulthood.

Evidence suggests that maternal hydration levels during pregnancy may be important in determining salt intake and a preference for salty foods in humans (Crystal & Bernstein, 1995; Crystal & Berstein, 1998; Shirazki, Weintraub, Reich, Gershon, & Leshem, 2007). Evidence shows that infants who had experience of moderate to severe mineral loss during gestation or in early infancy, through illness or dehydration, displayed an increase in salt preference in later childhood (Leshem, Maroun, & Weintraub, 1998; Shirazki *et al.,* 2007). Furthermore, there is evidence that suggests that salt sensitivity is negatively correlated to systolic blood pressure (SBP) in healthy and normotensive adolescents whose mothers reported significant vomiting in the first trimester of gestation (Málaga *et al.,* 2005). These individual differences can have significant implications for health outcomes. Several studies have shown that higher sodium intakes are associated with increased incidence of mortality from cardiovascular disease (CVD), particularly from stroke (He *et al.,* 1999; Umesawa *et al.,* 2008). Ikehara (2012) recently showed, in a large prospective cohort study of middle-aged men and women that a high salt preference was associated with a 20% increased risk of mortality from stroke compared with a low salt preference. These associations did not significantly change after adjustment was made for other cardiovascular risk factors including a participant's history of hypertension.

In addition to the effects of maternal–foetal nutrition, the impact of postnatal growth and infant feeding on long-term human health has also been investigated (Singhal & Lucas, 2004). Breast feeding, when compared with formula feeding, has been shown to have long-term beneficial effects on CVD risk factors such as blood pressure (Owen, Whincup, Gilg, & Cook, 2003; Martin, Gunnell, & Smith, 2005), insulin resistance (Owen, Martin, Whincup, Smith, & Cook, 2006), dyslipidaemia (Owen *et al.,* 2006; Owen *et al.,* 2008) and obesity (Owen *et al.,* 2005). Two systematic reviews (of twenty-six studies) have shown that both systolic and diastolic blood pressure are lower (effect size 0·5–1·5 mmHg) in breast-fed infants compared with formula-fed infants (Owen *et al.,* 2003; Martin *et al.,* 2005). However, this is a very modest effect and further research is needed to establish the role of duration of feeding on blood pressure. Any effect seen may be modest given the overall difference between those breastfed and formula-fed and questions would have to be raised as to whether these results would have any long-term clinical significance.

As infants move from an exclusive milk diet to a diet of mixed foods, the research focus on the impact of weaning and toddler diets on long-term human health has continued. The timing of the introduction of solid foods into the infant's diet is important for long-term health. The early introduction of solid food (before 15 weeks) is associated with heavier bodyweight and a greater percentage body fat at 7 years of age (Wilson *et al.,* 1998). However this study is an observational study thus making causation difficult to establish. Delaying the introduction of solid foods may also have long term consequences, contributing to the development of food allergies and altering food preferences (Harris, 2008).

The early years represent a pivotal time, during which long-term dietary habits are established, with potential life-long effects on appetite, obesity and other risk factors for CVD (Nicklas *et al.,* 1988; Carnell & Wardle, 2008). An earlier age of adiposity rebound, which is often established before the age of 5 years, and faster weight gain in pre-school children is a risk factor for later adiposity (Gardner *et al.,* 2009). These risks were found to track into later life (Nader *et al.,* 2006). The composition of the infant's diet during the early years is also important, as high intakes of protein and saturated fat can contribute to later obesity (Cowin & Emmett, 2000). A higher protein intake in the pre-school years is particularly associated with an increased risk of later obesity (Günther, Buyken, & Kroke, 2007).

Optimising the diets of women during pregnancy, infants and pre-school children could, therefore, be critical to the prevention of later diseases in adulthood. Much of the literature focuses on the nutritional quality of diets during these times. However, there is a growing body of evidence suggesting that the food choices a mother makes during her pregnancy may affect an infant's later acceptance of solid foods. It has been shown that flavours in the mother's diet can serve to heighten the acceptability of those flavours during weaning, (Mennella, Turnbull, Ziegler, & Martinez, 2005). Furthermore, a recent review found moderate tracking of eating behaviours from infancy to childhood, suggesting that certain aspects of eating behaviour are fairly stable for a given individual during childhood (Nicklaus & Remy, 2013). Therefore, an understanding of human taste, its development and its relationship with food preferences is vital for our understanding of current dietary patterns in society.

1.5 *Physiology of Human Taste*

What is referred to as taste in everyday language actually refers to mixture of taste, smell, irritation or feel that is perceived in the oral and nasal cavities. This thesis will focus upon the taste aspect only. A person can perceive thousands of different tastes, which are believed to be combinations of the 5 primary taste sensations: umami, salty, sweet, bitter and sour. Each of these sensations excites different taste receptors and has different recognition thresholds (Table 1.6). Taste occurs when the tastant activates the taste receptors and an afferent signal, using electrical impulses is sent to gustatory processing regions of the brain. At low concentrations, the afferent signal may be too weak and unable to produce a noticeable difference from a similar solution with the tastant. As the concentration of the tastant increases, the afferent signal strength will increase and will reach a level where it is discriminated from a solution of water but it is still not possible to identify the taste quality. This is known as the detection threshold. Actual identification of the tastant occurs when the concentration increases to a level that is high enough not only to activate the taste receptors but will also produce electrical impulses which can be carried via sensory neurons to the brain where they decoded and after which the taste quality can be identified (Liem, Miremadi, & Keast, 2011).

Table 1-6 Examples of Stimuli and molar Thresholds for tasting the Primary Taste Sensations

Taste	*Substance*	*Taste Receptors*	*Detection Threshold*
Salt	NaCl**	EnaC	0.01 M
Sweet	Sucrose	T1R2, T1R3	0.01 M
Umami	Glutamate	T1R2, T1R3, mGluR4	0.0007 M
Bitter	Quinine	T2Rs	0.000008 M
Sour	HCl*	PKD Channels	0.0009 M

*HCl- Hydrochloric Acid ** NcCl- Sodium Chloride ENaC-Epithelial Sodium channels T1R2-Taste receptor type 1 member 2; T1R3-Taste receptor type 1 member 3; mGluR4= metabotropic glutamate receptor 4; PKD Channel-**polycystic kidney disease**-like ion channel.

The identities of the specific chemicals that excite different taste receptors are still not fully known. One of the most common misconceptions about taste is the concept of a taste map where sensitivity to different tastes is thought to correspond to specific areas of the tongue. In reality, there are little regional differences in taste sensitivity. In mammals, taste buds (Figure 1.1) are located throughout the oral cavity, in the pharynx, the laryngeal epiglottis and at the entrance of the oesophagus. Most taste receptor cells are components of taste buds, which are clustered on three types of papillae (i.e., fungiform, foliate, and circumvallate) located on the tongue (Chandrashekar, Hoon, Ryba, & Zuker, 2006). More recently taste receptors have been found in other parts of the body such as the stomach, intestine and pancreas, where it is believed that they aid the digestive process by influencing appetite and regulating insulin production, as well as in the trachea and sperm, where their actions are poorly understood (Trivedi, 2012; Li, 2013).

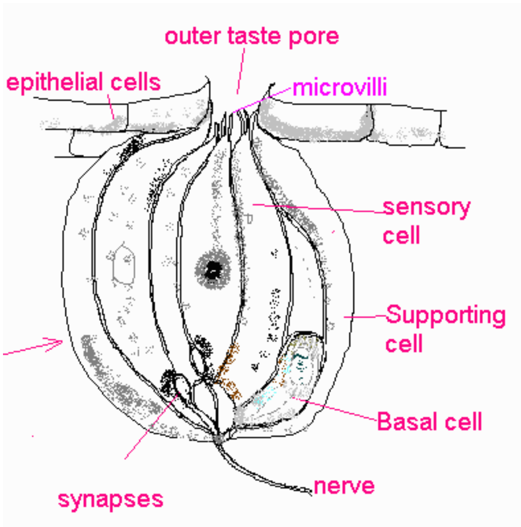


Figure 1-1 Drawing of a cross-section through a taste bud showing the different cells. Supporting cells - contain microvilli that appear to secrete substances into lumen of taste bud. Sensory receptor cells - has peg-like extensions projecting into lumen. At the base of the taste bud, afferent taste nerve axons invade the bud with each fibre typically synapsing with multiple receptor cells within the taste bud (Source: Tim Jacob, Cardiff University).

The taste receptors at the apical end of the taste receptor cells are exposed to the internal environment in the oral cavity (Figure 1.1). When food or drink enters the mouth, chemicals from those foods activate taste receptors. The chemical signal is converted to an electrical signal and sent via the seventh, ninth and tenth cranial afferent nerve fibres to the primary gustatory processing regions of the brain, the medulla. From there, information is relayed (1) to the somatosensory cortex for the conscious perception of taste and (2) to the hypothalamus, amygdala and insula, giving the so-called "affective" component of taste. This is responsible for the behavioural response, e.g. aversion, gastric secretion and feeding behaviour (Figure 1.2) It is not fully understood how the brain interprets the input from these signals and tells us what we are tasting (Lodish *et al.,* 2013).

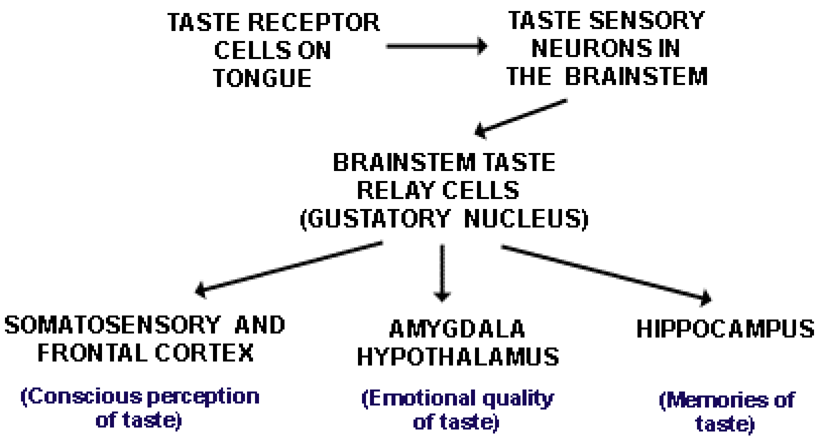


Figure 1-2 The pathway of taste molecules from taste receptor cells on the tongue to the specific sections of the brain showing the different parts involved in the conscious perception of taste as well as the emotion and memories often attached to tastes. (Source: Tim Jacob Cardiff University)

1.5.1 Salt Taste

Salty tastes are elicited by ionized salts. The quality of the taste varies somewhat from one salt to another as salts elicit other taste sensations besides saltiness. The cations of the salts are mainly responsible for the salty taste, but anions can also contribute to this taste to a lesser extent. When Na^+ (Sodium) ions enter the receptor cells via amiloride-sensitive epithelial Na^+ channels, ENaC (Lindemann, 2001), it causes a depolarisation of the cell and Ca^{2+} enters through voltage-sensitive Ca^{2+} channels. This causes transmitters to be released and results in increased firing in the gustatory nerve (Figure 1.2). This has been confirmed in recent studies where knocking out critical ENaC sub-units in taste buds impaired salt taste detection in mice (Chandrashekar *et al.,* 2010).

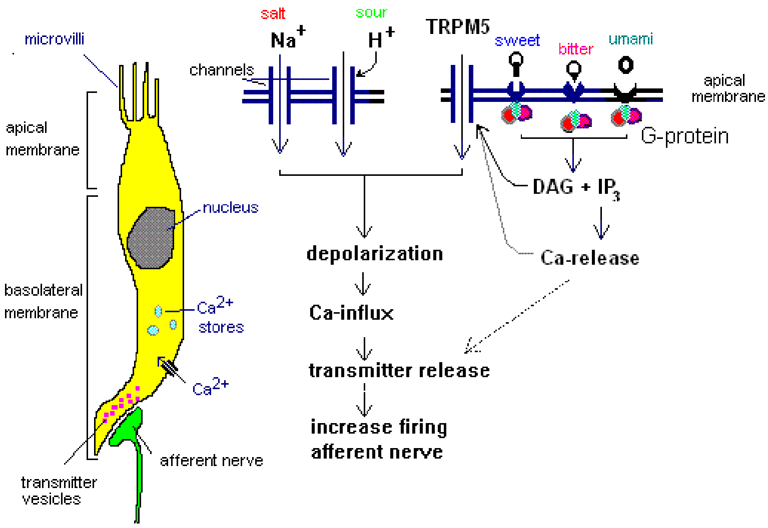


Figure 1-3. Drawing of a cross section through a taste receptor cell. It also shows how taste transduction occurs for each of the five basic tastes- salt, sour, sweet bitter and umami. Taste molecules fit into receptors on the microvilli at the top of the cell, causing electrical changes that release transmitters onto the nerve endings at the bottom of the cell. The nerve carries these taste messages to the brain. (Source: Tim Jacob Cardiff University).

1.5.2 Sweet Taste

Sweet taste is not caused by any single class of chemicals. Some of the types of chemicals that cause this taste include sugars, glycols, alcohols, aldehydes, ketones, amides, esters and amino acids. In the taste pore membrane, there are receptors T1R2 and T1R3 that bind glucose. By glucose binding to the receptors, a G-protein is activated, which in turn activates phospholipase C (PLC-ß2). Phospholipase C generates intracellular messengers which activate the TRPM5 channel and cause cells to depolarise. The depolarisation causes calcium to enter the cell and release transmitters, which cause the increases in the firing of the gustatory nerve (Figure 1.3).

1.5.3 Umami Taste

The taste sensation known as umami is imparted by a number of amino acids and ribonucleotides e.g. monosodium glutamate or MSG. It was first identified by Kikunae Ikeda at the Imperial University of Tokyo in 1909. Its existence has been debated among scientists for many years and its description was only translated into English in 2002 (Ikeda, 2002). Therefore, there has been a lack of research in this area until recently. Initially it was thought that the metabotropic glutamate receptor (mGluR4) mediated umami taste by binding to the receptor which activated a G-protein, increasing intracellular $Ca2^{+}$. However, more recently it has been found that the binding to T1R1 and T1R3 receptors mediate umami taste, by activating the non-selective cation channel TRPM5 and causing cells to depolarise. This depolarisation causes calcium (Ca^{2+}) to enter the cell and release transmitters which cause the increases in the firing of the gustatory nerve (Figure 1.2; Nelson *et al.,* 2002). However, in studies where T1R3 have been knocked out in mice, preference for umami has been detected (Yasumatsu *et al.,* 2009), suggesting that umami taste may be more complex and is likely to mediated through multiple types of taste receptors. Other candidate umami receptors that have been identified are G protein-coupled glutamate receptors (Nelson *et al.,* 2002; San Gabriel & Uneyama, 2012), though understanding of the mechanisms is still in its infancy.

1.5.4 Bitter Taste

Like sweet taste sensations, bitter tastes are not caused by any single type of chemical agent. The substances that give rise to bitter tastes are almost entirely organic substances. Two particular classes of substances are especially likely to cause bitter taste sensations viz.; (1) long-chain organic substances that contain nitrogen and (2) alkaloids (Lindemann, 2001). These alkaloids include many of the drugs used in medicines, such as quinine, caffeine, strychnine and nicotine. Some substances that taste sweet at first have a bitter aftertaste. This is true of saccharin, which makes this substance objectionable to some people. The bitter taste, when it occurs in high intensity, usually causes the person or animal to reject these foods. This was important in evolutionary terms as many deadly toxins found in poisonous plants are alkaloids, which all cause an intensely bitter taste. Aversion to these foods would have protected infants from eating dangerous substances (Drewnowski & Rock, 1995). Research suggests that sweet, umami and bitter tastes converge on a common transduction channel, the transient receptor potential channel TRPM5 (Chandrashekar *et al.,* 2006) via phospholipase C (PLC). It has been shown that PLC and TRPM5 are co-expressed with T1Rs and T2Rs and are vital for sweet, amino acid, and bitter taste transduction. Activation of T1R or T2R receptors by their respective taste molecules would stimulate G proteins, and in turn PLC (PLC-ß2). The activation of PLC generates two intracellular messengers inositol trisphosphate (IP3) and diacylglycerol (DAG) from the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) and opens the TRPM5 channel, resulting in the generation of a depolarising receptor potential (Figure 1.2; Zhang *et al.,* 2003).

1.5.5 *Sour Taste*

Acidic stimuli are the unique sources of sour taste. The intensity of the taste sensation is approximately proportional to the logarithm of the hydrogen ion concentration i.e. the more acidic the acid, the stronger the sour taste sensation. Initially, it was believed that the mechanism for detecting sour taste was similar to that which detected the salty taste; with either H^+ ions blocking K^+ channels causing a depolarisation, or with H^+ ions entering the cell through epithelial Sodium (ENaC) channels. Additionally, two acid-sensing channels - the PKD2L1 and PKD2L3 channels (Huang *et al.,* 2006; Ishimaru *et al.,* 2006) have been proposed as possible sour taste receptors. These channels are members of the transient receptor potential channel (TRP) family and are non-selective cation channels and are permeable to both Na^+ and Ca^{2+} (Figure 1.2). However, mice studies have shown that mice lacking PKD2L3 remain capable of detecting sour tastes (Nelson *et al.,* 2010). Chaudhari & Roper (2010) suggest that Type III cells in taste buds, which are plasma membrane channels that are modulated by cytoplasmic acidification such as certain K^+ channels, may be more likely sour taste receptors. However, more research is needed to understand these mechanisms further.

1.6 *Ontogeny of Primary Tastes*

In the human foetus, collections of cells resembling immature taste buds can be seen as early as the seventh or eighth week of gestation. Between weeks twelve and fourteen, the cells along these buds elongate and form taste pores. However, it is not until the second trimester (approximately weeks 13-15) that the adult form of the taste bud is recognisable. As the second trimester progresses, these taste buds continue to develop in complexity. From approximately week twenty onwards, no more changes in the morphology of the taste buds occur while these taste buds are thought to be fully functional by the third trimester (Bradley & Stern, 1967).

1.6.1 Evidence of Taste Preference in Infants.

In order to protect infants from ingesting harmful substances and to promote the consumption of nutritious substances, they already have a sophisticated sense of taste at or before birth (Cowart & Beauchamp, 1986; Rosenstein & Oster, 1988). Studies of premature infants of gestational age between 6-9 months have also shown that they are able to express differential responses to taste stimuli, particularly sweet and bitter tastes (LeCanuet & Schaal, 2002). Newborn infants are responsive to taste stimuli. Infants have an innate preference for sweet flavours, as demonstrated by positive facial expressions upon tasting. By comparison, sour substances trigger negative facial reactions and infants reject solutions that have sour tastes (Steiner, 1977). As they grow older this reaction is modified. Schwartz *et al.* (2009) showed a decrease in preference of sweet flavours and an increase in preference for salt flavours over the first year of life. Furthermore, they looked at infants aged 3, 6 and 12 months and found that at each age, sweet and salty tastes were the most preferred tastes, reactions to umami was neutral and sour and bitter were the least accepted tastes. However, during the first year, inter-individual variability increased for all tastes except salt.

Because infants cannot yet communicate verbally, sensory testing with them requires an indirect approach. Methodologies for the measurement of food preferences or acceptance in infants and toddlers have received little attention in the literature. Taste acceptance of infants and toddlers have been studied using a variety of behavioural measures like facial expressions (Ganchrow, Steiner, & Daher, 1983; Mennella, Jagnow, & Beauchamp, 2001; Schwartz, Issanchou, & Nicklaus, 2009), suckling patterns (Crook & Lipsitt, 1976), intake (Blossfeld *et al.,* 2007; Crystal & Berstein, 1998; Schwartz, Issanchou, & Nicklaus, 2009) or their mother's perception of their preferences (Blossfeld *et al.,* 2007; Wallace, Inbar, & Ernsthausen, 1992), whereby the primary caretaker (typically the mother) interpreted the behaviour of the child as he/she tasted the food, and rated acceptance on a traditional hedonic scale (Guinard, 2000). However, it is difficult to compare studies as methods vary widely. Table 1.7 describes the cognitive ability of infants from birth to 18 month, showing the difficulties in assessing taste preference in this age group and demonstrating how researchers rely on indirect methods of assessment.

Table 1-7 The Cognitive skills of infants adapted from the ASTM's Committee 18 on Sensory Evaluation (ASTM International).

Skill/Behaviour	*Birth -18 months*
Language –Verbal, Reading, Writing, Vocabulary	Pre-verbal. Rely of facial expressions. Cannot read. Cannot write. Uses sounds. Very few words.
Attention Span	Gauged by eye contact.
Reasoning	Limited to pain and pleasure.
Decision making	Does not make complex decisions.
Understanding scales	Does not understand scales.
Motor skills	Possesses some gross motor skills. No fine motor skills.
Recommended Evaluation Techniques	Behaviour Observations. Diaries. Consumption or Duration Measurements.
Adult involvement	Primary Caregiver. Trained Observer. Experimenter.

Steiner (1977) was the first to publish photographs of newborns' facial reactions to strong solutions of sucrose, citric acid, quinine sulphate, sodium, glutamate or water (Figure 1.4) and successfully demonstrated that infants can perceive tastes from birth and that taste preferences are mostly innate. The following sections will discuss in detail these reactions for all the basic tastes- salt; sweet; umami; bitter and sour.

Figure 1-4 Examples of newborns' facial reactions towards sweet solution (0.04M sucrose), sour solution (0.24M citric acid), bitter solution (0.00007M quinine sulfate), and umami solution (0.5% monosodium or potassium glutamate) in comparison to a neutral solution of distilled water. Taken from Steiner, 1977.

1.6.2 Salt Taste

There has been much discussion and variability encountered in the literature regarding infant salt preference (Crystal & Berstein, 1998). Salt taste preference has not been linked to any specific gene; therefore varying exposure to salt appears to provide a convincing explanation for wide variations in salt preferences (Wise, Hansen, Reed, & Breslin, 2007). At birth, salt does not reliably elicit either a consistent distinctive facial expression (Steiner, 1977) or a differential intake that would indicate that salty substances are distinguished from water or that salt is a preferred taste. Indeed, no reliable preference for the salt taste appears until approximately 4 months post-partum (Beauchamp, Cowart, Mennella, & Marsh, 1994). The relatively late appearance of the preferential response for salt has been interpreted by some as evidence that salt preference is due to previous exposure to salt in foods. However, other research has questioned that interpretation. Harris (1990a) found that 4 month old infants had a preference for salt in food, which could not have been based on experience as all infants up to that had been exclusively breastfed (human milk has a relatively low sodium content). The author suggested that infants may have an innate preference for salt that only appears on sensory maturation.

Stein *et al.* (2006) showed a relationship between birth weight and salty taste acceptance in infants and young children and found a significant association between lower birth weight and higher salt taste acceptance at 2 months. They found that 2 month old infants manifested a wide range of responses to salt taste stimuli, ranging from high acceptance to strong rejection. While authors suggest that such differences could conceivably be attributed to random variation, the strong inverse association of salt acceptance with birth weight is consistent with an impact of unidentified prenatal or early postnatal events influencing development of salt taste sensitivity, avidity or both (Stein, Cowart, & Beauchamp, 2006).

1.6.3 Sweet Taste

The sensory appeal of sweetness is both innate and universal. There are good adaptive reasons why humans may have evolved innate preferences for sweet tastes, as sweetness usually signals energy dense foods. Newborn infants, without any prior exposure to tastes, have shown a preference for sugar solutions compared to water (Steiner, 1977), with the facial response being described as one of facial relaxation and sucking (Rosenstein & Oster, 1988). They can also discriminate between different kinds of sugars (for example glucose and fructose), as well as different concentrations of the same sugars (Desor, Maller, & Turner, 1973). Interestingly, sucrose is used for pain management in infants and children as it is thought to have an anaesthetic effect. It is not fully understood how it works but it is thought to have a similar pathway to opioids, as it was shown in rat models that Naloxone, an opioid antagonist, actually blocked the effect of sucrose (Zempsky & Schechter, 2003).

Children's food preferences are often guided by taste alone and studies have shown that in pre-school children food preferences are determined by two factors – familiarity and sweetness (Birch, 1992; Birch, 1979; Aldridge, Dovey, & Halford, 2009). Although this preference is innate, it is soon modified by experience and becomes increasingly context specific. Very young infants show a preference for sweetened water; however, this preference was only maintained several months later among infants whose mothers continued to feed them sweetened water. This effect was specific to water and did not generalise to other beverages (Beauchamp & Moran, 1982). Experience can also teach children that some foods are appropriate contexts for sweetness whereas others are not. Pre-school children repeatedly given tofu - either plain, salted, or sweetened, came to prefer the version that was most familiar to them (Sullivan & Birch, 1990). This would suggest that sweet tastes are preferred in children - but only in familiar food contexts.

The preference for sweet tastes remains high during childhood but reduces as children mature (Desor & Beauchamp, 1987). There are also individual differences in the degree of sweetness preferred (Desor *et al.,* 1973). These differences may reflect differences in experience and may or may not correspond to dietary recommendations. Furthermore, experience may modify preference as children get older. Liem *et al.* (2004) demonstrated that repeated short-term exposure to orangeade with high concentrations of sucrose significantly increased children's preference for this orangeade while no increase in preference was detected in adults. Other research has shown that children whose mothers routinely added sugar to their diet preferred higher levels of sugar in apple juice compared to children whose mothers reported never adding sugar (Liem *et al.,* 2004). This shows the importance of introducing healthy foods and flavours to children at a young age to increase exposure to these foods and indicates the important influence mothers have on their child's diet.

1.6.4 Umami Taste

Umami substances have a subtle taste even at high concentrations and can also be difficult to distinguish from salty tastes, as sodium is also found in monosodium glutamate (MSG) (Lindemann, 2001; Kim, Breslin, Reed, & Drayna, 2004). Research has shown that, in adults and children, MSG solutions are unpalatable but the addition of MSG to foods increases their palatability. It appears that umami must be experienced in the context of other flavours to be liked i.e. it is a flavour enhancer rather than a pleasant flavour itself (Wardle & Cooke, 2008), though further research to understand this flavour is needed. To date, very little is known about the variability in umami taste perception among humans. The complete DNA sequence of T1R1 and T1R3 receptor genes suggests there may be some variation between populations but the relationship between possible polymorphism and umami taste perception has not yet been explored (Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009).

1.6.5 Bitter Taste

Infants show negative facial expressions to bitter tastes. Newborn infants react to bitter stimuli by mouth gaping, which is often accompanied by an elevation of the tongue in the back of the mouth (Rosenstein & Oster, 1988). These actions block swallowing and allow the liquid to drain from the mouth. It was also the strongest rated response given by infants compared to those given for sweet, salty and sour. However, bitter taste may also be modified through experience. Kajiura *et al.* (1992) showed that while newborn infants did not reliably reject bitter tastes, these rejections were evident in older infants (14-180 day old infants).

Genetic differences also contribute to the variability in human perception of bitter substances. Duffy & Bartoshuk (1996), in a review of existing evidence on bitter taste reactions, showed that among adults, there are individual differences in sensitivity to the bitter substances 6-n-Propylthiouracil (PROP) and phenylthiocarbamide (PTC), which can be explained by genetic differences. Those who have the two recessive alleles are considered as non-tasters (approximately 30% of a population) and those who have one or both dominant alleles are considered as tasters. Tasters were also shown to have a greater number of fungiform papillae on the tongue compared to non-tasters.

Adult PROP tasters perceive stronger bitterness in caffeine (Hall, Bartoshuk, Cain, & Stevens, 1975) and potassium chloride, the salt substitute, (Bartoshuk, Rifkin, Marks, & Hooper, 1988) when compared to non-tasters. Many vegetables are bitter tasting and are disliked for this reason. Taste sensitivity to PROP has been shown to influence food preference and reported intake in young children, with PROP tasters showing lower acceptance of raw broccoli (Keller, Steinmann, Nurse, & Tepper, 2002) and spinach (Turnbull & Matisoo-Smith, 2002). One study showed that non-taster children consume more vegetables overall, particularly the vegetables that are bitter tasting, compared to taster children (Bell & Tepper, 2006). These studies suggest that the PROP bitter-taste phenotype contributes to the development of vegetable acceptance and consumption patterns during early childhood.

1.6.6 Sour Taste

Little is known about inter-individual variation in sour taste perception (Garcia-Bailo *et al.,* 2009). An early genetic study of twins failed to show any evidence of heritability for the threshold at which individual could detect sourness (Kaplan *et al.,* 1967). However, in more recent years, another twin study has shown that genetic factors do play a role in recognition thresholds for sour tastes (Wise *et al.,* 2007). This suggests that genetics do play a role in sour taste perception but that any potential relationship between polymorphisms in these genes and sour taste perception needs to be investigated further.

There is a lack of research into the area of sour taste development and preference and little is known about the ontogeny of sour taste preferences. Negative gusto facial reactions to sour substances have been reported in infants (Steiner, 1977) and they reject solutions having sour tastes (Desor *et al.* 1973). Newborns with no taste experience respond differentially to sour and bitter stimuli, demonstrating that they can discriminate between these two taste sensations. Lip pursing and rapid sucking movements are the most commonly elicited facial response made by infants in reaction to sour flavours (Rosenstein & Oster, 1988). It is suggested that the reason for these movements is to compress the cheeks against the gums, thus increasing salivation in the mouth. This would dilute the sour solution, making it more palatable, as naturally occurring sour substances, like sweet substances, are likely to be nutritious and not toxic (Birch, 1999).

It is only in the last decade that researchers have started to examine links between perception of sour taste and food preferences, specifically if these preferences change with experience during the early years of life. Research by Liem & Mennella (2002) and Mennella & Beauchamp (2002) suggests that experience with sour tasting hydrolysed protein formula during infancy can modify the natural aversive reaction to sour taste amongst infants. Hydrolysed protein formula is given to babies who have an intolerance or allergy to cow's milk. It is described as having a bitter and sour taste as well as an unpleasant odour, due to the presence of free amino acids. Children aged between 4-5 years, who were fed hydrolysate formulas as infants, were more likely to prefer the odour and flavour of the hydrolysed formula and were less likely to make negative facial expressions during testing. Additionally, those children with experience of the sour tasting formula also had a higher preference for sour flavoured juices when compared to children without experience of the hydrolysed formula (Mennella & Beauchamp, 2002).

Additional work by Liem *et al.* (2004) revealed that, at 5-9 years of age, some children display heightened sour preferences independent of their experiences with hydrolysed formula. Researchers suggest that a preference for strong sour stimuli is related to children's willingness to try unfamiliar foods (Liem & Mennella, 2003; Liem *et al.,* 2004) and their fruit consumption (Liem & Mennella, 2003; Blossfeld *et al.,* 2007). Liem & Mennella (2003) demonstrated that sour taste preferences are heightened during childhood and that these preferences are related to children's food preference and habits. They found that those children who preferred extremely sour tastes tended to experience a greater variety of fruits when compared to other children. Liem *et al.* (2004) observed that preference for sour taste was not related to differences in rated sour intensity. However, those who preferred sour taste had a higher salivary flow.

Blossfeld *et al.* (2007) found a positive relationship between 18 month old infants who accepted highly sour tastes and a diet high in fruit intake and variety. Infants who accepted the sourest solutions also had a higher fruit intake at 6 months and a significantly higher increase in their fruit intake from 12 to 18 months (Blossfeld *et al.,* 2007). This study offered the first evidence that variations in sour taste acceptance exist between older infants. Furthermore, it suggested that some older infants do not automatically reject sour tastes. Since newborns appear to have a negative reaction to sour tastes, research is needed into possible critical periods in the development of these variations in sour taste preference. Through repeated exposure to predominately sour flavoured foods during these periods, it might be possible to overcome the child's innate aversive reaction to sour stimuli and thus improve fruit intake. Such a change, we will see, may yield many benefits for the child, and for society.

1.7 *Development of Food Preferences*

The maternal diet provides a great deal of knowledge to the foetus about available foods in the outside environment. After the birth of her infant, maternal choice with regard to early infant feeding practices (for example, breast feeding versus formula feeding) will impact on an infant's development and health. Moreover, decisions regarding the timing of weaning and, in turn, the foods introduced will also impact on the infant's development. Figure 1.5 gives an overview of the types of learning that can occur during various stages of infant development, viz. prenatal, neonatal and weaning. The next sections will consider each of these stages and explore how early learning experiences can affect food preference.

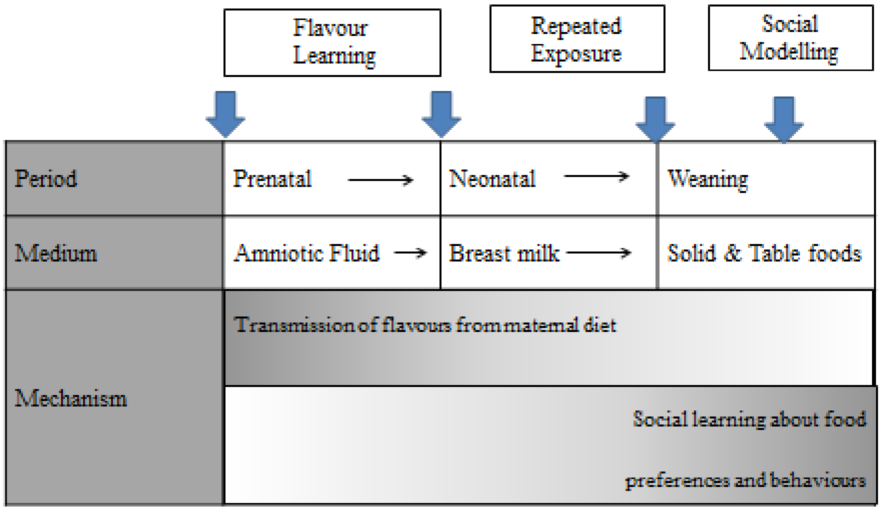


Figure 1-5 Types of flavour learning that can occur during various stages of infant development: prenatal, neonatal and weaning periods. Adapted from (Mennella & Ventura, 2010)

1.7.1 Prenatal Learning

The foetus develops surrounded by amniotic fluid, which is a rich source of sensory exposure. Amniotic fluid has been shown to contain flavours that resemble the flavour of the food previously eaten by the mother. Studies have shown that garlic (Mennella, Johnson, & Beauchamp, 1995), cumin, fenugreek and curry (Hauser, Chitayat, Berns, Braver, & Muhlbauer, 1985) odours have been identified by adult researchers in the amniotic fluid of pregnant women after they ingested oil of garlic capsules and spicy foods. Furthermore, experiences with flavours prior to birth have led to increased enjoyment and preference for these flavours at birth and weaning. For example, exposure to dietary transmitted flavours such as garlic or anise in amniotic fluid has been shown to influence the newborn's facial, mouthing, and orienting responses to those odours immediately post-partum (Hepper, 1988; Schaal, Marlier, & Soussignan, 2000).

Whilst in the womb, the foetus ingests almost one litre of amniotic fluid per day and it is suggested that the first experiences of flavour occur prior to birth. Early experiments demonstrated that foetuses swallowed more amniotic fluid following an injection of saccharin into the amniotic cavity (DeSnoo 1937, as cited by Saliba, Wragg, & Richardson, 2009). Later, Lilley (1972) reported that injections of bitter tasting poppy seed oil (a Lipiodol-radio-opaque substance) reduced foetal swallowing in foetuses aged 34-39 weeks. These studies suggest that taste buds in the human foetus may be functional prior to birth, particularly in the third trimester, allowing the foetus to taste flavours in utero. Mennella *et al*. (2001) examined the influence of repeated prenatal exposure to carrot juice on infant taste preference and found that women who consumed carrot juice for three consecutive weeks during their third trimester of pregnancy gave birth to infants who exhibited fewer negative facial expressions when first introduced to carrot-flavoured cereal in comparison to those who had no prenatal exposure to carrots.

Overall, this evidence suggests that the human foetus has the ability to detect and remember flavour information from the pregnant mother's diet during the third trimester. Furthermore, it provides strong evidence that prenatal flavours experienced by the foetus can improve the acceptance and enjoyment of similarly flavoured foods during weaning. This evidence raises the possibility that a pregnant mother's diet during late gestation may have a role in the acquisition of food and flavour preferences in infants as it has been established that experience with dietary flavour begins before birth.

1.7.2 Neonatal Learning

1.7.2.1 Breast Milk

Studies of human milk report that its flavour is predominately sweet and contains volatile food odours, which vary from mother to mother (Mennella *et al.,* 2001; Forestell & Mennella, 2007). Amniotic fluid and breast milk are regarded as sharing a commonality in their flavour profiles with the foods, spices and beverages eaten by the mother (Mennella *et al.,* 2005). It is thought that "*breast milk may bridge the experiences with flavours in utero to those in solid foods*" and may enhance acceptance of these foods during the weaning period (Mennella *et al.,* 2005). Forestell & Mennella (2007) found that breastfeeding only conferred an advantage in initial acceptance of a food - if mothers ate the food regularly. Furthermore, breastfed infants' acceptance of cereal was enhanced when it was prepared with their mothers' milk, while their willingness to accept the flavoured cereal correlated to their mothers' reported willingness to try novel foods and flavours (Mennella & Beauchamp, 1997; Mennella & Beauchamp, 1999).

Breastfeeding is associated with higher intakes of new foods. The combination of breastfeeding and high variety feeding experiences were associated with the greatest intake of new foods (Maier, Chabanet, Schaal, Leathwood, & Issanchou, 2008). A recent review by Remy *et al.* (2013) found moderate but significant associations between mode of milk feeding, complementary feeding practices and later eating patterns. Breastfeeding duration is positively associated with food variety later: it is associated with variety of free food choices among 2 to 3 year-old children (Nicklaus, Boggio, & Issanchou, 2005), with healthy eating habits at 2 years (Abraham, Godwin, Sherriff, & Armstrong, 2012), with food variety at 2 years (Scott, Chih, & Oddy, 2012), with fruit consumption at 6–8 years (Skinner, Carruth, Bounds, Ziegler, & Reidy, 2002b) and with a healthy eating patterns (consumption of meat, fruits and vegetables) at 2–8 years (Grieger, Scott, & Cobiac, 2011). Exclusive breastfeeding for at least 3 months is associated with a higher consumption of vegetables at 4 years (Burnier, Dubois, & Girard, 2010).

1.7.2.2 Infant Formula

In direct contrast to breast milk, formula milk has a consistent, unchanging flavour. Most mothers who use formula often feed their infant one type of formula milk and, therefore, these infants are exposed only to one constant flavour. The influence of the infant's milk feeding regimen on the acceptance of an infant's first pureed vegetable has been examined. Although breastfed and formula-fed infants initially had similar levels of intake of novel vegetables, after several exposures, breastfed infants increased their intake much more dramatically than formula-fed infants (Sullivan, 1994). Formula flavours are due to the composition and processing involved and can range in flavour depending on the product. Studies show that the type of formula consumed by infants can affect taste preferences (Mennella & Beauchamp, 1996; Gerrish & Mennella, 2001; Mennella & Beauchamp, 2002; Mennella, Forestell, Morgan, & Beauchamp, 2009). One particular study showed that infants with experience of a vanilla flavoured formula showed a greater preference for vanilla flavoured foods as adults (Mennella & Beauchamp, 1996).

Formulas made from hydrolysed casein, consumed by formula fed infants who cannot tolerate cow's milk protein and other intact proteins, differ greatly in taste compared to usual infant formulas. Mennella *et al.* (2005) reported that infants fed on one or another brand of hydrolysate formula significantly preferred the formula that was familiar to them. Other studies have shown these preferences can be long lived. Children aged 4-10 years, who were exposed to hydrolysates during their infancy, exhibited more positive responses to the sensory attributes associated with them (sour taste and aroma) than infants without such experiences (Liem & Mennella, 2002; Mennella & Beauchamp, 2002; Sausenthaler *et al.,* 2010). Infants aged between 4-9 months of age, who consumed hydrolysed casein formulas ate significantly more savoury, bitter and sour tasting cereals than those who were consuming breast milk or cow based formula (Mennella & Beauchamp, 2009). The balance of research indicates that taste experiences in milk feeding affect future taste preferences. Furthermore, breast milk has been shown to offer a rich source of sensory experience for the infant. Since the flavours of all types of formulae are constant and unchanging and lack the sensory information from a mother's diet, formula fed infants are deprived of an important learning experience prior to weaning. The impact of this loss upon later food habits and flavour preference is yet to be determined but has the potential to have a profound effect on obesity. The thesis will now explore the next stepping stone in the development of the infant and its taste preferences.

1.7.3 Learning during Weaning

Weaning usually begins with the introduction of smooth pureed foods (Stage 1). Infants who are typically 6 months old will progress to thicker purees, soft lumps and soft finger foods (Stage 2). At 9 months, infants will progress to mashed and chopped foods with finger foods (Stage 3) and by the age of one year they will typically be eating a diet comprised of family food (Stage 4). Appendix A has a detailed description of these four stages. The introduction of pureed foods accustoms the infant to taking food from a spoon, which is an important skill. Initially, foods are generally offered before milk feeds; 1-2 times per day. Bland tasting and savoury foods are often initially offered and parents are advised to avoid sugary and salty foods (Dunne, Farrell, & Kelly, 2011).

The texture and appearance of food and the perception of flavour are important influences in the development of the senses (Lawless, 1985), and thus affect food preferences. However, because infants innately display facial expressions of distaste to caregivers in response to certain foods, caregivers may be reluctant to continue trying these foods and hence limit their exposure to infants. Neophobia – the rejection of unfamiliar foods and its reduction – plays an important role in the shaping the food patterns of young children. At weaning all foods are new, and therefore neophobia has a significant role. If infants are not introduced to chewable foods at the recommended age they may be less likely to accept new textures at later ages and the variety of their diet may be limited. This may apply particularly to foods such as meat or hard fruits that require chewing. Northstone *et al.* (2001) found the age at which infants were introduced to lumpy solids affected later food preference. Those infants who experienced delayed introduction of lumpy foods (10 months or later) were more difficult to feed and had more definite likes and dislikes. Moreover, they were less likely to be having family foods at 15 months when compared to those introduced to lumpy solids between 6 and 9 months. Research examining food preferences has identified a link between early exposure to certain foods and subsequent food acceptance. It also suggests that the foundations for lifelong dietary habits are formed early in life (Fox, Reidy, Novak, & Ziegler, 2006). Furthermore, children who from the earliest age have plentiful opportunities to sample a variety of healthy foods appear to have healthier diets throughout childhood (Cooke, 2007) and are more willing to sample new foods (Pelchat & Pliner, 1986).

The three periods when infants are exposed to and thus potentially influenced by different flavours in early life; in utero, post-natally through milk feeding and at the weaning from milk to solid foods; show the importance of early interventions to promote healthy eating. The importance of exposure to flavours is seen as early as the third trimester of gestation. At this time, the foetus, equipped with a functional taste system, receives information about the flavours of its mother's diet through the amniotic fluid (Bilkó, Altbäcker, & Hudson, 1994). Learning continues after birth, initially through the flavours of the milk consumed and then through repeated exposure to foods and flavours at weaning. As previously outlined, research suggests that flavour preferences learnt early in life not only affect taste preferences in the short term (Mennella *et al.,* 2001) but may also be important for taste preference in the longer term (Mennella & Beauchamp, 1996).

1.8 *Link between Food Preference & Disease*

Children in developed countries have a wide variety of food to choose from - both healthy and unhealthy. Furthermore, children are increasingly allowed to make their own food choices (McGinnis, Gootman, & Kraak, 2006) but do not necessarily have the motivation or knowledge to make healthy choices (Hart, Bishop, & Truby, 2002). Research suggests that, alongside inactivity (Crooks, 2000; O'Brien *et al.,* 2007) and interactions between genes and environment (Olden, 2006; Wardle & Cooke, 2008), the choice of food children eat plays a significant role in the development of obesity and other related chronic diseases.

Obesity results from an imbalance between energy intake and energy expenditure. It has been declared by the World Health Organisation (WHO) to be one of the top five global risk conditions for health worldwide (WHO, 2013a). The International Obesity Task Force estimates that, globally, at least 155 million school-age children (about one in 10 children) are overweight or obese. Of those, around 30-45 million are classified as obese, accounting for 2-3% of the world's children aged 5-17 (WHO, 2009; International Obesity Taskforce, 2012). Globally, there are an estimated 43 million children under the age of five who are classified as either overweight or obese (WHO, 2013a). Most recent Irish figures indicate that 26% of nine year olds and 25% of three year olds were either overweight or obese (Williams *et al.,* 2010; Williams *et al.,* 2013). This is consistent with previous studies in this area (Whelton *et al.,* 2007). Numerous studies indicate that overweight and obesity in childhood tend to persist into later life (Ong & Loos, 2006). Accordingly, high prevalence levels in early childhood represent a worrying trend, not only in terms of the quality of life and health of the children involved but also in terms of the planning, delivery and cost of healthcare in the future.

Of great concern is the fact that weight status is related to social class in the study referred to above, 33% of nine-year-olds from semi-skilled/unskilled manual groups were obese or overweight compared with 22% from the professional/ managerial group (Williams *et al.,* 2010). This effect is already evident at three years of age; 5% of children from the professional/managerial and non-manual/skilled manual groups were classified as obese or overweight while 7% of children from the semi-skilled/unskilled group were classified as obese or overweight and 9% of children from the 'never worked' group were classified as obese or overweight (Williams *et al.,* 2013).

Childhood obesity is associated with a number of health problems, including hypertension (Figueroa-Colon *et al.,* 1997), asthma (Liu, Kieckhefer, & Gau, 2013) and fatty liver disease (Kinugasa *et al.,* 1984) during childhood and is also associated with premature mortality in adulthood (Reilly & Kelly, 2010). However, given the recent explosion in numbers of children classified as overweight and obese, of most concern is the associated health problems of hyper-insulinanaemia and increased risk of type 2 diabetes. Studies have found a 45% increase in the number of new paediatric type 2 diabetes cases annually (American Diabetes Association, 2000; Fagot-Campagna *et al.,* 2000; Ludwig & Ebbeling, 2001). As childhood populations become increasingly overweight, this figure is expected to increase dramatically (Pinhas-Hamiel *et al.,* 1999; Lobstein, Baur, & Uauy, 2004). The full burden of this childhood obesity crisis will not be seen until this generation of children become adults and many of the obesity-related conditions such as increased rates of heart disease, diabetes, certain cancers, gall bladder disease, osteoarthritis and endocrine disorders will be seen in young adult populations (Lobstein *et al.,* 2004).

Given the rapidly growing epidemic of obesity and associated chronic diseases, it is difficult to estimate the potential demands on future health services. Currently, it is estimated that medical expenditure on those on the obesity spectrum accounts for 6% of the total health expenditure in Europe (WHO, 2007). In Ireland, the cost of caring for those deemed overweight and obese in 2009 was estimated at €1.13 billion. More than a third (35%) of these costs were direct healthcare costs, and 65% were indirect costs. Overall, these direct costs represented 2.7% of the total healthcare costs for 2009 in Ireland (Dee *et al.,* 2013). Given the grave implications for the individual, and for society at large (in the economic and social spheres), it is of fundamental importance to identify any antecedents that may contribute to the emergence of these debilitating and life threatening conditions. In the next section I will discuss how children's diets may play a role in the development and thus, the prevention of these conditions.

The increase in fast food consumption among children over the past few decades has been identified as a particular area of concern (Guthrie, Lin, & Frazao, 2002). Fast food has been defined as *"readily available, energy dense meals, snacks foods and drinks, which tend to be consumed often and are frequently offered in large portion sizes"* (World Cancer Research Fund & American

Institute for Cancer Research, 2007). Evidence suggests that there has been a major shift in dietary behaviour of children (Poti & Popkin, 2011), with in excess of 30% of U.S. children eating fast food on any given day (Bowman, Gortmaker, Ebbeling, Pereira, & Ludwig, 2004). A recent systematic review concluded that high levels of meals eaten outside the home represent a risk factor for higher energy and fat intake and lower micronutrient intake (Lachat *et al.,* 2012). Moreover, children's consumption of these high fat, energy dense foods are positively related to higher BMI and obesity (Ricketts, 1997; Crooks, 2000; Maffeis, 2000).

In a review of its evidence on the contribution of sugary drinks to the increased prevalence of overweight and obesity, the WCRF & AICR (2007) concluded that there was substantial and consistent evidence that drinks containing sugars, including sucrose and high-fructose corn syrup, were a probable cause of weight gain. Subsequent to that research, several meta-analyses and systematic reviews provided further evidence that sugar-sweetened beverage consumption promotes weight gain in children and adults (Malik, Schulze, & Hu, 2006; Vartanian, Schwartz, & Brownell, 2007; Malik, Willett, & Hu, 2009; Malik, Pan, Willett, & Hu, 2013; Olsen & Heitmann, 2009). The International Health Behaviour in School-aged Children (HBSC) Study stated that 32% of boys and 25% of girls reported consuming at least one soft drink daily (Currie *et al.,* 2008). The same study showed in Ireland that 26% of Irish school aged children consumed at least one soft drink daily (Nic Gabhainn, Kelly, & Molcho, 2007).

There is a clear association between soft drink intake and increased energy intake, as people consuming soft drinks fail to compensate for the energy consumed in soft drinks and have higher food intakes than those who do not consume soft drinks (Vartanian, Schwartz, & Brownell, 2007). Fiorito *et al.* (2010) observed that, relative to girls who were not consuming soda beverages at age 5 years, soda consumers had higher subsequent soda intake, lower milk intakes, higher intakes of added sugars, lower protein, fibre, vitamin D, calcium, magnesium, phosphorous, and potassium from ages 5 to 15 years.

The World Cancer Research Fund recommends being as lean as possible within the normal range of body weight, being physically active, limiting the amount of energy dense foods and avoiding sugary drinks as important cancer prevention measures (World Cancer Research Fund & American Institute for Cancer Research, 2007). Since research suggests that the development of healthy food preferences can happen very early in life, targeting these early formative years may reduce the risk of obesity and other chronic diseases later in life. O'Brien (2007) suggests *"It is only through nurturing the formation of good habits during the early stages of life that we have any chance of reversing the alarming obesity trend that we simply could not have predicted 20 years ago"*.

1.9 *Sour Taste & Fruit Consumption*

A large body of epidemiological evidence suggests that a high fruit intake helps to promote health and prevent chronic disease such as obesity, cardiovascular disease, diabetes and cancer (Steinmetz & Potter, 1996; Ness & Powles, 1997; Ford & Mokdad, 2001; Bazzano, Serdula & Simin Liu, 2003; He, Nowson & MacGregor, 2006; He, Nowson, Lucas & MacGregor, 2007; Boeing *et al.,* 2012). Although many fruits contain sugar, they are a rich source of vitamins and minerals and a good source of fibre (Thurnham, Bender, Scott, & Halsted, 2000). Many fruits have a low energy density as a result of their high content of water, low content of energy and high content of dietary fibre (Drewnowski, 2004). Eating these fruits is considered to increase satiety (Rolls, Ello-Martin & Tohill, 2004). Many fruits also contain flavonoids, a group of phytochemicals which recently have been shown to have anti-obesity properties (Hughes *et al.,* 2008). As a result of these properties, diets which are high in levels of fruit and vegetables are encouraged. WHO/FAO (2005) recommends the intake of a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases and obesity.

Given the importance of fruit for a healthy diet, many governments have targeted fruit and vegetable consumption e.g. the 5-a-day message (NHS, 2011; Food Safety Authority of Ireland, 2012). In most Western countries, large population groups, including children and adolescents, eat far less than the recommended amount of fruits and vegetables (Currie *et al.,* 2008; Kelly, Gavin, Molcho & Nic Gabhainn, 2012). Several studies have shown that a child's intake of fruit and vegetable tracks into adolescence (Kelder, Perry, Klepp & Lytle, 1994; Resnicow *et al.,* 1998; Lytle, Seifert, Greenstein & McGovern, 2000) and that those food preferences and eating habits established in childhood and adolescence tend to be maintained into adulthood (Mikkilä, Räsänen, Raitakari, Pietinen & Viikari, 2005; Ashcroft, Semmler, Carnell, Van Jaarsveld & Wardle, 2008; Northstone & Emmett, 2008). This emphasises the importance of promoting fruit consumption in early life as an important public health issue. The latest Irish HBSC survey (2010) showed that 18% of boys and 22% of girls aged between 10-17 years of age reported eating fruit more than once a day (Kelly *et al.,* 2012). This figure has remained stable over the past number of years (19.4% in 2006; (Currie *et al.,* 2008). The recent Growing up in Ireland study reported that 78% of 9-year old children had eaten at least one portion of fruit daily (Williams *et al.,* 2010). A recent initiative in Irish schools-Food Dudes- show that provision of fruit and vegetables to children substantially increases children's fruit and vegetable consumption over a twelve months period (Horne *et al.,* 2008).

Numerous studies in Ireland indicate that dietary quality is strongly patterned by socio-economic status (Kelleher, Lotya, O'Hara, & Murrin, 2008), a trend which has also been reported by the recent Growing Up in Ireland study, where the higher the mother's level of education, the more fruit and vegetables the child ate. Approximately, 94% of 3-year old children whose mother had a degree level qualification had taken at least one portion of fruit in the preceding 24-hour period. This figure dropped to 82% among those with a lower secondary education or less (Williams *et al.,* 2013). This trend continued to be evident among the 9 year cohort, where 86% of nine-year-olds whose mothers were third level graduates ate at least one portion of fruit in the day before their interview, compared to 71% of children whose mother had a lower secondary education or less (Williams *et al., 2*010). Higher intakes of fruit in children have been shown to be associated with reduced intakes of total dietary fat, saturated fat and cholesterol (Dennison, Rockwell, & Baker, 1998). Other studies have shown that eating fruit as a substitute for energy dense foods can protect against obesity and is a better predictor of a healthy BMI than vegetables (Lin & Morrison, 2002).

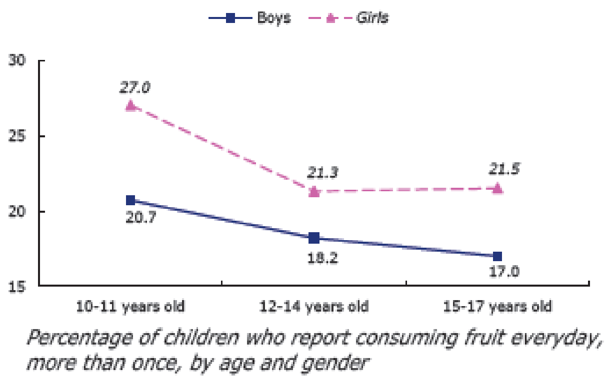


Figure 1-6 Daily fruit consumption among 10 and 17-year-olds in Ireland reported in the HBSC 2010 (Kelly *et al.,* 2012). Overall 20% of children reported eating fruit more than once.

Previous research has shown that infants' and children's preference for sour tastes has been linked to fruit consumption (Liem *et al.,* 2006), with individuals who had the highest sour taste preference eating the most fruit. Understanding why some children eat more fruit than others is important if we are to better promote fruit consumption from an early age. Fruit variety and consumption patterns are influenced early in life by such factors as breastfeeding duration and early fruit exposure and variety (Skinner *et al.,* 2002a; Liem *et al.,* 2004) in children as well as infants (Blossfeld *et al.,* 2007). Therefore early introduction of fruit into an infant's diet may potentially play a role of food preference development. Given the link between sour tastes and fruit consumption, investigation into how critical periods early in life may play a role in the development of sour taste preferences and may help promote healthy eating practices are important. Studies have shown a strong association between the availability of fruit and vegetables in the home and children's consumption of these foods (Resnicow *et al.,* 1997; Hearn *et al.,* 1998; Ding *et al.,* 2012).

1.10 *Conclusion*

Skinner and colleagues found that food preferences at 2-4 years of age were predictive of food preference at 8 years of age (Skinner *et al.,* 2002b). This finding demonstrates how important early exposure to food is to the formation of eating habits later in life. The dietary patterns that are formed in childhood are carried into adulthood (Birch, 1999; Lytle *et al.,* 2000). It is, therefore, established that acquisition of good eating habits starts early on in childhood.

Newborn infants have a functional taste system which can distinguish between the basic tastes, particularly sweet, bitter and salty (Steiner, 1977). These food preferences are both innate and learned (Birch & Sullivan, 1991; Mennella *et al.,* 2001). Despite the large amount of research dedicated to children's and infants' preferences for these tastes, little research has been carried out exploring the ontogeny of sour taste preferences until relatively recently. These studies are important as there is still much uncertainty as to what drives any potential differences in sour taste preferences of young infants.

Knowledge about the development of sour taste acceptance early in life can be exploited to improve fruit intake of children, which could help tackle the obesity epidemic. James (2006) highlighted the importance of nutrition before pregnancy, during pregnancy and in the first two years of life in the fight against the global obesity epidemic, while Philipsen & Philipsen (2008) stated that *"the fight for healthy childhood weight begins early—perhaps even before the baby is born."* This research sets out to understanding the role of diet, in particular fruit consumption, during these critical periods of development for the infant - in utero, through breast milk and at weaning. In order to determine if a link with fruit consumption and sour taste preferences in infants occurs, we must first examine the diet of mothers in the third trimester of pregnancy (Chapter 3); the diet of breastfeeding mothers (Chapter 4); early infant feeding practices from birth to six months (Chapter 5) and at 12 months (Chapter 6). In particular, this study will focus on the link between fruit consumption and sour taste acceptance at 6 and 12 months (Chapter 7) while other factors will also be considered.

CHAPTER 2. GENERAL METHODOLOGY

2.1 *Introduction*

The overall aim of this study was to investigate whether diet during pregnancy and early infant feeding practices are important for the development of sour taste preference during the first year of life. The methods used to meet the aims and objectives of this study are described in this chapter. Consistent methods were performed on the sample throughout the study. The study investigator was a research dietitian. The work reported in this thesis includes the recruitment of volunteers, conducting the dietary assessments, the taking of anthropometric measurements, the calculation of sour taste acceptance scores of infants at two time points; six months and twelve months, data entry, and analysis. All roles were undertaken by the investigator.

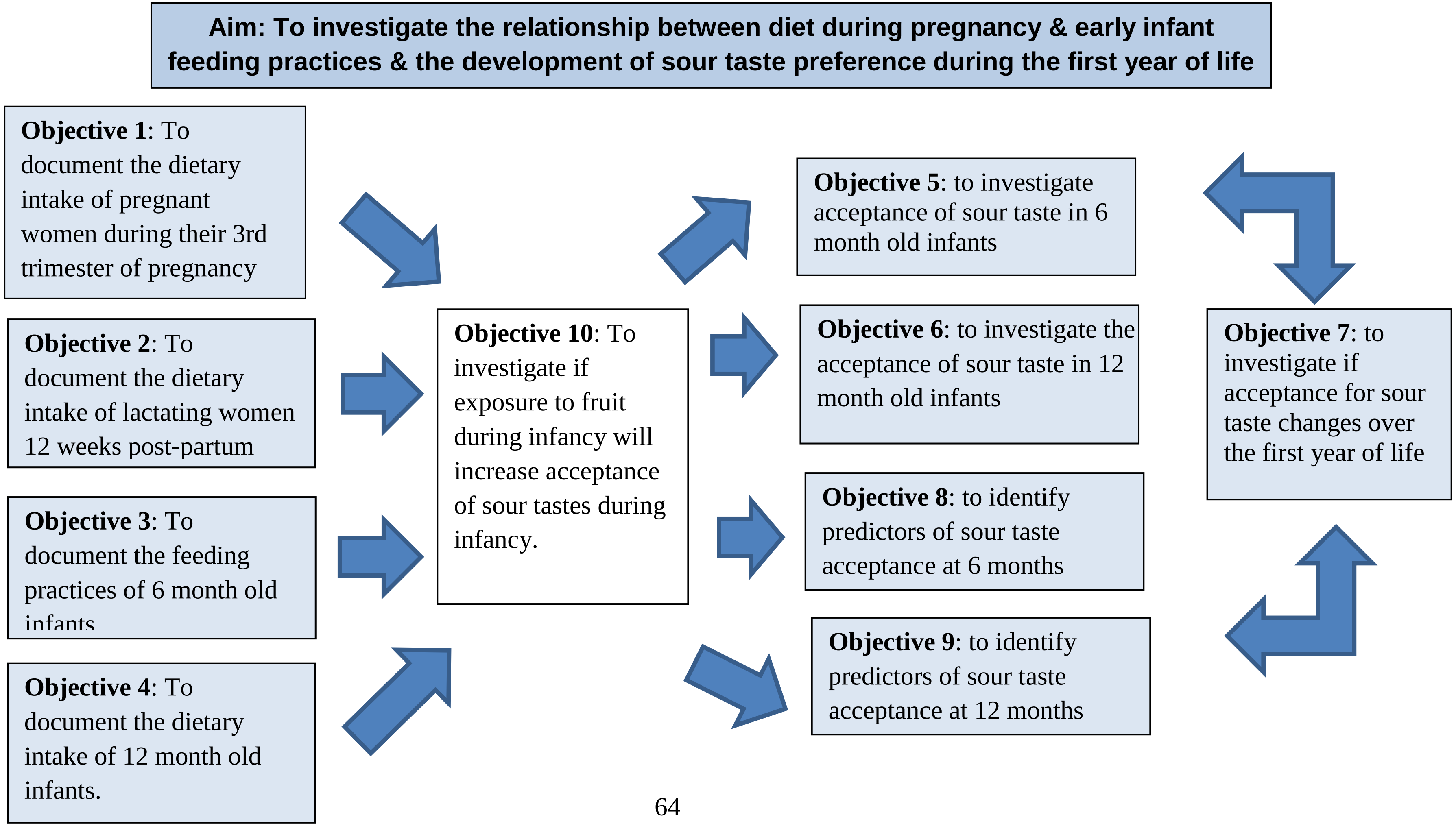
2.2 *Ethical Issues*

Ethical approval for this study was obtained from the Ethics Committees of both Dublin City University (REC reference: DCUREC/2009/050, approved on the 6th July 2009) and The Coombe Women and Infants University Hospital (reference number 2009/11, approved on August 22nd 2009). Following verbal consent, mothers received an information sheet detailing the study aim, the procedures and the requirements of the project. When mothers agreed to take part a copy of the written consent form was forwarded to them to sign. Copies of the information sheet and written consent form can be seen in Appendix B & C. All participants were required to sign a consent form before they began their involvement in the study. Participants were assured of confidentiality at all times throughout and were assured that they were free to withdraw at any time, without explanation. In order to ensure the confidentiality of data, participants were issued unique codes which connected to their personal details, their food diaries, their infant assessment forms and video recordings. All personal details were stored securely. Care was taken to ensure that they were held separately from the food diaries, the video data and infant assessment forms. All data were entered onto the researcher's computer and encrypted to ensure confidentiality of all electronic records. Only members of the research team had access to hard copies of the data, which were stored in a locked filing cabinet designated for project use only.

2.3 *Overview & Study Design*

Our study was designed as a prospective, longitudinal study, involving the recruitment of pregnant women and the monitoring of their infants until 12 months of age. Eligible mother-infant dyads were followed up at 6 months and 12 months post-partum. The study protocol stipulated that infants had to be followed up within 18 days of these time points. All efforts were made to comply with this timeframe. However, owing to difficulties arranging meetings with mothers, for a number of reasons including the availability of mothers and infant illnesses, some follow-ups occurred outside of these dates (n=3). Recruitment of mothers took place from September 2009 to December 2010. Follow-up of mother/infant pairs began in March 2010 and continued until March 2012.

Figure 2-1   The Study's Aim and Objectives



2.3.1 Setting

The study population was recruited primarily from the Coombe Women and Infants University Hospital in Dublin 8, Ireland. More participants were recruited from private antenatal classes conducted by midwives throughout the Greater Dublin area; through pregnancy forums from the websites www.rollercoaster.ie and www.activelink.ie; through local GP surgeries and foetal screening clinics in the Dublin area. The Coombe Women and Infant University Hospital is one of the three large maternity hospitals in Dublin where 8,709 infants were born in 2011. The hospital cares for pregnant women from a wide geographical area, including south and south-western Dublin as well as the surrounding counties. Most recent information indicated that 66.4% of mothers attending the Coombe reported their residence in the Dublin area while 32.8% of mothers were recorded as residing in the rest of Leinster (Fitzpatrick *et al.,* 2012). Thus, the majority of study participants lived in the Dublin region. However, some mothers lived in the Leinster region; -including Wicklow, Kildare, Meath, Kilkenny and Westmeath. The investigator sought to primarily recruit mothers who were close to the Dublin area to facilitate ease of follow-up. However, any mother who was interested in being involved in the study was allowed to participate and the study included three mothers who were living in the Munster region.

2.4 *Recruitment of Participants*

2.4.1 Hospital Recruitment

Recruitment of the convenience sample of women involved the investigator attending both morning and afternoon sessions of all public, semi-private and private clinics in the Coombe. This limited the selection bias towards any particular day of the week or time of clinic. Using the seating arrangements in each clinic, each pregnant mother attending a particular clinic was approached and informed of the purpose of the study and the level of involvement required of them to participate in the study. Although pregnant women were made aware that the study aimed to investigate whether diet during pregnancy or early infant feeding practices influenced the development of sour taste in infants, they did not receive any specific advice on their diet during or after pregnancy. Furthermore, they did not receive any advice on infant feeding or weaning post-partum. Mothers were advised that any questions concerning diet and feeding should be addressed to their G.P., midwife or public health nurse. To avoid biased reporting, the investigator introduced herself as a researcher working in DCU and care was taken to avoid prompting answers towards infant feeding or weaning practices.

All mothers were assured of complete confidentiality and all responses to questionnaires and dietary records were numerically coded. There was no financial incentive for participation in the study. However, mothers were informed that they could receive a copy of the dietary assessment at the end of the study if they requested it. They were also informed that any information would be used to improve our understanding of taste preference in infants, thus helping to improve advice given to future parents.

Hospital staff and midwives were aware that this study was being undertaken but were not involved in the recruitment of participants. Following verbal consent, mothers were given a patient information leaflet, detailing the study's aims and requirements with a written consent form. Following completion of the written consent form, mothers were given a self-administered questionnaire, which provided the investigators with demographic data and contact details for the participants for the purpose of the study follow-up.

2.4.2 Recruitment outside of the Hospital

It was decided to recruit mothers outside of the Coombe Women and Infant University Hospital to achieve an appropriate recruitment sample size. Other avenues of recruitment were explored. Popular websites, namely, www.rollercoaster.ie and www.actvielink.ie were contacted, requesting permission to post information regarding the study on their Mums-to-be forum. The investigator also contacted two Foetal screening Centres in Dublin and local GP offices, where leaflets and posters (Appendix D & E) were given to provide a general outline of the study as well as the contact details of the main investigator should women be interested in participating in the study. Mothers who expressed an interest and gave verbal consent, were sent a patient information leaflet, detailing the study's aims and requirements with a written consent form (Appendix B & C).

2.4.3 Inclusion Criteria upon Recruitment

- Pregnant women $\geq$ 12 weeks gestational age.
- Mothers who at the time of recruitment were planning to reside in Ireland for at least 12 months post-partum.
- Mothers who were willing to consent to follow-ups at time points up to 12 months post-partum.
- Mothers who were over 18 years of age.
- Infants had to be healthy, born at term ($\geq$ 37 weeks gestational age) and weighing $\geq$2.5 kg at birth.

2.4.4 Exclusion Criteria upon Recruitment

- Mothers under the age of 18 years of age (as required by ethic committee
- Any mother experiencing a 'high risk' pregnancy as defined by early bleeding in pregnancy warranting regular scanning and having any medical condition requiring obstetric monitoring, as reported by the mother.
- Infants requiring a medically prescribed diet post-delivery e.g. a metabolic disorder.
- Infants requiring medical treatment for any illness that would be considered an anomaly in a normal population of infants.

2.4.5 Sample Size

The investigator consulted with Dr Michael Parkinson, DCU, who has extensive experience in predicting participant numbers in population studies. Using the G*Power 3 programme (Dias *et al.,* 2013), it was estimated that a sample of 55 individuals would be required to conduct multiple regression analyses with 4 predictor variables, given a power of 0.80, a significance level of 0.05, and a medium effect size ($f^2 = 0.15$). This approach complied with the previous study in this area (Blossfeld *et al.,* 2007). Therefore, to ensure adequate statistical power and to allow for a potentially high drop-off rate, it was aimed to recruit approximately 140 pregnant women. It was estimated that the drop-out rate would be around 30-50%, given the longitudinal nature of the study. Previous longitudinal studies with infants and children have also reported drop rates between 12% and 32% (Nolan, Schell, Stark, & Gómez, 2002; Nicklaus *et al.,* 2005; Senn *et al.,* 2005; Blossfeld *et al.,* 2007).

In order to try and recruit a wide socio-economic spread of participants, the study aimed to recruit the majority of its subjects from public antenatal classes. However, poor interest was shown among those attending these clinics. Unfortunately, this proved to be an obstacle in my attempts to achieve this diversity (Chapter 3).

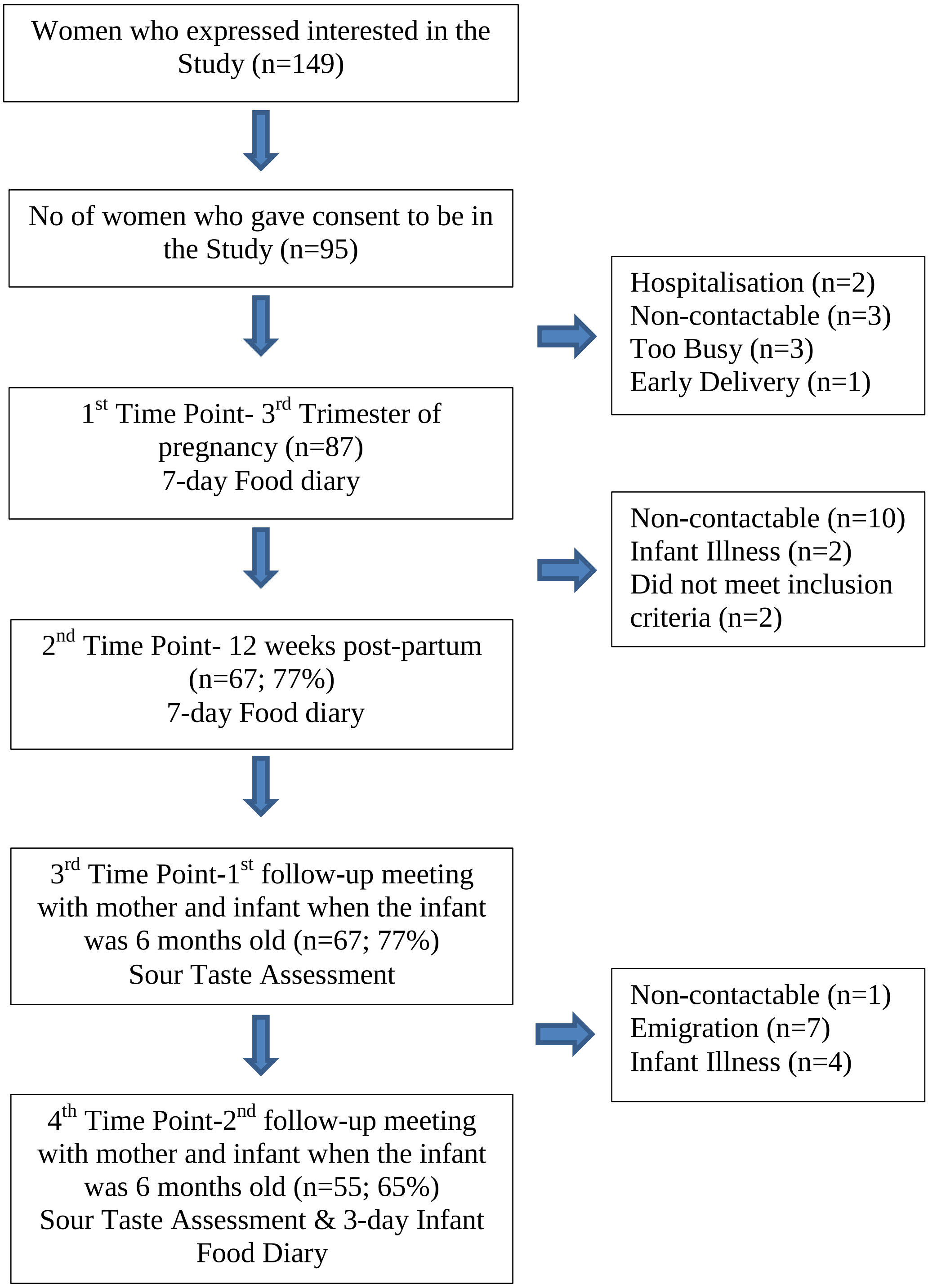
2.4.6 Non-Respondents

During recruitment, 149 mothers expressed an interest in the study and information material was sent to them. Of those, ninety-five returned consent forms (64%) during pregnancy with the intention of participating in the project. Non-participants offered a variety of reasons for non-participation, which included; the study was deemed too demanding or time-consuming; they were unwilling to be followed up post-partum; they had concerns about video-taping; they were overburdened regarding pregnancy or their partners were unhappy about participation in the study. Ultimately, by the first time point eighty- seven mothers had agreed to participate in the study.

The reasons for this drop-out at this stage were; hospitalisation and/or complications during the last trimester of pregnancy (n=2); non-contactable (n=3); too busy (n=4) and early delivery (n=1). However, despite considerable effort, it was not possible to retain all participants in the study over the course of the 12 months. By the fourth time point (12 months) 55 of the 87 mothers (63%) were still in the study (Figure 2.2). Other longitudinal studies with infants and children have also reported drop rates between 12% and 32% (Nolan, Schell, Stark, & Gómez, 2002; Nicklaus *et al.,* 2005; Senn *et al.,* 2005; Blossfeld *et al.,* 2007).

The biggest group drop-out occurred in the period between the third trimester and the immediate post-partum period. The majority of mothers who dropped out at this time either felt that the time commitment was too much, or were considered non-contactable (n= 10); were concerned with an illness of the infant (n= 2) or did not meet the inclusion criteria (n=2). Five national mother infant dyads emigrated during the study and two non-national mother-infant dyads returned to their home countries, making follow-up impossible. Non-compliance with testing procedure totalled three and five infants at 6 and 12 months respectively.

Figure 2-2 Flow-chart of Attrition Rates in the Study



2.5 *Follow- up with Mothers during their 3rd Trimester of Pregnancy*

2.5.1 Mothers' Anthropometric & Demographic Information

Socio-demographic and anthropometric data (e.g. self-reported height and pre-pregnancy weight) were collected using a self-completed postal questionnaire, which the women completed when they joined the study. Mothers reported their pre-pregnancy weight and height. From these measurements, their body mass index (BMI kg/m^2) was calculated and they were then categorised as underweight (<18.5 kg/m^2), a healthy weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) or obese (≥ 30.0 kg/m^2) (WHO, 2013a). Data regarding mothers' education and employment status as well as their partners' status were also recorded in the questionnaire. The CSO (1996) has classified the entire population into the following social class groups, which are defined on the basis of occupation.

Table 2-1 Social Class as defined by the CSO (1996)

Social Class	*Occupation*
1	Professional workers
2	Managerial and technical
3	Non-manual
4	Skilled manual
5	Semi-skilled
6	Unskilled
7	All others gainfully occupied & unknown

2.5.2 Dietary Assessment during Pregnancy

Participants were asked to complete their first 7-day food diary during their 8th month of pregnancy (Appendix F). Mothers were telephoned during the start of their 8th month of pregnancy and their contact details were checked before sending them the food diary.

Written instructions were sent to mothers explaining the procedure for completion of diaries, while all participants were advised by telephone on recording all food and beverage intake by a dietitian. Participants were requested to estimate portion sizes with the aid of measuring cups, spoons, or glasses, or by calculating weight or volume as indicated on packaging labels. If the portion size was not recorded clearly on individual food diaries it was quantified by the research dietitian using the Average Portion Size measure according to the Food Standards Agency (Food Standards Agency, 2002a). Mothers were informed that they could contact the investigator if they had any difficulties in completing the food diary. A stamped, addressed envelope was provided to facilitate each food diary's return. Mothers were given three weeks to complete the questionnaire and diary. After 21 days, mothers who had not returned the forms were contacted by the investigator and reminded to return the completed forms. The completed food diaries were reviewed by the researcher, a registered dietitian for any errors and omissions in the recording of food intake. If there were any identified, the participants were contacted to answer any questions that emerged e.g. Ham sandwich- how exactly was it made.

2.5.3 Assessment of Energy Underreporting

Basal Metabolic Rate (BMR) is defined as *"a standardised metabolic state corresponding to the situation at thermo-neutrality when food and physical activity have minimal influence on metabolism"* (Scientific Advisory Committee on Nutrition, 2012). Participants were asked to record their pre-pregnancy weight, which was used to assess their estimated Basal metabolic Rate (BMR), using Henry's equation (Henry, 2005) which is based on gender, age (years) and weight (kg) (Appendix L). BMR was higher in pregnant women during the third trimester of pregnancy; therefore 25% was added to their estimated BMR to account for this increase. Goldberg's method was used to predict levels of energy underreporting using the ratio of energy intake to estimated BMR (Black *et al.,* 1991). A ratio of ≤1.2 may indicate underreporting and a ratio of < 0.9 is a sign of definite underreporting (Goldberg *et al.,* 1991).

2.6 *Follow-up with Mothers after the Birth of their Infants*

Mothers were contacted at 12-16 weeks post-partum. The investigator attempted to contact mothers at different times during the day - including week and weekend days. Up to five attempts were made to contact mothers before they were deemed 'uncontactable'. In cases where telephone numbers provided by participants were invalid, a letter was posted to the home address requesting that they call the investigators. Following a non-reply to the letter, the mother was excluded from the study follow-up. Mothers who were contacted were asked to complete a second food diary. The protocol as described previously, with regard to training, completing and checking the food diaries, was also followed here.

2.6.1 The Collection of Breastfeeding Data

The method of infant feeding from birth as well the current method at the time of contact was established for each of the women during a follow-up telephone interview at twelve weeks. The definitions of exclusive and partial breastfeeding in the present study were in accordance with WHO breastfeeding definitions.

Partial Breastfeeding was defined as *"infants who receive breast milk in combination with formula feeds or other non-human milk feeds and/or solid food."*

(World Health Organization, 1991; World Health Organization, 2004).

Exclusive Breastfeeding was defined as *"the practice of feeding only breast milk (including expressed breast milk) and allows the baby to receive vitamins, minerals or medicine. Water, breast milk substitutes, other liquids and solid foods are excluded."*

(World Health Organization, 1991; World Health Organization, 2004).

The *"mixed feeding"* category included infants who received breast milk in combination with formula feeds or other non-human milk feeds and/or solid food. Breastfeeding "*duration*" denotes the number of days during the first six months for which exclusive or any breastfeeding continued during the study time frame. If mothers said they were exclusively breastfeeding, it was always ensure whether they included other supplementary fluids or solid foods in the feeding regime was checked to clarify that they only provided breast milk to their infants. At the end of the interview, the investigator repeated the recorded feeding practices back to mothers and confirmed the accuracy of the data. Each Infant's feeding status was also confirmed at the 6 month assessment meeting.

2.6.2 Anthropometric Measurements

At the 12-week time point, mothers were contacted by telephone and asked for their infant's birth measurements as recorded by health professionals at the hospital. All mothers reported birth weight and some reported birth length. None of the mothers reported head circumference measurements. These measurements were also confirmed at the six month meeting with mother and infant dyads. During this visit, anthropometric measurement of the infant's weight, length and head circumference was taken by the investigator. Details of the methodology used to take these measurements are discussed in Section 2.7.2.

2.7 *Follow-up Meetings with Mother & Infant Dyads*

Infants were met at two time points post-partum. These occurred at six and twelve months of age. Infants were followed up within 18 days of these time points, as discussed previously (n=3 were follow-up outside this period). The purpose of these meetings was to test the infants' acceptance of different levels of sourness and to take infants' anthropomorphic measurements. When infants turned 6 months old, the investigator then called their mothers and arranged a date and time within the next 14 days to meet in their home or in DCU, wherever the mother felt most comfortable. All of the mothers opted to meet the investigator in their own home. This was also repeated when the infants were 12 months old. Initially, in a pilot group, testing was repeated in reverse order after a 5 minutes break. However, infants tired easily and did not comply with the testing protocol. Instead in a sub-sample of ten infants, the procedure was repeated the next day to ensure consistency with results at each time point.

This first meeting comprised of three stages:

- **Stage 1-** Collection of information about the infant's feeding practice.
- **Stage 2-** Collection of anthropometric information on the infant.
- **Stage 3-** Examination of the infant's preference for sour flavours.

2.7.1 Stage 1 – Collection of Information about Infant's Feeding Practices

During the first stage of the meeting, mothers were interviewed to collect information about the infants' birth anthropometric measurements and the mothers' infant feeding practices (Appendix H). As part of this assessment, mothers were asked about their infants' current and past milk-feeding status as well as the solid foods included in their infants' diet at that time. Mothers were also asked about the source of their weaning information.

2.7.1.1 Introduction of Solid Food

At the six-month visit, mothers were asked details about when their infants had been introduced to solid food. Mothers were then asked to list any foods their infant had tried at least once during the period prior to the visit. In particular, mothers were asked about any fruit introduced to their infant. This information was recorded by the investigator. In order to help mothers recall, the investigator asked the mothers if foods were tried from each of the food groups. Furthermore, at the end of the interview, the investigator repeated the recorded foods back to mothers in order to confirm the accuracy of their reports.

2.7.2 Stage 2 - Anthropometric Measurements

Following completion of the interview with mothers in their home, the investigator recorded the infant's anthropometric measurements, comprising of weight, length and head circumference. The three measurements followed the WHO's international child anthropometric assessment standards (WHO Multicentre Growth Reference Study Group., 2006; de Onis *et al.,* 2007). All measurements were carried out by the same individual; therefore, it was only necessary to assess the intra-observer variability co-efficient of variation (CoV) of the techniques.

2.7.2.1 Weight

Naked weights with a clean nappy were taken using a high specification portable calibrated Seca 834 baby scales, with a weighting capacity of 20 kg and a graduation of 10 g (Figure 2.3). The weighing scales were placed on a level fixed surface. Infants are measured on the scales wearing only a dry nappy. The nappy was then weighed separately and subtracted from the infant's weight. Three consecutive weight measurements were taken and the average of the three weights was recorded, in line with established international practice for anthropometric measurements (WHO, 2008). To estimate the CoV, ten measurements were taken from one infant. This allowed to us to calculate the error due to movement of the infant as the infants were active when they were being weighed. The intra-observer variability for weight measurements was 0.34%.

Figure 2-3 The Seca 834 Portable Baby Scales used in this study.

2.7.2.2 Length

Supine length was measured using a mobile Seca 210 measuring mat which is designed specifically for infants, with a measuring range of between 10 cm- 99 cm, with graduations of 5 mm (Figure 2.4). Mothers were involved in this measurement and were asked by the investigator to holds their infant's head, the infant looking directly upward with the crown of the head in contact with the headpiece in the Frankfort Horizontal Plane. Accurate readings were ensured by avoiding any irregularities – making sure that the infants' heads were not tucked in against their chest, or indeed stretched too far back. Mothers were also asked to distract the infant and ensure a steady head position. The investigator pressed gently down on the infant's knees to ensure that the two legs were straight and the infant's full length was measured for an accurate length measurement. Then, using the moveable foot piece at the other end of the measuring mat, the investigator slid the foot piece up the mat and positioned it firmly against the infant's heels. The infant's heels were facing upwards to prevent hyperextension of the legs and inaccurate measurements. Three length measurements were taken to the nearest 5 mm and the average measurement was recorded. It was important that these measurements are done by a trained individual to ensure that the correct procedure was followed and every effort was made to avoid causing any distress to the infant. To estimate the CoV, ten measurements were taken on one infant. As length measurements can cause distress for the baby, it was completed as two sets of five measurements, five at the start of the interview and five again at the end of the interview. The co-efficient of variation for length was 0.37%

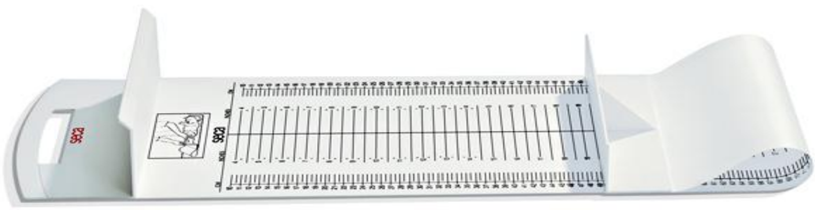


Figure 2-4 The Mobile Seca 210 Measuring Mat used in this study.

2.7.2.3 Head Circumference:

The head circumference was measured using a paediatric Seca 201 measuring tape (Figure 2.5). The measuring tape has a range of 0-205 cm with a graduation of 1 mm. Head circumference measurements were taken with the infant sitting on the mother's lap while the investigator placed the tape around the infant's head. The circumference was measured from the midway point between the infant's eyebrows and hairline at the front of the head and the occipital prominence at the back of the head. This was to ensure that the maximum circumference was measured. Three length measurements were taken to the nearest mm and the average measurement was recorded. Ten measurements of the head circumference were made of one infant to estimate the CoV, using the Seca 201 measuring tape. The intra-observer variability for head circumference was 0.12%

Figure 2-5 The Paediatric Seca 201 Measuring Tape used in this study.

2.7.3 Stage 3- Sour Taste Acceptance Measurements

Following completion of the infant's anthropometric measurements, infants were rested for 5 minutes. This allowed time for the investigator to explain the procedure thoroughly to the mother and to set up the equipment for the assessment of their infant's acceptance of sour tastes.

We examined a number of aspects of the Sour Taste Assessment, viz.

- The Environment
- The Solutions
- The Assessment

2.7.3.1 The Environment

Mothers were asked not to feed their infants for 2 hours before their appointment so that they would be tested approximately 0.5 – 1 hour before their next scheduled feeding. This was done to ensure that intake and responses during the test session were not affected by extreme hunger or satiation (Berridge, 1991), but rather reflected infants' hedonic responses to the solution. All of the meetings with the mothers and their infants were held in the infants' own homes. The infants were placed in their usual feeding positions, i.e. either in their mother's arms or in an infant chair or high chair. This was to ensure that the infants were comfortable and relaxed. Infants' acceptance of the different solutions was assessed by measuring their intakes of different flavoured drinks, which were given sequentially over short fixed time periods. This technique has been used in previous studies of sensory testing with infants and toddlers (Beauchamp, Cowart, & Moran, 1986; Cowart & Beauchamp, 1986; Crystal & Berstein, 1998; Blossfeld *et al.,* 2007).

2.7.3.2 The Solution

In order to test the infants' acceptance of varying levels of sourness, the infants were presented with 4 test solutions, varied in their concentration of citric acid. The test solutions used in the assessment were made up by diluting 10 ml blackcurrant squash

(Ribena; GlaxoSmithKline Nutritional Healthcare, Brentford, Middlesex, UK) in 200 ml of cooled boiled water (for sterilisation) with varying levels of citric acid ($C_6H_8O_7$) to create sourness in the solutions. The blackcurrant squash does produce a slightly sweet taste. This helps to ensure no rejection of the base solution (Blossfeld *et al.,* 2007). The solutions had either no citric acid (0.00M; base solution) or different concentrations of citric acid (0.013M, 0.029M and 0.065M; $C_6H_8O_7$ Sigma-Aldrich Ireland, Dublin, Ireland). A new solution was made up on each occasion. Blackcurrant squash was used in the drinks to avoid infants' rejection of the base solution, by giving the base solution slightly sweet, pleasant taste. Research has shown that infants have an innate preference for sweet flavours (Steiner, 1977). Although all infants in this study were not familiar with this squash, none of the infants rejected the base solution.

2.7.3.3 The Assessment

The investigator carried out all assessments in order to avoid any inter-variability in testing protocol. The infants were recorded using either Panasonic VDR-D250 DVD Camcorder or Flip Video Camera (Figure 2.6). The video camera was placed, using a stand, approximately 2 meters in front of the Mother-infant dyad and the camera lens was zoomed so that the video image contained the face and upper torso of the infant as much as possible. This also allowed the investigator to remain out of direct sight of the infant during the assessment, thus avoiding distraction. The investigator was still able to see the assessment in order to judge when to terminate the session.

Figure 2-6 Panasonic VDR-D250 DVD Camcorder (left) and Flip Video Camera (Right) used in this study

All of the solutions were at room temperature and were presented using 4 identical Avent 9 oz bottles (Philips), a different bottle for each solution (Figure 2.7). If the infants were unfamiliar with these bottles the solution was presented in their own cup or bottle to ensure that they would drink the solution. In this case there was no difference in the rest periods given to infants to avoid any irregularities in testing.

Figure 2-7 Avent infant feeding bottles used in this study

When the camera was set up and the infant was ready and comfortable the test commenced. Mothers were instructed to offer each solution, by placing the bottle to the infant's mouth and lightly rubbing the nipple against the lips until the infant voluntarily accepted. If the infant refused to drink he/she was offered one of their own toys to increase compliance with the testing protocol. The mothers were advised to avoid giving any cues to their infants regarding the palatability of the solutions they were giving. Feeding ended when the infants rejected at least three consecutive offers of the solution as decided by the investigator or they had had been feeding for 1 minute. In this manner, infants determined the pacing of the session and how much they consumed. Cues that the infant had rejected the solution were; closing the mouth firmly; pushing the bottle away; turning their head firmly away from the bottle or spitting the solution out. Immediately after each drink, mothers were asked to rate their infant's reaction to the solution using a *Likert* scale of perceived like/dislike (Appendix I), in which higher numbers reflected greater liking.

Infants were presented with 50 ml of each solution starting with the base solution (0.00M citric acid). The remaining solutions were given in a random order and the mother was blinded to the order of the solutions. The solutions were presented to the infants for 60 seconds or until rejection, whichever came first. An interval of 2 minutes was given between each presentation to allow the infants to rest. This method was based on similar studies with older infants (Blossfeld *et al.,* 2007). The investigator spoke only to indicate the start and end of each test time. The maximum amount of solution that the infant could drink was 50 ml; however, none of the infants finished the solution in the allotted time frame. The total amount of solution drank was determined by weighing the bottle or drink container before and after the infant had tasted it. The amount of solution taken was calculated to the nearest ml and any spillage or leaking on a bib was also recorded, by weighing the bib before and after. Amount of solution was measured using a Salter electric digital scales model 1036, with a measuring range of between 0.0- 5000 g, with graduations of 0.1 g.

2.7.4 Twelve month Follow-up

Mothers were contacted by telephone at approximately two weeks before the infants' 1st birthday to arrange a follow-up appointment. At the 12-month visit, mothers were asked details about their infants' current feeding patterns; particularly whether they were breastfeeding at this time and how often they were. This information was recorded by the investigator. Furthermore, at the end of the interview, the investigator repeated the recorded feeding practices back to mothers in order to confirm the accuracy of the data. Caregivers were provided with a food diary and asked to record detailed information regarding the amount and types of all foods, beverages and nutritional supplements consumed, as well as the amount of food left over by the child over 3 consecutive days. Caregivers were asked to keep these food diaries during two typical weekdays and one typical weekend day. They were asked to record the infants' intakes using household measures (measures such as teaspoon, tablespoon, fluid ounces, pints etc.). Where applicable, the cooking methods used, brand names of the foods consumed, the packaging size and type and details of recipes were also recorded. Estimated intakes were recorded as food served and left over. Caregivers were given a timeframe of 10 days during which they could complete the food diary. Printed instructions and a sample food diary were provided to the participant on each occasion to help minimise errors. The investigator also gave detailed verbal instructions to participants regarding the recording of the infant's food and drink intake during each meeting. It was explained that if the mother felt that their infant was sick or not eating well on a particular day, she should stop recording and repeat it on a new day. For those infants in day-care, I asked that the crèche staff complete the diary for food and beverages that the infant consume during their time at the crèche. Crèches provide meal plans as well as daily logs to mothers regarding their infants' daily activity and diet, including amount eaten, as a matter of routine, thus facilitating the recording of intakes. Crèches were provided with written instructions regarding the reporting of infants' diet. I also offered to contact the crèches if they had any difficulties or questions regarding completing the diary. Participants were also given a stamped self-addressed envelope in which to return completed food diaries to the investigator. Participants were telephoned to remind them about the diaries if they had not returned them after 14 days(n=9).

Breast milk was not quantified in the food record. Instead, a "breastfed" entry was selected and 1 serving was entered for each breastfeeding occasion. The amount of breast milk for breastfed infants was estimated using the following method having consulted the available literature (Dewey, Finley, & Lönnerdal, 1984; Kent, Mitoulas, Cox, Owens, & Hartmann, 1999; Briefel *et al.,* 2010). When breast milk was pumped and fed to a child in a bottle, the "human milk" option in Dietplan 6.4 (Forestfield Software Ltd., 2011) was selected and it was quantified similarly to all other drinks. Consulting the available literature, I assigned breast milk volumes based on the child's age in months and the total amount or volume of other milks (infant formula, cow's milk, or soy milk) reported over the course of the food record. It was calculated as 89 ml per feed to a daily maximum of 600 ml. Nutritional composition information for infant formula and other commercial baby foods was obtained from manufacturers' information.

Dietplan 6.4 (Forestfield Software Ltd., 2011) was used to analyse daily intakes of energy, nutrients and food items obtained from the food diaries. Mean nutrient intakes were calculated from foods only. During this study, modifications were made to the food composition database to include recipes of composite dishes, nutritional supplements, fortified foods, infant specific products and generic Irish foods that were commonly consumed. It was stressed to participants that they should not try to change or 'improve' their infant's diet during the recording period. At the end of the recording period, participants were asked whether their child's food intake had been the same as usual, less than usual, or more than usual during the recording period and were asked to explain why this might have been. They were also asked if there were any items consumed during the recording period which had not yet been written down. Details on such items were then recorded by the researcher in the food diary.

2.8 *Data Handling & Analysis*

To ensure patient confidentiality all mothers were assigned an identification number thereby avoiding the use of any potentially identifying information during analysis. All food diaries, questionnaires and data collection sheets had this identification number. Contact details were securely filed separately from the study results and only the investigating team had access to them. All details and results were stored in a locked filing cabinet that only members of the research team had access to. Documents will be stored for a period of five years and then destroyed by Dr. Tracey Harrington. All results were presented in terms of the entire data set and any potentially identifying information was omitted.

The use of video recording was solely for the purposes of the analysis of infants' facial expressions. Only members of the research team viewed these tapes. Video recordings were stored until the project was completed and will be destroyed by Dr. Tracey Harrington after 5 years. Investigators viewed the videotapes and determined the length of feed and the frequency of the infant's negative facial expressions (e.g. nose wrinkling, brow lowering, upper lip raising, gaping, head turning) when they were tasting the different drinks. The infants' total intake of each solution was calculated by weighing each container before and after presenting it to the infant.

2.8.1 Nutritional & Statistical Analysis of Food Diaries

Nutrient analysis of food and beverage intake was performed using Dietplan 6.4, dietary analysis software (Forestfield Software Ltd., 2011), which is based on McCance & Widdowson's 6th edition food tables (Food Standards Agency, 2002b) as well as updated information from food manufacturers. Supplement usage was not included in the nutrient estimations. Fifty per cent of all information entered was randomly rechecked for errors. Ten per cent of the analysed records were double checked with the original data food records for accuracy. Missing nutrient data were added where possible, using a combination of food labels, which were collected by participants, for unusual foods and manufacturer's information. The research dietitian was solely responsible for the collection, quantification, coding, entry and checking of the food diaries. Statistical analysis of the data, using Statistical Package for the Social Science (SPSS) version 19.0 (IBM SPSS, 2010) included means, standard deviations (SD), medians and interquartile ranges (IQR) of average daily intakes for energy, protein, total fat, saturated fat, as well as vitamins and minerals considered important for each group- pregnant women, lactating women and 12-month old infants.

The results of women's dietary diaries during pregnancy analysed were compared to the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) (Rogers, Emmett, & Study Team, 1998), which looked at the diet of 11,923 pregnant women at 32 weeks gestation using a semi-quantitative food frequency questionnaire (FFQ). My results were also compared to the Irish National Adult Nutrition Survey (NANS) 2008-2010, for females aged 18-59, which used 4-day semi-weighed food record (Irish Universities Nutrition Alliance, 2011) and the Irish Recommended Daily Allowances (RDA), the Average Requirements (AR) Lowest Threshold Intakes (LTI) for women aged 19-59 years including the increment for pregnancy where appropriate (Food Safety Authority of Ireland, 1999).

The maternal dietary data generated from women at 12 weeks post-pregnancy were compared to Recommended Daily Allowances (RDA)(Food Safety Authority of Ireland, 1999), and two Irish national surveys; the Survey of Lifestyle Attitudes & Nutrition in Ireland (SLAN) 2007 (Harrington *et al.,* 2008) and the National Adult Nutrition Survey 2008-2010 (NANS) 08-10 (Irish Universities Nutrition Alliance, 2011). The percentage of participants consuming more than the EAR was examined to determine the nutritional adequacy of this group's diet. Participants' adherence to some of the Irish Dietary Guidelines such as those for dairy produce, fruit and vegetables was investigated as these foods provide many of the increased requirements for vitamins and minerals needed during lactation.

Finally, the data generated from the intakes of the infants in the study were compared to results from the recent National Pre-School Nutrition Survey (NPNS) (Walton, 2012) and the UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC), 2011 (Lennox, Sommerville, Ong, Henderson H., & Allen, 2013). The NPNS investigated the habitual food and beverage intake of a sample of 500 children living in the Republic of

Ireland aged 12 to 59 months inclusive. Food intake was estimated using a four-day weighed food record (Walton, 2012). The DNSIYC investigated the food consumption, nutrient intakes and nutritional status of infants and young children aged 4 to 18 months (n=2638), living in private households in the UK, using a 4-day estimated food diary (Lennox *et al.,* 2013).

2.8.2 Video Data

All of the data were downloaded from the flip camera or copied from the discs of the Panasonic camera to the investigator's computer. Details regarding the date, timing and the infant ID were recorded. The files were converted to suitable format (.mp4 files) for use with a video analysis program using Super © (http://www.erightsoft.com/SUPER. html).

To calculate the duration of drinking of the novel solutions and the number of negative responses, the videos of infants were slowed to 5-20% of their normal speed, and length of drink and negative responses coded using a frame-by-frame analysis by means of Observer™ XT 10.5 (Noldus Information Technology, 2012). Facial responses at the beginning of feeding are thought to be reflective of hedonic responses in animals (Berridge, 1996) and pre-verbal human infants (Soussignan *et al.,* 1997; Rosenstein & Oster, 1988; Mennella *et al.,* 2001; Steiner, Glaser, Hawilo, & Berridge, 2001). Because facial expressions of distaste have been shown to be more discriminating than facial expressions of liking (e.g., smiling) in gauging infants' hedonic responsiveness (Mennella *et al.,* 2001; Forestell &

Mennella, 2007; Mennella, 2009), the researcher analysed the frequency of the negative responses to the solutions (Table 2.2).

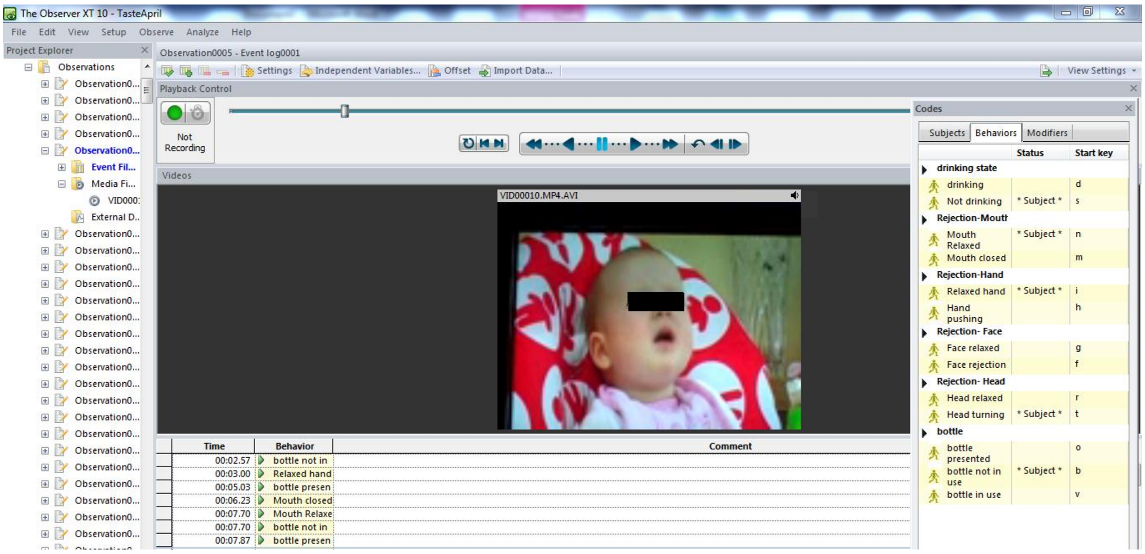
Table 2-2 Coding Scheme for Observer XT 10.5

Behaviour	*Key Code**	*Initial Event*
Drinking State		
Drinking	D	
Not drinking	S	Default behaviour
Mouth Position		
Mouth Relaxed/open	N	Default behaviour
Mouth closed	M	
Hand Position		
Relaxed	I	Default behaviour
Pushing/Pulling	H	
Face Position		
Relaxed	G	Default behaviour
Rejection**	F	
Head Position		
Relaxed	R	Default behaviour
Turning/ Avoiding	T	
Bottle Position		
Bottle presented to infant	O	
Bottle not in use/out of view	B	Default behaviour
Bottle in use by infant/in mouth	V	

*Key pressed on the computer keyboard to record each behaviour **Includes eye squinting, nose wrinkling, frowning, upper-lip raise, and gaping

For all of the analyses, each of the behaviours was considered separately. When the researcher identified a behaviour that is described on the coding scheme (Table 2.2), the key was pressed on the computer keyboard that was associated with the behaviour (e.g., "d" for "drinking"). The Observer XT 10.5 system (Noldus Information Technology, 2012) then registered the occurrence of the behaviour in the event log and automatically assigned a timestamp (Figure 2.8). The duration of behaviour was calculated for the elapse of time between the beginnings of two mutually exclusive behaviours. Behaviours were organized into groups according to the criterion that behaviours in the same group cannot occur at the same time as each other, i.e. all behaviours are mutually exclusive. The advantage of having mutually exclusive behaviours is that during coding there is no need to mark the end of a behaviour. When a new behaviour begins and is marked, the previously coded behaviour of the same group automatically stops. The default behaviour named in the behaviours list below is the behaviour that the Observer XT 10.5 (Noldus Information Technology, 2012) assumes to be active at the start of each observation.

Figure 2-8 Screen shot of Observer XT10.5, (Noldus Information Technology, 2012) where each observation is coded for negative responses to each solution



All coding was completed by the investigator over two four-week periods. All of the videos sessions were randomly coded and the investigator did not know which drink was being sampled on each occasion. Data regarding infant ID, drink concentration and age were added after all infants had been coded to avoid bias. The sound of the infants sessions were turned off to ensure that the infants' vocal sounds did not bias the analysis. The first clip which was coded was also taken as a random sample to assess intra-rater variability. Every two weeks the session was recoded by the investigator to test consistency. Therefore, over the coding period the session was coded 6 times.

Reliability analysis was run using the Observer XT 10.5 software (Noldus Information Technology, 2012). This monitors the number of agreements and disagreements in the coding of mutually exclusive behaviours. During the process all behaviours are given a timestamp and for comparison of event, I used a tolerance window of 1 second. Cohen Kappa ($\varkappa$), an overall measurement of agreements that is corrected for agreement by chance (Cohen 1960) and Pearson Rho (ϱ), a measure of the strength of the linear relationship between the numbers of disagreements in the coding of the video, was calculated for each pair – the initial coding session was compared to each subsequent session. Results showed $\varkappa=0.93$ ($p<0.001$) and $\varrho=0.89$($p<0.001$), showing very high consistency and agreement between the sessions. SPSS Version 19.0 (IBM SPSS, 2010) was used for all statistical analyses. The author was the only individual who entered the data into the SPSS database, fifty per cent of which were randomly re-checked for any errors.

Wilcoxon signed rank test was used to investigate if any differences were observed between the sour taste acceptance by the two sub-samples of infants (n=6) - one at 6 months and one at 12 months, whose sour taste acceptance was tested on two consecutive days using the three outcome variables: Rater's Liking Ratio (R-LR); Ingestion Ratio (IR) and Mother's Liking Ratio (M-LR). The analysis did not reveal any significant difference

($p>0.05$) between testing days for any of the three outcome variables:- Rater's Liking Ratio; Ingestion Ratio and Mother's Liking Ratio at any concentration at either time point.

2.8.3 Sour Taste Acceptance Scores

An acceptance score was created for each method. For each variable the acceptance scores for the base solution were the same for all infants (0.5) and served as a control. The acceptance scores for all other solutions could have a value equal to 0.5, which would show indifference; a value greater than 0.5, which would show acceptance or a value less than 0.5 which would show rejection of the solution. This method complied with approaches in previous studies (Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009). The scores spanned from 0 to 1. Using a proportional variable makes comparisons across ages and across individuals possible, eliminating individual- and age-related differences in sucking behaviour.

A **Rater's Liking Ratio** (R-LR) was calculated by dividing the Rater's Liking score given for that solution by the Rater's Liking score given for the base solution plus the Rater's Liking score given for the base solution.

For example: Rater's Liking Ratio for 0.013M *Citric Acid* =

$$\frac{Raters\ Liking\ Score\ 0.013M}{Rater's\ Liking\ Score\ for\ 0.013M + Rater's\ Liking\ Score\ for\ Base\ solution}$$

An **Ingestion Ratio** (IR) was calculated by dividing the intake of the solution (ml) by the intake of that solution plus the intake of the base solution.

For example: Ingestion Ratio for 0.013M *Citric Acid* =

$$\frac{Intake\ of\ 0.013M\ solution\ (mls)}{Intake\ of\ 0.013M\ solution\ (mls) + Intake\ of\ base\ solution\ (mls)}$$

A **Mother's Liking Ratio** (M-LR) based on the rating score given by their mother for each solution, was calculated by dividing the liking score given for that solution by liking score given for that solution plus the liking score given for the base solution.

For example: Mother's Liking Ratio for 0.013M Citric Acid =

$$\frac{Mother\ Liking\ Score\ 0.013M}{Mother's\ Liking\ Score\ for\ 0.013M + Mother's\ \ Liking\ Score\ for\ Base\ solution}$$

CHAPTER 3. DIETARY ASSESSMENT OF A POPULATION OF WOMEN DURING PREGNANCY LIVING IN IRELAND

3.1 *Introduction*

Pregnancy is a period of complex growth and development for the foetus. Given the potential impact that nutritional imbalance has upon the risk of morbidity and mortality for the infant, optimal nutrition is critical during pregnancy (Galtier-Dereure, Boegner, & Bringer, 2000; Godfrey & Barker, 2001; Rasmussen, Chu, Kim, Schmid, & Lau, 2008; Olson, Strawderman, & Dennison, 2009; McDonald, Han, Mulla, & Beyene, 2010). The impact of maternal nutrition can also impact the long term health of the infant as epidemiological, clinical and animal studies have linked over and under nutrition to the development of type 2 diabetes, cardiovascular disease, asthma and other chronic diseases later in life (Scholl *et al.,* 1995; Dereure *et al.,* 2000; Fitzsimon *et al.,* 2007; Galtier- Sagawa, 2010). A recent study showed evidence that maternal macronutrient intake during the third trimester of pregnancy has a stronger influence on a child's macronutrient intake at 10 years of age than maternal post-natal or paternal macronutrient intake. This influence is particularly pronounced with regard to fat and protein. The authors suggest that this could reflect in utero programming of offspring appetite by maternal diet during pregnancy (Brion *et al.,* 2010).

The nutritional content of diet is, therefore, critical for the developing foetus. National recommendations specific to pregnancy have been developed in most countries, with recommended increases in many essential micronutrients (Department of Health, 1991; Health Promotion Unit, 2006). In Ireland, it has been decided that additional energy is needed to meet the increased metabolic demands which exist during the last trimester of pregnancy, while many increases in micronutrients are also recommended throughout pregnancy, particularly for vitamin D, folate, iron and calcium (Food Safety Authority of Ireland, 1999). The majority of studies exploring maternal diet during pregnancy report that dietary energy intake does increase sufficiently to cover increased demands (FAO, 2004). However, uncertainties exist regarding optimal maternal fat deposition and possible reductions in maternal physical activity, which make absolute figures difficult to calculate. Despite these uncertainties, it is commonly accepted that in healthy, well-nourished women, total energy expenditure increases during gestation with additional energy expenditure being primarily attributed to an increase in absolute basal metabolic rate.

Increases in basal metabolism during pregnancy occur as a result of accelerated tissue synthesis, increased active tissue mass, and increased cardiovascular and respiratory demands. Based on studies of healthy, well-nourished, pregnant women who gave birth to healthy infants, the WHO (FAO, 2004) recommended an average increase in BMR over pre-pregnancy values of 5%, 10% and 25% for the first, second and third trimesters respectively. Additional energy requirements of between 1.0-1.7 MJ/day, depending on maternal BMI pre-pregnancy, are recommended to meet increased metabolic demands during the last trimester of pregnancy. These added energy needs can be met by modest increases in consumption of a balanced diet. However, there is no indication that recommendations for macronutrient intake, expressed as a percentage of energy intake, need to differ in pregnancy from those in the general population.

Antenatal clinics across the world provide leaflets encouraging women who are planning a pregnancy or are in the early weeks of pregnancy, to eat healthily and to avoid certain foods, such as shellfish and unpasteurised foods. However, little information is provided regarding the complications in pregnancy that are associated with calorie excess and macronutrient imbalance. Recent studies show that during pregnancy, many Irish women failed to meet the current national recommended guidelines for healthy eating, with less than half of the participants meeting the recommended intake for each food group in the food pyramid (O'Neill *et al.,* 2011). It is, therefore, important to identify at risk groups with poor dietary intake and nutritional status during pregnancy in order to target appropriate evidence based advice and education. There remains a lack of data surrounding the nutritional habits of pregnant women, especially during the third trimester, when growth is fastest and requirements are often at their highest. Pregnant women are excluded from national nutritional surveys and the many nutritional studies that examine pregnancy tend to concentrate on one nutrient (McGowan, Byrne, Walsh, & McAuliffe, 2011; Peña-Rosas & Viteri, 2009a; Peña-Rosas & Viteri, 2009b) or the first trimester (McGowan & McAuliffe, 2012; Murrin, Shrivistava, & Kelleher, 2010).

This chapter presents the dietary intakes of pregnant women in Ireland during their third trimester and provides a discussion of the findings. Result from this study will be compared to similar studies viz. the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) (Rogers *et al.,* 1998), the latest Irish National Adult Nutrition Survey (NANS) 2008-2010 (Irish Universities Nutrition Alliance, 2011), the Irish National Recommendations (Food Safety Authority of Ireland, 1999) and the Scientific Advisory Committee on Nutrition in the UK (SACN, 2012). I will determine whether the mothers in my study are meeting current nutritional guidelines and will examine the potential for under-reporting in my cohort.

3.2 *Methods*

The study design, recruitment and methodology for the dietary assessment of my cohort of pregnant women are provided in Chapter 2. Women completed a 7-day, semi- quantitative food diary during the 8th month of pregnancy. Data used in this chapter are presented using numerical descriptive statistics, including means with standard deviations and medians with interquartile ranges (IQR). Daily intakes of fruit and vegetable, dairy products and fish are presented using percentages and actual (n) values.

3.3 *Results*

3.3.1 Population Characteristics

Eighty-seven women completed food diaries during their third trimester of pregnancy. Participants did not receive any specific advice on their diet during pregnancy and were not asked to modify their diet during the recording period. None of the mother's had been referred for any specific dietary advice during the study period. Mothers' dietary intake was determined using a 7-day food record during the last trimester of pregnancy – in the period between weeks 33 to 37. Participants ranged between 26-44 years with a mean age of 35 years (± 4.3). The characteristics of participants are displayed in Table 3.1. Participants were predominantly Irish (81.6%, n= 71) while the remainder were British (3.4%, n=3), European (10.3%, n=8), Asian (1.1%, n=1), North American (2.2%, n=2) and African (1.1%, n=1). Participants were predominantly married (83.9%) or co-habiting (14.9%). All participants had completed second level education; 11.5% (n=10) had post-secondary qualifications; 42.5% (n=37) were educated to degree level and 46% (n=40) to postgraduate level (Post-Graduate Diploma; Masters or PhD). Participants were employed on a full-time (66.7%, n=58) or part time (14.9%, n= 13) basis while the remainder worked in the home (18.4%, n=16), with the majority of participants from social classes 1 & 2 (83.9%). The mean pre-pregnancy BMI was 23.3 kg/m^2 (± 3.2), 79.5% (n=69) of women were within the healthy range, while 15 % (n= 10) had a BMI ≥ 25 kg/m^2, 4.4 % (n=4) BMI ≥ 30 kg/m^2 and 1.1% (n=1) reported being under-weight (BMI=17.9 kg/m^2).

Table 3-1 Characteristics of the Study Participants (n=87)

Characteristic	*Mean/Median*	*SD/IQR*	*Range*
Age (years)†	35.1	4.3	26- 44
Pre-pregnancy Wt. (kg)‡	63.5	9	48- 108
Height (m)†	1.66	0.07	1.51- 1.85
Pre-pregnancy BMI (kg/m²)†	23.3	3.2	17.9- 35.3
Demographic Characteristic	*% of participants in each group*		
Ethnicity			
Irish	81.6% (n=71)		
UK	4.1% (n=3)		
Other Europeans	10.3% (n=9)		
Others	5.5 (n=4)		
Marital Status			
Married	83.9% (n=73)		
Living with Partner	14.9% (n=13)		
Single	1.1% (n=1)		
Maternal Highest Educational Qualification Level			
Post-grad	46% (n=40)		
Degree	42.5% (n=37)		
Higher education below degree	11.5% (n=10)		
Leaving Cert & below	0		
*Maternal Employment Status**			
Full-time	66.7% (n=58)		
Part-time	14.9% (n=13)		
Unemployed	9.2% (n=8)		
Economically inactive	3.4% (n=3)		
Social Class			
1 & 2	83.9% (n=73)		
3 & 4	12.6% (n=11)		
5 & 6	2.3% (n=2)		
7	1.1% (n=1)		
Pre-pregnancy Weight Status BMI			
Underweight	1.1% (n=1)		
Healthy Weight	79.5% (n=69)		
Overweight	15.0% (n=10)		
Obese	4.4% (n=4)		

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR) *Employment Status is based on the Standard Occupational Classification (CSO)

3.3.2 Daily Intakes of Macronutrients

Mean energy, macronutrient and dietary fibre intakes from the cohort are displayed in Table 3.2. Mean intakes for energy, protein, carbohydrate, fat and fibre were 2151 kcal (±427), 79g (±14.9) 276 g (±62.7), 90 g (±25.4) and 19 g (±6.3) respectively (Table 3.2). Results for energy intake reported in my study are higher than NANS 08-10 (1721 kcal/day) and ALSPAC Study (1839 kcal/day) (Irish Universities Nutrition Alliance, 2011; Rogers *et al.,* 1998). However, they were slightly lower than the latest SACN (2012) recommendations for energy during pregnancy.

Our results for dietary fibre intake were consistent with the results of the most recent national adult nutrition survey (19.2 g/day, IUNA 2011). However, the majority of mothers failed to meet the guidelines for the European Food Safety Authority recommendation of 25g/day (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010)

Table 3-2 Mean daily macronutrient intakes based on food dairies (n=87) in comparison to the results of the non-pregnant women aged between 18-64 from the NANS 2008-2010 (IUNA 2011) as well as ALSPAC Study of pregnant women (Rogers *et al.,* 1998) and the SACN (2012) recommendations for energy during pregnancy.

Nutrient	*Study Participants*	*SD*	*NANS 08- 10*	*ALSPAC Study*	*SACN 2011*
Energy -MJ/day	8.93	1.99	7.2	7.7	8.9 - 9.1*
Energy – kcal	2151	427	1721	1839	-
Protein –g	78.5	14.9	70.4	66.3	-
Total Fat –g	89.9	25.4	67.6	70.4	-
Carbohydrate –g	267.3	62.7	199.7	-	-
Fibre-g†	18.9	6.3	*17.3*	19.3	-

*SACN requirements calculated based on EAR MJ/d for a population of less active women aged 25-44yrs plus 0.8MJ increment for the cost of pregnancy †Fibre: Southgate method

The percentage contribution to food energy from carbohydrates was 49%, which is consistent with the latest national studies (Harrington *et al.,* 2008; Irish Universities Nutrition Alliance, 2011) as well as the national recommendations (Food Safety Authority of Ireland, 1999). Study participants consumed on average 15% of their energy from protein, which met the national guidelines for protein intake. They were marginally lower than the 17.6% reported in the latest national survey NANS 08-10 (Irish Universities Nutrition Alliance, 2011) but were higher than the ALSPAC study of pregnant women (14%; Rogers *et al.,* 1998). Similar percentage contributions to food energy from fat (37%) were reported among study participants and the NANS 08-10 study (Irish Universities Nutrition Alliance, 2011). This percentage contribution was higher than that reported in the ALSPAC Study (34%; Rogers *et al.,* 1998). The range of intakes for the study participants were between 18.5% and 54% and more than two-thirds of participants (69%) had dietary fat intakes above the recommended level of 35% (Table 3.3).

Table 3-3 The percentage contribution of each macronutrient to energy for study participants compared to the national recommendations (Food Safety Authority of Ireland (FSAI), 1999)compared to women aged between 18-64 years in the national study NANS 2008-2010 (Irish Universities Nutrition Alliance, 2011) as well as the ALSPAC Study of pregnant women (Rogers *et al.,* 1998).

Nutrient	*Study Participants*	*Irish Recommendations*	*NANS 08- 10*	*ALSPAC Study*
Protein	15%	10-15%	18%	14%
Carbohydrate	49%	50%	46%	-
Fat	37%	30-35%	37%	34%
Saturated Fat	15%	<10%	-	14%

*Some figures do not add up to 100% due to rounding errors in calorie conversion.

Given the high intakes of total fat among this group, contribution to energy intake from saturated fat intakes was considered. The average contribution of saturated fat to total energy intake found in this study was 15%, which is higher than the national dietary guidelines (Food Safety Authority of Ireland, 1999) (<10%) and is higher than intakes reported in the ALSPAC study for pregnant women (14%; Rogers *et al.,* 1998). Ninety-two per cent (n=81) of participants had saturated fat intakes of greater than 10% of total energy intake and 4.7% (n=4) of participants had intakes that were greater than 20% (Table 3.3).

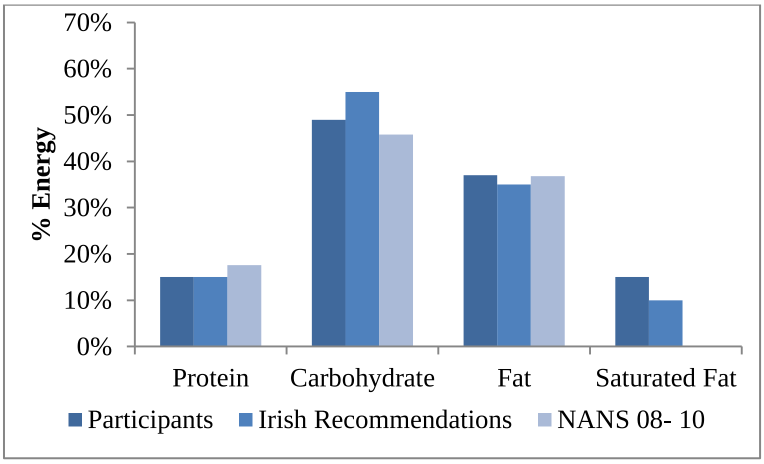


Figure 3-1 Percentage Energy Intake from Macronutrients for Participants compared with the National Recommendations (FSAI, 1999) and the results from the recent National Adult Nutrition Survey (NANS) 08-10 (IUNA, 2011).

3.3.3 Daily Intakes of Micronutrients

The approximate mean intakes of the main micronutrients were calculated for the study population and compared to current RDA, AR and LTI for women aged 19–50 with the addition, where appropriate, of an increment for pregnancy (FSAI, 1999). A significant percentage of participants failed to meet the recommended requirements (RDA)for calcium (73.6%), iron (81.6%), folate (98.9%), vitamin D (100%), vitamin C (25.3%) and riboflavin (31%). A higher percentage of individuals met the recommendation for vitamin B12 (98.9%) and zinc (93%) (Table 3.4).

Table 3-4 Mean group intakes, RDA including the increment for pregnant women; percentage of women below this higher RDA; the average requirements (AR) for non-pregnant women aged 19-64 the lowest threshold intake (LTI) for non-pregnant women aged 19-64 and percentage of women below the AR & LTI for selected nutrients (Food Safety Authority of Ireland, 1999).

Nutrient	*Study Participants Mean/Median*	*SD/IQR*	*RDA**	*% below RDA**	*AR*	*% below AR*	*LTI*	*% below LTI*
Calcium (mg/d)†	1023	263	1200	73.6	615	6.9	430	0
Iron (mg/d)†	12.5	3.5	15	81.6	10.8	31	7.5	3.4
Zinc (mg/d)†	9.4	2.1	7	6.9	5.5	1.1	4	1.1
*Vitamin D (μg/d)‡	2.46	1.98	10	100	-	-	-	-
Vitamin B12(μg/d)‡	4.85	1.67	1.6	1.1	1.0	0	0.6	0
Folate (μg/d)†	278.3	88.2	500	98.9	230	31	160	5.7
*Vitamin C (mg/d)‡	135	106	80	25.3	46	8	32	4.6
*Riboflavin (mg/d)‡	1.8	0.6	1.6	31	1.3	4.6	0.6	0

*The RDA shown in the table is the figure for women aged 19-50, with the addition where appropriate of an increment for pregnancy. †Normally distributed data presented as mean and standard deviation (SD). ‡Skewed data presented as median and interquartile range (IQR).

The approximate mean intakes of the main micronutrients were compared to data obtained from NANS 08-10 (Irish Universities Nutrition Alliance, 2011) and ALSPAC (Rogers *et al.,* 1998) studies. When compared to the NANS 08-10 study (Irish Universities Nutrition Alliance, 2011), the mean intakes of study participants were higher for sodium, calcium, magnesium, zinc and vitamin C but lower for all other vitamins and minerals. It is interesting to note that, when compared to the ALSPAC study of pregnant women during the last trimester of pregnancy (Rogers *et al.,* 1998) my participants compared very favourably, reporting higher intakes for all the micronutrients investigated (Table 3.5).

The average intake of salt was 7.3g daily, with a range of 3.4g- 13.3g daily. The RDA is 1.6 g/70 mmol sodium (4g salt) per day for adults (Food Safety Authority of Ireland, 2005). Due to high levels of salt reported in previous national studies, the Food Safety Authority of Ireland (Food Safety Authority of Ireland, 2005) agreed a population target of a maximum intake level of 6 g/day of salt. In this study, approximately 20% of participants reported intakes below this recommended maximum level. NANS 08-10 (Irish Universities Nutrition Alliance, 2011) study showed a lower mean daily intake of salt for women aged 18-64 years of 6.2 g/day (Table 3.5).

Table 3-5 Mean daily micronutrient intakes based on food dairies (n=87) in comparison to the results of the non-pregnant women aged between 18-64 from SLAN 2007 (Harrington *et al.,* 2008) and the NANS 08-10 (Irish Universities Nutrition Alliance, 2011) as well as pregnant women from the ALSPAC study (Rogers *et al.,* 1998).

Nutrient	*Study Participants*	*SD*	*NANS 08- 10*	*ALSPAC*
Sodium (mg)†	2906	721	2480	-
Calcium (mg)†	1023	263	824	953
Magnesium (mg)†	297	74	255	253
Iron (mg)†	12.5	3.5	13.7	10.4
Zinc (mg)†	9.5	2.1	9.0	8.3
Vitamin D (µg)‡	2.79	1.64	3.9	-
Vitamin E (mg)‡	10.0	3.8	12.5	8.4
Thiamin (mg)†	1.7	0.4	3.4	1.4
Riboflavin (mg)‡	1.8	0.6	3.3	1.7
Niacin (mg)†	20.5	5.8	24.7	15.8
Vitamin B6 (mg)‡	2.1	0.7	4.2	1.8
Vitamin B12 (µg)‡	4.9	1.7	8.0	-
Folate (µg)†	278	88	339	250
Vitamin C (mg)‡	135	106	141	80.3

†Normally distributed data presented as mean and standard deviation (SD). ‡Skewed data presented as median and interquartile range (IQR).

3.3.4 Intake of Fruit & Vegetables

The Irish National Guidelines recommend at least 5 portions of fruit and vegetables per day, with at least 2 portions being fruit and 3 portions being vegetables (Food Safety

Authority of Ireland, 2012). The mean number of portions of vegetable and fruit was 3.8 (±1.8), which is below the recommended guidelines (Table 3.6). Only 21.2% (n= 18) of my participants met the recommended guidelines of 5 portions of fruit and vegetables daily. The mean number of fruit portions consumed was 2.3 (±1.3), with approximately 58% of women (n=50) consuming the recommended two or more portions per day. The range in fruit intake was between 0.0 and 7.7 portions per day. Of most concern was the fact that 17.6% (n=17) were consuming less than one portion of fruit a day. Vegetable intake was lower than fruit intake among this group as the mean number of vegetable portions consumed daily was 1.5 (±0.8), which is half the recommended amount. Only 7.1% (n=6) were meeting their recommended 3-a-day of vegetables and 22.4% (n=19) of women reported eating less than one portion of vegetables per day.

3.3.5 Intake of Dairy Products

During the data collection period, the national dietary guidelines for pregnancy were 5 portions of dairy produce (e.g. milk, cheese and yoghurts) per day compared to the 3 portions per day recommended for the general population (Health Promotion Unit, 2006). These guidelines have changed subsequently, with the Department of Health and Children in 2012 now recommending 3 portions of diary produce per day for both pregnant and non-pregnant women (Food Safety Authority of Ireland, 2012). The mean number of dairy portions for my participants was 2.4 portions, which was less than half the recommended level of dairy for pregnant women in Ireland and was also below the new revised recommendations. Only one woman consumed 5 portions of dairy per day, while fewer than 30% of women consumed 3 portions of dairy per day (28.6%; n=25; Table 3.6).

Table 3-6 Study participants' mean number of daily/weekly servings of fruits, vegetables, dairy produce and oily fish (n=87).

Food Group	*Mean/ Median no. of portions*	*SD/IQR*	*Range*
*Fruits**	2.3†	1.3	0.0- 7.7
*Vegetables**	1.5†	0.8	0.2- 4.3
*Fruit & Vegetables**	3.8†	1.8	0.2- 10.8
*Dairy Produce***	2.4†	1.1	0.1- 5.9
*Oily Fish****	0.7‡	0.5	0.0- 3.5
No of portions	*% of participants consuming each no. of portions/day*		
Fruit			
< 1.0	17.6% (n=15)		
1.0-1.9	25.9% (n=22)		
2.0- 2.9	30.6% (n=27)		
≥3.0	27.1% (n=23)		
Vegetables			
< 1.0	22.4% (n=19)		
1.0-1.9	54.1% (n=47)		
2.0- 2.9	16.4% (n=14)		
≥3.0	7.1% (n=6)		
Fruit & Vegetables			
< 1.0	3.5% (n=3)		
1.0-1.9	9.4% (n=8)		
2.0- 2.9	20.0% (n=17)		
3.0- 3.9	23.6% (n=21)		
4.0-4.9	22.3% (n=20)		
≥ 5.0	21.2% (n=18)		
Dairy Products			
< 1.0	8.0% (n=7)		
1.0-1.9	33.4% (n=29)		
2.0- 2.9	29.7% (n=26)		
≥3.0	28.6% (n=25)		
Oily Fish			
< 1.0	47.1% (n=41)		
1.0-1.9	7.1% (n=6)		
2.0- 2.9	32.1% (n=28)		
≥3.0	13.8% (n=12)		

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR) *1 portion = 80 g of fruit or vegetables ** 1 portion = 30 g of cheese/ 200 ml of milk / 150 g of yogurt *** 1 portion = 90 g Fish

3.3.6 Intake of Oily Fish

As 100% of participants failed to meet the RDA for vitamin D, I examined dietary sources of vitamin D. Oily fish (e.g. herring, mackerel, salmon, sardines, tuna) provide good sources of vitamin D while some brands of breakfast cereal, margarines and milks are fortified with small amounts of vitamin D. Forty-nine women (56.3%) reported eating oily fish at least once a week (Table 3.6). The mean number of portions of oily fish per week was 0.75 (±0.89) with a range from 0-3.5 portions. The most common oily fish eaten was tinned tuna, with 28.7% of women (n=25) reporting to have eaten tuna over the week.

3.3.7 Supplement Usage

Participants were asked if they had taken any dietary supplements during their last trimester of pregnancy. The majority of women in the study (57.5%; n=50) reported never taking any dietary supplements during this period. Just over a third of women in the study (35.6%; n=31) reported taking a daily supplement, while the remaining women (6.9%; n=6) reported taking an iron tablet infrequently, saying they took them on days they felt "low or very tired". Of the women who reported taking a daily supplement, 64.5% (n=20) reported taking an iron supplement and 32.3% (n=10) reported taking a multivitamin supplement suitable for pregnancy. One woman reported taking folic acid daily, while another woman reported taking a vitamin C tablet. One woman reported taking a magnesium/calcium tablet daily as she had no dairy products in her diet. Seven women (22.5%) reported taking omega 3 tablets as well as their other supplements whilst 2 women taking iron tablets, also reported taking a vitamin D supplement (Table 3.7).

Table 3-7 Supplement Usage among Study Participants (n=87)

Daily Supplement Usage	*% of women taking a supplement*
Yes	35.6% (n=31)
No	57.5% (n=50)
Infrequently	6.9% (n=6)
Type of Supplement Used	*% of women taking each supplement**
Iron	22.9% (n=20)
Multivitamin	11.4% (n=10)
Omega 3	8.0% (n=7)
Vitamin C	1.1% (n=1)
Folic Acid	1.1% (n=1)
Vitamin D	2.3% (n=2)
Magnesium/Calcium	1.1% (n=1)

* Some figures do not add up to 100% as some women took more than one supplement ** Multivitamin was suitable for pregnant women

3.3.8 Under-reporting

Bias of reporting energy intake is a well-known problem in dietary surveys (Black *et al.,* 1991) and 38% (n=33) of women in this cohort reported an energy intake of less than 120% of BMR, indicating that they may have been under-reporting. Moreover, 8.3% (n=7) reported an energy intake of less than 90% of BMR adjusted for pregnancy, which is considered definitive under-reporting. The methodology for these calculations is based on pre-pregnancy BMI, using the Henry equation (Henry, 2005) and includes an added 25% to BMR in order to account for the average increase in BMR over pre-pregnancy values for the third trimester. This level of under-reporting is similar to other studies (Brion *et al.,* 2010; Goldberg *et al.,* 1991; Rogers *et al.,* 1998), which suggests that this population reflects a typical cohort.

3.4 *Discussion*

3.4.1 Macronutrient Intake

Despite 79.5% of women in this study reporting a healthy BMI, there was an imbalance in their intake of macronutrients, with high intakes of fats and low intakes of carbohydrates. Previous studies have shown that this imbalance results in a higher risk of developing impaired glucose tolerance for the mother (Moses, Shand, & Tapsell, 1997; Saldana, Siega-Riz, & Adair, 2004) and a smaller-for-gestational-age birth for the infant (Scholl, Chen, Khoo, & Lenders, 2004). EU recommendations suggest that dietary fat intake in pregnancy (as a percentage of total energy intake) should be as recommended for the general population (Koletzko, Cetin, & Brenna, 2007). However, in my cohort, higher than recommended intakes of total fat and saturated fat were observed. Of most concern is the finding that 92% of women reported having a saturated fat intake greater than 10% of their total energy intake. These fats can accumulate in adipose tissue (DeLany, Windhauser, Champagne, & Bray, 2000; Kien, Bunn, & Ugrasbul, 2005; Storlien *et al.,* 2001) and being obesogenic, (Hariri, Gougeon, & Thibault, 2010) contribute to increased risk of gestational diabetes (GDM) and large-for-gestational-age newborns (Luoto *et al.,* 2011). A recent review (Morisset *et al.,* 2010) suggested that high fat and low carbohydrate intake is associated with a higher risk for GDM, independent of pre-pregnancy BMI, although mothers with GDM were excluded from this present study.

The higher fat and saturated fat intakes in this cohort may indicate a lack of awareness of the importance of nutrient dense foods during pregnancy in order to meet increased micronutrient requirements. Maternal macronutrient intake during the third trimester of pregnancy has a stronger influence on child macronutrient intake at 10 years of age, particularly for fat intake, than maternal post-natal intake or paternal macronutrient intake (Brion *et al.,* 2010). It is a concern that this high fat diet was similar to participants' pre-pregnancy diet, which may therefore continue post-partum. For this reason, pregnancy may be an opportunity to identify these at-risk women and advise them during pregnancy, which will benefit both mother and child in the future.

3.4.2 Micronutrient Intake

3.4.2.1 Sodium

Eighty per cent of individuals had a salt intake that was twice the RDA for sodium. Their intake was higher than the latest national survey. Given that discretionary salt was not considered, the total salt intake amongst study participants may be even higher. In comparison to non-pregnant women, pregnant women find it more difficult to identify salt levels in solutions and prefer higher salt levels (Brown & Toma, 1986), which could explain their higher consumption level. This level of salt consumption could have long term health implications for mother and infant. Studies have shown that high levels of dietary salt in rats during intrauterine development can permanently alter the mechanisms that regulate cardiovascular function, blood pressure (Fereidoun, Ahmad, Ahmad, & Pouria, 2011) and kidney function (Koleganova *et al.,* 2011). However, this association is yet to be determined in humans.

3.4.2.2 Calcium

Approximately 75% of women were below the Irish RDA for calcium for pregnant women, though 93.1% of women met the AR of calcium for non-pregnant women. The mean intake for this cohort compared favourably to that reported in the latest national survey (Harrington *et al.,* 2008) as well as ALSPAC (Rogers *et al.,* 1998), suggesting that women may have tried to increase their intake of calcium during pregnancy. Calcium plays a crucial role in maintaining maternal bone health during pregnancy and ensuring adequate development of foetal teeth and bones. Calcium supplementation can reduce the risk of pre-eclampsia and of pre-term births (Sibai, 2011), showing the importance of calcium for a healthy pregnancy.

Dairy is a good source of dietary calcium and given that the mean number of servings of dairy produce consumed per day was 2.4, which is less than half the recommended levels, which would account for the low percentage of women meeting the RDA with the increment for pregnancy for calcium. During the data collection period, the national recommendation for dairy product consumption for pregnant women was 5 portions per day (Food Safety Authority of Ireland, 1999). However, the national recommendation has reverted to 3 portions per day which is the recommendation for the general population (Food Safety Authority of Ireland, 2012). This is due to more recent evidence suggesting that, during pregnancy, adaptive physiological changes in mineral metabolism such as elevated gastrointestinal absorption, decreased excretion and the skeleton acting as a reservoir to buffer shortfalls in mineral supply will occur, independent of maternal mineral supply in the range of normal dietary intakes (Prentice, 2003). However, these processes may not provide the sufficient levels of minerals necessary for foetal growth without increased dietary intake when women's intakes are marginal or sunlight exposure is low, given the importance of vitamin D and calcium absorption and utilisation during pregnancy.

3.4.2.3 Vitamin D

In this cohort, the dietary intake of vitamin D was well below the Irish recommendations for pregnant women and the levels reported in the latest national survey (Irish Universities Nutrition Alliance, 2011). Maternal vitamin D deficiency during pregnancy has been linked to maternal osteomalacia, reduced birth weight, neonatal hypocalcaemia and tetany (Food Safety Authority of Ireland, 1999). In animal studies, vitamin D deficiency was linked to hypertension (Li *et al.,* 2004). The main source of vitamin D is ultraviolet B rays on our skin provided by sunlight. However, Ireland's northerly latitude limits exposure to sunshine. Between November and March, there is inadequate quality and quantity of sunlight to enable sufficient production of vitamin D by the body, which requires us to rely more on dietary sources of vitamin D as well as our body stores. Oily fish, eggs and liver are the richest sources of vitamin D, and only 47.1% of the women in my study reported eating less than one serving of oily fish per week. Consumption of liver and raw or undercooked eggs is not recommended during pregnancy due to the high levels of vitamin A in liver and the risk of salmonella in eggs. Another of the most popular choices as a source of vitamin D, tuna fish, is also restricted due to high mercury levels (Food Safety Authority of Ireland, 2004). These factors further reduce Irish pregnant women's ability to meet their Vitamin D requirements during the winter months.

Current UK and Irish guidelines (Department of Health, 1991; Department of Health (DH), 2000; Food Safety Authority of Ireland, 1999) recognise the difficulty in reaching this target through food sources alone and, therefore, it is recommended that all pregnant women take a 10 μg vitamin D daily supplement. It is of concern that only 14.3% of women in this study reported taking vitamin D supplementation. Timing of supplementation should also be considered as a significant association was observed between low 25-hydroxyvitamin D [25(OH)D] concentrations in early pregnancy and subsequent pre-eclampsia (Bodnar *et al.,* 2007), while a significant association was found between maternal plasma 25(OH)D concentrations in mid-gestation and the risk of developing gestational diabetes mellitus (Clifton-Bligh, McElduff, & McElduff, 2008; Maghbooli *et al.,* 2007). Future recommendations of vitamin D supplementation may have to be adjusted with each trimester.

More recently, the Irish recommendations for vitamin D intake in adults have changed, suggesting that everyone aged 5-50 years and those pregnant or over the age of 51 years should take 5 μg and 10 μg vitamin D-3 daily supplement respectively (Food Safety Authority of Ireland, 2012). These new recommendations may encourage more discussion regarding the benefits and need for vitamin D supplementation in Ireland and may help compliance rates during pregnancy in the future years. However, the low compliance rates for peri-conceptual folic acid supplement usage seen in Ireland (Murrin *et al.,* 2007) indicate the difficulty which may lie ahead of attempts to increase compliance with this recommendation and indicate the need for continuous health promotion interventions during pregnancy.

3.4.2.4 Omega-3 Fatty Acids

Omega-3 fatty acids are essential fatty acids that can only be obtained from an individual's diet. Greenberg *et al.* (2008), in a review, suggest that omega 3 fatty acids are critical for foetal neurodevelopment and may also be important for the timing of gestation and for birth weight. During the last trimester of pregnancy, the foetus accrues about 50-70 mg a day of the omega-3 fatty acid docosahexaenoic acid (DHA), which is a primary structural component of the human brain (Innis, 2005). However, many women are unlikely to be consuming sufficient omega-3 fatty acids among this cohort. Nearly half of the women (47.1%) consumed less than one portion of oily fish per week and only 8% of women took an omega-3 supplement suitable for pregnancy. However, there are low intakes of oily fish in the general population in Ireland and therefore many women would not be used to eating oily fish. Concern over levels of mercury in oily fish and the guidelines to limit these fish during pregnancy (FSAI, 2004) may account for the low intakes reported. Greenberg *et al.* (2008) suggest that pregnant women should consume 2 servings of fish a week plus vegetable oils (e.g. flaxseed, canola and soybean) as well as suitable supplements. However, no official recommendations regarding supplementation have been developed as of yet.

3.4.2.5 Folate

Fruit and vegetables are an important source of many micronutrients such as folate and vitamin C. Among study participants, folate intake was below the Irish AR, when the increment for pregnancy was included. Folate, which is found in leafy green vegetables, is the only vitamin whose RDA is set at a substantially higher level for pregnancy than for lactation, which reflects the key role of folate in cell division and development. Of particular concern was the fact that 5.7% reported intakes that were below the LTI for non-pregnant women. However, in Ireland many foods are now voluntarily fortified with folic acid by food manufacturers and it is therefore possible that the level reported was underestimated. This conclusion appears to be supported by the latest report by the Implementation Group on Folic Acid Food Fortification in Ireland (Food Safety Authority of Ireland, 2009), which suggested that voluntary fortification had increased blood folate levels in Ireland, such that by 2007, 93% of women had erythrocyte folate levels considered adequate to protect against neural tube defects (NTD).

3.4.2.6 Iron

The role of iron supplementation during pregnancy is more controversial and routine supplementation during pregnancy is a matter of debate (Breymann, 2002). Foetal iron needs are met at the expense of the mother's own stores as the increased demand for iron by the foetus is met through the combined actions of mobilisation of maternal iron stores, increased dietary absorption and savings made in basal iron store losses due to cessation of menstruation. A recent Cochrane review (Peña-Rosas & Viteri, 2009b) found that, while either daily or weekly iron supplementation was effective in preventing anaemia and iron deficiency in the mother, they found no evidence of a significant reduction in substantive maternal and neonatal adverse clinical outcomes at term. They concluded that there was insufficient evidence to recommend routine iron supplementation during pregnancy.

The majority of women in this cohort (81.6%) failed to meet the recommended intake of dietary iron during their third trimester and many of them (77.1%) were not taking any oral supplementation, putting them at risk of iron deficiency anaemia (IDA). Some women in the study reported taking iron supplements irregularly or not at all due to their side effects. This highlights the importance of dietary sources of iron rich foods to prevent IDA. Moreover, vitamin C will increase the absorption of non-haem iron if both nutrients are consumed alongside each other. While the mean daily intake of vitamin C was well above Irish recommendations for most women, the timing of this consumption may also be important.

3.4.2.7 Fruit & Vegetables

In this study only 21.2% of women met the Irish guidelines of 5 portions of fruit and vegetables per day. This is similar to other studies, which have also reported low levels of fruit and vegetable consumption among pregnant women (Wen, Flood, Simpson, Rissel, & Baur, 2010; Wilkinson, Miller, & Watson, 2009). Consumption of fruit and vegetables can be positively associated with higher birth weight (Mikkelsen, Osler, Orozova-Bekkevold, Knudsen, & Olsen, 2006; Rao *et al.,* 2001). However, a more recent study showed that this positive association was significant only for vegetable intake (Ramón *et al.,* 2009). This association occurred mainly in higher vegetable intake during the first trimester for higher weight and during the third trimester for greater length.

3.5 *Strength & Limitations*

The use of 7-day estimated food diaries is a particular strength of this study as it reduces the possibility of recall bias. Irrespective of the data collection method used, under- and over-reporting is unavoidable in nutritional assessment (Black & Cole, 2000; Black & Cole, 2001). However, there is considerable evidence that a 7-day food diary suffers less measurement error than other methods (Bingham *et al.,* 1994). A recent Irish study showed that 59% of pregnant women under-reported their weight at antenatal booking, resulting in 12% of overweight women being classified as normal and 5% of obese women being classified as overweight (Fattah *et al.,* 2009). Therefore it is possible that some women also underreported their weight and were misclassified in this study which may have an effect on the level of predicted under-reporting.

The extent of adaptive changes in BMR in pregnancy is poorly understood; therefore, estimating individual BMR levels is difficult. A study examining the energy cost of pregnancy reported that, while variability between studies was low, the variability of individual women within each study was high (45-70%) making predictions of individual BMR during pregnancy difficult (FAO, 2004). Difficulties are further compounded as some studies show a reduction in BMR in well-nourished UK subjects (Prentice, Goldberg, Davies, Murgatroyd, & Scott, 1989); undernourished Gambian women (Lawrence, Coward, Lawrence, Cole, & Whitehead, 1987) and showed low increases in BMR in United States subjects (Kopp-Hoolihan, van Loan, Wong, & King, 1999). None of the women in the study had energy intakes below 90% BMR without the adjustment for pregnancy. Therefore it is difficult to say if the women were actually under-reporting. Clark and Ogden (1999) compared the food intake of pregnant versus non-pregnant women. They observed increased food intake among pregnant women along with lower body dissatisfaction, suggesting that a higher food intake is considered legitimate during pregnancy. This could account for lower than expected levels of definitive under-reporting. Given the difficulty in estimating BMR during the third trimester, it was decided to include all study participants in the analysis. In a comparison of the total group, including those described as definitive under-reporters during pregnancy, and the total group, excluding those described as definitive under-reporters during pregnancy, there was no significant difference in the mean intake of macronutrients.

Another limitation of the study is high educational and socioeconomic status of the women in the group. Given that studies in Ireland have linked poor nutrition to more socially disadvantaged groups (Kelleher *et al.,*2008), the results of this study is of concern. Previous research has shown that older; higher income; better educated; pregnant women have a better overall quality of diet (Bodnar & Siega-Riz, 2002; Murrin *et al.,* 2007). In this study many of these highly educated, older women were unable to meet the many of the nutritional recommendations for pregnancy such as fat, salt and vitamin D. The findings of this study and current literature suggest that these nutritional inadequacies may be problem within the whole population of pregnant women and possibly greater among those form socially disadvantaged groups.

3.6 *Conclusion*

The most significant change in women's behaviour during pregnancy occurs with regard to eating habits and food choices (Lewallen, 2004). Therefore, this period presents itself as an important opportunity to influence the health and nutrition of these women, both during their pregnancy and in the future. Research has shown that older, higher income, better educated pregnant women have a better overall quality of diet (Bodnar & Siega-Riz, 2002; Murrin *et al.,* 2007), suggesting that pregnant women from a low economic background should be targeted for dietary interventions. Overall, my research and literature offers convincing evidence that women from all socio-economic backgrounds would benefit from more education about the importance of healthy eating during pregnancy, in particular with regard to vitamin D. Healthcare professionals therefore have an important opportunity to influence the health and nutrition of women during pregnancy and further studies are needed to appraise current modes of communication.

CHAPTER 4. DIETARY ASSESSMENT OF A POPULATION OF WOMEN POST-PREGNANCY LIVING IN IRELAND

4.1 *Introduction*

The increased nutrient demands a mother experiences during pregnancy are met either by a well-balanced diet or by utilisation of body stores (Thomas & Bishop, 2007). It is important that all women in the period post-pregnancy have healthy, nutrient dense diets. Women post-partum must replace depleted nutrient stores as a result of the demands of pregnancy, especially if their diet during pregnancy has been lacking essential nutrients. While a mother's post-partum energy requirements will return to that of non-pregnant women, Qvist *et al.* (1986) found that, at term gestation, nearly half of their participants had low folate levels, which persisted during the post-partum follow-up.

Additionally, mothers who exclusively breastfeed must meet all the needs of their babies in addition to their own, and therefore breastfeeding mothers have higher macro- and micro-nutrients requirements than their non-pregnant peers (Department of Health, 1991; Food Safety Authority of Ireland, 1999). Therefore, optimal post-partum nutrition is vitally important as this period is characterised by increased nutrient requirements, in order to replace depleted nutrients for lactating mothers. Although a mother's nutritional needs during lactation are greater than those during pregnancy and for the general population, there are no special dietary recommendations for mothers during lactation.

The nutritional cost of lactation is dependent on the quantity of milk produced and the milk's nutritional content (Picciano, 2003). Much of a breastfeeding mother's increased energy requirements is met by utilising fat stores deposited during pregnancy (typically 2.7-3.6 kg), while some energy is conserved by changes in the mother's metabolism. It is estimated that breastfeeding mothers require an increased energy intake of approximately 1.92 MJ/day (Food Safety Authority of Ireland, 1999). More recently, EFSA (2013) estimated that women exclusively breastfeeding during the first six months post-partum have an additional energy requirement of 2.1 MJ/day (500 kcal/day) over pre-pregnancy, which takes into account a requirement of 2.8 MJ/day (670 kcal/day) for milk production and an energy mobilisation from maternal tissues of 0.72 MJ/day (170 kcal/day). However, a mother's ability to produce milk generally far exceeds actual milk production and increased demands by the infant are met by increased supply - irrespective of maternal energy intake (Daly & Hartmann, 1995).

During pregnancy and breastfeeding, the nutrient density of the maternal diet assumes great importance, as the increased demand for energy exceeds a prospective mother's needs for other nutrients (Picciano, 2003). Adequate nutrition, while critical during lactation, may not be easily achieved by diet alone. The adequacy of the maternal diet is often assumed when the infant is thriving and milk composition is within normal ranges. However, maternal adequacy should not be assumed from infant growth data as the nutrient content of human milk is often maintained at the expense of the mother's own nutrient stores (Institute of Medicine (IMO), 1991).

Studies have shown that low-income pregnant women in the US have a heightened awareness about healthy eating during pregnancy. However, this concern diminished after delivery and the majority of women returned to their, less healthy, pre-pregnancy diets (Havas *et al.,* 1998; Treiman *et al.,* 1996). Overall, there is a scarcity of information regarding the nutritional status of women post-pregnancy - both lactating and non-lactating (Doran & Evers, 1997; George, Milani, Hanss-Nuss, & Freeland-Graves, 2005; George, Hanss-Nuss, Milani, & Freeland-Graves, 2005) and the nutritional status of women in Ireland during lactation has not been reported in the literature.

This chapter presents the results of the dietary intake of women, 12 weeks post-partum, living in Ireland and provides a discussion of the findings. The data generated from this study were compared to Recommended Daily Allowances (RDA) (Food Safety Authority of Ireland, 1999), and the latest Irish national survey- National Adult Nutrition Survey 2008-10 (Irish Universities Nutrition Alliance, 2011). The percentage of participants consuming more than the EAR are examined to determine the nutritional adequacy of this groups' diet. To conclude the chapter, the study will consider participants' adherence to some of the Irish Dietary Guidelines, such as those for oily fish, dairy produce, fruit and vegetables, as these foods provide many of the increased requirements for vitamins and minerals needed during lactation (Food Safety Authority of Ireland, 2012).

4.2 *Methods*

The study design, recruitment and data collection for the follow-up of all mothers 12 week post-partum are discussed in detail in Chapter 2**.** Women completed a 7-day, semi-quantitative food diary approximately 12-weeks post-partum. Data used in this chapter are presented using numerical descriptive statistics; including means with standard deviations and medians with interquartile ranges (IQR). Daily intakes of fruits and vegetables, dairy products and fish are presented using percentages and actual (n) values.

4.3 *Results*

4.3.1 Population Characteristics

Table 4-1 Demographic & Anthropometric Characteristics of Participants in the Study (n=66)

Characteristic	*Mean/Median*	*SD/IQR*	*Range*
Age (years)†	35.2	4.1	26- 44
Pre-pregnancy Weight (kg)‡	65.3	11.2	50- 108
Height (metres)†	1.67	0.07	1.51- 1.80
Demographic Characteristic	*% Participants*		
Ethnicity			
Irish	78.8 (n=52)		
UK	4.5 (n=3)		
Other Europeans	10.6 (n=7)		
Others	6.1 (n=4)		
Marital Status			
Married	83.3 (n=55)		
Living with Partner	15.2 (n=10)		
Single	1.1 (n=1)		
Maternal Highest Educational Qualification Level			
Post-grad	45.5 (n=30)		
Degree	42.4 (n=28)		
Higher education below degree	12.1 (n=8)		
Leaving Cert & below	0		
Maternal Employment Status			
Full-time	73.8 (n=48)		
Part-time	13.6 (n=9)		
Unemployed	7.7 (n=5)		
Economically inactive*	4.6 (n=3)		
Social Class**			
1 & 2	86.3 (n=57)		
3 & 4	10.6 (n=7)		
5 & 6	1.5 (n=1)		
7	1.5 (n=1)		

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR) *Economically inactive (CSO) **Standard Occupational Classification (CSO)

Sixty-six women returned completed diaries at 12 weeks post-partum. The population characteristics are summarised in Table 4.1. Mothers were predominantly Irish nationals (78.8%, n=52) while the remainder were British (4.5% n=3); European (10.6% n=7); Asian (1.5% n=1); North American (3% n=2) and Australian (1.5% n=1). Mothers were predominantly married (83.3% n=55) or living with their partner (15.2% n=10). This cohort had a high educational status; with 45.5% (n=30) educated to a postgraduate level; 42.4% (n=28) educated to degree level; while a minority held qualifications less than degree level (12.1% n=10). The majority of mothers were employed (87.4% n=57), on either a full-time (73.8% n=48) or part-time (13.6% n= 9) basis and were from social class 1 & 2 (86.3%).

4.3.2 Daily Intakes of Macronutrients

The mean intakes for the study participants as a group for energy, protein, carbohydrate, fat and fibre were 2090 kcal (± 482), 72 g (± 17.4), 248 g (± 68.1), 89 g (± 23.2) and 17 g (± 9.1) respectively (Table 4.2). These results for energy intake were higher than the results from NANS 08-10 (1721 kcal/day) (Irish Universities Nutrition Alliance, 2011) and lower than the estimated requirement based on the recent SACN report (2011). The Goldberg's cut-off of ≤1.2 (Goldberg *et al.,* 1991) showed that 21.2% (n=14) of women in the group might be under-reporting energy in this study; as discussed in Chapter 2 (pp.76).

Participants were further divided based on infant feeding practices. Comparisons were made between those women exclusively breastfeeding (n=35) with those who were either partially breastfeeding (n=12) or formula feeding (n=15). This is because exclusively breastfeeding have higher requirements than the other groups in order to produce sufficient The mean intakes for women, who were exclusively breastfeeding, for energy, protein, carbohydrate, fat and fibre were 2159 kcal (±513), 71 g (±19.2), 254 g (±74.9) 94 g (±24.5) and 18 g (±9.9) respectively. This is compared to women who did not breastfeed or only partially breastfed, whose intake for energy, protein, carbohydrate, fat and fibre were 2013 kcal (±441), 74 g (±18.7), 242 g (±60.1), 84 g (±20.7) and 17 g (±8.6) respectively. There was no significant difference in intakes of any of the macronutrients between the two groups (Table 4.2). Exclusively breastfeeding mothers had intakes that were less than that recommended by the latest SACN report (SACN, 2012).

Table 4-2 Mean daily macronutrient intakes based on food dairies (n=66) for the total group, for those mothers exclusively breastfeeding (n=35) and for those mothers who were not breastfeeding & partially breastfeeding (n=31) as well as the results of the non-pregnant women aged between 18-64 from NANS 08-10 (Irish Universities Nutrition Alliance, 2011).

Nutrient	*All Mothers*	*SD/IQR*	*Exclusive BF Mothers (n=35)*	*SD/ IQR*	*None & Partial BF Mothers (n=31)*	*SD/IQR*	*P value****	*NANS 08- 10*	*SACN 2011**	*SACN 2011** EBF*
Energy -MJ/d	8.77	2.03	9.03	2.1	8.43	1.85		7.2	8.1-8.3	10.2-10.4*
Energy- kcal/d †	2090	482	2159	513	2013	441	.389	1721	-	-
Protein –g ‡	72	17.4	70	19.2	74	18.7	.93	70.4	-	-
Carbohydrate –g †	248	68.1	254	74.9	242	60.1	.66	199.7	-	-
Total Fat –g†	88.8	23.2	94	24.5	84	20.7	.196	67.6	-	-
Saturated Fat –g†	33.6	10.3	36	10.7	31	9.6	.183	-	-	-
Fibre- g ‡	17.3	9.1	17.8	9.9	17.1	8.6	.644	17.3		

*SACN requirements calculated based on EAR MJ/d for the population of less active women aged 25-44yrs ** SACN requirements calculated based on EAR MJ/d for the population of less active women aged 25-44yrs plus 2.1MJ increment for the cost of lactation ***Exclusively breastfeeding mothers were compared to those mothers who were not †Normally distributed data presented as mean and standard deviation (SD); differences assessed by paired samples t test. ‡Skewed data presented as median and interquartile range (IQR); differences assessed by Mann Whitney U test

The percentage contribution of each macronutrient to total food energy was calculated and compared to the National Recommendations for Irish Adults (Food Safety Authority of Ireland, 1999); NANS 2008-10 (Irish Universities Nutrition Alliance, 2011).

Table 4-3 The percentage contribution of each macronutrient to food energy for group based on food dairies (n=66) for the total group, for those mothers exclusively breastfeeding (n=35) and for those mothers who were not breastfeeding/ partially breastfeeding (n=31) as well as the results of the non-pregnant women aged between 18-64 from the NANS 08-10 (Irish Universities Nutrition Alliance, 2011).

Nutrient	*All Mothers*	*Partial or No breastfeeding (n=31)*	*Exclusively breastfeeding mothers (n=35)*	*Irish Recommend-ations*	*NANS 08- 10*
Carbohydrate	47%	48%	47%	50%	45.8%
Protein	15%	15%	15%	10-15%	17.6%
Fat	38%	37%	39%	30-35%	36.8%
Saturated Fat	15%	14%	15%	<10%	-

*Some figures do not add up to 100% due to rounding errors in calorie conversion.

On average, participants consumed approximately 15% of their energy from protein, which met the national guidelines. These figures were marginally lower than the reported intakes (17.6%) from NANS 2008-10 (Irish Universities Nutrition Alliance, 2011). Among study participants, the percentage contribution to food energy from carbohydrates was 47.3%, which is just below the national recommended level of 50% (Food Safety Authority of Ireland, 1999). Nearly two thirds of participants (64%; n=42) had carbohydrate intakes below the recommended level of 50%. Moreover, 59% of exclusively breastfeeding mothers and 69% of non-exclusively breastfeeding mothers had intakes below the recommended level of 50% for carbohydrates. This result appears consistent with the other studies, in which participants' average carbohydrate intake comprised 46-48% of total energy intake.

Fat provided on average 38.2% of my participants' food energy, with a range of intakes between 26.8% and 49.8%. This figure was higher than the fat intakes reported in the latest national study – 36.8% (Irish Universities Nutrition Alliance, 2011). The majority of mothers (75.8% n=50) had intakes which exceeded the generally recommended upper limit of 35% for the proportion of energy from fat. A significant contribution to energy intake from saturated fat intakes was also noted. The average contribution to total energy intake from saturated fat reported was 14.5%, which was higher than the national dietary guidelines (Food Safety Authority of Ireland, 1999) (<10%). Only 6.1% (n=4) of mothers had intakes that were less than the upper limit of 10% food energy from saturated fat. Nearly 8% (n=5) of mothers had saturated fat intakes that were above 20%, twice the maximum recommended level.

Our results for dietary fibre intake were consistent with the results of the most recent national adult nutrition survey (19.2 g/day, IUNA 2011). However, the majority of mothers failed to meet the guidelines for the European Food Safety Authority recommendation of 25 g/day (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010).

In a sub-analysis of the groups, women who were exclusively breastfeeding, had average contributions to total energy intake from protein, carbohydrate, total fat and saturated fat of 14.5%, 46.7%, 39% and 15% respectively. In comparison, non– or partially breastfeeding women reported intakes of 15.2%, 48%, 37.3% and 13.9% for protein, carbohydrate, total fat and saturated fat respectively.

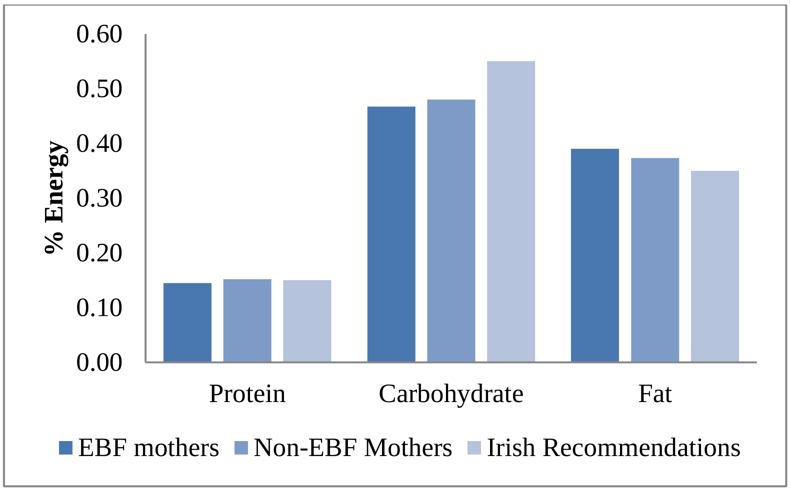


Figure 4-1 Percentage energy intake from macronutrients for exclusive breastfeeding (EBF) mothers, Non-exclusively breastfeeding mothers and National Irish recommendations (FSAI, 1999)

4.3.3 Daily Intakes of Micronutrients

The mean intake of the main micronutrients was calculated for the study population (Table 4.5) and were compared to data obtained from the latest national survey – NANS (Irish Universities Nutrition Alliance, 2011). In comparison to NANS, the mothers had lower mean intakes of sodium, calcium, magnesium, zinc, vitamin B6 and vitamin C. The groups were further divided based on infant feeding practices. There was no significant difference in micronutrient intakes between those women exclusively breastfeeding and those who were not exclusively breastfeeding (Table 4.4). Furthermore, consumption of micronutrients, which are particularly important during lactation, was compared to current Irish Recommended Dietary Allowances (RDA), Average Requirements (AR) and Lowest Threshold Intakes (LTI) for women aged 19–50, (FSAI, 1999) with the addition, where appropriate, of an increment for lactation (Table 4.5).

Table 4-4 Mean/Median daily micronutrient intakes based on food dairies (n=66) for the total group, for those mothers exclusively breastfeeding (n=35) and for those mothers who are not breastfeeding/ partially breastfeeding (n=31) as well as the results of the non-pregnant women aged between 18-64 from the latest national study- NANS 08-10 (Irish Universities Nutrition Alliance, 2011).

Nutrient	*Total Group*	*SD/ IQR*	*Exclusively breastfeeding mothers*	*SD/ IQR*	*Partial / No Breastfeeding*	*SD/ IQR*	*P value**	*NANS 08- 10*
Sodium (mg) †	2939	715	3058	794	2803	598	.121	2480
Calcium (mg) †	955	291.6	997.2	320.4	907.4	247	.287	824
Magnesium (mg) ‡	275	102.4	275.4	111.8	277.0	77.6	.509	255
Iron (mg) ‡	11.5	5.7	11.2	6.1	12.6	5.0	.617	13.7
Zinc (mg) ‡	8.9	3.4	8.0	2.8	9.3	2.2	.299	9.0
Vitamin D (µg) ‡	1.85	2.1	2.2	2.2	1.7	2.0	.195	3.13.9
Vitamin E (mg) ‡	9.67	4.3	9.1	5.1	10	4.5	.729	12.5
Thiamin (mg) ‡	1.5	0.7	1.5	0.7	1.58	0.7	.719	3.4
Riboflavin (mg) ‡	1.59	0.7	1.54	0.6	1.6	0.9	.705	3.3
Niacin (mg) ‡	19.8	7.6	19.6	6.92	20.4	7.8	.397	24.7
Vitamin B6 (mg) ‡	1.82	0.6	1.96	0.68	1.90	0.6	.612	4.2
Vitamin B12 (µg) ‡	4.28	1.7	4.45	1.74	4.08	1.74	.426	8.0
Folate (µg) ‡	240.3	141	223	151	264.8	145.9	.827	339
Vitamin C (mg) ‡	118	126	146.9	264	106.9	88.7	.088	141
Copper (mg) ‡	1.15	0.8	1.2	1.1	1.26	0.49	.299	1.3
Selenium (µg) ‡	37.4	22.3	37.1	20.4	37.3	24.9	.590	-
Iodine (µg) ‡	125	57	124	85.9	127	64.4	.555	-

*Exclusively Breastfeeding mothers' intakes were compared to those mothers not exclusively breastfeeding . †Normally distributed data presented as mean (SD); differences assessed by independent samples t test. ‡Skewed data presented as median and interquartile range (IQR); differences assessed by Mann Whitney U test *statistically significant at P<0.05

Table 4-5 Mean total group intakes, RDA including the increment for lactating women; the RDA for non-pregnant women aged 19-64; the average requirements (AR) for non-pregnant women aged 19-64; the lowest threshold intake (LTI) for non-pregnant women aged 19-64 and percentage of women below these cut-off points for selected nutrients (Food Safety Authority of Ireland, 1999)

Nutrient	*Group*	*SD/ IQR*	*RDA* Lact*	*% < RDA* Lact*	*RDA***	*% < RDA***	*AR***	*% < AR***	*LTI***	*% < LTI***
Calcium (mg/d) †	954.5	291.6	1200	81.8	800	33.3	615	14	430	0
Iron (mg/d) ‡	11.5	5.7	15	75.8	14	68.2	10.8	40.9	7.5	6.1
Zinc (mg/d) ‡	8.9	3.4	12	84.8	7	15.2	5.5	1.5	4	0
Vitamin D (μg/d) ‡	1.85	2.1	10	100	0-10	-	-	-	-	-
Vitamin B12 (μg/d) ‡	4.28	1.7	1.9	4.5	1.4	3.0	1.0	1.5	0.6	0
Folate (μg/d) ‡	240.3	141	400	90.9	300	66.7	230	50	160	10.6
Vitamin C (mg/d) ‡	118	126	80	28.8	60	13.6	46	9.1	32	4.5
Riboflavin (mg/d) ‡	1.15	0.8	1.7	60.2	1.3	27.3	1.1	16.7	0.6	0
Copper (μg/d) ‡	37.4	22.3	1.4	66.7	1.1	50	0.8	9.1	0.6	0
Selenium (μg/d) ‡	125	57	75	95.5	55	78.8	40	57.6	20	1.5
Iodine (μg/d) †	4.28	1.7	160	77.3	130	57.6	100	30.3	70	7.6

*Figure for women aged 19-50, with the additional increment for lactation ** Figures for non-lactating women aged 19-50 †Normally distributed data presented as mean (SD). ‡Skewed data presented as median and interquartile range (IQR);

Study participants had adequate intakes for many micronutrients – as indicated by the low percentage of mothers who had intakes below the Average Requirements (AR) for many of the micronutrients (Table 4.5). However, a significant number of inadequate intakes were observed for some key nutrients during pregnancy such vitamin D (100 % below 10μg), folate (50% <AR), selenium (57.6% <AR), iron (40.9% <AR) and iodine (30.3% <AR). Additionally, a sub-analysis showed that exclusively breastfeeding women had intakes of 223 μg folate; 11.2 mg iron; 2.2 μg vitamin D; 147 mg vitamin C; 997 mg calcium and 3058 mg sodium. In comparison, non- and partially breastfeeding women had intakes of 265 μg folate; 12.6 mg iron; 1.7 μg vitamin D; 107 mg vitamin C; 907.4 mg calcium and, 2083 mg sodium (Table 4.4).

Salt intakes were estimated based on the sodium content of foods recorded in the 7-day food diary. The average reported intake of salt was 7.5 g daily, with a range of 3.9 g–11.6 g. The recommended dietary allowance (RDA) is 1.6 g sodium (4 g salt) per day for adults (Food Safety Authority of Ireland, 2005). Of concern was that only a quarter of participants (23%; n=15) reported intake below the maximum recommended level of 6 g daily. This is of concern, as the proportion exceeding limits could be in fact greater as discretionary salt was excluded from these results. While this result compared favourably with NANS 08–10 showed a lower mean daily intake of salt for women aged 18–64 years of 6.2 g/day (Irish Universities Nutrition Alliance, 2011).

4.3.4 Intake of Fruit & Vegetables in mothers 12 weeks post-partum

Table 4-6 Mean number of daily servings of mothers form the fruit and vegetables and dairy shelves of the Irish food pyramid, and from oily fish (n=66).

Food Group	*Mean No. of Portions*	*SD*	*Range (portions)*
Fruits*	2.04	1.38	0.0- 7.5
Vegetables*	1.4	0.75	0.0- 3.5
Fruit & Vegetables*	3.5	1.8	0.1- 10.8
Dairy Produce†	2.23	1.09	0.2- 6.0
Oily Fish‡	0.9	1.3	0.0- 5.8

No of Portions	*% of Women Consuming*
Fruit	
< 1.0	13.6%
1.0-1.9	45.5%
2.0- 2.9	25.7%
≥3.0	15.2%
Vegetables	
< 1.0	30.%
1.0-1.9	485%
2.0- 2.9	16.7%
≥3.0	4.5%
Fruit & Vegetables	
< 1.0	4.5%
1.0-1.9	6.1%
2.0- 2.9	25.8%
3.0- 3.9	36.3%
4.0-4.9	15.2%
≥ 5.0	12.1%
Dairy Products	
< 1.0	15.2%
1.0-1.9	25.7%
2.0- 2.9	34.9%
≥3.0	24.2%
Oily Fish	
< 1.0	53%
1.0-1.9	6.1%
2.0- 2.9	10.6%
≥3.0	10.6%

*1 portion =80g of fruit or vegetables †1 portion = 30g of cheese/ 200ml of milk / 150g of yogurt ‡ 1 portion = 90g Fish

Irish National Guidelines recommend at least 5 portions of fruit and vegetables per day, with at least 2 portions being fruit and 3 portions being vegetables (Appendix K). The mean number of portions of vegetable and fruit for this group was 3.5 (±1.8), which is below the recommended guidelines. Only 12.1% of women met the recommended guidelines of 5 portions of fruit and vegetables daily. The mean number of fruit portions consumed was 2.0 (±1.4), with approximately 41% of women consuming the recommended two or more portions per day. The range in fruit intake was between 0.0 and 7.5 portions per day. Of most concern was that 13.6% were consuming less than one portion of fruit a day. Vegetable intake was lower than fruit intake among participants – the mean number of vegetable portions consumed daily was 1.4 (±0.7), which is less than half the recommended daily amount. The range in vegetable intake was between 0.0 and 3.5 portions per day. Only 3% were meeting their recommended 3-a-day of vegetables and 30.3% of women reported eating less than one portion of vegetables per day.

4.3.5 Intake of Dairy Products

The dietary recommendation for dairy produce (e.g. milk, cheese and yoghurts) is 3 portions per day for the general population (Food Safety Authority of Ireland, 2012). However, as there is an increase in the requirements for calcium during lactation in Ireland, during the study period there was a recommendation that pregnant and lactating women consume five portions from this food group. These guidelines have subsequently changed, with the Department of

Health and Children in 2012 now recommending 3 portions of diary produce per day for both pregnant and non-pregnant women (Food Safety Authority of Ireland, 2012). The mean number of dairy portions for the entire group was 2.2 portions, which was less than half the recommended level of dairy for lactating women at that time. It is also below the current recommendations for lactating and non–lactating women (Table 4.6).

4.3.6 Intake of Oily Fish

As 100% of participants failed to meet the RDA for vitamin D, I examined dietary sources of vitamin D. Oily fish (e.g. mackerel, salmon, tuna) provide good sources of vitamin D while some brands of breakfast cereal, margarines and milks are fortified with small amounts of vitamin D. The average intake of oily fish was less than one portion per week (0.9 ±1.3 portions), with a range of 0.0 to 5.8 portions for week. More than half (53%) of women reported consuming less than 1 portion of oily fish per week and similar what was reported during pregnancy, tinned tuna was the most common oily fish consumed.

4.4 *Discussion*

4.4.1 Macronutrient Intakes

This is the first Irish study to document the dietary intake of Irish mothers at 3 months post-partum. The findings from this study add to existing literature that shows that breastfeeding can be maintained at lower than recommended levels of energy (Hoppu, Kalliomäki, & Isolauri, 2000; Mackey, Picciano, Mitchell, & Smiciklas-Wright, 1998; Mackey & Picciano, 1999; Todd & Parnell, 1994). Evidence suggests that maternal dietary factors may have an impact on milk composition i.e. prolonged lactation in nutritionally depleted women may impact more on the composition of the breast milk than on the volume produced (Allen, 2005; Institute of Medicine (IMO), 2009). Another possibility is that results may also be reflective of under-reporting of dietary intakes.

Koletzko *et al.* (2007) suggest that dietary fat intake in lactation, expressed as percentage energy, should be as recommended for the general population. Among this cohort, the percentage of energy contributed from macronutrient sources did not correspond with the proportions recommended for Irish women, with fat and saturated fat intakes above dietary guidelines. This is of particular concern, as evidence suggests that breast milk fatty acids may have properties which could affect the development of atopic disease and that breast milk rich in saturated and low in n–3 fatty acids may be a risk factor for atopic dermatitis in infants (Hoppu *et al.,* 2000; Hoppu, Rinne, Lampi, & Isolauri, 2005). Furthermore, the high intake of saturated fat reported by breastfeeding and non–breastfeeding mothers is an indication of a generally unbalanced diet, which may have long term consequences for both the mother and her infant. Saturated fat is believed to be more obesogenic (Hariri *et al.,* 2010) and, therefore, there is the potential that these energy dense diets may contribute to the retention of weight gained during pregnancy.

4.4.2 Micronutrient Intakes

Maternal micronutrient deficiencies during lactation can cause a major reduction in the concentration of some nutrients in breast milk, with subsequent infant dietary deficits. The micronutrients most affected by maternal status are iodine, selenium, thiamine, riboflavin, vitamin B6, vitamin B12, vitamin C as well as the fat soluble vitamins A, D, E and K (Allen & Graham, 2003). Moreover, high intakes of sodium was reported among this group. The main micronutrients will now be considered in more detail in the following sections.

4.4.2.1 Sodium

High intakes of dietary sodium (salt) are associated with an increased risk of total cardiovascular disease and stroke (Strazzullo, D'Elia, Kandala, & Cappuccio, 2009), which are the single highest cause of death (41%) in Ireland. High blood pressure is one of the major modifiable causal factors in the development of cardiovascular disease. Mindful of the risks to long-term health, the WHO has recommended salt reduction as one of the top three priority actions to tackle the global non-communicable disease crisis (Beaglehole *et al.,* 2011).

The results from the latest National Nutrition Survey showed that the average intake for women aged 18–64 is 6.2 g/day (Irish Universities Nutrition Alliance, 2011). In Ireland, the FSAI (2010) has advised that all adults should have a maximum intake of 4 g of salt per day (1.6 g/day for sodium; (Food Safety Authority of Ireland, 2005). My findings show that 98.5% (n=65) of mothers reported a daily intake greater than the recommended 4 g/day. In fact, only one woman had an intake of 4 g/day or below. In thisstudy, discretionary salt (salt added in cooking and at table)was excluded, which is estimated to account for approximately 15–20% of total dietary sodium intake, therefore my results may be underestimated. Furthermore, the dietary analysis package – Dietplan 6.4 (Forestfield Software Ltd., 2011) uses the Food Composition Tables (Food Standards Agency, 2002b) and does not account for recent salt reductions made by the food industry since the publication of these tables, which may counter the underestimation in those foods where brand names were not given and therefore could not be checked from food labels.

4.4.2.2 Calcium

Lactation programmes an obligatory skeletal calcium loss irrespective of maternal calcium intake, but this lost calcium is completely restored to the maternal skeleton after cessation of breastfeeding (Kovacs, 2005). The results from my study, using an independent t-test, show no significant difference in daily calcium intakes between exclusively breastfeeding mothers (997.2

mg/d ±320) and mothers who partially breastfed or formula fed infants (907 mg/d ±247; p=0.29). However, the average intakes for both groups were higher than the average intake reported in the NANS 08-10 for non-lactating women (824 mg/d). Maternal calcium loss during lactation is estimated at 280–400 mg/day and can reach up to 1000 mg/day, which is approximately three times higher than that during pregnancy (Sowers, 1996). Given its importance, the RDA for calcium in lactation is increased from 800 mg/day to 1200 mg/day. A majority of women reported calcium intakes that were below the RDA during lactation (81.8%; n=54).

During the data collection period, the national dietary guidelines for lactation were 5 portions of dairy produce (e.g. milk, cheese and yoghurts) per day compared to the 3 portions per day recommended for the general population. These guidelines have changed since, with the Irish Department of Health in 2012 now recommending 3 portions of diary produce per day for both lactating and the general adult female population (Food Safety Authority of Ireland, 2012). A particular concern for this group was that 14% of women reported levels below the AR for non-pregnant women and that the mean number of dairy food portions was 2.3 portions, which is below the recommended three servings a day for women in the general population. These results are consistent with the most recent national survey (NANS), where calcium intakes were below the AR in 16% of women aged 18–64 years (Irish Universities Nutrition Alliance, 2011). Overall, inadequate calcium intake may contribute to reduced bone mass and increased susceptibility to osteoporosis in the long term in these women, irrespective of infant feeding method.

4.4.2.3 Vitamin D

One of the fat-soluble vitamins, vitamin D plays a major role in regulating calcium absorption and is essential for good bone health from conception. It is also involved in calcium metabolism, which forms strong bones. Therefore, it is suggested that vitamin D is particularly important for growing children as well as for pregnant and lactating women. It is particularly important at a time when their exposure to sunshine is limited (Mughal, Salama, Greenaway, Laing, & Mawer, 1999). The incidence of rickets has risen in infants and toddlers in Ireland and other developed countries over the past decade (Food Safety Authority of Ireland, 2007; Gordon *et al.,* 2008; Ward, 2005) which demonstrates the importance of this micronutrient.

Newborn infants are potentially at risk of vitamin D insufficiency as their vitamin D stores at birth are dependent on the vitamin D status of their mother, which is often insufficient (Muldowney, 2010). Even when mothers have optimal vitamin D status, newborns will accrue about 50–60% of their mother's vitamin D stores at birth and these stores are usually depleted by approximately 8 weeks of age (Hollis & Wagner, 2004). Thereafter, vitamin D is derived from infants' diet, sunlight and supplementation. Since Ireland is located 51º to 55º North, little or no vitamin D can be produced from sunlight from September to May (Cashman *et al.,* 2008).

Study participants reported low intakes of vitamin D during their pregnancy, with all of them failing to meet the 10 μg daily requirement for vitamin D (Chapter 3). Post-pregnancy, these women continued with their low dietary intake of vitamin D, with a mean intake of 2.38 μg/day for the entire group and 2.5 μg/day amongst those exclusively breastfeeding. Again, none of the women reported consuming the 10μg/day recommendation. Other studies have reported similar high rates of vitamin D insufficiency, both in pregnancy and infancy, with deficiencies reported in 5–50% of pregnant women and 10–56% of breastfed infants (Mulligan, Felton, Riek, & Bernal-Mizrachi, 2010; O'Riordan, Kiely, Higgins, & Cashman, 2008; Wagner & Greer, 2008).

The results from this study are much lower than those reported in the recent national study (Irish Universities Nutrition Alliance, 2011), where the mean intake of vitamin D was nearly twice that reported here (4.2 μg/day). As previously discussed, it is difficult to obtain sufficient vitamin D through diet alone. Because of concerns about low levels of vitamin D status and increasing reports of childhood nutritional rickets in Ireland, recent Irish policy now recommends that all infants, irrespective of how they are fed, should be given a daily vitamin D supplement of 200 IU (5 μg/day) from birth to 12 months of age (Health Service Executive (HSE), 2010). The results of this study would reinforce the need for this recommendation in Ireland.

4.4.2.4 Omega–3 Fatty Acids

Oily fish is an important source of Omega–3 fatty acids such as docosahexaenoic acid (DHA), which is a critical component of cell membranes – especially in the brain and retina. Infants accumulate DHA into the central nervous system up to the age of 18 months (Denomme, Stark, & Holub, 2005; Szajewska, Horvath, & Koletzko, 2006). DHA accumulation in the brain begins during pregnancy and continues during the first years of life, reaching a total brain DHA deposition of about 4 g by 2–4 years of age (Koletzko *et al.,* 2008). European recommendations suggest that lactating women should aim to achieve at least a DHA intake of 200 mg per day. By way of comparison, a portion of salmon contains approximately 260 mg of DHA, showing how difficult it is to achieve this through diet alone (EFSA, 2012). The mean intake among the study participants was 0.9 portions of oily fish per week, with 53% (n=35) of the women reporting no oily fish intake. Low intakes may also be due to concerns about mercury levels in oily fish. Given the low intakes observed in this and other studies, we may need to assess omega-3 recommendations and discuss how women may increase consumption by including other sources of omega–3 fatty acids in their diet, for example, enriched eggs and flaxseeds.

4.4.2.5 Folate

Lactating women also have an increased need for folate in the breastfeeding period in order to support the exponential growth of the breastfed infant. The folate content of breast milk is relatively independent of maternal folate status (Tamura, Yoshimura, & Arakawa, 1980; Mackey & Picciano, 1999), and breastfed infants are usually well protected against folate deficiency (Ek & Magnus, 1979; Smith, Picciano, & Deering, 1985; Salmenperä, Perheentupa, & Siimes, 1986). The mother, as a consequence, may be at risk of folate deficiency. Of most concern was that 10.6% of participants in my study failed to even meet the LTI for non-pregnant women, putting themselves at high risk of folate deficiency. This concurred with the findings of Todd & Parnell (1994), who also reported low intakes of folate in breastfeeding mothers. Given the role of folate in the prevention of neural tube defects, it is vital that healthcare professionals stress the importance of improving folate intakes in these women who are capable of becoming pregnant. This support should include educating these women about foods rich in folate – perhaps encouraging the use of a folic acid-containing supplement. However, it must be noted that my study, calculated nutrient intakes from foods sources alone and did not consider supplement intake and therefore my results may be underestimated. Moreover, the dietary analysis package – Dietplan 6.4 (Forestfield Software Ltd., 2011) uses the Food Composition Tables (Food Standards Agency, 2002b) and does not account for the recent voluntary fortification of some products in Ireland by the food industry after this period, which may compound this underestimation.

4.4.2.6 Iron

Iron deficiency remains the most common nutrient deficiency in both the developing and developed world. It is widely acknowledged that iron deficiency remains a problem for older infants and toddlers in many countries (Lawson, Thomas, & Hardiman, 1998; Hay, Sandstad, Whitelaw, & Borch-Iohnsen, 2004; Yang *et al.,* 2009; Baker & Greer, 2010). In the UK there are no recommendations for increasing iron intake during lactation, where iron losses (secreted in breast milk) are compensated for by the amenorrhoea associated with lactation (Department of Health, 1991). In Ireland a 1 mg/day increase is recommended during lactation (Food Safety Authority of Ireland, 1999). In this study, the mean daily intake was 13 mg/day, which was lower than the RDA for iron during lactation and is also lower than the reported intakes for non-lactating women in the two cited national studies. Exclusive breastfeeding mothers had lower intakes when compared to the entire study population, though this did not reach significance. The majority of mothers failed to meet the RDA for lactating (75.8%) and non-lactating women (68.2%). More than 40% of had intakes below the AR of iron and 6.1% had intakes below the LTI for iron; putting these women at risk for iron deficiency anaemia.

4.4.2.7 Vitamin C

Vitamin C helps the absorption of non-haem iron if both nutrients are consumed at the same time. Vitamin C consumption is particularly important given the low intakes of iron by mothers. Vitamin C output in milk is highly variable and responsive to levels of maternal intake. The mean daily intake in this study was 201 mg/day, which is well above the recommended daily allowance of vitamin C in Ireland during lactation (60 mg/day). The majority of mothers in my study (97%) had intakes above the RDA for vitamin C (Food Safety Authority of Ireland, 1999), which would suggest adequate intake in the diet of this group.

4.5 ***Strengths & Limitations***

One of the strengths of the study is the prospective method of the dietary assessment – the seven day estimated food diary. The 7-day food diary is considered the gold standard for estimating food intake (Bingham *et al.,* 1994).Under-reporting is always a risk in dietary surveys. Using Goldberg's cut-offs of 1.2 (Goldberg *et al.,* 1991), approximately 22% (n=14) of women were considered to under-report energy intake in this study. It was decided that these women would still be included in the analysis as women during the post-partum period are often actively trying to lose excess weight which they gained during pregnancy. It is therefore possible that they may have restricted their energy intake in order to achieve weight loss rather than under-reporting their energy intake, though this was not checked with the participants in this study. One study showed that between 40–50% of women requested professional advice to lose weight after the birth of their infants (Rössner & Ohlin, 1995). The level of possible under-reporting among participants is below the levels reported in a previous national study – SLAN 2007, which reported that 37% of women under-reported their energy intake (Harrington *et al.,* 2008). It is also possible that those participants who took part in the study had an interest in nutrition as well as the subject matter of the overall study; therefore, they may have been more motivated to accurately record their diets compared to the national studies. Another strength of this study is in its focus on the whole maternal diet. Most previous studies in this period of the lifecycle have only considered a single nutrient in isolation or focused on the infant's nutritional status in this period. Focusing on one nutrient limits our understanding of nutrient–nutrient interaction as one nutrient may be a marker for other nutrients inadequacies (for example, iron and folate deficiencies often coexist).

4.6 *Conclusion*

Overall, the findings suggest that lactation may be maintained on lower levels of energy intake than is currently recommended. Regardless of infant feeding methods my results show that many women are having an unbalanced diet – with high levels of fat and salt in their diets and are also consuming low levels of many essential vitamins and minerals, possibly increasing their long term risk of developing heart disease, certain cancers and osteoporosis.

Fruit and vegetables are an important source of many vitamins and minerals. Reported intakes of vegetables, dairy products and oily fish were below the recommendations for health and improvements in these areas may help in lowering saturated fat intakes while increasing the nutrient density of mothers' diets. Under the WHO (2001) recommendations, mothers are encouraged to exclusively breastfeed their infants for the first six months of life. While breastfeeding rates are still low in Ireland, they are increasing (The Economic and Social

Research Institute - Health Research and Information Division, 2012). In light of increases in breastfeeding rates in Ireland, more information and advice for all segments of the population is needed. Advice for such women should target food sources for nutrients that are not currently being met viz. calcium, vitamin D, iron. Given that parenthood is a time of great change and parents may be more receptive to dietary advice, it creates an opportunity for health professionals to discuss healthy eating, which may promote better long term health for both mother and infant. Since recommendations for lactating women are essentially the same as for other adults, this may be a good time to target healthy eating interventions and education. Further research is needed to identify groups of mothers in Ireland who are at nutritional risk who could benefit from targeted nutrition intervention programmes.

CHAPTER 5. INFANT FEEDING PRACTICES DURING THE FIRST SIX MONTHS OF LIFE

5.1 *Introduction*

Although breastfeeding rates slumped in the middle of the 20th century in most Western countries, they have begun to recover, particularly among the affluent classes in the last thirty years (Curtin, 1954; Kalapesi, 1974; McAndrew *et al.,* 2012). The infants most likely to be breastfed are those from families living in relatively affluent circumstances with well-educated parents, or families from minority ethnic backgrounds (Bolling, Grant, Hamlyn, & Thornton, 2007; Brown, Raynor, Benton, & Lee, 2010; Kelly & Watt, 2005). The Republic of Ireland has one of the lowest rates of breastfeeding in the developed world (Department of Health, 1994; 2005) and has been slow to increase levels of breastfeeding, with approximately 57% of mothers giving some breast milk (either exclusively or partially) at hospital discharge (The Economic and Social Research Institute - Health Research and Information Division, 2012). The WHO (2001) and the Irish Department of Health and Children (1994; 2005) have strongly endorsed breastfeeding; noting the multitude of benefits that it provides to infants, mothers, families and communities. These benefits include improved nutritional, immunological, psychological, economical, and environmental outcomes. Good quality studies of conditions such as gastroenteritis, respiratory disease, sudden infant death syndrome, and otitis media for infants, and breast cancer for mothers, have shown that all of these conditions are more prevalent when infants are not breastfed (Howie *et al.,* 1990; Wilson *et al.,* 1998; Möller *et al.,* 2002; Horta *et al.,* 2007; Ip *et al.,* 2007; Quigley, Kelly, & Sacker, 2007; Duijts, Ramadhani, & Moll, 2009). Recent studies have shown an increased risk of poorer cognitive development and behavioural problems in children who were not breastfed (Kramer *et al.,* 2008; Heikkilä *et al.,* 2011; Quigley *et al.,* 2012).

Despite a large public health campaign in Ireland promoting the benefits of breast milk over the past decade, there has only been a 14.4% increase in the number of women exclusively breastfeeding at hospital discharge; percentages in absolute terms increased from 42% of women in 2002 to 46% in 2011 (The Economic and Social Research Institute - Health Research and Information Division, 2012). Breastfeeding duration is more difficult to compare as no national data are collected in Ireland with regard to duration of breastfeeding. However, studies show that, despite many women initiating breastfeeding at birth, there is a high dropout rate in the early period of breastfeeding (Kelly & Watt, 2005; Tarrant *et al.,* 2011; The Economic and Social Research Institute - Health Research and Information Division, 2012). However, rates are still low in comparison to other European countries, though rising every year. A recent report of breastfeeding support services in Ireland showed that there is still a need to change breastfeeding culture in Ireland. In particular, it was felt that public health nurses need to be more accessible to breastfeeding mothers and breastfeeding support home visits needed to be prioritised, giving more support services that are appropriate and responsive to mothers changing needs if breastfeeding rates are to continue to improve in Ireland (Leahy-Warren, Mulcahy & Phelan 2009).

In Ireland, there has been very low adherence to the WHO (2001) infant feeding guidelines for exclusive breastfeeding and delay of the introduction of solid food until 6 months. Previous Irish research has shown that the majority of infants have been weaned before 4 months (Freeman, 1996; Twomey *et al.,* 2000; Tarrant *et al.,* 2010). Not only is the timing of solid food introduction important but the types of foods introduced can be have long term consequences for infant health. Research examining food preferences has identified a link between early exposure to certain foods and subsequent food acceptance (Mennella & Trabulsi, 2012) and suggests that the foundations for lifelong dietary habits are formed early in life (Fox *et al.,* 2006). Furthermore, children who from the earliest age have had plentiful opportunities to sample a variety of healthy foods have been found to have healthier diets throughout childhood (Cooke, 2007) and were more willing to sample new foods (Pelchat & Pliner, 1986). Food preferences at ages 2 and 4 years old have been shown to be major predictors of later food preferences at 8 years of age (Skinner *et al.,* 2002b).

This chapter presents the results of the infant feeding practices during the first 6 months of life and provides a discussion of the findings. National data in this area are lacking, as statistics regarding breastfeeding are only collected at hospital discharge (The Economic and Social

Research Institute - Health Research and Information Division, 2012). Many studies support the hypothesis that as well as exposure to flavours in the womb, flavours transferred in breast milk positively shapes infants food preferences at weaning (Hausner, Nicklaus, Issanchou, Mølgaard, & Møller, 2009; Maier *et al.,* 2008; Mennella *et al.,* 2001) and variety of foods later (Scott *et al.,* 2012). In particular, fruit consumption in the 1st year of life has been linked to sour taste preference in 18 month infants (Blossfeld *et al.,* 2007), For this reason, infant fruit experience during this time was of particular interest to this study. Exposure to fruit or fruit consumption discussed in this chapter will be used in later chapters to assess their relationship to differences in preference for sour tastes in infants. I will report on levels of breast and formula fed infants amongst the study; at birth, at 12 weeks and at 6 months. I will compare these results to the national figures recorded at hospital discharge. Since no national figures regarding breastfeeding duration are recorded in Ireland, I will compare my results to National UK figures and to results from a large Dublin based study of infant feeding practices during the first year of life (Tarrant, Younger, Sheridan-Pereira, White, & Kearney, 2009; Tarrant *et al.,* 2010). To conclude the chapter, the study will examine participants' adherence to some of the international recommendations on complementary feeding, such as the WHO (2001) and ESPHAGN (2008) recommendations.

5.2 *Methods*

The study design, recruitment, and the inclusion and exclusion criteria for the follow-up of all mothers and infant dyads are given in Chapter 2. Data used in this chapter are presented using numerical descriptive statistics including means with standard deviations and medians with interquartile ranges (IQR). Infant feeding method and number of fruits tried were collected from all mothers and are presented using percentages and actual (n) values.

5.3 *Results*

5.3.1 Infant Characteristics at Birth

Table 5-1 Infant Characteristics at 6 months

Infant Characteristics	
Gender	
-Male	54.5% (n=36)
-Female	45.5% (n=30)
Birth Weight (kg; n=65)*	3.61 (0.45) †
Birth Length (cm; n=41)*	51.8 (2.8) †
6 month Weight (kg; n=65)*	8.21 (0.91) †
6 month Length (cm; n=64)*	69.75 (3.38) ††
6 month Head Circumference (cm; n=57)*	44.73 (1.38) †
Duration of EBF (wks.; n=47)	20 (10) ††

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR). *At birth length & height was determined by health professionals in the hospital and self-reported by mothers. At 6 months weight, length & head circumference was measured by researcher during home visits.

The population characteristics of the infants in the study are described in Table 5.1. Nearly 55% of the infants were male (54.5%; n=36), while 45.5% (n=30) were female. The ethnicity of the cohort was predominantly Caucasian (97%; n=64), with two infants (3%; n=2) classified as Eurasian. The mean birth weight was 3.61 kg (±0.45), with a range of 2.75–4.48 kg. One baby was excluded from the study as he/she was born below 2.5kg as per exclusion/inclusion criteria (Section 2.4.3; pp.71). The mean birth length was 51.8 cm (±2.8), with a range of 46.0–58.0 cm. Only 62.1% (n=41) of mothers reported a birth length while the remainder (37.9%; n=25) reported that no birth height was given to them and/or recorded by the hospital. At six months, infant's mean weight was 8.21 kg (±0.91) with a range of 5.5–10.6 kg, while the median length was 69.75 cm (±3.38), with a range of 62.5–78.0 cm. There was no length recorded for two infants at 6 months due to non-co-operation, which can happen in human studies, especially those involving infants and children. The mean measurement for head circumference was 44.7 cm (±1.38; n=57). No head circumference measurement was recorded for 9 infants as no accurate measurement could be taken.

5.3.2 Infant Feeding

Table 5-2 Breastfeeding Rates at birth and 12 weeks compared to the national rates of breastfeeding at hospital discharge

Infant Feeding Method	*Subjects at Birth (n= 66)*	*Subjects at 12 weeks*	*National Level at Discharge from Hospital (2012)*
Exclusively Breastfeeding	71.2% (n=47)	59.1% (n=39)	46.6%
Partial Breastfeeding	19.7% (n=13)	18.2% (n=12)	8.6%
Formula Feeding	9.1% (n=6)	22.7% (n=15)	44.8%

Infant feeding initiation patterns are summarised in Table 5.2 accompanied by the most recent national figures. More than seventy per cent of mothers in my study initiated exclusive breastfeeding at birth (71.2%; n=47), and another 19.7% (n=13) partially breastfed. Only 9.1% (n=6) of women reported using formula milk exclusively. This is in stark contrast to the latest national figures, which reported that only 46.6% of infants were exclusively breastfed at hospital discharge, while 8.6% were partially breastfed and 44.8% received only formula milk (Figure 5.1).

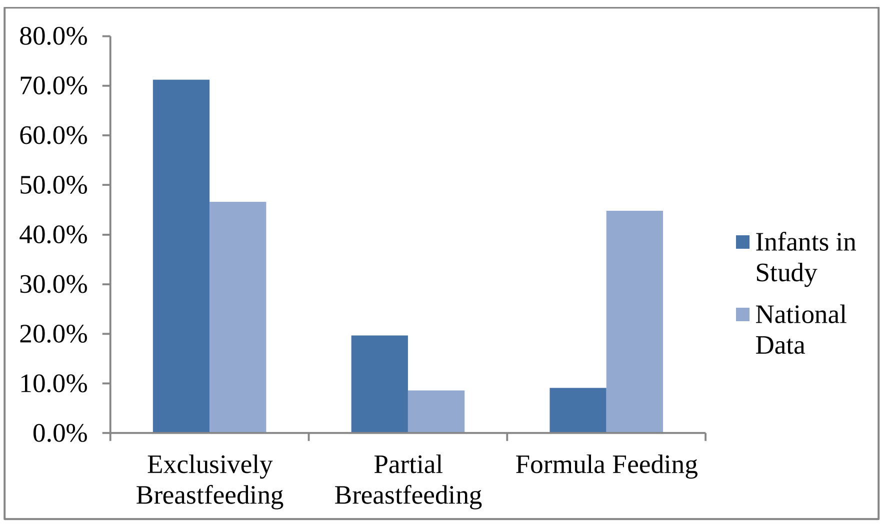


Figure 5-1 Feeding status of infants at birth compared to the latest National Data (ERSI, 2012)

Of those mothers in my study who were exclusively breastfeeding at birth, the average duration was 20 weeks (±10; Table 5.1). At twelve weeks, 59.1% (n=39) of infants were exclusively breastfed, 18.2% (n=12) were partially breastfed and 22.7% (n=15) were having formula milk only. Those mothers that had stopped exclusive breastfeeding at this point had completely ceased breastfeeding and moved their infants on to formula milk (Table 5.2).

Table 5-3 Feeding status of infants at 6 months

Feeding Status at 6 months	*Percentage of Subjects (n=66)*	*Percentage of infants (Tarrant 2008)(n=401)*
Exclusively Breastfed	3.0% (n=2)	0.2% (n=1)
Partially Breastfed: Breastfed with solid food	36.4% (n=24)	3.2% (n=13)
Partially Breastfed: Breastfed with formula milk &/or solid food	13.6% (n=9)	6.2% (n=25)
Formula fed	1.5% (n=1)	-
Formula fed with solid food	45.5% (n=30)	90% (n=361)

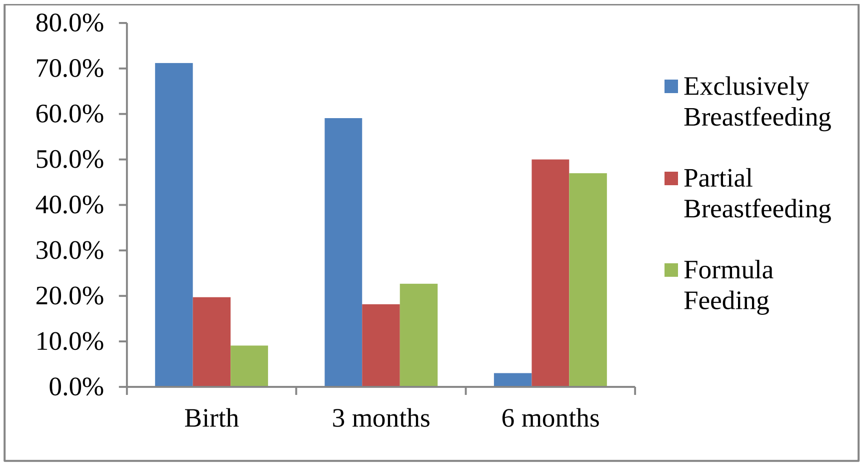


Figure 5-2 Feeding methods of the infants in this study at birth, 3 months and 6 months

At six months, only two mothers (3.0%) reported exclusively breastfeeding their infant in adherence with the WHO recommendation for infant feeding (WHO, 2001). However, there was also a significant number of women who were partially breastfeeding with solid food only (36.4%; n=24) and partially breastfeeding (13.6%; n=9) with some formula milk included with solid food. On a positive note, half of the study's infants received some breast milk (50.0%; n=31) at 6 months. This figure is higher than most other studies found (Tarrant *et al.,* 2012; Twomey *et al.,* 2000). There are no comparable national statistics for this age group; however Tarrant *et al.* (2009) examined a large sample of Dublin-born infants' feeding status at 6 months (Table 5.3) and showed that 90% of infants were receiving no breast milk and only 9.6% of infants were receiving any breast milk by 6 months.

5.3.3 Weaning

Table 5-4 Solid Food Initiation and the foods tried by the infants

Solid Food Initiation	
Age at 6 month visit (weeks; n=66)	6.3 (±0.4) †
Age starting solids (weeks; n=66)	22 (±4.0) ††
Total number of fruit tried at least once by 6 month visit (n=66)	5.5 (±3) ††
Total number of foods tried at least once by 6 month visit (n=66)	13 (±5.6) †

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR)).

The mean age of the infants at the 6-month visit was 6.3 months (±0.4). By this time point, these infants had all been introduced to solid foods. The median age for the introduction of solid foods was 22 weeks (±4.0), with a range of 16–27 weeks. The mean number of foods introduced by this visit was 13 (±5.6) with a range of 1–25 foods (Table 5.4). One infant had not been introduced to solid food until 27.3 weeks as he had an infection. This infant was met only after he started solid food. Figure 5.3 shows the percentage of infants introduced to solid foods compared to the recent study by Tarrant *et al.* (2010a). Three infants (4%) among my group were introduced to food at 16 weeks and none were introduced before this age. This is stark contrast to other studies (Tarrant *et al.,* 2010).

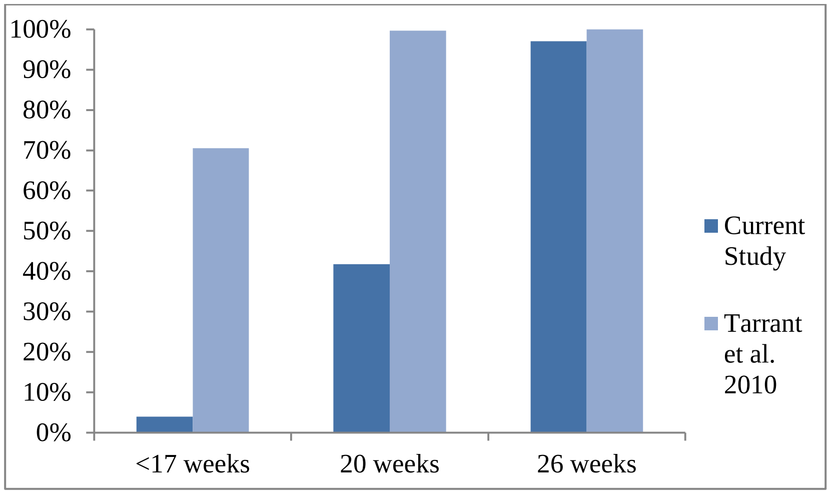


Figure 5-3 Age at which solid foods was introduced in this study and a recent Dublin based study (Tarrant *et al.*, 2010).

Our study also considered the number of fruits introduced to infants around the time of weaning. The median number of fruits introduced to infants by 6 months of age was 5.5 (±3.0). Of the fruits introduced to the infants at this time, the most common were Pear (54.5%; n=36); Apple (50%; n=33); Banana (50%; n=33); Berries (30.3%; n=20) and Mango (21.2%; n=14; Table 5.5). Commercial fruit pots were responsible for the frequent amounts of berries and mango reported by mothers.

Table 5-5 Top five fruit consumed by infants at 6 months

Fruit	*% Infants consuming it at least once*
Pear	54.5% (n=36)
Apple	50.0% (n=33)
Banana	50.0% (n=33)
Berries	30.3% (n=20)
Mango	21.2% (n=14)
Citrus	12.2% (n=8)

5.4 *Discussion*

The WHO recommends that all infants are exclusively breastfed until 6 months and that breastfeeding should continue until the baby is at least 2 years old (WHO, 2001). However, figures show that Ireland has one of the lowest rates of breastfeeding in Europe. Recent studies show that less than 50% of women were exclusively breastfeeding at discharge from hospital (Williams *et al.,* 2010; The Economic and Social Research Institute - Health Research and Information Division, 2012). In this study 71.2% of women initiated exclusive breastfeeding (n=47) and another 19.7% were partially breastfeeding at hospital discharge (n=13), which was much higher than the most recent Irish national data collected at hospital discharge (The Economic and Social Research Institute - Health Research and Information Division, 2012). The Latest National Infant Survey reports that exclusive breastfeeding is more common amongst mothers over the age of 25, with the highest proportion (50.2%) in the 30–34 year age group. Those women resident in Dublin city and county recorded figures higher than other areas, with more than 50% of mothers exclusively breastfeeding, as did women among the higher professional occupations (63.0%) compared to unemployed mothers (27.8%, Begley *et al.,* 2009). This may account for the comparatively high levels of women in my study who initiated exclusive breastfeeding and is consistent with other studies that showed higher levels of breastfeeding amongst women who are older, well-educated and living in the least deprived areas (Dyson, McCormick, & Renfrew, 2005; Gudnadottir *et al.,* 2006; Begley *et al.,* 2009; McAndrew *et al.,* 2012).

Breastfeeding duration is more difficult to compare as no national data are collected in Ireland with regard to duration of breastfeeding. In this study, of those exclusively breastfeeding, the median duration was 20 weeks (±10). At twelve weeks, the majority of mothers were still breastfeeding (Table 5.2). At six months, 50% of mothers were reportedly providing some breast milk. The most common reason stated by the women for the cessation of exclusive breastfeeding was the introduction of solid food. Studies in Ireland and the UK showed that women who initiate breastfeeding stop after a few weeks (Williams *et al.,* 2010; Tarrant *et al.,* 2011; McAndrew *et al.,* 2012). The recent Growing-Up in Ireland study showed that the mean cessation time point for breastfeeding was at 11 weeks for Irish-born mothers. Moreover, those women who were educated to Leaving Certificate level stopped breastfeeding after 10 weeks on average, while those who completed their third level education stopped breastfeeding at 14 weeks on average (Williams *et al.,* 2010). Another Irish study also found low breastfeeding initiation rates among Irish nationals (47.1%) as well as a dramatic decline in breastfeeding rates over time, with only 12.7% women exclusively breastfeeding by 12 weeks (Tarrant *et al.,* 2011). Given the demographic of my study's population, the participants' age and high level of educational attainment could account for the continued high level of exclusive breastfeeding reported at 12 weeks (59.1%; n=39). Also, given the nature of the overall study, participation bias by mothers interested in breastfeeding is a possibility, which may also account for the high breastfeeding rate at 12 weeks.

Age, education and marital status can affect both incidence and duration of breastfeeding. Studies have found that maternal age is a strong determinant of breastfeeding duration, with older women breastfeeding for longer (Kuan *et al.,* 1999; Dubois & Girard, 2003). In a review, Callen & Pinelli (2004) consistently found that married women had a higher incidence and duration of breastfeeding. Furthermore, the literature supports a strong socio-economic gradient with breastfeeding duration, with particular reference to the positive influence of higher maternal education levels (Bulk-Bunschoten *et al.,* 2001; Mikiel-Kostyra, Mazur, & Boltruszko, 2002). A recent systematic review, found that women with high education levels breastfed more often and for a longer duration (Thulier & Mercer, 2009).

In accordance with national and international weaning guidelines, the weaning process commences at six months of age approximately when the volume of milk ingested by exclusively breastfed infants becomes insufficient to meet their nutritional requirements (European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), 2008; WHO, 2001). Irish-based research undertaken prior to this study indicates that significant deficiencies existed with regard to weaning practices among mothers during the first year of life (Twomey *et al.,* 2000; Tarrant *et al.,* 2010a). The recent study by Tarrant *et al.* (2010) showed that 70.5% of infants received complementary foods before 16 weeks and that 99.7% had been introduced to solid food by 20 weeks.

Studies suggest that flavour experiences and food preferences during infancy track into childhood and adolescence (Beauchamp & Mennella, 2009; Liem & Mennella, 2002; Mennella & Beauchamp, 2002). Moreover, the timing of the first introduction of solids has been shown to be a potentially important determinant of subsequent health (Wilson *et al.,* 1998). It is, therefore, essential to research and report on the proportion of infants weaned onto solids during the first 6-months of life. The Department of Health and Children (2005) follows the WHO (2001) guidelines for complementary feeding and suggests that the introduction of solid food should be delayed until 6 months. The ESPGHAN Committee recommended that weaning should not be introduced before 17 weeks and not later than 26 weeks (European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), 2008). In my study all of the infants were weaned after this minimum recommended time of 4 months. My findings compare very favourably to previous Irish research which reported that 79% (Twomey *et al.,* 2000) and 70.5% (Tarrant *et al.,* 2010) of infants were introduced to solid food by 4 months. Data from this study, therefore, suggest that it is possible for mothers to delay weaning beyond 17 weeks, with no negative impacts.

The median age for commencing complementary foods in this study was 22 weeks (±4.0), Tarrant *et al.* (2010) found that 99.7% of infants had been weaned by 20 weeks. Previous research suggests that greater compliance with weaning guidelines is observed in mothers who attempt to initiate breastfeeding (Tarrant *et al.,* 2010). Recent work by the Growing-up in Ireland Study has found that mothers with the highest education levels (degree level) introduce solid foods later (20.1 weeks) when compared with those who have lower levels of education (secondary level - 17.8 weeks) (Williams *et al.,* 2010) and may account for the later introduction of solid food seen among participants in this study. While only two mothers in my study followed the WHO (2001) guidelines and exclusively breastfed until 6 months, 53% of infants (n=35) were still receiving some breast milk. The delayed introduction of solid foods until 6 months occurred with six infants (9.1%), two being exclusively fed, while three infants were receiving a combination of breast and formula and one was receiving formula only up to this point. Infants who were not breastfed at 6 months were consuming infant formula milk and none of the mothers reported the use of cow's milk as a sole milk source.

5.5 *Strengths & Limitations*

The positive features of this study included the strict use of breastfeeding definitions and the fact that one investigator conducted the interviews with mothers, which reduced any inter-observer variation that may have occurred had a team of investigators been involved. While there was an initial fall-off in participation from initial contact during pregnancy to post-pregnancy follow up at 12 weeks (22.3%), all of the mothers contacted at 12 weeks continued to participate at 6 months. However, since the sample size was relatively small, and composed mainly of older, well-educated, married women, it was not representative of a national sample of mothers. Therefore, results cannot be generalised to the general population of pregnant women.

Parents of breastfed infants, when compared to the parents of formula fed infants, have been shown to have more positive attitudes towards breastfeeding and to be more knowledgeable about the health benefits and nutritional superiority of breastfeeding (Shaker, Scott, & Reid, 2004; Sloan, Sneddon, Stewart, & Iwaniec, 2006; Ward, 2005). Given the subject matter of this study – nutrition and infant feeding, mothers that are interested in nutrition and health may also be more likely to breastfeed their infants and would be more likely to participate in this study. Therefore, there is a strong possibility that the high breastfeeding rates observed may be specific and localised to mothers involved in this study. Long-term recall of breastfeeding data has been found to be inaccurate (Bland *et al.,* 2003) and it is possible that the reporting of the feeding status of some infants was influenced by maternal memory bias. However, the accuracy of the feeding status at 12 weeks and 6 months can be assured since the investigator contacted all of the mothers by telephone at 12 weeks and met with all mothers at 6 months.

5.6 *Conclusion*

Our results suggest that national efforts to promote breastfeeding for the first six months in have been somewhat successful, at least for the mothers recruited in this study However, public health policy makers in Ireland may need to evaluate the feasibility of promoting the WHO (2001) recommendations for all infants in Ireland. Whilst the majority of women in this study initiated breastfeeding and all of them meet the minimum time period for the introduction of solid foods, only three mothers were exclusively breastfeeding at 6-months. This indicates an extremely low compliance with the WHO (2001) recommendation. However, 50% of infants were still receiving some breast milk at this stage. Virtually all health care professionals would agree that any breastfeeding is better than none and that an infant who is combination fed will still reap many benefits from breast milk. Given the high success among this cohort of older, well educated working women in initiating breastfeeding, perhaps future public health campaigns may need to target messages about the duration of breastfeeding and weaning guidelines, while campaigns targeting younger and less educated women may need to emphasize the benefits of any breast milk to help initiation rates. For this sample of infants who are growing up during a period of increasing obesity prevalence, aggressive public health interventions should be considered, targeting the first year of life as a primary priority.

CHAPTER 6. THE DIETS OF A POPULATION OF 12-MONTH IRISH INFANTS

6.1 *Introduction*

It is well recognised that the period from conception to two years has far-reaching effects on health that impact from early childhood to adult life (Ravelli *et al.,* 1998; Godfrey & Barker, 2001; Barker, 2012). The spiralling prevalence of childhood obesity, now known to affect toddlers (WHO, 2012), has its origins in poor infant feeding practices (Owen *et al.,* 2005). In addition, later health issues such as diabetes, obesity and heart disease, may be partly due to the inadequacy of the mother's diet during pregnancy and may be also due to how they were fed as babies, particularly during the first year of life (Barker, 1997; Roseboom *et al.,* 2000; Koletzko, *et al.,* 2009). It is therefore important to understand what young children are eating.

During the first year of life, babies triple their birth weight and double their surface area, making this a period of very rapid growth which is never repeated during the lifecycle. At this time, infants' nutrient requirements in relation to their body weight are also greater when compared to adults. For example, at 12 months of age, an infant's protein requirements are double, vitamin C requirements are five-fold and iron requirements 6.5 fold those of an adult on a per kilogram the body weight basis (Department of Health, 1991). These high requirements mean that children of this age are particularly vulnerable to nutritional deficiency.

This chapter presents the dietary intakes of 12 month old infants living in Ireland and provides a discussion of the findings. Average daily intakes of total energy were compared to age and sex specific UK reference values taken from the Scientific Advisory Committee on Nutrition (SACN) energy report by calculating the Estimated Average Requirement (EAR) for each child in the study based on body weight (SACN, 2012). The median physical activity level (PAL) value was adjusted for growth. Macronutrient and micronutrient intakes for infants were compared to the 1991 COMA report on Dietary Reference Values for Food Energy and Nutrients for the United Kingdom (Department of Health, 1991), the 1999 Recommended Dietary Allowances for Ireland (Food Safety Authority of Ireland, 1999) and with two large studies viz. the recent Irish National Pre-School Nutrition Survey (NPNS) (Walton, 2012) and the UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC), 2011 (Lennox *et al.,* 2013). The percentage of participants consuming more than the EAR was examined to determine the nutritional adequacy of this group's diet. To conclude the chapter, the study considered the participants' adherence to some of the Irish Dietary Guidelines for infants such as those for fruit and vegetables and dairy produce. These foods provide many of the essential vitamins and minerals needed for healthy growth (Food Safety Authority of Ireland, 2011).

6.2 *Methods*

The study design and data collection for the follow-up of all 12-month old infants is discussed in detail in Chapter 2. Mothers completed a 3-day, semi-quantitative food diary when the infant was approximately 12 months of age (n=55). Mothers were asked to keep these food diaries during two typical weekdays and one typical weekend day. Data used in this chapter are presented using numerical descriptive statistics; including means with standard deviations and medians with interquartile ranges (IQR). Daily intakes of fruit and vegetable, dairy products and fish are presented using percentages and actual (n) values.

6.3 *Results*

6.3.1 Population Characteristics

Table 6-1 Infant Characteristics

Infant Characteristics	
Gender	
-Male	54.5% (n=30)
-Female	45.5% (n=25)
12 month Weight (kg; n=55)*	10.7 (±1.9) ††
12 month Length (cm; n=55)*	79.0 (±4.5) †
12 month Head Circumference (cm; n=50)*	47.8 (±1.95) ††
% of infants receiving any breast milk at 12 months	27% (n=15)

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR)

* At 12 months weight, length & head circumference was measured by researcher during home visits.

The population characteristics of the infants at 12 months of age are described in Table 6.1. The median weight and head circumference was 10.7 kg (±1.9) and 47.8 cm (±1.95) respectively. The median infant length was 79 cm (±4.5). When compared with the UK-WHO Growth Standard (RCPCH/WHO/Department of Health, 2013) for their age and sex, 90% of boys and 83.8% of girls were above the 50th percentile for length; 86.6% of boys and 75% for girls were above the 50th percentile for weight and 100% of boys and 92% of girls were above the 50th percentile for head circumference measurements. More than a quarter (27%) of infants were still receiving some breast milk at this age.

6.3.2 Daily Intakes of Macronutrients of 12-month old Infants

SPSS (IBM SPSS, 2010) Version 19.0 was used for all statistical analyses. Data were tested for normality and are presented using numerical descriptive statistics including means with standard deviations (SD) and medians with interquartile ranges (IQR). As there was no statistical differences in the macronutrient intakes of males and females (p>0.05), results were combined for the group. Infant feeding methods, which were collected from all mothers and confirmed using the food record, are presented using percentages and actual (n) values.

Table 6-2 Mean daily macronutrient intakes based on food dairies (n=55) in comparison to the results of infants aged 12 months from the National Pre-School Nutrition Survey (NPNS, 2012)

Nutrient	*Study Participant*	*NPNS 2010*	*DNSIYC 2011*
Energy– MJ/day ††	4.3 (±0.8)	4.2 (±0.9)	4.07 (±0.87)
Energy- Kcal ††	1020 (±190)	1005 (±222)	967 (±208)
Protein- g ††	35.6 (±11.85)	39.2 (±10.3)	37.7 (±10.2)
Carbohydrate- g †	137.21 (±20.95)	126.3 (±31.7)	126 (±29)
Total sugars –g †	71.86 (±16.15)	69.6 (±21.8)	66 (±18.6)
NMES-g ††	2.7 (±4.68)	43.4 (±19.9)**	19.8 (±12.1)**
Total Fat- g†	43.5 (±7.4)	38.1 (±11.0)	38.2 (±10.6)
Sat fat-g†	19.62 (±4.93)	17.7 (±5.8)	17.5 (±5.8)
Fibre- g††	9.06 (3.98)	10.5 (±3.7)	7.3 (±2.7)

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR). ** Difference assessed by Wilcoxon sign-rank test (Statistically significant at *P*<0.05)

The approximate mean intakes of macronutrients for the study population are presented in Table 6.2 and compared to data obtained from the NPNS (Walton, 2012) and DNSIYC (Lennox *et al.,* 2013). The mean intakes for the study participants for energy, protein, carbohydrate and fat were 1020 kcal (±190), 35.6 g (±11.85), 137.2 g (±20.95) and 43.5 g (±7.4) respectively (Table 6.2). These results for energy intake are comparable with the results from the NPNS which reported a mean daily food energy intake of 1005 kcal/day (Walton, 2012) and were slightly higher than the DNSIYC results, which reported a mean daily food energy intake of 967 kcal. Non-milk extrinsic sugar intake was significantly lower among the study group 2.7 g (±4.68) compared to the NPNS 2010 43.4 g (±19.9) (Walton, 2012), and the DNSIYC 2011, 19.8 g (±12.1) (Lennox *et al.,* 2013).

Table 6-3 The percentage contribution of each macronutrient to food energy for the group compared to one year old results from the National Pre-School Nutrition Survey (Walton, 2012) and 12-18 month olds from the DNSIYC 2011 (Lennox *et al.,* 2013)

Nutrient	*Participants*	*NPNS 2010*	*DNSIYC 2011*
Protein	14%	16%	16%
Carbohydrate	52%	50%	49%
Fat	37%	34%	35%
Sat Fat	17%	16%	16%

*Some figures do not add up to 100% due to rounding errors in calorie conversion.

The percentage contribution of each macronutrient to total food energy was calculated and compared to the recent national pre-school nutrition survey (Walton, 2012). Among study participants, the percentage contribution to food energy from carbohydrates was 52%, which was consistent with the above study. Participants consumed approximately 14% of their energy from protein, which was marginally lower than the reported intakes from the national surveys (15%; NPNS; 16% DNSIYC). Among the study participants, total fat provided 37% of food energy, with a range of intakes between 25% and 46%. The average contribution to total energy intake from saturated fat reported in this study was 17%, which was similar to the NPNS and DNSIYC figure of 16 % (Walton, 2012; Lennox *et al.,* 2013).

Table 6-4 Comparisons of the median daily intake of energy (IQR) from the infants to recommendations within the SACN energy report 2012.

Gender	*Study Median EAR*	*% exceeding EAR*	*% exceeding EAR DNSIYC 2011*
Males	3.57 (±0.43)	86.7% (n=26)	88% (n=27)
Females	3.39 (±0.76)	72.0% (n=18)	88% (n=22)

Calculation based on body weight and age in years. Intakes are compared to the physical activity level (PAL) adjusted for growth for boys and girls (SACN, 2012).

The average daily intakes of total energy were also compared to age and sex specific UK reference values taken from the Scientific Advisory Committee on Nutrition (SACN, 2012) for energy. The percentage of infants calculated as the EAR for energy was similar for boys and girls, at approximately 87% and 72% respectively (Table 6.4). It should be noted that 50% of the population are expected to have requirements exceeding the EAR.

6.3.3 Daily Intakes of Micronutrients

Table 6-5 Mean daily micronutrient intakes based on food dairies (n=66) in comparison to the results of one year olds in the Irish NPNS (Walton, 2012); 12-18 month old in the UK DNSIYC 2010 (Lennox *et al.,* 2013) and well as the UK EAR for 1-3year olds in the UK (DoH 1991) and the Irish RDA for 1-3 year olds (FSAI 1999).

Nutrients	*Study Results*	*UK EAR*	*Irish RDA*	*NPNS 2010*	*DNSIYC 2011*
Sodium (mg)***	1031 (±408)†	-	-	918 (±341)	907 (±348)
Potassium (mg)	1752 (±312)†	-	800	1716 (±435)	1599 (±437)
Magnesium (mg)	144 (±27)†	65	80	143 (±38)	135 (±37)
Iron (mg)	10.1 (±4.7)‡	5.3	8	7.0 (±3.0)	6.4 (±2.7)
Zinc (mg	5.4 (±0.8)†	3.8	4	5.4 (±1.8)	5.4 (±1.6)
Calcium (mg)	809 (±175)†	275	800	840 (±297)	790 (±260)
Vitamin D (μg)	2.5 (±5.2) ‡	-	10	4.2 (±5.2)	2.6 (±2.8)* 3.9 (±3.9)**
Thiamin (mg)	0.93 (±0.3)†	0.3	-	1.0 (±0.4)	0.85 (±0.26)
Riboflavin (mg)	1.4 (±0.4)†	0.5	0.8	1.6 (±0.7)	1.49 (±0.53)
Niacin (mg)	9.7 (±5.6)‡	-	-	10 (±4.5)	16.4 ± (4.6)
Vitamin B6 (mg)	1.0 (±0.3)†	-	-	1.2 (±0.6)	1.1 (±0.4)
Vitamin B12 (μg)	2.9 (±2.1)‡	0.4	0.7	4.1 (±2.0)	3.7 (±1.7)
Folate (μg)	143 (±32)†	50	100	159 (±80)	144 (±41)
Vitamin C (mg)	71 (±49)‡	20	45	75 (±44)	62.5 (±34.5)
Copper (mg)	0.5 (±0.2)‡	-	0.4	0.5 (±0.2)	0.5 (±0.2)

†Normally distributed data presented as Mean (SD). †† Skewed data presented as Median (IQR). *Vitamin D intake does not include values for breastfed children as the vitamin D content of breast milk is not known. **Vitamin D intake including values for breastfed children excluding the contribution from breast milk as it is unknown. ***Underestimate of total sodium intake as sodium from discretionary salt added in cooking or at the table is excluded.

The approximate mean intakes of the main micronutrients for the study population are reported above (Table 6.5). As there was no statistical differences in the micronutrient intakes of males and females (p>0.05), results were combined. Adequacy of intake of vitamins and minerals were assessed by determining the percentage of infants with intakes below the UK Estimated Average Requirements (EAR) for the selected nutrients (FSAI, 1999). Intakes of most vitamins and minerals were adequate – as indicated by the low percentage of infants with intakes below the EAR. None of the participants had intakes below the UK EARs for calcium, magnesium, thiamin, riboflavin, vitamin B12 or C. Only one person (1.8%) had an intake below the EAR for zinc and four infants (7.1%) had an intake below the EAR for iron. My data were also consistent with the recent NPNS (Walton, 2012) and DNSIYC (Lennox *et al.,* 2013) studies with regard to all micronutrients except iron, which was higher among the study group, and vitamin D, which was lower (2.5 μg) than that reported in the national studies (4.2 μg– NPNS 2010; 3.9 μg –DNSIYC 2011). In the absence of a general consensus on the EAR for vitamin D, the percentage of children with intakes of less than 5 μg and 1 μg were determined. A majority of infants (67.3%; n=37) had intakes below 5 μg. It was of particular concern that 45.5% (n=25) had intakes below 1 μg.

6.3.4 Under-reporting

Under-reporting of food consumption is known to be a problem in all dietary surveys, although it is generally considered to be less of an issue for younger children than adults. It is not known to what extent it is a problem for this age group. By comparing the infants' Basal Metabolic Rates (BMR), with estimated cut-offs, to their reported energy intake, I identified one case where definite underreporting of energy intake took place and this infant was excluded from the analysis. However, it should be noted that this figure of under-reporting is much lower than other studies (Lioret *et al.,* 2011).

6.4 *Discussion*

The WHO recommends that breastfeeding should continue until the baby is at least 2 years old (WHO, 2001). Given the low numbers initiating breastfeeding in Ireland (The Economic and Social Research Institute - Health Research and Information Division, 2012) and the high dropout rate in the early weeks (Tarrant *et al.,* 2009), breastfeeding at 6 months is relatively unusual (Twomey *et al.,* 2000; Tarrant *et al.,* 2008), while 12 months is extremely rare. No studies in Ireland have looked at rates of breastfeeding among this age group nationally. In this study 25% of infants still received some breast milk at 12 months, which compares very favourably to the UK DNSIYC, where only 8% of infants were still receiving some breast milk at ages 12–18 months. This result is unsurprising, given the relative high rates of breast feeding among my study population at 6 months (Chapter 5).

6.4.1 Macronutrient Intake

Milk/formula was the most important source of energy (31.5%) in my study. This result is similar to the NPNS (Walton, 2012), in which it was found that milk/formula provided 29% of total energy. Other important sources of energy were meat, bread, breakfast cereals, fruit & fruit juices, biscuits & cakes and yoghurt. Infants' intakes of protein, carbohydrate and fat, accounted for 14.2%, 52%, and 37% of total energy intake. The main sources of carbohydrates in the diet were milk/formula, fruit and breakfast cereals, while the main sources of fat in the diet were milk/formula and meat. The current advice for adults and children over 5 years is to consume a diet in which about 35% of their daily energy needs are provided by the fat in food. This recommendation does not apply to children under the age of five, due to the importance of dietary fat as a source of fat-soluble vitamins, essential fatty acids and energy for this age group while consuming a manageable volume of food. ESPGHAN (2008) recommends that fat should not fall below 25% of total energy intake for this age group. In this study, none of the infants reported intakes below 25% (Agostoni *et al.,* 2008).

Given the high percentage of infants in this study who were still receiving some breast milk, these results may underestimate intakes of fat and energy. Mandel *et al.* (2005) showed that mothers, who have been lactating for more than one year, had human milk with significantly increased fat and energy contents, compared with milk expressed by women who have been lactating for shorter periods. While there is no current recommendation for the percentage of dietary energy which should come from saturated fat in infants, there is a fine balance between consuming sufficient fat and consuming excess intakes of saturated fat.

High levels of high fat, high sugar foods should be discouraged to help reduced the risk of overweight, obesity and tooth decay. In this study, nearly 5% of energy came from foods on the top shelf of the food pyramid – those high in sugar and fat e.g. cakes, chocolate and biscuits. Similarly, the NPNS 2010 found that 6% of 12 months olds' energy intake came from cakes and confectionary (Walton, 2012). These results are of concern, since Skinner and colleagues (2001) found that food preferences did not change from toddlers to school-aged children and demonstrated how important early exposure to food is with regard to the formation of eating habits later in life. It is suggested that flavour preferences learned early in life not only affect taste preferences in the short term (Mennella *et al.,* 2001) but may also be important for taste preference in the longer term (Mennella & Beauchamp, 1996).

The UK RNI for protein for 1–3 year olds is 14.5 g/day (Department of Health, 1991). In my study the median intake was 35.6 g/day, more the twice the RNI requirements. Participants consumed approximately 14% of their energy from protein, which was marginally lower than the reported intakes from the national surveys (15.2%; NPNS and 15.6% DNSIYC). While these figures are lower than the national figure, it may be of concern, since Gunther *et al.* (2007), found that a higher intake of animal protein, especially dairy, at 1 year of age was associated with an unfavourable body composition (higher BMI and relative fat mass) at 7 years of age. Other studies have associated high protein intakes in infancy with rapid growth velocity, which may have adverse effects in later life (Rolland-Cachera *et al.,* 1995; Ong & Loos, 2006; Koletzko, von Kries, Closa *et al.,* 2009).

Dietary fibre as part of a varied, balanced diet is essential for good health at all ages. However, no specific dietary recommendations for fibre are given for children aged less than 2 years, as these foods should not be encouraged at the expense of more energy rich foods which are required to adequate growth. Among my group, the median intake of fibre was 9.1 g/day (±3.98), which was slightly lower than the Irish NPNS study (10.5 g/day) but higher than the UK DNSIYC study (7.3 g/day, ±2.7) (Walton, 2012; Lennox *et al.,* 2013).

6.4.2 Micronutrient Intake

Intakes of most vitamins and minerals were adequate, as indicated by the small proportion of infants with intakes below the EAR. It should be noted that 50% of the population are expected to have requirements below the EAR. The general adequacy of these infants' diets was to be expected, given the parent's socioeconomic status. They were older, college-educated mothers from higher socio-economic groups and had the resources to provide the necessary foods to meet the nutrient needs of a growing child. The diets of this cohort greatly exceeded the RDAs for this age group. For example, the average intakes for magnesium, iron, folate and vitamin C were 180%, 126%, 143% and 157% above the RDA respectively. These results may be due to the fact that a third of infants (n=22) were drinking formula milk or human milk (n=15) as their main drink at 12 months. These drinks have higher levels of iron compared to whole cow's milk, which 27% (n=15) had as their main drink at 12 months. The two micronutrients of most concern among this cohort were sodium and vitamin D.

6.4.2.1 Sodium

The FSAI recommends an intake of no more than 6 g salt per day for adults. However, in reality, Irish intakes are approximately 75% higher than this (Giltinan *et al.,* 2011). The Dietary Reference Values (DRV) set for sodium are based on physiological requirements for infants, with the RNI set at 500 mg/day for this age group (DoH, 1991). The FSAI recommends less than 800mg (2 g salt) for children aged 1 to 3 years. Mean sodium intake for the infants in the study was 1031 mg/day, more than twice the RNI and exceeding the recommendation for this age group, with nearly three quarters of infants (74.5%; n=41) exceeding the 2 g salt/day recommendation. The mean intake of salt was 2.6 g salt/day with the NPNS study also having higher than recommended levels of salt at 2.3 g/day (Walton, 2012). Of concern is that only 9.1% (n=6) infants had intakes below the RNI, while 54.6% of infants had intakes that were twice the RNI (FSAI, 1999). Early salt intakes may have persistent long-term effects on blood pressure independent of salt intakes later in life (Strazzullo, Campanozzi, & Avallone, 2012). A randomised controlled trial of infants in the first 6 months of life reported that those in the lower sodium group had systolic blood pressure which was significantly lower than those who had received the higher sodium diet at 15 years follow up and that this was independent of their current sodium intakes (Geleijnse *et al.,* 1997). The results in the present study may also be underreported, as possible discretionary salt added at the table and during cooking was not considered in the analysis. Many parents reported giving their child processed meats such as ham or sausages over the recording period, which would be high in salt. Also, four mothers reported using gravy and/or stock cubes during this time when feeding their infants. These foods are high in salt and are discouraged for this age group (FSAI, 2012).

6.4.2.2 Vitamin D

Although, in general, my cohort's diets were nutritionally adequate, the study identified several potential problems with vitamin D intake with 45.5% of infants having intakes of less than 1 μg per day. Dietary vitamin D is important for the deposition of calcium in the skeleton as it is unlikely that infants can depend on exposure to sunlight for vitamin D synthesis. Given their levels of education, the mothers in this study were most likely aware of the fortification of milk with vitamin D. However, it is unclear as to whether they knew that most other dairy foods are not fortified. Cheese and yoghurt, which provide calcium but not vitamin D, were frequently served to 12 month old infants, providing approximately 8% of total energy intake. Although the calcium intake of the group met the RDA, consumption of vitamin D did not, and both nutrients are essential for normal bone development. However, the vitamin D content of breast milk is not known and, for this reason, recorded infant consumption may be underestimated. It is difficult to get enough vitamin D through food alone, while the other main source of vitamin D is direct sunlight on the skin, although this will vary by the degree of exposure of the infants' skin to summer sunshine and sun exposure is not advisable for infants. Only one infant was given a vitamin D supplement during this time. This low level of supplementation may have come about as the FSAI (2011) recommendation of routine supplementation of vitamin D to all infants under the age of 12 months was published during the study and some of the babies would have reached 12 months by that stage.

6.5 *Strengths & Limitations*

A particular strength of this study was the implementation of a 3-day food diary to estimate food intakes of the infants. This prospective method reduces the possibility of recall bias. Among infants, measurement of food intake has been recognised as a difficult task as researchers have to rely on parents' reports during a time when feeding may be stressful (Lanigan, Wells, Lawson, Cole, & Lucas, 2004). Currently there is a lack of research evaluating methods of dietary intake in infants and young children. Four general dietary assessments methods have been reported in infant research: 24-hour dietary recall, food diary, diet history and food frequency questionnaire. To date, most studies have used 24-hour recall (Skinner *et al.,* 1997; Nolan *et al.*, 2001; Devaney *et al.,* 2004), as it is less time consuming for participants than other dietary assessment methods. However, an infant's food intake can vary considerably from day to day and a single 24-hour dietary recall may not account for daily variation in food choices. For this reason, assessments of an individual's dietary habit by considering only one day can prove problematic. To overcome this limitation, the current study utilised a 3- day semi-quantitative dietary diary at 12 months to assess infants' diets. The infants had a 3-day food diary as opposed to a seven day as previous research of infants' diet has shown that between-subject variability in dietary intake is greater than within-subject variability, therefore fewer days are required to assess dietary intake in infants (Lanigan *et al.,* 2004). The 3-day food diary in the present thesis proved sufficient to assess between subject variations for the study's infants. The potential for some mis-reporting needs to be borne in mind when interpreting findings from this survey. However, based on BMR and Goldberg cut-offs (Goldberg *et al.,* 1991), only one participant was considered to be definitively underreporting and was excluded from the analysis. This may be due to the fact that my participants had an interest in nutrition as well as the subject matter of the overall study and therefore may have been more motivated to accurately record their infants' diets. They were also advised that they could receive feedback on their infants' diets if they wished after the study was completed, which may have motivated them to be more accurate. Another limitation of the dietary data is that breast milk cannot be precisely measured, but the volume rules, I applied, are based on the available peer-reviewed articles from highly industrialized countries (Dewey *et al.,* 1984; Kent *et al.,* 1999; Briefel *et al.,* 2010).

6.6 *Conclusion*

Generally, healthy infants can achieve recommended intakes of micronutrients from food alone; with the exception of vitamin D. Dietitians and other Healthcare Professionals should encourage caregivers to use foods rather than supplements as the primary source of nutrients in children's diets. However, some concern still arises with regard to the infants' low intakes of vitamin D and high intakes of energy, protein and salt. Further research is needed to look at supplementation levels of vitamin D in infants in Ireland to ascertain if the new guidance for vitamin D supplementation is being followed and if it is adequate for this group. Further education regarding the possible long-term effects of high intakes of salt and protein in infant diets and the high levels of salt in such foods as ham, processed meats, breakfast cereals and breads is needed among parents of this age group.

CHAPTER 7. INFANTS' SOUR TASTE ACCEPTANCE AT SIX & TWELVE MONTHS OF LIFE

7.1 *Introduction*

While infants are born with an innate preference for sweet tastes and an aversion to sour and bitter tastes (Steiner, 1977; LeCanuet & Schaal, 2002), exposure to fruits in the third trimester of pregnancy, via the mother's diet or through breast feeding is thought influence the acceptance of sour taste in infants. Mennella *et al.* (2001) have shown that infants whose mothers took carrot juice daily in the third trimester of pregnancy or during lactation were more willing to accept carrot flavoured cereals. After birth, breast milk continues to supply a great deal of sensory information with regard to the types of food consumed in the mothers' diet. Exposure to different flavours is thought to enhance acceptance of these foods during the weaning period (Mennella *et al.,* 2005). Liem & Mennella (2002) and Mennella & Beauchamp (2002) have shown that infants exposed to sour tasting hydrolysed protein formula during early infancy could overcome their innate rejection of sour tastes. Moreover, Blossfeld *et al.* (2007) found that sour taste acceptance in toddlers (18 months) was linked to earlier fruit consumption patterns at 6 and 12 months, which supports the hypothesis that early exposure to fruit can influence sour taste acceptance.

Due to infants' inability to communicate verbally, researchers have to rely on indirect measures of preference and acceptance (Guinard, 2000). Like adults, infants control their facial muscles to express primary emotions and are, therefore, well equipped to convey a wide range of emotional states in response to pleasant and unpleasant tastes (Ekman & Oster, 1979). Throughout evolution it is thought that non-verbal signals are given by infants to warn caregivers that the infants may be eating something harmful (Babchuk, Hames, & Thompson, 1985). However, such reactions to sour flavours may discourage mothers from feeding certain foods to their children. Taste preference and acceptance in infants' and toddlers are usually studied using behavioural measures like facial expression (Ganchrow *et al.,* 1983; Rosenstein & Oster, 1988), suckling patterns (Crook & Lipsitt, 1976) or intake quantities (Blossfeld *et al.,* 2007; Crystal & Berstein, 1998; Schwartz, Issanchou, & Nicklaus, 2009). The development of sour taste preference in infants has been explored previously, using Ingestion Ratios (IR) (Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009) and a Liking Ratio (LR), the experimenter's judgment of the infant's liking, using a 5 point scale, at low concentration levels (Schwartz, Issanchou, & Nicklaus, 2009). Schwartz *et al.* (2009) found that by using IR, infants at 3 months rejected sour tastes but were indifferent to them at 6 and 12 months. In contrast, using LR, they found that sour tastes were rejected at all ages – at 3, 6 and 12 months.

Given that there is no gold standard approach to assess taste acceptance in infants, it is difficult to compare results and different methods may pick up on different cues regarding taste and therefore produce differing results. For this reason it may be necessary to use a combination of methods. Facial responses at the beginning of feeding are thought to be reflective of hedonic responses in animals (Berridge, 1996) and pre-verbal human infants (Mennella *et al.,* 2001; Rosenstein & Oster, 1988; Soussignan *et al.,* 1997; Steiner, 1977). Facial expressions of distaste have been shown to be more discriminating than facial expressions of liking (e.g., smiling) in gauging infants' hedonic responsiveness (Forestell & Mennella, 2007; Mennella *et al.,* 2001; Mennella & Beauchamp, 2009). For the purposes of this study, I used a Rater's Liking Ratio (R-LR), using video analysis, in conjunction with techniques that assess mother's rating score (M-LR) and the amount consumed by the infant (IR) to assess if infants' sour taste acceptance can be linked to exposure to fruit or other factors. This thesis will seek to determine the degree of acceptance of sour taste in my cohort at 6 and 12 months and determine if changes occur over this period. Exposure to fruit early in life will also be examined to determine whether this increases acceptance of sour tastes during infancy and whether other factors are associated with this.

7.2 ***Methods***

The methods used in this chapter are discussed in detail in Chapter 2. In order to test the infants' acceptance of varying levels of sourness, infants were presented with 4 test solutions, with varying citric acid concentrations (0.00M; 0.013M; 0.029M; 0.065M). The infants' feeding sessions were recorded using a video camera, which was placed approximately 2 metres in front of the Mother-infant dyad so that the video image contained the face and upper torso of the infant as much as possible. Feeding sessions ended when the infants had been feeding for 1 minute or rejected at least three consecutive offers of the solution, as decided by the experimenter. Immediately after each drink, mothers were asked to rate their infant's reaction to the solution using a Likert scale of perceived like/dislike (Appendix I); higher numbers reflecting greater liking. This was used to calculate the Mother's Liking Rating (M-LR) score. The infants' total intake of each solution was calculated by weighing each container before and after presenting it to the infant and was used to calculate the Ingestion Rating (IR) score.

The videotapes were then viewed to determine the length of feed and the frequency of the infant's negative facial expressions (e.g. nose wrinkling, brow lowering, upper lip raising, gaping, head turning) when they were tasting the different drinks. This was used to assess the Rater's Liking Ratio (R-LR). Video analysis involved slowing the video down and coding it frame by frame for non-verbal cues including; 1) facial expressions; 2) hand pushing; 3) head turning and 4) duration of drinking. The data were independently analysed and were revisited several times. A score was calculated taking into account the length of time the infant was drinking and the number of negative and positive non-verbal cues the infant exhibited. Section 2.8.3 discusses of Sour Taste Acceptance scores in detail (pp. 99–100). A value equal to 0.5 indicated indifference to the solution, while a value greater than 0.5 showed acceptance and a value less than 0.5 showed rejection of the solution.

SPSS (IBM SPSS, 2010) Version 19.0) was used for all statistical analyses. Initial exploration of the data was carried out prior to analysis, in order to check for accuracy of data input, the amount of missing values, the presence of univariate outliers, and to ensure goodness of fit between the distribution of study variables and the assumptions of multiple regression, following guidelines set out by Tabachnick and Fidell (2007). Any potential outliers for each variable were identified by looking for standardized scores in excess of ± 3.29. Frequency histograms were examined to identify any variables that deviated from normality. Skewness, Kurtosis, and Kolmogorov-Smirnov values were also examined as further indications of non-normal distribution. Variables that were not normally distributed in the sample and/or had outliers that exceeded the ± 3.29 cut-off score were transformed to better meet the assumptions of multiple regression (Table 7.1). No outliers were identified in the data and as a result, no cases were removed from the analyses.

Table 7.1 Transformations performed on non-normally distributed variables at each time point.

Variable	6 months	12 months
R-LR 4	square root	-
IR 3	square root	-
IR 4	-	square root
M-IR3	square root	square root
M-IR4	-	square root

R-LR- Rater's Liking Ratio, IR- Ingestion Ratio; M-LR- Mother's Liking Ratio

Multiple regression output was also examined for normality, linearity and multicollinearity. An inspection of the residual histograms and scatterplots for each regression analysis revealed that none of these assumptions appeared to be violated. All variance inflation factors (VIF) were less than 10 and tolerance values were greater than 0.50, indicating that there was no multicollinearity in the data (Tabachnick & Fidell, 2007). Durbin-Watson scores were between 1 and 3 in value, suggesting that the assumption of independent errors was met (Field, 2009). Using a p <0.001 criterion for Mahalanobis distance, no multivariate outliers were identified in the data and all Cook's distances were less than 1, suggesting that no individual cases were influencing the models being tested (Stevens, 2002). As a result, no cases were removed from the analyses.

7.3 *Results*

7.3.1 Subject Characteristics

The population characteristics of the infants at 6 months are described in Chapter 5 (Table 5.1; pp. 160) and at 12 months are described in Chapter 6 (Table 6.1 – pp. 177). Of the sixty-seven infants who participated in the sour taste acceptance assessment at 6 months, 4 were excluded from the analysis as they did not comply with the experimental procedures and refused to sample the different solutions on three separate occasions. At 12 months, out of the fifty-five infants who participated in the sour assessment, five were excluded from analysis as they did not comply with the experimental protocol after several attempts.

Descriptive statistics were calculated for all predictor and outcome variables at 6 and 12 months, and are displayed in Appendix M & N respectively. Preliminary ANOVAs were performed to assess whether there were differences in stimuli concentration order in outcome variables (Rater's Liking Ratio -R-LR; Ingestion Ratio -IR; Mother's Liking Ratio -M-LR). As the analysis did not reveal any significant influence, data were combined for further analyses. Furthermore, no correlations were observed between birth weight, birth height, weight, height and age at testing time points and infants' taste acceptance variables. Given a lack of any identified correlation, infants were combined across all these variables for further analysis.

7.3.2 Infants can Distinguish Sour Tastes at Six and Twelve Months.

A one-way repeated measure ANOVA was conducted to compare sour taste preference over the three concentrations of citric acid at 6 and 12 months. The mean concentration was plotted for each variable (Figures 7.1 & 7.2). At 6 months a significant decrease in mean sour taste acceptance (Figure 7.1) was seen across all concentrations for each outcome variable, suggesting mean acceptance decreased significantly with each increasing concentration (as reported below ($p<0.0001$).). Using Cohen (1988) guidelines, these results suggest a very large effect using all methods**.** These results suggest that increasing critic acid concentration does significant reduce infant's acceptance of the drinks at 6 months.

R-LR: Wilks' Lambda = 0.47, F (2, 62) =35.4, $p<0.0001$, (partial eta squared= 0.54);

IR: Wilks' Lambda = 0.31, F (2, 60) =66.9, $p<0.0001$, (partial eta squared= 0.69);

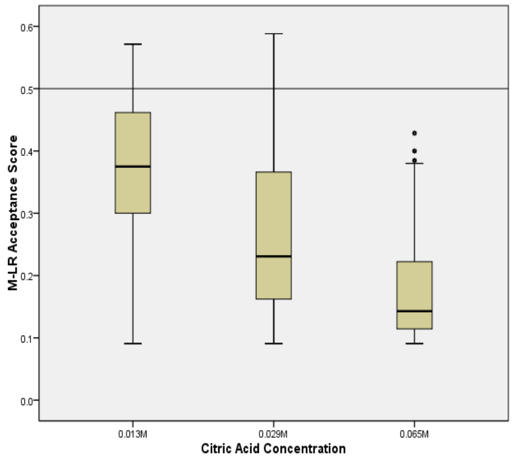
M–LR: Wilks' Lambda = 0.28, F (2, 62) =79.3, $p<0.0001$, (partial eta squared= 0.72)

Similarly, at 12 months a significant decrease in mean sour taste acceptance ($p<0.001$) was seen across all concentrations for each outcome variable (Figure 7.2), suggesting mean acceptance decreased significantly with each increasing concentration as reported below ($p<0.0001$). Using Cohen (1988) guidelines, these results suggest a very large effect using all methods**.** These results suggest that increasing critic acid concentration does significant reduce infant's acceptance of the drinks at 12 months.

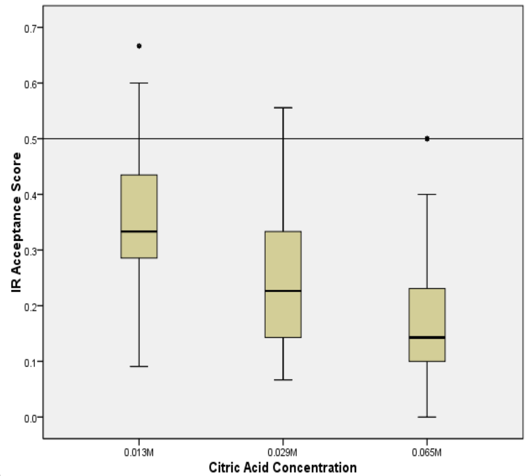
R-LR: Wilks' Lambda = 0.43, F (2,48)=31.6, $p<0.0001$ (partial eta squared= 0.57)

IR: Wilks' Lambda = 0.18, F (2,48)=108.0, $p<0.0001$ (partial eta squared= 0.82)

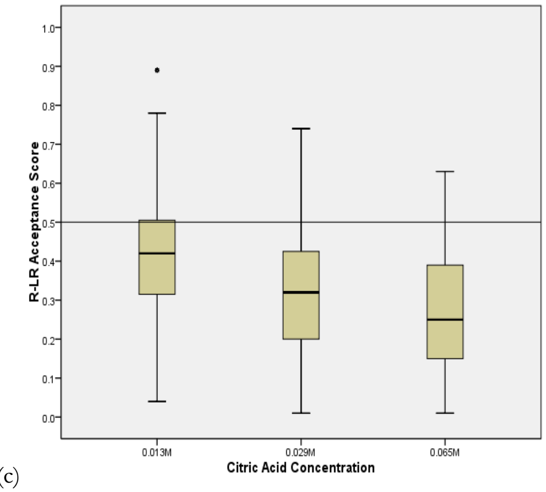
M-LR: Wilks' Lambda = 0.23, F (2,49)=85.6, $p<0.0001$ (partial eta squared= 0.78)



(a)



(b)



(c)

Figure 7-1 Sour taste acceptance score across the three methods (a) Mother Liking Ratio (L-LR) (b) Ingestion ratio (IR) and (c) Rater's Liking Ratio (R-LR) in infants at 6 months of age. The reference line on the Y axis shows the acceptance score at baseline (0.5). Infants are considered to have accepted the solution if they scored 0.5 or higher. Infants are considered to have rejected the solution if they scored less than 0.5. For each box plot the bottom and the top of the box are the 25th and 75th percentiles and the line within the box is the median. The whiskers extend from the box as far as the data extend to a distance of at most 1.5 times the interquartile range. Any values more extreme than this are marked by a *

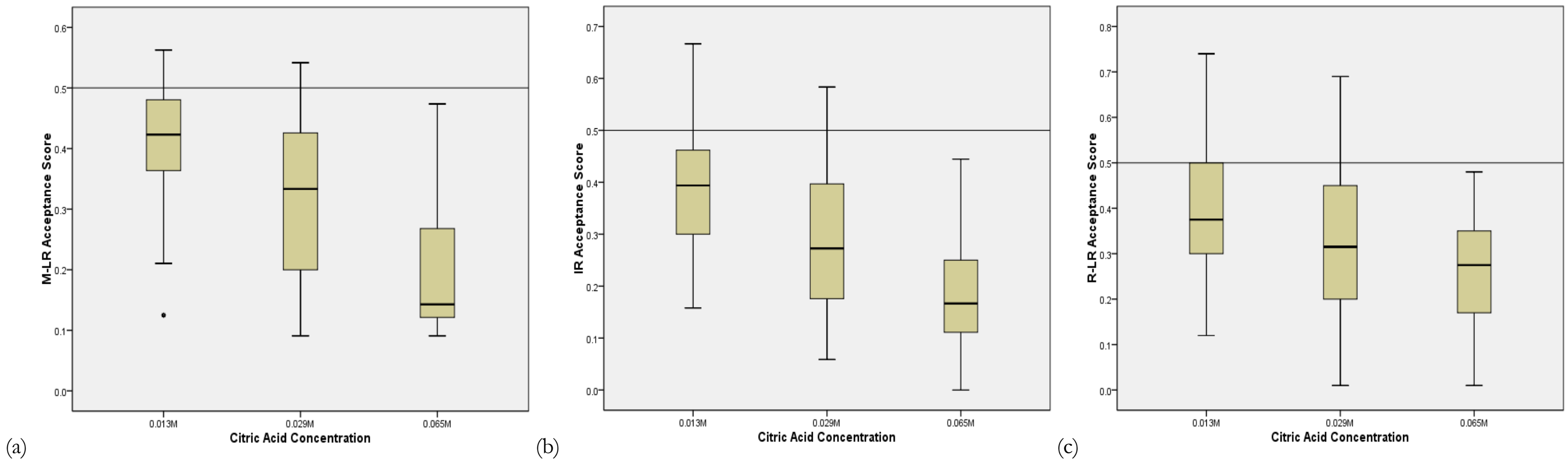


Figure 7-2 Sour taste acceptance score across the three methods (a) Mother liking Ratio (L-LR) (b) Ingestion ratio (IR) and (c) Rater's Liking Ratio (R-LR) in infants at 12 months of age. The reference line on the Y axis shows the acceptance score at baseline (0.5). Infants are considered to have accepted the solution if they scored 0.5 or higher. Infants are considered to have rejected the solution if they scored less than 0.5. For each box plot the bottom and the top of the box are the 25th and 75th percentiles and the line within the box is the median. The whiskers extend from the box as far as the data extend to a distance of at most 1.5 times the interquartile range. Any values more extreme than this are marked by a *

0.013M Citric Acid

The mean acceptance score at 6 months of 0.013M citric acid using each of the three methods; R-LR, IR & M-LR were 0.42 (±0.17); 0.36 (± 0.11) and 0.37 (±0.10) respectively. At twelve months, the mean acceptance score, using each of the three methods- R-LR, IR & M-LR, were 0.39 (±0.15); 0.39 (± 0.12) and 0.41 (±0.09) respectively. Upon analysis of the total group at 6 months using a one-way t test, infants significantly rejected solutions at 0.013M concentration over base solution (0.00M citric acid) using all three methods R-LR ($t_{63} = 4.0$, $p<0.001$); IR ($t_{63} = 9.2$, $p<0.001$) and M-LR ($t_{63} = 9.5$, $p<0.001$). Furthermore, at 12 months similar results were reported, with infants also rejecting this solution at 12 months when examined under all methods; R-LR ($t_{50} = 5.3$, $p<0.001$); IR ($t_{50} = 6.7$, $p<0.001$) and M-LR ($t_{50} = 7.3$, $p<0.001$) at 0.013M citric acid. However, for all outcome variables at both ages, the data revealed large inter-individual differences with some infants showing preference (6 months: 3.2–25.6%; 12 months: 4–20 %) or indifference (6 months: 1.6–15.6%; 12 months: 4–8%) for this concentration over the base solution (Table 7.2).

0.029M Citric Acid

The mean acceptance score at 6 months of 0.029M citric acid using each of the three methods; R-LR, IR & M-LR were 0.33 (±0.17); 0.25 (± 0.12) and 0.26 (±0.12) respectively. At twelve months, the mean acceptance score, using each of the three methods- R-LR, IR & M-LR, were 0.33 (±0.15); 0.29 (± 0.14) and 0.31 (±0.11) respectively. All infants significantly rejected sour tastes at 0.029M concentration over base solution (0.00M citric acid) at 6 months; R-LR (t_{63} = 8.4, p<0.001), IR (t_{63} = 16.0, p<0.001) and M-LR (t_{63} = 15.8, p<0.001) and at 12 months R-LR (t_{50} = 8.5, p<0.001), IR (t_{50} = 11.0, p<0.001) and M-LR (t_{50} = 9.6, p<0.001). For all 0.029M citric acid solution outcome variables, the data revealed inter-individual differences at both time points, with some infants demonstrating a preference (6 months: 1.6–12.7%; 12 months: 1.6–4.8 %) or indifference (6 months: 4–8 %; 12 months: 4–8%) for this concentration over the base solution (Table 7.2).

<u>0.065M Citric Acid</u>

The mean acceptance score at 6 months of 0.065M citric acid using each of the three methods; R-LR, IR & M-LR were 0.26 (±0.16); 0.18 (± 0.12) and 0.18 (±0.09) respectively. At twelve months, the mean acceptance score, using each of the three methods- R-LR, IR & M-LR, were 0.26 (±0.13); 0.18 (± 0.10) and 0.20 (±0.11) respectively. We saw rejection of the 0.065M solution by all infants compared to the base solution (0.00M citric acid) using all three methods at 6 months; R-LR (t_{63} = 11.8; P<0.001), IR 6 (t_{63} = 21.3; P<0.001) and M-LR (t_{63} = 26.4; P<0.001) as well as at 12 months R-LR (t_{50} = 13.6; P<0.001) IR (t_{50} = 22; P<0.001) and M-LR (t_{50} = 18.1; P<0.001). A few inter-individual differences were observed at 6 months using R-LR and IR but all of the infants showed rejection using the M-LR rating at 6 months. For all 0.065M citric acid solution outcome variables at 12 months, no preference or indifference were observed in any individual (Table 7.2).

Table 7-2 Percentage Preference and Indifference for Sour Tastes at 6 and 12 months.

Variable		*6 months*			*12 months*		
		0.013M	*0.029M*	*0.065M*	*0.013M*	*0.029M*	*0.065M*
Preference	R-RLR	25.6% (n=16)	12.7% (n=10)	8.0% (n=5)	20% (n=10)	8.0% (n=4)	0
	IR	11.2% (n=7)	1.6% (n=1)	1.6% (n=1)	12.0% (n=6)	4.0% (n=2)	0
	M-LR	3.2% (n=2)	3.2% (n=2)	0	4.0% (n=2)	4.0% (n=2)	0
Indifference	R-RLR	1.6 % (n=1)	1.6 % (n=1)	0	6.0% (n=3)	8.0% (n=4)	0
	IR	6.5% (n=4)	4.8% (n=3)	3.2% (n=2)	11.8% (n=6)	5.9% (n=3)	0
	M-LR	15.6% (n=10)	1.6 % (n=1)	0	19.6% (n=10)	4.0% (n=2)	0

R-LR- Rater's Liking Ratio, IR- Ingestion Ratio; M-LR- Mother's Liking Ratio

7.3.3 Using All Three Methods, Sour Taste Preference does not change over the First year of Life.

A one-way repeated measure ANOVA was conducted to evaluate whether sour taste acceptance changed over time (Figure 7.3). Overall, there was no statistically significant change in acceptance levels between 6 and 12 months of age (p=0.49). Using each method individually, no significant differences between the two time points were seen for R-LR (p=0.5) and IR (p=0.38). However, there was a statistically significant difference in M-LR (p=0.04), with mothers perceiving a higher acceptance of sour flavours for their infants at 12 months compared to 6 months of age.

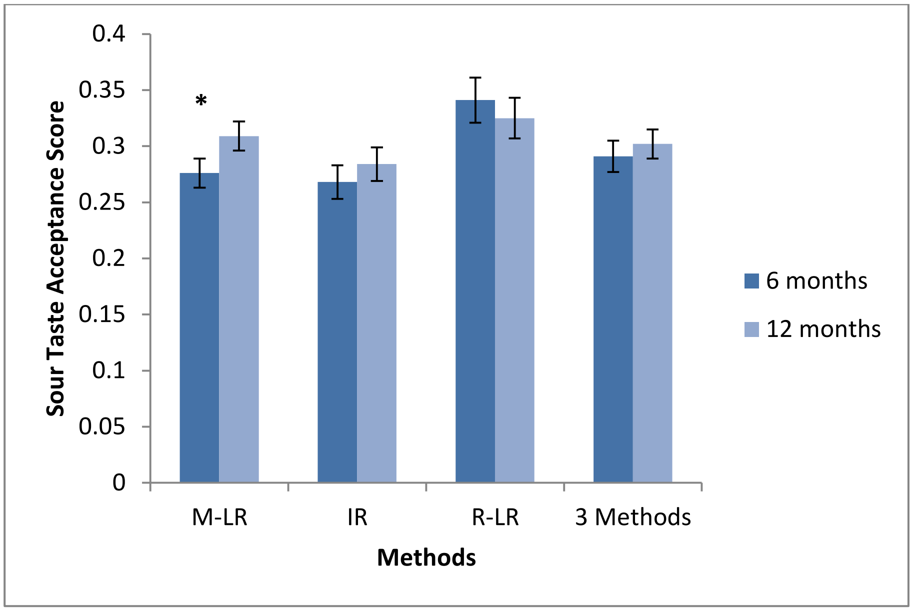


Figure 7-3 Comparison of the mean acceptance score of the three methods M-LR (Mother's Liking Ratio), IR (Ingestion Ratio) and R-LR (Rater's Liking Ratio) as well as for overall acceptance in assessing sour taste acceptance at 6 months and 12 months. *p<0.05

7.3.4 Rater's Liking Ratio of Sour Taste Acceptance was significantly higher compared to Mother's Liking Ratio and Ingestion Ratio. While Mother's Liking Ratio saw changes in Sour Taste Acceptance between Six and Twelve Months.

A repeated measure ANOVA was used to investigate if there was a difference in the results achieved by the different methods used to assess sour taste acceptance at each time point. The mean scores and standard deviation for 6 and 12 months were plotted as presented in Figure 7.4 and 7.5.

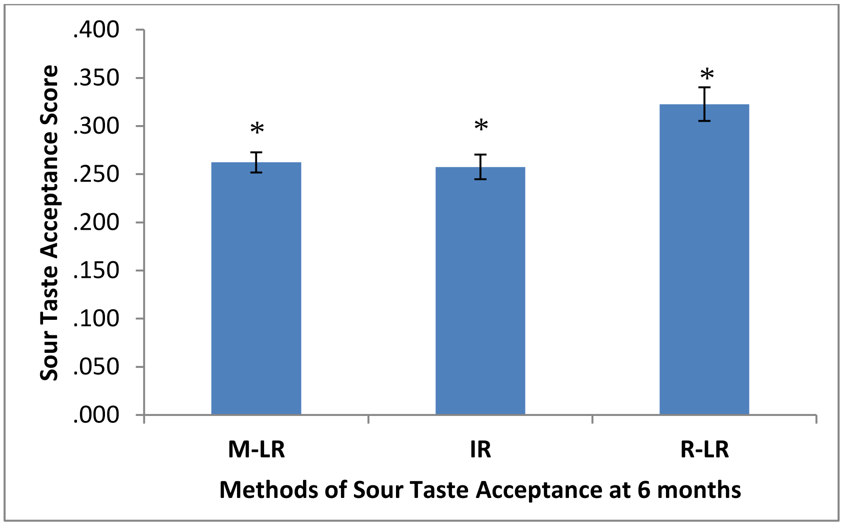


Figure 7-4 Comparison of the mean acceptance score of the three methods used in assessing sour taste acceptance at 6 months- M-LR (Mother's Liking Ratio), IR (Ingestion Ratio) and R-LR (Rater's Liking Ratio). R-LR rated sour taste acceptance were significantly higher than the two other methods M-LR and IR (*p<0.05). There was no statistical difference between sour taste acceptance scores of the M-LR and IR.

At 6 months the Rater's Liking Ratio (R-LR) score of sour taste acceptance was significantly higher when compared to the Mother's Liking Ratio (M-LR) score ($p<0.001$) as well as the Ingestion Ratio (IR) score ($p=0.001$), with a mean difference of 0.06 and 0.07 respectively. There were no statistically significant differences between acceptance scores using Mother's Liking Ratio and Ingestion Ratio ($p=0.71$).

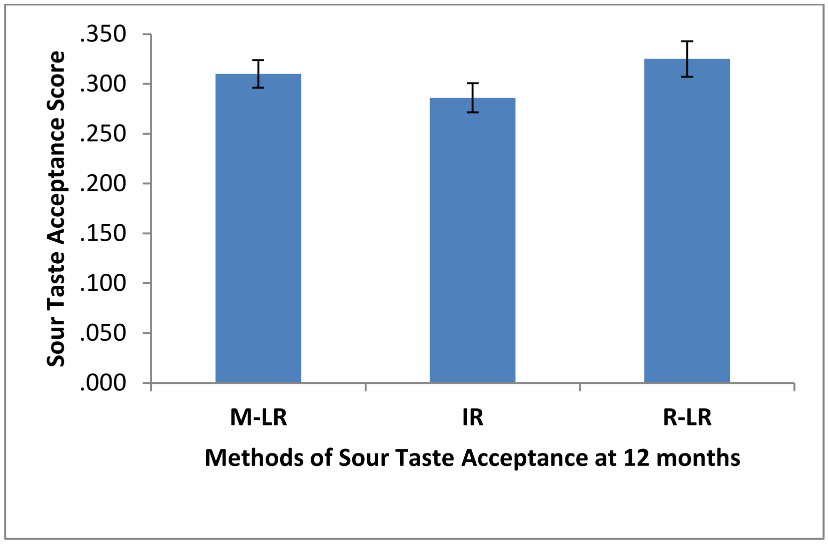


Figure 7-5 Comparison of the mean acceptance score of the three methods used in assessing sour taste acceptance at 12 months- M-LR (Mother's Liking Ratio), IR (Ingestion Ratio) and R-LR (Rater's Liking Ratio). R-LR rated sour taste acceptance significantly higher than M-LR. There were no statistically significant differences between R-LR and IR or for M-LR and IR.

At 12 months, the Rater's Liking Ratio score of sour taste acceptance was significantly higher than the Mother's Liking Ratio score ($p<0.001$), with a mean difference of 0.04. There was no statistically significant differences between the Rater's Liking Score and Ingestion Ratio ($p=0.10$) or between the Mother's Liking Ratio and Ingestion Ratio ($p=0.34$).

We also investigated the relationship between the different methodologies at each age. Mother's Liking Ratio was poorly related to the Rater's Liking Ratio and Ingestion Ratio methods at 6 months for each concentration (R^2 =0.12–0.34; Table 7.3). In contrast, the Rater's Liking Ratio and Ingestion Ratio was strongly related (R^2 =0.51–0.77; Table 7.3) at each concentration at 6 months. The relationship between the three methods of sour taste acceptance of infants at 6 months of age for 0.065M citric acid is shown in Figure 7.6. (Appendix O displays detailed graphs of the relationship between each method at each concentration as well as the corresponding R^2 value).

Table 7-3 The relationship between each method (M-LR, IR , R-LR) at each concentration (0.013M, 0.029M, 0.063M) at 6 months reported as R^2 value

Variable	*M-LR*	*IR*
0.013M M-LR	-	
0.013M IR	0.34*	-
0.013M R-LR	0.29*	0.77*
0.029M M-LR	-	
0.029M IR	0.12*	-
0.029M R-LR	0.16*	0.51*
0.065M M-LR	-	-
0.065M IR	0.18*	-
0.065M R-LR	0.19*	0.63*

Note: R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$;

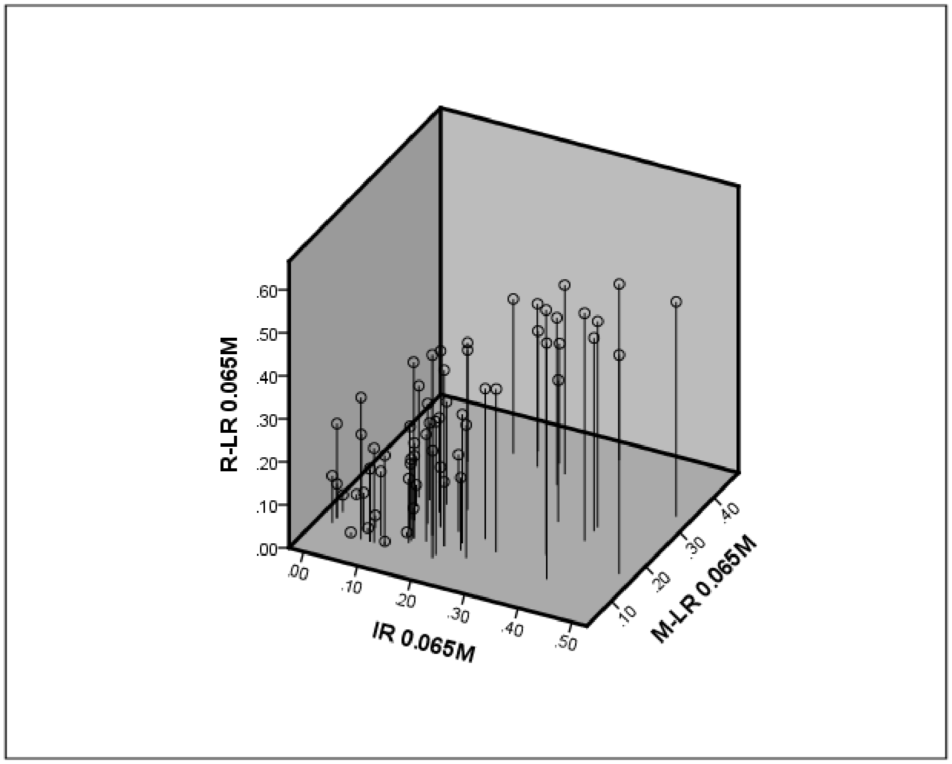


Figure 7-6 Relationship between the 3 methods (R-LR, IR, M-LR) of assessing sour taste acceptance in infants at 6 months of age for 0.065M citric acid.

Similar relationships for the three methods were seen at 12 months, with the Mother's Liking Ratio poorly related to the Rater's Liking Ratio and Ingestion Ratio methods at 12 months for each concentration (R^2= 0.09–0.24). Rater's Liking Ratio and Ingestion Ratio were moderately to strongly related (R^2= 0.43–0.80) at each concentration at 12 months (Table 7.4). The relationship between the 3 methods of sour taste acceptance of infants at 12 months of age for

0.065M citric acid is shown in Figure 7.7. (Appendix P displays detailed graphs of the relationship between each method at each concentration as well as the corresponding R^2 value).

Table 7-4 The relationship between each method (M-LR, IR , R-LR) at each concentration (0.013M, 0.029M, 0.063M) at 12 months reported as R^2 value.

Variable	M-LR	M-LR
0.013M M-LR	-	-
0.013M IR	0.19*	-
0.013M R-LR	0.24*	0.80*
0.029M M-LR	-	
0.029M IR	0.21*	-
0.029M R-LR	0.22*	0.63*
0.065M M-LR	-	-
0.065M IR	0.17*	-
0.065M R-LR	0.09*	0.43*

Note: R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$;

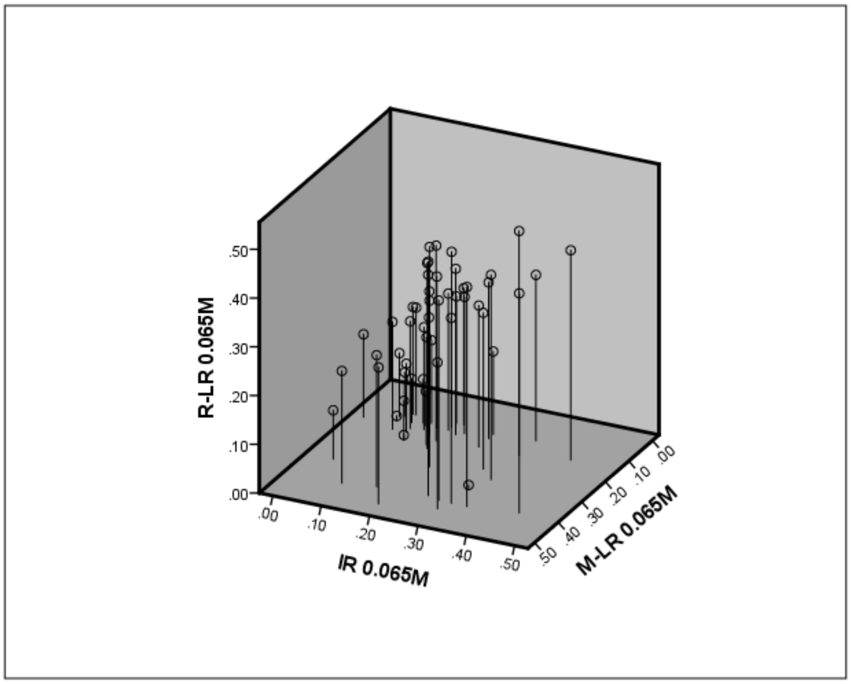


Figure 7-7 Relationship between the 3 methods (R-LR, IR, M-LR) of assessing Sour Taste Acceptance of Infants at 12 months of age for 0.065M citric acid.

7.3.5 Sour Taste Preference in Infants is associated with Length of Breastfeeding, Gender & Fruit Intake during Pregnancy at Six Months & Fruit Consumption at Twelve Months.

7.3.5.1 Multivariate Analyses at 6 months

A Multivariate analysis was completed to investigate possible factors associated with sour taste acceptance at six months, using all methods. A number of factors (length of time since solid food began, mothers' daily fruit intake during pregnancy, length of time exclusively breastfeeding and gender) that might influence sour taste acceptance at 6 months were examined. The length of time since solids had been introduced had no significant effect on sour taste preference at 6 months ($p=0.07$). It was hypothesised that mothers' daily fruit intake during pregnancy would have a positive effect on sour taste acceptance at 6 months. Therefore, the data were divided into 2 groups; low intake (≤ 2 portions of fruit per day) and high intake of fruit per day (>2 portions of fruit per day). There was a statistically significant positive effect for the number of fruits consumed by mothers during pregnancy on infant's sour taste acceptance at 6 months $F(2,48)=5.65$ ($p=0.02$), partial eta squared=0.11, which suggests a moderate effect (Cohen 1988 pp. 284–7). Figure 7.8 shows that infants whose mothers had more than two portions of fruit per day during pregnancy had significantly higher acceptance scores for sour tastes (Acceptance Score=0.24 $\pm$0.02) when compared to infants whose mothers had two or less portions of fruit per day at 6 months during pregnancy (Acceptance Score=0.30; ±0.02), with the mean difference in acceptance being 0.06 (± 0.03).

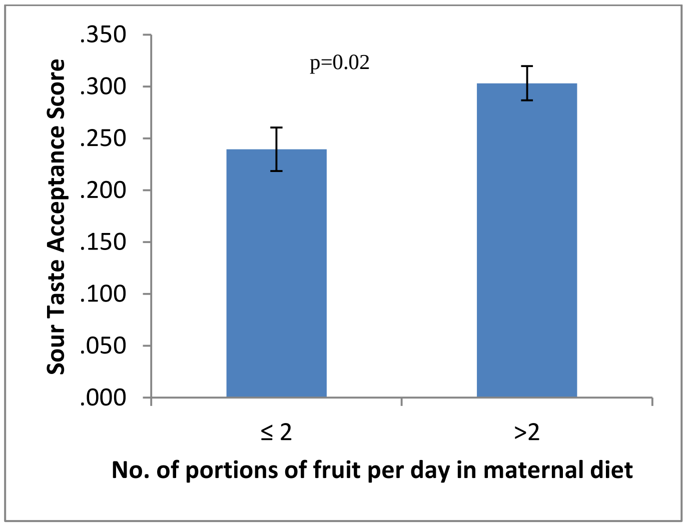


Figure 7-8 Mean sour taste acceptance compared to mother's fruit intake during pregnancy (n=64).

The effect of breastfeeding on sour taste acceptance was also examined. Analysis demonstrated that exclusive breastfeeding had a positive effect on sour taste acceptance. The data showed that length of exclusive breastfeeding had a significant positive effect on sour taste acceptance at 6 months, F (2, 48) =4.01 (p=0.025) partial eta squared 0.149, which suggested a large effect (Cohen, 1988; pp284–7). Those infants that were exclusively breastfed for less than 1 week had a lower acceptance of sour tastes (Acceptance Score=0.22 ±0.02) than infants who were exclusively breastfed for greater than one week but less than or equal to 20 weeks (Acceptance

Score 0.28; ±0.02; p=0.03) and those who were exclusively breastfed for greater than 20 weeks (Acceptance Score 0.31; ±0.03; p=0.01). There was no significant difference in sour taste acceptance between length of exclusive breastfeeding in those infants that were exclusively breastfed for >1 week but less than 20 weeks and those who were exclusively breastfed for >20 weeks (p=0.39; Figure 7.9).

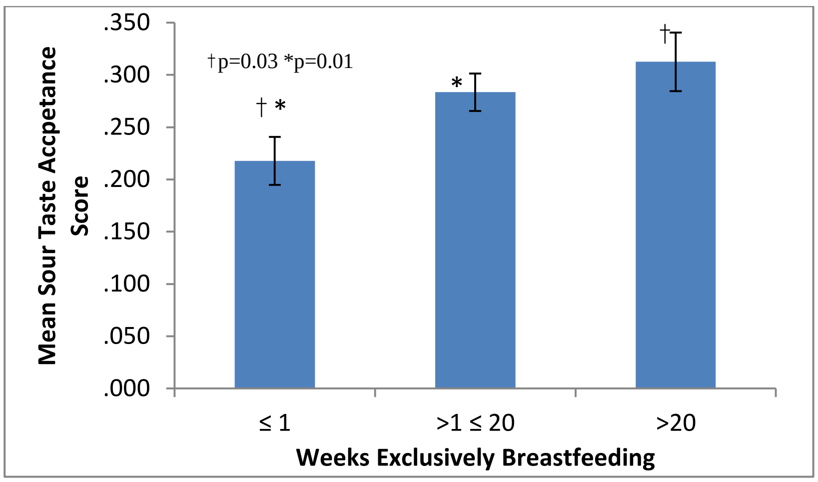


Figure 7-9 Sour taste acceptance scores of infants at 6 months compared across levels of exclusive breastfeeding (n=64)

The effect of gender on sour taste acceptance was also examined. There was a statistically significant effect of gender on sour taste acceptance at 6 months F (2.48) =5.27 (p=0.018) partial eta squared 0.116, which suggested a moderate effect (Cohen 1988; pp284-7). Figure 7.10 shows that females had a significantly lower acceptance of sour tastes when compared to males at 6 months, with the mean difference in acceptance being 0.07 (±0.03).

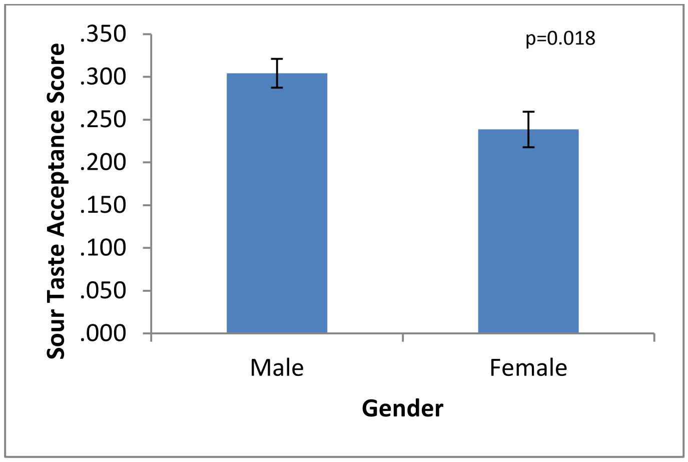


Figure: 7-10 Comparison of sour taste acceptance of males and females at 6 months (p=0.018)

7.3.5.2 Exclusively Breastfed Infants at 6 months

A sub-analysis was completed to investigate possible factors associated with sour taste acceptance at 6 months in infants who were exclusively breastfed for at least 6 weeks (n=43). Gender (p=0.07), infants' fruit intake at 6 months (p=0.13) or the number of weeks eating solid food (p=0.23) had no significant effect on sour taste acceptance at 6 months in those infants exclusively breastfed for at least 6 weeks. By comparing males and females the median number of weeks of exclusive breastfeeding were 16 weeks (±24) and 9 weeks (±26) respectively, though this was not statistically different p=0.774, due to the wide interquartile ranges for both groups. It was hypothesised that mothers' fruit intake during breastfeeding would have a positive effect on sour taste acceptance at 6 months. However, there was no significant effect of mother's fruit intake during breastfeeding (p=0.16) on sour taste acceptance at this age (Figure 7.11).

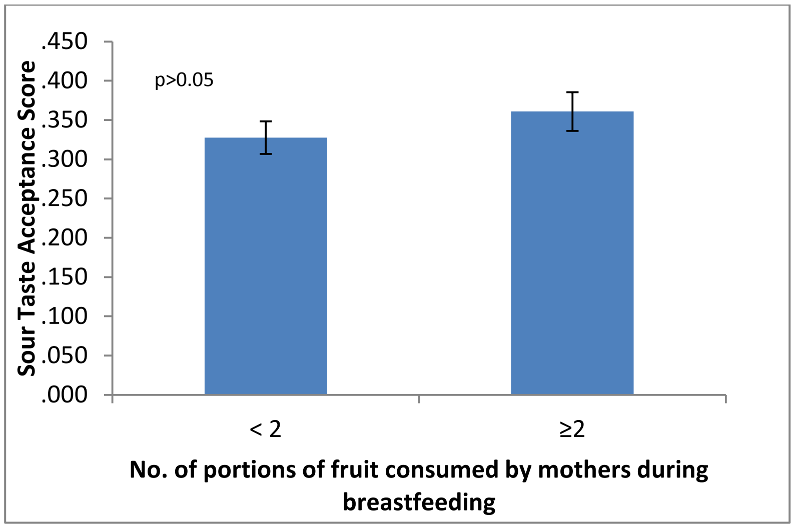


Figure 7-11 Sour taste acceptance scores of exclusively breastfed infants at 6 months (n=43), where mothers consumed varying amounts of fruit during this time. There was no significant effect between sour taste acceptance and fruit consumption in breastfeeding mothers.

However, length of exclusive breastfeeding continued to have a significant effect on sour taste acceptance in those infants whose mothers exclusively breastfed for at least 6 weeks. Mean acceptance scores were higher in infants who had been breastfed exclusively for more than 20 weeks (0.37) compared to those who had been exclusively breastfed for 20 weeks or less (0.26; p <0.01; Figure 7.12).

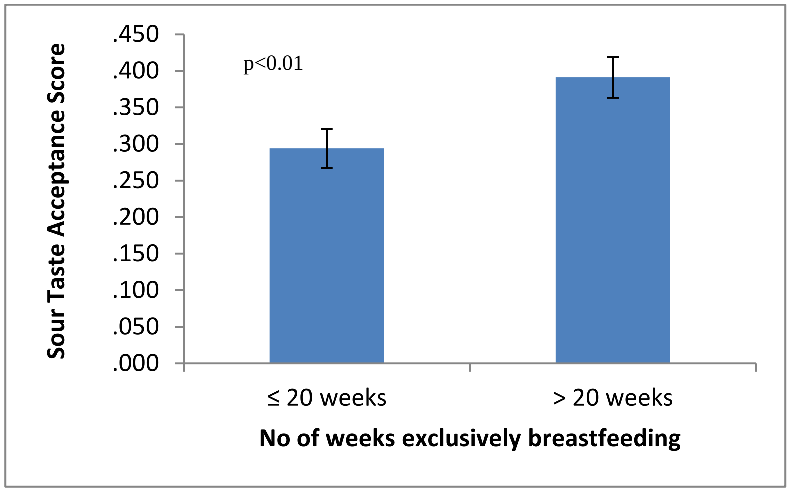


Figure 7-12 Sour taste acceptance scores of for two groups of exclusively breastfed infants at 6 months (n=43).

7.3.5.3 Multivariate Analyses at 12 months

A repeated measures ANOVA was completed to investigate possible factors associated with sour taste acceptance at 12 months. A number of factors (length of time since solid food was taken; Mothers' fruit intake; length of time breastfeeding; gender and infants' own fruit intake) which could influence sour taste acceptance at 12 months were examined. Gender (p=0.36) or length of exclusive breastfeeding (p=0.86) had no significant effect on sour taste preference at 12 months.

The effect of an infant's own fruit intake at 12 months on sour taste acceptance was also examined. Infants' daily fruit intakes were quantified in grams using the completed food diaries. Using decision tree analysis, two distinct clusters of infants were identified, those that had intakes <116 g per day and those with intakes >116 g per day. Following further division sub-groups were formed based on infants' fruit intake, comprised of two groups; high (>116 g) and low (<116 g). Figure 7.13 shows a significant positive effect of infant's fruit intake at 12 months on their sour taste acceptance. Those infants who consumed more fruit at 12 months had a higher acceptance for sour tastes when compared to those who consumed less fruit ($p \leq 0.05$).

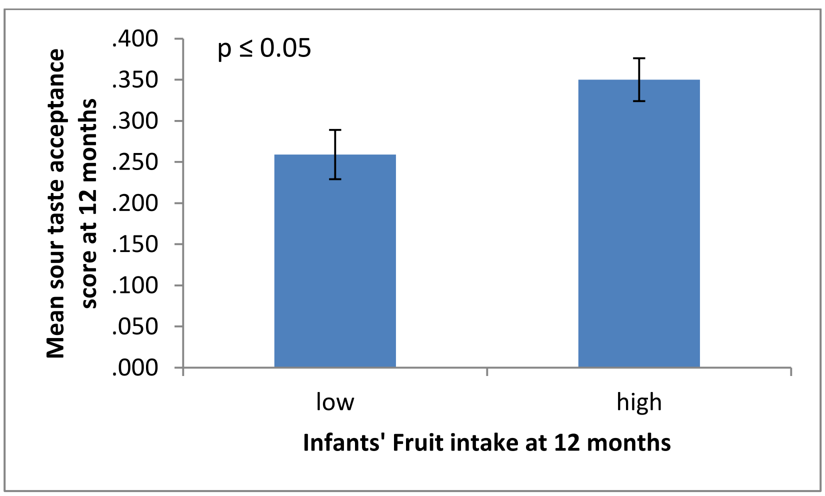


Figure 7-13 Sour Taste Acceptance Scores in Low and High Fruit Consumers at 12 months of age (n=50).

7.3.5.4 Predictors of Sour Taste Acceptance at 6 months

Potential predictive variables of sour taste acceptance with significant relationships were chosen from the correlation matrix and their predictive strengths were examined using multiple regression (Table 7.5). A multiple regression was conducted to predict acceptance of each solution from; weeks exclusively breastfed; mothers' intake of fruit during pregnancy and the number of weeks eating solid food. The results of the linear regression analyses are summarised in Tables 7.6 –7.8. In each case, the unstandardized and standardized beta values presented, along with the R^2 and adjusted R^2 for the total model are reported.

Table 7-5 Correlation Matrix between predictor and outcome variables at 6 months

	Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	No. wks eating solid food	-												
2.	No. Fruit Tried	.218*	-											
3.	Age starting solids (wks)	-.575**	-0.16	-										
4.	Length EBF (wks)	.128	0.25*	0.24	-									
5.	No of Fruit eaten during pregnancy	-.034	0.19	-0.14	0.24*	-								
6.	R- LR 0.013M	-.306**†	0.11	0.01	0.17	.01	-							
7.	R-LR 0.029M	-.166	0.09	-0.03	0.33**	0.0	0.69**	-						
8.	R-LR 0.065M	-.198†	-0.06	-0.05	0.32**	0.04	0.52**	0.73**	-					
9.	IR 0.013M	-.325**†	0.04	0.02	0.12	0.08	0.88**	0.54**	0.38**	-				
10.	IR 0.029M	-.179†	0.11	-0.06	0.36**	0.18	0.61**	0.72**	0.56**	0.54**	-			
11.	IR 0.065M	-0.132†	0.09	-0.11	0.31**	0.09	0.5**	0.64**	0.73**	0.46**	0.66**	-		
12.	M-LR 0.013M	-0.09†	0.17	-0.10	0.27*	0.14	0.54**	0.41**	0.37**	0.58**	0.37**	0.34**	-	
13.	M-LR 0.029M	-0.71†	0.14	0.03	0.38**	0.28	0.35**	0.40**	0.42**	0.26**	0.39**	0.27**	0.57**	-
14.	M-LR 0.065M	-0.061†	0.22	-0.03	0.38**	0.37**	0.33**	0.37**	0.42**	0.32**	0.43**	0.42**	0.34**	0.60**

Note: R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$; ** $p \leq .01$;

Table 7-6 Summary of linear regression analyses predicting sour taste acceptance of 0.013M concentration at 6 months using the three methods- R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*			*Ingestion Ratio (IR)*			*Mother's Liking Ratio (M-LR)*		
	B	*SE B*	*Β*	*B*	*SE B*	*β*	*B*	*SE B*	*Β*
No. wks. eating solid food	-.021	.010	-.274*	-.005	.006	-.097*	-.018	.007	-.318
Length EBF (wks.)	.002	.002	.131	.003	.001	..254	.001	.002	.078
No of Fruit eaten during pregnancy	-.005	.016	-.038	-.006	.010	.076	.003	.012	.037
	R^2*=0.095,* ***Adj. R²=0.047*** *F*(3, 57) = 2.0 (p=0.13)			R^2*=0.092,* ***Adj. R²=0.044*** *F*(3, 57) = 1.93 (p=0.14)			R^2*=0.113,* ***Adj. R²=0.065*** *F*(3, 55) = 2.35 (p=0.08)		

Note: * $p \leq .05$; ** $p \leq .01$ Adj- Adjusted. R-LR= Rater's Liking Ratio. IR= Ingestion Ratio. M-LR= Mother's Liking Ratio. The model was not predictive of sour taste acceptance at the level of 0.013M citric acid.

Table 7-7 Summary of linear regression analyses predicting sour taste acceptance of 0.029M concentration at 6 months using the three methods- R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*			*Ingestion Ratio (IR)*			*Mother's Liking Ratio (M-LR)*		
	B	*SE B*	*Β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
No. wks. eating solid food	-.012	.010	-.159	-.010	.007	-.185	.002	.007	.027
Length EBF (wks.)	.005	.002	.335*	.004	.001	.361**	.004	.001	.304*
No of Fruit eaten during pregnancy	-.010	.015	-.084	-.005	.011	.059	.018	.011	.198
	R^2=0..136, ***Adj. R²=0.091*** F(3, 57) = 3.0 **(p ≤0 .05)**			R^2=0.187, ***Adj. R²=0.142*** F(3, 55) = 4.21**(p ≤ 0.01)**			R^2=0.16, ***Adj. R²=0.115*** F(3, 57) = 3.61**(p ≤ 0.05)**		

Note: * $p \leq .05$; ** $p \leq .01$. Adj- Adjusted. R-LR= Rater's Liking Ratio. IR= Ingestion Ratio. M-LR= Mother's Liking Ratio. The model was predictive of sour taste acceptance at the level of 0.029M citric acid.

Table 7-8 Summary of linear regression analyses predicting sour taste acceptance of 0.065M concentration at 6 months using the three methods- R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*			*Ingestion Ratio (IR)*			*Mother's Liking Ratio (M-LR)*		
	B	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
No. wks. eating solid food	-.018	.009	-.248*	-.003	.003	-.148	-.001	.005	.023
Length EBF (wks.)	.006	.002	.376**	.001	.001	.329*	.003	.001	.300*
No of Fruit eaten during pregnancy	-.011	.014	-.091	.000	.004	-.005	.020	.008	.298*
	R^2=0.204, ***Adj.R^2=0.162*** *F*(3, 57) = 4.86 **(p ≤0 .01)**			*R^2=0.136,* ***Adj.R^2=0.091*** *F*(3, 57) = 3.0**(p<0.05)**			*R^2=0.223* ***Adj. R^2=0.182*** *F*(3, 57) = 5.44 **(p<0.01)**		

Note: * *p* ≤ .05; ** *p* ≤ .01. Adj- Adjusted. R-LR= Rater's Liking Ratio. IR= Ingestion Ratio. M-LR= Mother's Liking Ratio. The model was predictive of sour taste acceptance at the level of 0.065M citric acid.

Using linear regression analysis of factors predicting R-LR, IR & M-LR acceptance at 0.013M citric acid, the prediction model was not statistically significant for any of the outcome variables and did not predict acceptance at 0.013M concentration (Table 7.6). In contrast, using linear regression analysis of factors predicting R-LR, IR & M-LR acceptance at 0.029M citric acid, the three prediction models R-LR, IR and M-LR were statistically significant F (3, 57) = 3.0 ($p<0.05$), F (3, 55) = 4.21 ($p<0.01$) and F (3, 57) = 3.61 ($p<0.05$) respectively. The model accounted for approximately 9–14.2% of the variance in acceptance of the 0.029M citric acid solution (Table 7.7). Acceptance was primarily predicted by length of exclusive breastfeeding for all models, with it uniquely accounting for 8.7% 10.5% and 12% of the variance in the M-LR, R-LR, and IR models respectively.

Similarly, the findings from the linear regression analysis of factors predicting R-LR, IR & M-LR acceptance at 0.065M citric acid found that all three prediction models; R-LR; IR and M-LR were statistically significant (F (3, 57) = 4.86 ($p<0.01$); F (3, 57) =3.0 ($p<0.05$) and F (3, 57) =5.44 ($p<0.01$) respectively). The models accounted for approximately 9–18% of the variance in acceptance of the 0.065M citric acid solution (Table 7.8). Again acceptance was primarily predicted by length of exclusively breastfeeding for all models with it uniquely accounting for 8.5%; 10.8% and 13.3% of the variance in the M-LR, IR and R-LR models respectively. Other significant predictors were number of weeks eating solid food (6.2% of the variance) for the R-LR model and number of portions of fruit eaten by mothers during pregnancy (8.4% of the variance) for M-LR model, thus providing further support for the hypothesis that the flavours of the mothers' diet during pregnancy may be transmitted to their baby through amniotic fluid.

Overall, this provides evidence that exclusive breastfeeding can predict 8-13% of the variance in acceptance of extreme sour tastes in 6 month old infants. It also suggests that the number of portions of fruit eaten by mothers during pregnancy and the number of weeks eating solid foods may predict some variance in acceptance at extreme citric acid levels (0.065M). Any addition of gender as a variable to the analysis did not improve the model and gender did not independently predict sour taste using any method at any concentration level. Exclusive breastfeeding continued to largest and only significant factor in predicting the variance in the model. This adds weight to the suggestion that exclusive breastfeeding may be confounding the gender effect observed at six months.

7.3.5.5 Predictors of Sour Taste Acceptance at 12 months

From existing literature, it was suggested that fruit intake of the infant may be related to sour taste acceptance at 12 months (Blossfeld *et al.,* 2007). Given the correlation between the length of exclusive breastfeeding at 6 months and sour taste acceptance, I also considered possible correlations at 12 months. However, no correlation was found and this was excluded from the regression analysis. Correlations between these independent variables and the outcome variables are reported in Table 7.9. Hierarchical multiple regression analyses were conducted for each of the 12-month outcome variables of taste acceptance. Acceptance at 6 months for the corresponding outcome variable at 12 months was controlled for in the first step. The number of fruits tried by the infant at 6 months and the amount of fruit consumed in grams by the infants at 12 months were added in the next step.

Table 7-9 Correlation Matrix between predictor and outcome variables at 12 months

	Variable	1	2	4	5	6	7	8	9	10	11	12	13
1.	No of fruit tried at 6 months	-											
2.	Fruit intake 12 months	.253	-										
4.	Vitamin C 12 months	.334*	.455*	-									
5.	Length of exclusive breastfeeding	.278*	.384*	.149	-								
6.	R- LR 0.013M	.298*	.231	.290	.087	-							
7.	R-LR 0.029M	.192	.230	.178	.125	0851**	-						
8.	R-LR 0.065M	.183	.359	.22	-.100	.612**	.749**	-					
9.	IR 0.013M	.264	.372*	.267	.025	.893**	.753**	.612**	-				
10.	IR 0.029M	.047	.052	-.049	.093	.706**	.793**	.246**	.597**	-			
11.	IR 0.065M	.177	.347	.177	.001	.555**	.640**	.652**	.598**	.666**	-		
12.	M-LR 0.013M	.187	.020	.076	-.027	.486**	.464**	.322**	.435**	.376**	.219	-	
13.	M-LR 0.029M	.183	.047	.196	-.033	.577**	.470**	.392**	.404**	.458**	.318**	.650**	-
14.	M-LR 0.065M	.187	.020	.076	-.007	.308**	.251**	.297**	.297**	.244**	.410**	.406**	.636**

Note: R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$; ** $p \leq .01$;

Table 7-10 Summary of linear regression analyses predicting sour taste acceptance of 0.013M concentration at 12 months using R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*				*Ingestion Ratio (IR)*				*Mother's Liking Ratio (M-LR)*			
	B	*SE B*	*B*	*⊿R²*	*B*	*SE B*	*B*	*⊿R²*	*B*	*SE B*	*β*	*⊿R²*
Step 1				**.061***				.034				.064
Outcome variable 6 months	.172	.093	.252*		.166	.110	.183		0.21	0.16	0.261	
Step 2				.074				.082				.005
Fruit tried 6 months	.007	.006	.140		.006	.005	.082		0.002	.008	.050	
Fruit intake 12 months	.001	.000	.201		.000	.000	.212		0.45	.000	.035	
	R^2 = .135, **Adj. R^2 = .092**				R^2 =.034, **Adj. R^2 = .019**				R^2 = .069, **Adj. R^2 = .042**			
	$F(3, 47)$ = 3.1*****				$F(3, 47)$ = 2.6				$F(3, 47)$ = 0.62			

Note: Adj- Adjusted; R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$; ** $p \leq .01$

Table 7-11 Summary of linear regression analyses predicting sour taste acceptance of 0.029M concentration at 12 months using R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*				*Ingestion Ratio (IR)*				*Mother's Liking Ratio (M-LR)*			
	B	*SE B*	*B*	*⊿R²*	*B*	*SE B*	*β*	*⊿R²*	*B*	*SE B*	*β*	*⊿R²*
Step 1				**.076***				**.076***				.046
Outcome variable 6 months	.264	.099	**.343***		.274	.129	**.278***		.222	.195	.210	
Step 2				.075				.008	.000	.000	.128	.016
Fruit tried 6 months	.005	.006	.099		.003	.006	.045		.000	.011	.009*	
Fruit intake 12 months	.001	.000	.239		.000	.006	.065					
	R^2 = .076 **Adj. R^2 = .062**				R^2 = .07, **Adj. R^2 = .061**				R^2 = .065, **Adj. R^2 = .051**			
	$F(3, 47) = 3.5$*				$F(1, 47) = 1.84$				$F(3, 47) = 0.55$			

Note: Adj- Adjusted; R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$; ** $p \leq .01$

Table 7-12 Summary of linear regression analyses predicting sour taste acceptance of 0.065M concentration at 12 months using R-LR, IR & M-LR.

Variable	*Rater's Liking Ratio (R-LR)*				*Ingestion Ratio (IR)*				*Mother's Liking Ratio (M-LR)*			
	B	*SE B*	*Β*	*⊿R²*	*B*	*SE B*	*β*	*⊿R²*	*B*	*SE B*	*β*	*⊿R²*
Step 1				.002				.115				.036
Outcome variable 6 months	.077	.079	0.124		0.26	.118	**0.385***		.224	.147	.197	
Step 2				.098				.137				.020
Fruit tried 6 months	.004	.009	.094		.007	.009	.140		.001	0.006	.159	
Fruit intake 12 months	.001	.000	**.286***		.001	.000	.295		.000	.000	.197	
	R^2 =0 .100, **Adj. R^2 = .055**				R^2 =0 .252, **Adj. R^2 = .159**				R^2 = .036, **Adj. R^2 = .020**			
	$F(3, 47)$ = 2.2				$F(3, 48)$ = 2.7				$F(3, 48)$ = 1.31			

Note: Adj- Adjusted; R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio. * $p \leq .05$; ** $p \leq .01$

The results of the hierarchical regression analyses are summarised in Tables 7.9 – 7.11 for acceptance of 0.013M; 0.029M and 0.065M citric acid concentration for each outcome variable. In each case, the unstandardised and standardised beta values presented were taken from the final regression model with both steps entered, along with the R^2 and adjusted R^2 for the total model are reported. The outcome variable for the corresponding acceptance scores at 6 months were controlled for in the first step of the regression model predicting acceptance at 12 months.

The prediction model was not statistically significant for Ingestion Ratio or Mother's Liking Ratio at any concentration (0.013M; 0.029M and 0.065M) and did not predict acceptance at these concentrations. In contrast, the prediction model, using the Rater's Liking Ratio, was statistically significant at 0.013M citric acid concentration $F(3, 47) = 3.1$ ($p<0.05$), with this model accounting for 9% of the variance and the 0.029M citric acid concentration $F(3, 47) = 3.5$ ($p<0.05$), with this model accounting for 6% of the variance. The only significant predictor of both these models was the R-LR acceptance score at 6 months, accounting for 6.6% and 9% of the variance at 0.013M and 0.029M respectively. However, the prediction model was not statistically significant for Rater's Liking Ratio at 0.065M concentration and did not predict acceptance at this concentration.

7.4 *Discussion*

This study provides the first evidence that infants can detect extreme sour tastes at 6 and 12 months and also demonstrates that they can distinguish between different concentrations of sourness and modify their behaviour accordingly. Moreover, this research suggests that rejection of sour taste remained stable from 6 months to twelve months, a result which was consistent with that found by Schwartz *et al.* (2009). My study also demonstrated that, between the age of 6 months and 12 months, some infants accept extreme sour tastes. This finding has previously been shown only in toddlers and older children (Liem & Mennella, 2003; Blossfeld *et al.,* 2007). The different methods employed to detect sour taste preference in infants were compared and significant differences were observed between methods. Finally, sour taste preference in infants was linked to different factors at 6 and 12 months.

While, the mean acceptance of the sour tastes was less than the base solution, indicating rejection at 6 and 12 months, the study shows there were large variations in acceptance levels within the cohort. At 6 months, 23% readily accepted at least one of the two higher concentrations of citric acid when assessed by a least one method. At 12 months 13.7% readily accepted these high sour tastes. Blossfeld *et al.* (2007), using the same concentrations as this study, showed similar variability in sour taste acceptance in 18 month old toddlers, with 23% of the study population showing higher acceptance of at least one of the two highest concentrations when compared to the base solution. More recently, Schwartz *et al.* (2009) in a study of similarly aged infants (3–12 month olds) found even greater variability within groups at all ages, with 48% and 44% of infants at 6 and 12 months respectively showing a preference for the sour solutions over water. However, Schwartz *et al.* (2009) used much lower concentrations of citric acid (0.006M) than this current study.

Given the identified variability in infant's responses to sour taste, consideration will be given to potential explanations for this variance. One hypothesis suggests that some infants accept higher sour stimuli from birth onward, which allow them to accept these solutions at 6 months. Another explanation posits that a developmental shift in sour taste perception takes place before the age of 6 months, a shift which is independent of experience. There is some evidence to suggest that this may be the case. It has been shown that an infant's salt preference changes during the early months of life, from indifference at birth to demonstrating a preference for salty tastes by the age of 4–6 months (Harris, Thomas, & Booth, 1990b). However, research into salt preference may not be appropriate for consideration of sour taste preference as in the case of sour taste there is an innate rejection of sour flavours by newborns (Desor *et al.,* 1973; Steiner, 1977), which continues through the first year of life. My study, along with Schwartz *et al.* (2009) would appear to refute the suggestion that sour taste preference evolves over the first year of life, given the stability it found in sour taste acceptance over the first year of life, which is now discussed in detail.

An alternative hypothesis suggests that variability in sour taste acceptance could be genetically linked. While no genetic variations in taste sensitivity have been identified for sour taste as of yet, genetic variations have been identified for other tastes, such as bitter, where genetic variations of 6-n-proylthiouracil (Zhang *et al.,* 2003) have been linked with preferences for bitter tasting food in children (Bell & Tepper, 2006). This particular hypothesis may be further explored in the coming years. As the receptors for sour taste preference have been identified relatively recently (Huang *et al.,* 2006), it is possible that genetic variations in sour taste acceptance may yet be discovered.

Previous studies suggest that there is a link between fruit intake and sour taste acceptance in infants (Blossfeld *et al.,* 2007) and older children (Liem & Mennella, 2003). Amniotic fluid contains volatile chemicals transmitted from the mothers' diet to their infants and research indicates that infants can detect these flavours, suggesting that memories are formed from these early experiences (Mennella *et al.,* 1995; Mennella *et al., 2*001; Schaal *et al.,* 2000). Studies have shown that exposure to anise, garlic, ethanol, carrot and vanilla aid acceptance of these tastes by infants (Lipchock, Reed, & Mennella, 2011).

In this study, a positive relationship was found between the amount of fruit eaten by mothers during their third trimester of pregnancy and their infants' sour taste acceptance. Mothers who had eaten more than 2 portions of fruit per day during the third trimester of their pregnancy had infants who were more accepting of sour tastes at 6 months. In fact, the number of portions of fruit consumed by mothers during pregnancy uniquely accounted for between 8.4% of the variance in sour taste acceptance for the highest concentrations of citric acid (0.065M) at 6 months using Mothers' Liking Ratio. This is the first evidence that fruit intake during pregnancy is associated with sour taste acceptance and strengthens the hypothesis that acceptance might be shaped by the infant's experience prior to birth.

After birth, infants rely solely on a milk diet for the first few months of life. Mennella (1999) suggests that breast milk is a flavour bridge that connects the flavours learned through amniotic fluid and that of the infant's weaning diet. Forestell & Mennella (2007) suggested that breastfed infants were more accepting of fruits and vegetables, if their mother regularly ate these foods themselves than formula fed infants.

In this study, length of exclusive breastfeeding was positively associated with sour taste acceptance. Those infants who were breastfed for longest were most accepting of sour taste. This was true for both the entire group and in a sub-analysis of those exclusively breastfed for at least 6 weeks. In fact, length of exclusive breast feeding uniquely accounted for 8–14% of the variance in sour taste acceptance among infants at 6 months for the two highest concentrations of citric acid (0.029M and 0.065M). This strengthens the hypothesis that acceptance might be shaped by the infant's early feeding experience. This is the first evidence that suggests that duration of breastfeeding is associated with sour taste acceptance.

Previous research that found that duration of breast feeding did not influence sour taste acceptance in the first year of life (Schwartz *et al.,*2012). A possible explanation for the contradicting result in this thesis may be found in the very low concentrations of citric acid used by Schwartz *et al.* (2009). In a similar vein, exclusive breastfeeding was not predictive of acceptance among the lowest concentration of citric acid in the study but was predictive of acceptance at the highest two concentrations. A possible explanation for the lack of a relationship between sour taste acceptance at low levels and exclusive breastfeeding may be that the taste variation in the solution was too small to be attended to and did not drive differential consumption for infants used to eating some solid foods.

Despite the positive association between sour taste acceptance and length of exclusive breastfeeding, I found no association between levels of mothers' fruit consumption during lactation and sour taste acceptance at 6 months. One explanation for this finding is that breastfed children are more open to trying novel tastes because they have been exposed to a diversity of flavours via breast milk early in life (Birch, 1998) or that similar flavours in mothers' milk help acceptance of these new foods during weaning (Forestell & Mennella, 2007; Mennella *et al.,* 2001). This finding may be explained by the fact that previous studies used strong flavours (Hepper, 1988; Schaal *et al.,* 2000) or large quantities of the same flavour on a daily basis - one example being carrot juice (Forestell & Mennella, 2007; Mennella *et al.,* 2001).

In reality, women's diets consist of many different flavours, which alter daily and are experienced in varying amounts. Overall, the flavour of breast milk is sweet, and therefore sour flavour notes may be low in the milk. Among exclusively breastfeeding mothers, 66% reported consuming ≤ 2 portions of fruit per day (Chapter 4) compared to 39% of women during pregnancy who reported consuming ≤ 2 portions of fruit per day (Chapter 3). Given the unequal numbers of women with high fruits intakes during lactation compared to numbers of women with low intakes of fruits, there may not have been enough variance in the amount of fruit consumed by mothers at this time to detect differences within the smaller sample size in this sub-analysis (n=42). Furthermore, transfer rates of flavour compounds into breast milk have not yet been identified for fruit and therefore might not be similar to placental transfer. Therefore it is possible that much higher numbers of participants will be needed to observe differences in fruit intake during lactation than compared to pregnancy. Future studies, which consider the flavour compounds of the breast milk of women given fixed amounts of a certain fruits daily, may help to elucidate this further.

Blossfeld *et al.* (2007) found that fruit consumption at 6 and 12 months were predictive of sour taste preference at 18 months. In this study, there was no correlation between the number of fruits tried at 6 months and sour taste preference. This finding may be explained by the fact that most infants had only been eating solid food for a few weeks, with 32% having been exposed to solid foods for less than 2 weeks. These infants would have only tried some fruits once, and would have had these fruits in very small quantities (e.g. one teaspoon). Furthermore, the most common fruits reported by mothers were banana, commercial fruit pots or stewed apple and pear. These fruit flavours are predominantly sweet while only one mother reported that their infant had tried citrus fruits.

This is the first study to suggest gender difference in taste preference. Among my cohort, males accepted extreme sour tastes more than females at 6 months ($p=0.018$). One explanation is that these differences are due to genetics. However, previous studies have shown no gender differences in sour taste acceptance (Blossfeld *et al.* 2007; Schwartz, Issanchou, & Nicklaus, 2009) or in the other primary tastes such as bitter tastes, where genetic differences are better understood (Schwartz, Issanchou, & Nicklaus, 2009). These studies had similar sample sizes to the current study (Blossfeld *et al.* 2007 $n=53$; Schwartz, Issanchou, & Nicklaus, 2009 $n=45$). Furthermore, there was no association with gender observed at 12 months in the current study. However, one study showed that adult women were supertasters of bitter tastes more frequently (Bartoshuk, Duffy, & Miller, 1994). Another explanation is that gender differences seen are related to early infant feeding practices as the effect of gender is only seen at 6 months at not 12 months. While the median number of weeks of exclusive breastfeeding for males were 16 weeks (± 24) and for females 9 weeks (± 26) respectively, it was not statistically different ($p=0.774$). This may be due to the wide range in length of exclusive breastfeeding in the two groups (0-26 weeks for both groups). Therefore, further research is needed into the effect of gender on taste.

At 12 months, fruit intake was positively associated with sour taste acceptance. At this stage, infants would have been eating solid foods, including fruit, for many months and would have eaten them in larger quantities than at 6 months. This may explain the relationship between infant fruit consumption and sour taste preference at 12 months and its absence at 6 months. It is also possible that some individuals had a genetic preference for sourness and, therefore, accepted fruit more readily at weaning contributing to the relationship between sour acceptance and fruit consumption. It is not possible to explore this further under the auspices of this study, but future studies could further establish the accuracy of these suggestions.

While exclusive breastfeeding duration was predictive of higher acceptance of extreme sour flavours at 6 months, this relationship was no longer observed at 12 months. This might be related to the fact that in previous research, breastfeeding conferred an advantage in initial acceptance of a food – if mothers ate the food regularly. Once weaned, an infant's preference was based on experience (Forestell & Mennella, 2007). This assumption was supported in the current study by the finding that breastfeeding no longer showed any advantage at 12 months but that higher fruit intake at 12 months was associated with higher sour taste acceptance.

For obvious ethical reasons, infants in the present study were not randomly assigned to different feeding regimens and, therefore, my conclusions can only be tentative. In comparisons of breastfed infants with formula fed infants, it is accepted that there can be other differences. Not only are breastfed infants perceived differently by their mothers (Glynn *et al.,* 2007) but they are typically introduced to solid food at a later age (Danowski & Gargiula, 2002). Moreover, breastfeeding rates are related to social class (Williams *et al.,* 2010), with those in higher classes more likely to breastfeed. Studies have shown that social class is related to nutritional quality and fruit consumption (Kelleher, Lotya, O'Hara, & Murrin, 2008; Murrin *et al.,* 2007; Williams *et al.,* 2010). A strength of this study is that the overwhelming majority (88%) were from higher social classes (Chapter 3 & 4), removing much of this differential.

However, grouping breastfed infants on the basis of flavour experiences early in life presents other difficulties in interpretation, as there is a great degree of individual variation in the taste quality of breast milk, thus the flavour experienced by infants varies due to the transmission of volatiles from the mothers' diet to breast milk. Any interpretation of these findings is limited, as breast milk was not analysed for possible taste compounds in the present study. Future studies which investigate flavour compounds in breast milk, through evaluation by a sensory panel of experts' perceived taste or by chemical analysis of the composition of taste compounds, would add greatly to the interpretation of these findings.

The current study used methodologies specifically designed to objectively measure infants' behavioural responses to novel solutions and are similar to other studies' approaches. (Blossfeld *et al.,* 2007; Mennella *et al.,* 2001; Mennella *et al.,* 1995; Schwartz, Issanchou, & Nicklaus, 2009). However, this is the first study to use three methods of measurement in analysing infants' acceptance of sour tastes. By doing so, I was able to consider both ingestion levels and taste reactivity (Liking). Berridge (2000) suggested that patterns of taste reactivity reflect the hedonic or aversive impact of a taste, (i.e. its palatability). While palatability is often correlated with both the amount ingested and sensory qualities of a taste (e.g. sourness or sweetness), Berridge argues that in many situations this correlation can be disrupted and taste reactivity is separable from ingestion patterns. Therefore, it was deemed important not just to rely on ingestion as a method to assess acceptance as done in previous studies (Blossfeld *et al.,* 2007). Videotaping infants and rating their taste reactivity allowed us to pick up individuals, which I might have missed by assessing ingestion alone, with 4 individuals assessed as liking 0.013M by R-LR that were not picked up by assessment of ingestion alone.

Previous video techniques have used only facial expressions, known as Facial Action Units, as measurement of acceptance and had the disadvantage of not accounting for other non-verbal cues (Forestell & Mennella, 2012). The video technique used in this study (R-LR) was performed by videoing the infant feeding and then slowing the video down and coding it frame by frame for non-verbal cues including facial expressions, hand pushing and head turning as well as length of time the infants drank for, giving it a possible advantage over previous techniques. Moreover, data can be analysed by independent researchers and can be revisited several times after the initial assessment has taken place. It also takes into account the length of time the infant is drinking in addition to the number of negative and positive non-verbal cues. The calculation of these scores is described further in Chapter 2.7 (pp99–100). Videotaping allowed us to record facial expressions such as eye squints, nose wrinkling and gaping, while also recording other non-verbal cues e.g. pushing away of the bottle. This allowed us to observe a range of non-verbal negative behaviours cues and quantify their number during the testing session. Negative expressions demonstrated that all infants were able to recognise the sour taste in the solutions.

We also used the amount drunk to calculate acceptance as with previous studies (Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009). This gave us an objective measure of consumption. However, it does not account for non-verbal cues, which may influence a mother when introducing new foods to their infant. Finally, I used Mothers Liking Ratio (M-LR), as a third method of assessment as it is possible that infants' mothers, who know their infants best, are able to pick up on non-verbal cues that no other method picked up. Using a proportional variable (i.e. Liking Ratio) made comparisons between individuals possible. However, Mothers' Liking Ratio showed a weak relationship between Ingestion Ratio (R^2=0.12–0.34) and Rater's Liking Ratio (R^2=0.16–0.29). In contrast, Rater's Liking Ratio showed a much stronger relationship between Ingestion Ratio (R^2=0.51–0.77).

It notable that, in this current study, Mothers' Liking Ratio (M-LR) of sour taste acceptance did increase from 6 to 12 months. One potential explanation for this change may be that mothers had become more familiar with their infants trying novel flavours by 12 months of age and thus did not rate rejection of these novel tastes as strongly as they did at 6 months. Moreover, M-LR had a weak relationship with the other two methods (R-LR, IR) at both time points (Table 7.3 & 7.4). This may be because the mothers are able to pick up cues from their infants that the other methods are unable to capture. Both IR and R-LR showed a much stronger relationship. This may be due to the fact that both methods considered the amount ingested by the infants.

From an evolutionary perspective, facial expressions served as a non-verbal signal to parents that their infant may be ingesting something potentially harmful. Parents would have used these cues to avoid feeding infants poisonous substances (Babchuk, Hames, & Thompson, 1985). However, in today's society, negative reactions by infants to new tastes and flavours, may discourage parents from feeding their infant these flavours (e.g. fruits and vegetables). Although infants may not initially like a particular flavour, they can eventually learn to accept and like it, often after it is presented 8–12 times (Forestell & Mennella, 2007; Gerrish & Mennella, 2001; Sullivan & Birch, 1994). Given the differences between the M-LR results and the other two methods, parents may need better understanding regarding this normal course of taste acceptance. Previous research showed that approximately a quarter of parents drew premature conclusions about their infant's dislike of foods, often after fewer than two exposures (Carruth, Ziegler, Gordon, & Barr, 2004). As a result of parents' reluctance to persevere with foods after having negative reactions during feeding, infants may not learn to like some fruits. (Forestell & Mennella, 2012) suggest that parents should focus not only on their infant's facial reactions but also on their infant's willingness to continue eating. They suggest that parents should also allow multiple opportunities to taste novel flavours. My study adds weight to this recommendation.

7.5 *Strengths & Limitations*

Due to infants' inability to communicate verbally, researchers have to rely on indirect measures. Therefore, I aimed to control experimentally a number of factors that could bias infants' behaviour during acceptance tests, using a number of approaches. Firstly, infants' acceptance of sourness was assessed by three methodologies. The use of all three methodologies gave my research a more complete picture of the cohort's varying responses to stimuli. Secondly, to avoid any bias, mothers were unaware of the study hypothesis and, in the case of sour assessment, did not know which taste and what concentrations were offered to the infants at the time. Thirdly, several efforts were made to ensure that infants and their mothers complied with the study protocol and achieved external validity. One of the problems involved deciding upon how to present the drinks to the infant's. At 6 months, most infants accepted their drinks from the bottle described in Chapter 2 but at 12 months, many more infants were fussy regarding the cup/bottle used. Therefore, I decided to let the mother choose the cup/bottle in which the solutions should be offered to the infants, similar to the approach by Blossfeld *et al.* (2007). Acceptance scores, as measured by ingestion rate, were calculated as a ratio to avoid any differences in absolute amounts. Using different containers to offer the solutions may have contributed to some inconsistencies in the data; however, my results show clearly that differences in sour acceptance between infants existed.

Finally, the sensory tests with infants were conducted in the infant's own home. Carrying out sensory tests with infants successfully requires that the child feels comfortable in the research environment (Pooper & Kroll 2003). For the assessment, the mothers always did the feeding, to avoid bias by an unknown person. Mothers were asked not to give any cues to their infants during the test session and they were also blinded to what concentration of acid was being fed to their infants. To ensure that mothers complied with the testing protocol and to observe the feeding of their infants, the investigator was always present during the session but out of sight of the infant. This protocol was utilised in an attempt to achieve external validity of the study.

Attrition is a major obstacle in conducting any longitudinal research. Families who agreed to participate were dedicated and highly motivated. By the final time point (12 months) 55 of the 87 mothers (65%) were still in the study. Reasons for withdrawals are previously described in Chapter 2. These finding are similar to other longitudinal studies with infants and children which reported dropout rates up to a third of participants (Nolan *et al.,* 2001; Nicklaus *et al.,* 2005; Senn *et al.,* 2005; Blossfeld *et al.,* 2007). In addition, 3 infants at 6 months and 5 infants at 12 months did not comply with the research protocol and were removed from the analysis. This resulted in a reduction in the sample size from 64 participants at 6 months to 50 participants at 12 months. The smaller sample size is likely to have reduced the power and reliability of the findings observed at a multivariate level for the follow-up time points. Although every effort was made to enrol subjects from different socio-economic backgrounds, the high number of well-educated older women involved could have biased the research outcomes as they might have been more health conscious and considerate in their diet and feeding methods than the general population. However, while the population of this study was quite homogenous in terms of their socio-economic status education and age, high variability in their own diets and that of their infants' food intake and taste preference was found. As a result, strong conclusions about what factors have significant influences on taste acceptance could be drawn.

7.6 *Conclusion*

The present study is the first to demonstrate that at age 6 and 12 months some infants accept high concentrations of sourness, something previously shown only in toddlers (18 month olds) or in older children (5–12 year olds). This research also suggests that acceptance of sour tastes may be related to length of exclusive breastfeeding as well as gender and amount of fruit consumed during pregnancy. Furthermore, the data suggest that the acceptance of sour tastes at 6 months is related to fruit intake during pregnancy, length of exclusive breastfeeding and gender, with males having higher sour taste acceptance. At 12 months, fruit consumption by the infants was positively associated with sour taste acceptance. Given the relationship between fruit consumption and sour taste acceptance early in life, it is especially important to understand this relationship in a context of developing healthy food patterns early in life, in a world of increasing childhood obesity.

CHAPTER 8. GENERAL DISCUSSION

8.1 *Introduction*

The main aim of the study was to examine factors that influence sour taste development in infants. This was in order to test the hypothesis that mothers' diet *in utero* and postnatally, as well as infants' diets in relation to fruit consumption, would have a positive impact on sour taste acceptance in their infants. In order to test this hypothesis, one needs to investigate the early environment of the foetus and infant, *in utero* and through breast milk. Infants are born with an innate preference for sweet flavours and an innate aversion to bitter and sour tastes (Birch, 1998; Steiner, 1977). An infant's development of sour taste preference is important due to its link to high fruit consumption from an early age (Blossfeld *et al.,* 2007; Liem & Mennella, 2003). The study's subsidiary aims sought to examine the diets of mothers during pregnancy; after delivery; and to examine the diet of the infants themselves.

This study is the first to establish an association between maternal fruit intake and sour taste acceptance in young infants. Regular fruit consumption in the maternal diet during pregnancy and length of exclusive breastfeeding are positively associated with sour taste acceptance at 6 months. Males were found to be more accepting of sour tastes than females, a finding that has not been previously reported (Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009). Infants who consumed high quantities of fruit at 12 months were found to be more accepting of sour tastes; however, there was no association with exclusive breastfeeding, gender or maternal fruit intake. These novel findings contribute significantly to the literature on sour taste development, by broadening the understanding of the factors associated with the development of sour taste acceptance in the first year of life. This final chapter will discuss the overall findings of the study in relation to the current literature and implications and recommendations for future research will be given.

8.2 *General Discussion*

A novel finding of this study is that regular high fruit intake (>2 portions) in the maternal diet during the third trimester of pregnancy is positively associated with sour taste acceptance in infants at 6 months. Whilst in the womb, the foetus, which has a functioning taste system by 20 weeks (Bradley & Stern, 1967), is surrounded by amniotic fluid, which it ingests daily (Lilley, 1972). Amniotic fluid contains flavours that resemble the flavour of the food eaten by the mother (Hauser *et al.,* 1985). It has been suggested that foetal learning about foods regularly eaten by the mother through frequently transmitted flavours in amniotic fluid may be a fundamental feature of dietary learning for humans (Mennella & Trabulsi, 2012). A previous study illustrated that mothers' who drank carrot juice daily for three weeks during the third trimester of pregnancy, had infants who showed greater acceptance of carrot flavoured cereal at weaning compared to infants without this familiarisation (Mennella *et al.,* 2001). Similarly, newborns regularly exposed to anise or garlic flavours through the maternal diet during the last trimester of pregnancy, showed a preference of these odours at birth compared to infants unfamiliar with these flavours (Mennella *et al.,* 1995; Schaal *et al.,* 2000).

The results of this current study further support the hypothesis that flavour acceptance is shaped by foetal experiential learning prior to birth. The association established in this study between high maternal fruit intakes on a daily basis and sour taste acceptance may have significant implications for an infant's future dietary habits. The early sensory experience during late gestation may provide a bridge facilitating the acceptance of similar foods to those eaten by the mother during pregnancy, thus promoting the transition to healthy or less healthy table foods. Therefore, whether a foetus first becomes familiar with flavours of fruit may depend on a mother's dietary choices during pregnancy. As food habits established during infancy persist into later childhood and adolescence (Skinner *et al.,* 2002b; Nicklaus, Boggio, Chabanet, & Issanchou, 2004), early consistent experiences with fruit *in utero* may increase infants' acceptance of these fruits.

This is the first study of its kind to demonstrate that length of exclusive breastfeeding is associated with higher sour taste acceptance. Mothers who exclusively breastfed for more than 20 weeks had infants that were more accepting of sour tastes at 6 months when compared to those who breastfed for less than 20 weeks. Previous studies by Schwartz *et al.* (2012) have indicated that duration of exclusive breastfeeding had a positive association on umami taste acceptance (Schwartz *et al.,* 2012); however, no relationship was observed with the other 4 basic tastes; sour; bitter; salt; and sweet. A possible reason for their findings may lie in the low concentrations used to test acceptance; for example, infants were indifferent to sour and bitter solutions compared to water. It is, therefore, possible that the stimuli were not detected by infants due to the low concentrations. This conclusion is consistent with the findings in this current study as the length of exclusive breastfeeding was not predictive of sour taste acceptance at the lowest concentration (0.013M). In contrast length of exclusive breastfeeding was predictive of sour acceptance at the two highest concentrations of citric acids (0.029M and 0.065M). By offering a range of sourness (4 solutions with varying citric concentrations) to the infants, it allowed a more complete analysis of sour tastes acceptance in this study.

In addition to the use of low concentrations there were differing protocols used for my study compared to Schwartz *et al.* (2009). Schwartz *et al.* (2009; 2012) presented 20 bottles to infants with low concentrations of the five basic tastes. Given the difficulties keeping young infants' attention on task, fatigue could have influenced these results. These solutions were given by a researcher, an unfamiliar adult, which may have further influenced the results. Furthermore, Schwartz *et al.* (2012) only considered the relationship between breastfeeding and infants' intakes (IR), and did not consider other non-verbal cues. In contrast, the current study focused solely on sour tastes and three methods were used. Each infant was presented with 4 drinks with varying concentration of citric acid, similar to those used in previous studies (Blossfeld *et al.,* 2007; Liem & Mennella, 2003; Liem *et al.,* 2004). They were given by their mother in their own environment, thus ensuring that the infant was as relaxed as possible. It is the contention of this thesis that the protocol as well as the multiple measurements of acceptance followed adds weight to its findings.

While maternal fruit consumption during pregnancy was positively associated with sour taste acceptance at 6 months, no association between mothers' fruit consumption during lactation and their infant's sour taste acceptance was observed. Breast milk contributes a unique complex flavour experience to infants during lactation (Ganchrow & Mennella, 2003; Hausner *et al.,* 2009), as the flavours experienced by infants vary due to the transmission of volatiles (molecules which give food its flavour and aroma) from the mothers' diet to breast milk (Hausner *et al.,* 2009). However, this apparent divergence in the results of pre-natal and post-natal learning may be explained by a higher transfer rate of volatile compounds across the placenta than across the epithelial cells in the breast, resulting in higher concentrations of the flavour compounds in amniotic fluid in comparison to breast milk, as hypothesised in previous research (Sörgel *et al.,* 2003; Hausner *et al.,* 2008). Data comparing amniotic fluid and breast milk transfer rates for flavour compounds do not exist in the current literature. However, the externally derived compound, acrylamide, (a possible carcinogenic compound derived from heating starchy food) has been shown to transfer across the placenta in higher concentrations than for breast milk (Sörgel *et al.,* 2003). A similar effect may be observed with regard to sour volatiles and, as such, there may be higher concentrations in amniotic fluid, which could account for the present study's findings.

This study identified gender difference in taste preference. Among the current cohort, males were found to have accepted extreme sour tastes more than females at 6 months. Gender differences in the current findings may be explained by an anomaly in the data due to sampling bias. Among this cohort there was a trend towards longer duration of exclusive breastfeeding in males (16 weeks ± 24) compared to females (9 weeks ±26), though this difference was not statistically significant. Had there been similar numbers of males and females breastfeeding for equal amounts of time, this finding may not have been observed. Supporting this is the fact that effect of gender was seen at 6 months but not at 12 months. Furthermore, previous studies have shown no gender differences in sour taste acceptance among infants (Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009), but the role played by genetics in sour tastes acceptance is poorly understood (Garcia-Bailo *et al.,* 2009). In bitter tastes, genetic differences are better understood and genetic differences contribute to the variability in human perception of bitter substances such 6-n-Propylthiouracil (PROP; Keller *et al.,* 2002; Turnbull & Matisoo-Smith, 2002). However, previous studies have shown no gender linked differences in bitter tastes in infants and children (Schwartz, Issanchou, & Nicklaus, 2009; Mennella, Pepino & Reed, 2005). However, one study in adults showed that adult women are considered more sensitive to bitter tastes (supertasters) more frequently than males (Bartoshuk *et al.,* 1994). Given these findings, further work in this area is needed to understand these results.

The current study found that, while regular exposure to fruit *in utero* and length of exclusive breastfeeding were positively associated with sour taste acceptance at 6 months, these factors were no longer predictive of higher acceptance of extreme sour flavours at 12 months. Existing literature indicates that infants who experience a greater variety of flavours at the start of weaning display a higher acceptance of novel foods than infants who are exposed to homogenous favours (Gerrish & Mennella, 2001; Maier *et al.,* 2008; Mennella, Nicklaus, Jagolino, & Yourshaw, 2008). Furthermore, Forestell & Mennella (2007) argued that the initial advantages conferred by breastfeeding must be followed by continued repeated exposure to fruits and vegetables after solids are introduced. In this study, sour taste acceptance was positively associated with fruit intake at 12 months. This adds to the current literature which indicates that sour taste acceptance is positively associated with fruit consumption (Blossfeld *et al.,* 2007; Schwartz *et al.,* 2012). It is thought that there is a critical period (1-3 years), during which direct exposure is most likely to lead to the acquisition of taste preferences (Skinner *et al.,* 2002a). The early introduction or exposure to fruits and vegetables is positively associated with increased intake and variety of these foods consumed later in childhood (Cooke *et al.,* 2004; Skinner, Carruth, Bounds, & Ziegler, 2002a).

Previously, infants' taste acceptance has been evaluated using a range of measurements; either singularly or in combination. Some examples include; facial expressions (Ganchrow *et al.,* 1983; Rosenstein & Oster, 1988; Soussignan *et al.,* 1997; Forestell & Mennella, 2007; Mennella *et al.,* 2009; Forestell & Mennella, 2012); intake quantities (Crystal & Berstein, 1998; Blossfeld *et al.,* 2007; Schwartz, Issanchou, & Nicklaus, 2009); and perception scores by mother or independent persons (Mennella & Beauchamp, 2002; Mennella *et al.,* 2004;

Schwartz, Issanchou, & Nicklaus, 2009). This study has clearly shows that statistical differences exist between different methods of investigating taste acceptance in non-verbal infants. In this study, three measurements were taken together in order to assess acceptance, as there is no gold standard of measurement. These included physiological measurements (measurement of intake- IR); Mother perception (M-LR) and measurement using video of non-verbal cues (R-LR) such as facial, head and hand movements. While there are differences in each of the three approaches to rating, using a mix of methodologies yielded some benefits. For example, R-LR was able to identify additional individuals' sour taste acceptance (n=4) which would not have been identified with ingestion alone. R-LR and IR related strongly to each other compared to these methods and M-LR. Berridge (2000) argues that, in many situations, the relationship between taste reactivity is separable from ingestion patterns and as such reliance on one method may bias results. Previously, Blossfeld *et al.* (2007) relied on the amount ingested by the infants as a measure of acceptance and may have reported skewed results by being unable to consider additional behavioural responses by the infants.

Previously, Facial Action Coding System (FACS) has been used in some tastes acceptance studies (Forestell & Mennella, 2007; Mennella, Forestell, Morgan, & Beauchamp, 2009; Forestell & Mennella, 2012) but this system may misinterpret those infants who may show dislike of substances but continue consuming the product or those infants who may have expressed strong aversion using other non-verbal cues such as kicking and pushing with feet or hands. Recently, Mennella & Trabulsi (2012) recognised the limitations of using

FACS alone and suggested that further studies which consider additional measurement such as other body movements may be needed in give a better understanding of the infant's responses to flavours. In this study R-LR scores took account of these non-facial cues in addition to facial reactions. In combination with other measurements (IR & M-LR), this may represent an improvement on previous approaches. Systematic review of the methods used in taste acceptance is needed to help towards creating a consensus with regard to a gold standard approach for use in future taste acceptance research.

Similar difficulties in assessing non-verbal infants have been found in other areas of behavioural research such as pain assessment. In this area, a coding system known as Neonatal Facial Coding System (NFCS) has been used (Stevens, Johnston, Petryshen, & Taddio, 1996; Taddio, Katz, Ilersich, & Koren, 1997; Botvinick *et al.,* 2005). This is a simplified version (10 of the 98 basic units) of FACS which is considered the gold standard of facial measurements (Ekman & Friesen, 1978; Grunau, Johnston, & Craig, 1990; Grunau *et al.,* 1998). While NFCS, in pain assessment, has the ability to discriminate between intensities of distress it cannot distinguish between different types of distress for example pain–related and non-pain-related distress (Ahola-Kohut & Pillai Riddell, 2009). Other studies have used other measurements such as crying; variations in heart rate; blood pressure; oxygen saturation; breathing patterns and body movements (Sweet & McGrath, 1998; Bellieni *et al.,* 2004; Bellieni, 2012). In a systematic review of infant pain assessment tools, Duhn & Medes (2004) concluded that, while the use of multiple measurements provided a stronger measure, it still does not fulfil all criteria for an ideal measure.

The current study shows that there are many opportunities to influence sour taste acceptance during the first year of life. The research findings indicate that the frequency of exposure to fruit from the maternal diet in pregnancy; length of exposure to breast milk and the infant's own fruit intakes at 12 months led to increased sour taste acceptance. Therefore, the study's subsidiary aims sought to examine the diets of mothers during pregnancy; after delivery; and to examine the diet of the infants themselves. Therefore, there needs to be a focus on dietary intakes during these critical times. In the present study mothers were consuming higher than recommended intakes of fat, saturated fat and salt and lower than recommended intakes of vitamin D during both pregnancy and lactation. These dietary patterns were repeated for salt and vitamin D in their infants' diets at 12 months. Therefore, some infants may be exposed to an unhealthy diet even prior to weaning. Obesogenic eating patterns, characterised by high consumption of energy-dense, nutrient-poor foods, can start before 6 months of age (Tarrant *et al.,* 2010). Hence the need to reinforce the importance of introducing flavours of food that are both healthy and nutrient-dense. One such fundamental dietary principle is the promotion of the intake of fruit from an early age.

Vitamin D consumption was consistently low across all dietary periods (pregnancy; lactation and 12 month olds). It is a vital nutrient required for normal skeletal development, and its deficiency can lead to rickets, poor linear growth, motor delay, bone fragility and hypocalcaemic seizures (Wharton & Bishop, 2003). In recent, vitamin D deficiency and associated rickets are re-emerging as major public health issues worldwide (Holick, 2006). Previous studies have found that approximately 50% of pregnant women and 56% of breastfed infants in Ireland had insufficient vitamin D (O'Riordan *et al.,* 2008; Mulligan *et al.,* 2010). As infants are reliant on their mothers' vitamin D stores prior to birth and during breastfeeding (Donovan, 2009), the high rates of breastfeeding amongst this study cohort could put the study's infants at risk of vitamin D deficiency.

It was assumed that younger and less educated mothers are less likely to meet the national recommendations for healthy eating (FSAI, 2012) and therefore should be targeted for dietary interventions during pregnancy (Bodnar & Siega-Riz, 2002; Freisling, Elmadfa, & Gall, 2006; Murrin *et al.,* 2007). The current study consisted of older, well educated women, who were working and generally came from higher socio-economic groups. Despite this, the majority of participants' diets did not meet many of the national recommendations for healthy eating. Interestingly, 76% of mothers initiated breastfeeding at birth, a result which was higher than the national average (The Economic and Social Research Institute - Health Research and Information Division, 2012). However, this is in line with other Irish studies of older, well-educated mothers (Walton, 2012, The Economic and Social Research Institute - Health Research and Information Division, 2012). While the initiation of breastfeeding is on the increase, early cessation of breastfeeding continues to be a problem, with a number of studies indicating that many Irish and UK mothers who initiated breastfeeding at birth had ceased by 6 weeks (Williams *et al.,* 2010; Tarrant *et al.,* 2011; McAndrew *et al.,* 2012). In a study of Irish mothers reported that the main reasons participants gave for early breastfeeding cessation were; maternal tiredness (26%); restriction of their freedom (23%); and their own perception of inadequate milk supply (17%; Tarrant *et al.,* 2008). The cohort yielded unusual results with regard to the high number of women breastfeeding for long periods – with 50% of infants at 6 months and 27% of infants at 12 months receiving some breast milk. Again, this could be due to the profile of the study's participants or due to the fact that the messages of the benefits of prolonged breastfeeding are reaching a receptive audience. Further investigation into why some mothers continue to feed for longer periods may provide data that could further inform health promotion initiatives.

To date, the majority of the nutritional literature available to Irish parents with regard to pregnancy and their infants' early months concentrates on the nutritional qualities of mothers' diets (Health Promotion Unit, 2006) and of breast milk (HSE, 2013) but does not address the sensory quality of foods, such as taste, during early infancy (Health Promotion Unit, 2012; HSE, 2013). This study, along with other research, provides preliminary evidence that regular exposure to particular flavour compounds early in life has the potential to influence infants' dietary and fruit patterns beyond infancy (Mennella *et al.,* 2001; Liem & Mennella, 2003; Forestell & Mennella, 2007), indicating the importance of providing specific education regarding the sensory qualities of fruits to both health professionals and parents. As this research has demonstrated, the maternal diet is predictive of the types of foods and tastes an infant is presented with early in life. For this reason, interventions that address the maternal diet and feeding habits, during pregnancy and post-partum, may enable infants to develop taste preferences that support healthy eating in later life. This study's findings suggest that all women, irrespective of demographics, should receive information regarding best practice for healthy eating in pregnancy and lactation. Furthermore, the findings suggest that information should also highlight the risks associated with excessive fat and salt consumption, something that is lacking in current information. Existing research and the current study suggest that sensory aspects of breast milk (such as transmission of volatiles) and duration of breastfeeding may play a role in facilitating sour taste development. Furthermore, the current study showed that repeated exposure to fruit in the maternal diet in the third trimester of pregnancy facilitated sour taste acceptance at 6 months. These benefits are not currently highlighted in existing information provided to pregnant women (Health Promotion Unit, 2006) and may also help to improve breastfeeding initiation and duration rates.

Recent consistent messages regarding the benefits of breastfeeding to both mother and infants has helped increase breastfeeding initiation rates in Ireland over the last decade (from 42% to 57%) (The Economic and Social Research Institute - Health Research and Information Division, 2012). As evidenced in this study and others, the message regarding the importance of breastfeeding has been effective with some groups, particularly those with third level education and those from higher socio-economic standing (Williams, Greene, McNally, Murray, & Quail, 2010; The Economic and Social Research Institute - Health Research and Information Division, 2012). However, less consistency has reported with regards to weaning. Many women in this study were recruited on mother and infant forums, where mothers often discuss weaning and feeding issues and report confusion in the information available on weaning (Porter & Ispa, 2012). This has not been helped by inconsistent recommendations internationally (WHO, 2001; ESPGHAN, 2008; BDA, 2013). A recent Irish study reported that only 7% of GPs and 50% of Practice Nurses in Ireland provided appropriate literature with regard to correct weaning practices (Allcutt & Sweeney, 2010). Therefore, reliance on commercial infant formula websites and peer-led forums, allows for potential misinformation and confusion among mothers at this important time. The findings of this thesis and current literature suggest that all parents should be provided with specific and targeted information on healthy eating during pregnancy and lactation, and the introduction of solid foods to infants. Clear, consistent evidence-based guidance, for the first 1000 days - from conception to 2 years - is required to ensure that key areas are covered with all parents. A national information pack would ensure the provision of accurate, consistent and practical information for all stages to all parents. Recently the website http://www.first1000days.ie provides much of this information to parents. However, again we are relying on commercial companies (Danone Baby Nutrition)to provide this information.

Results from the literature and this study show that the current information readily available such as – 'Healthy eating in Pregnancy' and 'How to spoon feed your child' may not be enough to improve infants' diets during these critical times (Health Promotion Unit 2006; 2012). Kramer *et al.* (2000) and Anderson (1995; 2001) found no evidence that routine advice on how to improve nutrition during pregnancy was successful in altering dietary intake. Kafatos *et al.* (1989) found that individual nutritional counselling during by trained researchers fortnightly home visits was associated with a significant improvement in dietary intake during pregnancy. This intensive intervention, whilst successful, may not be realistic on a national level. As previously mentioned, while all women may benefit from nutritional advice during pregnancy, education may be more successful if individualised messages are developed in a format, medium and language designed to appeal to specific target groups (Watson & McDonald, 2009). Public health nurses are ideally placed to provide information regarding mother's diets post-partum, to support breastfeeding, to help with weaning and to provide guidance with regard to vitamin D supplementation. However, an appraisal of current methods of education and an audit of the skills and resources available to provide an effective support is also required. Further opportunities may arise to improve communication with mothers. The recent growth in smart mobile phone and tablet applications may open potential new and exciting avenues for development of targeted advice for groups of women during this critical time. For a modest outlay in terms of software development, expectant mothers could receive real-time nutritional guidance with regard to their diet.

8.3 *Future Studies*

This study observed a relationship between maternal fruit intake in pregnancy and sour taste acceptance at six months. No such association was found between infants' sour taste acceptance and their mothers' diets during lactation. As previously mentioned, the composition of volatiles in mothers' milk varies according to maternal diet (Hausner *et al.,* 2008). However, volatiles have been analysed chemically in relatively few studies (Shimoda, *et al.,* 2000; Bingham, Lavin, & Acree, 2003; Buettner, 2007; Hausner *et al.,* 2008; Hausner *et al.,* 2009). Moreover, maternal food intake has not been recorded or controlled during milk collection in any of the above studies. Future studies that chemically analyse breast milk would be beneficial in cohorts of low and high fruit intake in order to identify specific volatiles related to fruit consumption.

Previous research into flavour transmission has shown flavour peaks 30 minutes to 3 hours after ingestion of breast milk in a range of flavour compounds (Mennella *et al.,* 1991a; Mennella *et al.,* 1991b; Mennella *et al.,* 1999). Studies which compare mothers who are given high intakes of a particular fruit versus mothers eating low fruit may help in the identification of compounds related to fruit. Furthermore, consideration could be given to the timing of these flavour transmissions. Future research, by examining these compounds, might investigate whether these specific compounds affect acceptance of sour tastes in infants. This could lead to a greater understanding if there is a causal relationship between particular compounds in the diet and infants' acceptance of sour tastes.

Other research could examine any potential benefits of partial breastfeeding (mixture of breast and formula milk). In Ireland, many infants are partially breastfed (ERSI, 2012; Williams *et al.,* 2010) and gain some exposure to varying flavours through breast milk. The results of the current study observed a positive association between length of exclusive breastfeeding and sour taste acceptance. Furthermore, current literature suggests breastfeeding improves the initial acceptance of novel foods at weaning (Forestell & Mennella, 2007; Mennella *et al.,* 2001). It would be of interest to consider the effects of partial breastfeeding on infant's sour taste acceptance. In this study, due to sample size, it was not possible to divide formula fed and partially breastfed infants into separate groups. Given that length of exclusive breastfeeding was positively associated with sour taste acceptance, it would be of interest to consider repeating this study in a larger group and consider the effects of long durations of partial breastfeeding on infants' acceptance of sour taste and whether these differ from those infants exclusively breastfed.

In addition, it is also important to consider the effects of different types of fruit and to quantify frequency of exposure needed to affect sour taste acceptance. Previous randomised control trials considered the effect of carrot flavours (daily consumption of carrot juice for three weeks) in the maternal diet during pregnancy or lactation on the acceptance of carrot flavour cereal at weaning (Mennella *et al.,* 2001). However, the authors did not explore whether these carrot flavours influenced the acceptance of other similar flavoured vegetables. Mennella *et al.* (2008) showed that at weaning an infant's repeated exposure to a particular fruit (pear) at weaning for eight consecutive days increased the infant's consumption of that fruit. Furthermore, infants who ate a variety of fruit over the eight days also increased their intake of pears although they had no direct experience during the exposure period. This demonstrated the importance of repeated exposure in increasing acceptance of similar flavours. It was beyond the scope of the current study to isolate particular fruits and their relationship with sour taste acceptance. Interventions adopting a similar approach to the carrot study, which consider an infant's experiences of specific fruits – *in utero* and during lactation – and explore any association between their acceptance and an infant's acceptance of other fruits would further contribute to the existing research.

Finally, in this current study, infants had some experience with solid foods at 6 months which could influence data at 6 months. Government recommendations encourage mothers not to introduce solid foods before at least 17 weeks (Department of Health and Children, 2005). The Growing-up in Ireland study showed that mothers introduced solid foods to their infants during the fourth month of life approximately (19 weeks; Williams *et al.,* 2010). It would, therefore, be of interest to repeat this study at an earlier time-point – for example before 17 weeks to ascertain whether the results could be confirmed at this age. By doing so, this would allow for the infants to have several months of exposure to breast milk, while further limiting the chances that they had been exposed to solid foods.

8.4 *Conclusion*

The results of this thesis suggest that sour taste acceptance is explained in some part by an infant's early exposure, through their mothers' fruit intake during pregnancy, post-partum through breastfeeding, and through their own early experience with fruit. Given the increase in childhood obesity in Ireland (Williams *et al.,* 2010; Williams *et al.,* 2013) and other developed countries (International Obesity Taskforce, 2012; WHO, 2009), the importance of developing early healthy eating habits has assumed great importance. It is not enough to solely examine children's diets from a nutritional perspective; consideration of the sensory world in which infants develop their food and taste preferences is required as these preferences can have profound effects on their diets and possible future health outcomes. Greater understanding of the factors associated with the development of sour tastes and the process by which healthy and unhealthy food patterns evolve can offer an important tool in the promotion of fruit intake from an early age and hence encourage healthier eating patterns from infancy through to adulthood. This thesis contributes to the literature in this area and offers potential avenues for future research and potential interventions in an area of increasing importance in today's obesogenic environment.

References

Abraham, E. C., Godwin, J., Sherriff, A., & Armstrong, J. (2012). Infant feeding in relation to eating patterns in the second year of life and weight status in the fourth year. *Public Health Nutrition, 15*(09), 1705-1714.

Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., . . . Rigo, J. (2008). Complementary feeding: A commentary by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition, 46*(1), 99-110.

Ahola Kohut, S., & Pillai Riddell, R. (2009). Does the neonatal facial coding system differentiate between infants experiencing pain-related and non-pain-related distress? *The Journal of Pain, 10*(2), 214-220. http://dx.doi.org.remote.library.dcu.ie/10.1016/j.jpain.2008.08.010

Aldridge, V., Dovey, T. M., & Halford, J. C. G. (2009). The role of familiarity in dietary development. *Developmental Review, 29*(1), 32-44. DOI: 10.1016/j.dr.2008.11.001

Allcutt, C., & Sweeney, M. (2010). An exploration of knowledge, attitudes and advice given by health professionals to parents in Ireland about the introduction of solid foods. A pilot study. *BMC Public Health, 10*(1), 201.

Allen, L. H. (2005). Multiple micronutrients in pregnancy and lactation: An overview. *The American Journal of Clinical Nutrition, 81*(5), 1206S-1212S.

Allen, L. H., & Graham, J. M. (2003). Assuring micronutrient adequacy in the diets of young infants. In F. M. Delange, & K. P. J. West (Eds.), *Micronutrient Deficiencies in the First Six Months of Life.* (pp. 55-88). Basel: Vevey/S: Karger AG.

American Diabetes Association. (2000). Type 2 diabetes in children and adolescents. *Pediatrics, 105*(3), 671.

Anderson, A. S. (2001). Pregnancy as a time for dietary change? *Proceedings of the Nutrition Society, 60*(04), 497-504.

Anderson, A. S., Campbell, D. M., & Shepherd, R. (1995). The influence of dietary advice on nutrient intake during pregnancy. *British Journal of Nutrition, 73*(02), 163-177.

Ashcroft, J., Semmler, C., Carnell, S., Van Jaarsveld, C., & Wardle, J. (2008). Continuity and stability of eating behaviour traits in children. *European Journal of Clinical Nutrition, 62*(8), 985-990.

ASTM International. ASTM E1871 - 10 standard guide for serving protocol for sensory evaluation of foods and beverage. Retrieved from http://www.astm.org/Standards/sensory-evaluation-standards.html

Babchuk, W. A., Hames, R. B., & Thompson, R. A. (1985). Sex differences in the recognition of infant facial expressions of emotion: The primary caretaker hypothesis. *Ethology and Sociobiology, 6*(2), 89-101.

Baker, R. D., & Greer, F. R. (2010). Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics, 126*(5), 1040-1050.

Barbieri, M. A., Portella, A. K., Silveira, P. P., Bettiol, H., Agranonik, M., Silva, A. A., & Goldani, M. Z. (2009). Severe intrauterine growth restriction is associated with higher spontaneous carbohydrate intake in young women. *Pediatric Research, 65*(2), 215-220.

Barker, D. J. P. (1997). Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition, 13*(9), 807-813.

Barker, D. J. P. (Ed.). (2000). *Influences on development and disease in later life. NIH monograph series*. New York: Marcel Dekker.

Barker, D. J. P. (2012). Developmental origins of chronic disease. *Public Health, 126*(3), 185-189.

Barsai, M. E. (Ed.). (2003). *Human nutrition A health perspective.* (2nd ed.). London: Arnold.

Bartoshuk, L. M., Duffy, V. B., & Miller, I. J. (1994). PTC/PROP tasting: Anatomy, psychophysics, and sex effects. *Physiology & Behavior, 56*(6), 1165-1171.

Bartoshuk, L. M., Rifkin, B., Marks, L. E., & Hooper, J. E. (1988). Bitterness of KCl and benzoate: Related to genetic status for sensitivity to PTC/PROP. *Chemical Senses, 13*(4), 517-528. doi:10.1093/chemse/13.4.517

Baxter, S. D., Thompson, W. O., & Davis, H. C. (2000). Fourth-grade children's observed consumption of, and preferences for, school lunch foods. *Nutrition Research, 20*(3), 439-443. doi: 10.1016/S0271-5317(00)00136-6

Bayley, T. M., Dye, L., Jones, S., DeBono, M., & Hill, A. J. (2002). Food cravings and aversions during pregnancy: Relationships with nausea and vomiting. *Appetite, 38*(1), 45-51. doi: 10.1006/appe.2002.0470

Bazzano, L. A., Serdula, M. K., & Simin Liu MD, S. (2003). Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Current Atherosclerosis Reports, 5*(6), 492-499.

Beaglehole, R., Bonita, R., Horton, R., Adams, C., Alleyne, G., Asaria, P., . . . Casswell, S. (2011). Priority actions for the non-communicable disease crisis. *The Lancet, 377*(9775), 1438-1447.

Beauchamp, G. K., Cowart, B. J., Mennella, J. A., & Marsh, R. R. (1994). Infant salt taste: Developmental, methodological, and contextual factors. *Developmental Psychobiology, 27*(6), 353-365.

Beauchamp, G. K., Cowart, B. J., & Moran, M. (1986). Developmental changes in salt acceptability in human infants. *Developmental Psychobiology, 19*(1), 17-25.

Beauchamp, G. K., & Moran, M. (1982). Dietary experience and sweet taste preference in human infants. *Appetite, 3*(2), 139-152.

Beauchamp, G. K., & Mennella, J. A. (2009). Early flavor learning and its impact on later feeding behavior. *Journal of Pediatric Gastroenterology and Nutrition, 48*, S25-S30.

Beaudry, M., Dufour, R., & Marcoux, S. (1995). Relation between infant feeding and infections during the first six months of life. *The Journal of Pediatrics, 126*(2), 191-197.

Begley, C., Gallagher, L., Clarke, M., Carroll, M., & Millar, S. (2009). The national infant feeding survey 2008. *Dublin: Health Service Executive,*

Bell, K. I., & Tepper, B. J. (2006). Short-term vegetable intake by young children classified by 6-n-propylthoiuracil bitter-taste phenotype. *American Journal of Clinical Nutrition, 84*(1), 245-251.

Bellieni, C. V., Sisto, R., Cordelli, D. M., & Buonocore, G. (2004). Cry features reflect pain intensity in term newborns: An alarm threshold. *Pediatric Research, 55*(1), 142-146.

Bellieni, C. V. (2012). Pain assessment in human fetus and infants. *The AAPS Journal, 14*(3), 456-461.

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: Microstructure of affective taste reactivity patterns. *Neuroscience & Biobehavioral Reviews, 24*(2), 173-198.

Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience & Biobehavioral Reviews, 20*(1), 1-25.

Bilkó, Á, Altbäcker, V., & Hudson, R. (1994). Transmission of food preference in the rabbit: The means of information transfer. *Physiology & Behavior, 56*(5), 907-912.

Bingham, S. A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K. T., . . . Day, N. E. (1994). Comparison of dietary assessment methods in nutritional epidemiology: Weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *British Journal of Nutrition, 72*(04), 619-643.

Bingham, P. M., Lavin, E., & Acree, T. (2003). Odorants in breast milk. *Archives of Pediatrics & Adolescent Medicine, 157*(10), 1031.

Birch, L. L. (1992). Children's preferences for high-fat foods. *Nutrition Reviews, 50*(9), 249-255.

Birch, L. L. (1998). Development of food acceptance patterns in the first years of life. *Proceedings of the Nutrition Society, 57*(04), 617-624.

Birch, L. L. (1999). Development of food preferences. *Annual Review of Nutrition, 19*(1), 41-62. Retrieved from http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.nutr.19.1.41

Birch, L. L., & Sullivan, S. A. (1991). Measuring children's food preferences. *Journal of School Health, 61*(5), 212-214.

Birch, L. L. (1979). Dimensions of preschool children's food preferences. *Journal of Nutrition Education, 11*(2), 77-80.

Black, A. E., & Cole, T. J. (2001). Biased over-or under-reporting is characteristic of individuals whether over time or by different assessment methods. *Journal of the American Dietetic Association, 101*(1), 70-80.

Black, A., & Cole, T. (2000). Within-and between-subject variation in energy expenditure measured by the doubly-labelled water technique: Implications for validating reported dietary energy intake. *European Journal of Clinical Nutrition, 54*(5), 386-394.

Black, A., Goldberg, G., Jebb, S., Livingstone, M., Cole, T., & Prentice, A. (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. evaluating the results of published surveys. *European Journal of Clinical Nutrition, 45*(12), 583-599.

Bland, R., Rollins, N., Solarsh, G., Van den Broeck, J., & Coovadia, H. (2003). Maternal recall of exclusive breast feeding duration. *Archives of Disease in Childhood, 88*(9), 778-783.

Blossfeld, I., Collins, A., Boland, S., Baixauli, R., Kiely, M., & Delahunty, C. (2007). Relationships between acceptance of sour taste and fruit intakes in 18-month-old infants. *British Journal of Nutrition, 98*(05), 1084-1091.

Bodnar, L. M., Catov, J. M., Simhan, H. N., Holick, M. F., Powers, R. W., & Roberts, J. M. (2007). Maternal vitamin D deficiency increases the risk of preeclampsia. *Journal of Clinical Endocrinology & Metabolism, 92*(9), 3517-3522.

Bodnar, L. M., & Siega-Riz, A. M. (2002). A diet quality index for pregnancy detects variation in diet and differences by sociodemographic factors. *Public Health Nutrition, 5*(06), 801-809.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., . . . Schulze, M. (2012). Critical review: Vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition, 51*(6), 637-663.

Bolling, K., Grant, C., Hamlyn, B., & Thornton, A. (2007). Infant feeding survey 2005.

Botvinick, M., Jha, A. P., Bylsma, L. M., Fabian, S. A., Solomon, P. E., & Prkachin, K. M. (2005). Viewing facial expressions of pain engages cortical areas involved in the direct experience of pain. *NeuroImage, 25*(1), 312-319.

Bowen, D. J. (1992). Taste and food preference changes across the course of pregnancy. *Appetite, 19*(3), 233-242. doi: 10.1016/0195-6663(92)90164-2

Bowman, S. A., Gortmaker, S. L., Ebbeling, C. B., Pereira, M. A., & Ludwig, D. S. (2004). Effects of fast-food consumption on energy intake and diet quality among children in a national household survey. *Pediatrics, 113*(1), 112-118. doi:10.1542/peds.113.1.112

Bradley, R. M., & Stern, I. B. (1967). The development of the human taste bud during the foetal period. *Journal of Anatomy, 101*(4), 743-752.

Breymann, C. (2002). Iron supplementation during pregnancy. *Fetal and Maternal Medicine Review, 13*(1), 1-29.

Briefel, R. R., Kalb, L. M., Condon, E., Deming, D. M., Clusen, N. A., Fox, M. K., . . . Reidy, K. C. (2010). The feeding infants and toddlers study 2008: Study design and methods. *Journal of the American Dietetic Association, 110*(12, Supplement), S16-S26. http://dx.doi.org/10.1016/j.jada.2010.09.005

Brion, M. J. A., Ness, A. R., Rogers, I., Emmett, P., Cribb, V., Smith, G. D., & Lawlor, D. A. (2010). Maternal macronutrient and energy intakes in pregnancy and offspring intake at 10 y: Exploring parental comparisons and prenatal effects. *The American Journal of Clinical Nutrition, 91*(3), 748-756.

British Dietetic Association. (2013). *The British Dietetic Association specialist paediatric group position statement complementary feeding: Introduction of solid food to an infants diet* . BDA-British Dietetic Association. Retrieved from: http://www.bda.uk.com/policies/WeaningPolicyStatement.pdf

Brown, A. E., Raynor, P., Benton, D., & Lee, M. D. (2010). Indices of multiple deprivation predict breastfeeding duration in England and Wales. *The European Journal of Public Health, 20*(2), 231-235.

Brown, J. E., & Toma, R. B. (1986). Taste changes during pregnancy. *The American Journal of Clinical Nutrition, 43*(3), 414-416.

Brundage, S. C. (2002). Preconception health care. *American Family Physician, 65*(12), 2507-2514.

Buettner, A. (2007). A selective and sensitive approach to characterize odour-active and volatile constituents in small-scale human milk samples. *Flavour and Fragrance Journal, 22*(6), 465-473.

Buijsse, B., Feskens, E. J. M., Schulze, M. B., Forouhi, N. G., Wareham, N. J., Sharp, S., . . . Tjonneland, A. (2009). Fruit and vegetable intakes and subsequent changes in body weight in european populations: Results from the project on diet, obesity, and genes (DiOGenes). *American Journal of Clinical Nutrition, 90*(1), 202-207.

Bulk-Bunschoten, A., Bodegom, S., Reerink, J., Jong, P., & Groot, C. (2001). Reluctance to continue breastfeeding in the netherlands. *Acta Paediatrica, 90*(9), 1047-1053.

Burnier, D., Dubois, L., & Girard, M. (2010). Exclusive breastfeeding duration and later intake of vegetables in preschool children. *European Journal of Clinical Nutrition, 65*(2), 196-202.

Butte, N. F., Wong, W. W., Ferlic, L., Smith, E. O., Klein, P. D. & Garza, C. (1990) Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. *Pediatrics Research.* 28:631-640.

Butte, N. F., Wong, W. W., Hopkinson, J. M., Heinz, C. J., Mehta, N. R., & Smith, E. O. B. (2000). Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. *The American Journal of Clinical Nutrition, 72*(6), 1558-1569.

Callen, J., & Pinelli, J. (2004). Incidence and duration of breastfeeding for term infants in Canada, United States, Europe, and Australia: A literature review. *Birth, 31*(4), 285-292.

Carnell, S., & Wardle, J. (2008). Appetite and adiposity in children: Evidence for a behavioral susceptibility theory of obesity. *American Journal of Clinical Nutrition, 88*(1), 22-29.

Carruth, B. R., Ziegler, P. J., Gordon, A., & Barr, S. I. (2004). Prevalence of picky eaters among infants and toddlers and their caregivers' decisions about offering a new food. *Journal of the American Dietetic Association, 104*, 57-64.

Cashman, K. D., Hill, T. R., Lucey, A. J., Taylor, N., Seamans, K. M., Muldowney, S., . . . Horigan, G. (2008). Estimation of the dietary requirement for vitamin D in healthy adults. *The American Journal of Clinical Nutrition, 88*(6), 1535-1542.

Cattaneo, A., Burmaz, T., Arendt, M., Nilsson, I., Mikiel-Kostyra, K., Kondrate, I., . . . Fallon, M. (2009). Protection, promotion and support of breast-feeding in europe: Progress from 2002 to 2007. *Public Health Nutrition, 13*(6), 751-756.

Cattaneo, A., Yngve, A., Koletzko, B., & Guzman, L. R. (2005). Protection, promotion and support of breast-feeding in europe: Current situation. *Public Health Nutrition, 8*(01), 39-46.

Center for Disease Control and Prevention (2013) Retrieved December /21st, 2013, from http://www.cdc.gov/parasites/toxoplasmosis/

Chalmers, B., Levitt, C., Heaman, M., O'Brien, B., Sauve, R., & Kaczorowski, J. (2009). Breastfeeding rates and hospital breastfeeding practices in Canada: A national survey of women. *Birth, 36*(2), 122-132.

Chandrashekar, J., Hoon, M. A., Ryba, N. J., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature, 444*(7117), 288-294.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J., & Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. *Nature, 464*(7286), 297-301.

Chaudhari, N., & Roper, S. D. (2010). The cell biology of taste. *The Journal of Cell Biology, 190*(3), 285-296.

Clark, M., & Ogden, J. (1999). The impact of pregnancy on eating behaviour and aspects of weight concern. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 23*(1), 18-24.

Clifton-Bligh, R., McElduff, P., & McElduff, A. (2008). Maternal vitamin D deficiency, ethnicity and gestational diabetes. *Diabetic Medicine, 25*(6), 678-684.

COMA. (1994). *Weaning and the Weaning Diet, Report of the Working Group on the Weaning Diet of the Committee on Medical Aspects of Food Policy.* London: HMSO.

Cooke, L., Wardle, J., Gibson, E., Sapochnik, M., Sheiham, A., & Lawson, M. (2004). Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public Health Nutrition CAB International, 7*(2), 295-302.

Cooke, L. (2007). The importance of exposure for healthy eating in childhood: A review. *Journal of Human Nutrition and Dietetics, 20*(4), 294-301.

Coulthard, H., Harris, G., & Emmett, P. (2009). Delayed introduction of lumpy foods to children during the complementary feeding period affects child's food acceptance and feeding at 7 years of age. *Maternal & Child Nutrition, 5*(1), 75-85.

Cowart, B. J., & Beauchamp, G. K. (1986). The importance of sensory context in young children's acceptance of salty tastes. *Child Development, 57*(4), 1034-1039.

Cowin, I., & Emmett, P. (2000). Cholesterol and triglyceride concentrations, birthweight and central obesity in pre-school children. ALSPAC study team. avon longitudinal study of pregnancy and childhood. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 24*(3), 330-339.

Crook, C. K., & Lipsitt, L. P. (1976). Neonatal nutritive sucking: Effects of taste stimulation upon sucking rhythm and heart rate. *Child Development, 47*, 518-522.

Crooks, D. L. (2000). Food consumption, activity, and overweight among elementary school children in an appalachian kentucky community. *American Journal of Physical Anthropology, 112*(2), 159-170.

Crystal, S. R., & Berstein, I. L. (1998). Infant salt preference and mother's morning sickness. *Appetite, 30*(3), 297-307.

Crystal, S. R., & Bernstein, I. L. (1995). Morning sickness: Impact on offspring salt preference. *Appetite, 25*(3), 231-240.

CSO (1996). Central statistics office- surveys and methodology. Retrieved from http://www.cso.ie/en/surveysandmethodology/

Currie, C., Gabhainn, S. N., Godeau, E., Roberts, C., Smith, R., Currie, D., . . . Rasmussen, B. (2008). *Inequalities in young people's health: Health behaviour in school-aged children (HBSC) international report from the 2005/2006 survey. Vol. 5*. Copenhagen: World Health Organization, 2008.

Curtin, M. (1954). Failure to breastfeed: A review of feeding history of 1007 infants. *Irish Journal of Medical Science, 6*, 447-451.

Daly, S. E., & Hartmann, P. E. (1995). Infant demand and milk supply. part 1: Infant demand and milk production in lactating women. *Journal of Human Lactation, 11*(1), 21-26.

Danowski, L., & Gargiula, L. (2002). Attitudes and practices regarding the introduction of solid foods to infants. *Family Practice, 19*(6), 698-702.

Darwin C. (1877) Biographiche skizze eines kleinen kindes. *Kosmos.* 367–376.

De Boo, H.A., & Harding, J. E. (2006). The developmental origins of adult disease (barker) hypothesis. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 46*(1), 4-14.

de Onis, M., Onyango, A. W., Borghi, E., Siyam, A., Nishida, C., & Siekmann, J. (2007). Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization, 85*, 660-667.

Dee, A., Perry, I., O'Neill, C., Doherty, E., Callan, A., Kearns, K., . . . Sweeney, M. (2013). PP47 the cost of overweight and obesity on the island of Ireland. *Journal of Epidemiology and Community Health, 67*(Suppl 1), A68-A68.

DeLany, J. P., Windhauser, M. M., Champagne, C. M., & Bray, G. A. (2000). Differential oxidation of individual dietary fatty acids in humans. *American Journal of Clinical Nutrition, 72*(4), 905-911. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0033806851&partnerID=40&md5=b3fe3353c9638e58ec3faeed124ca33a

Dennison, B. A., Rockwell, H. L., & Baker, S. L. (1998). Fruit and vegetable intake in young children. *Journal of the American College of Nutrition, 17*(4), 371-378.

Denomme, J., Stark, K. D., & Holub, B. J. (2005). Directly quantitated dietary (n-3) fatty acid intakes of pregnant canadian women are lower than current dietary recommendations. *The Journal of Nutrition, 135*(2), 206-211.

Department of Health (Ed.). (1991). *Report on health and social subjects. Dietary reference values for food, energy and nutrients for the United Kingdom*. London: HMSO.

Department of Health. (1994). *National breastfeeding policy for Ireland*. Dublin: Department of Health.

Department of Health (DH) (Ed.). (2000). *Folic acid and the prevention of disease. report on health and social subjects. 50*. London: The Stationary Office.

Department of Health and Children. (2003). Policy change in breastfeeding guidelines. Health promotion unit, department of health and children. Retrieved March 4th, 2010, from http://www.dohc.ie/press/releases/2003/20030805.html

Department of Health and Children. (2005). *Breastfeeding in Ireland: A five-year strategic action plan.* Dublin: Department of Health and Children.

Desor, J., Maller, O., & Turner, R. E. (1973). Taste in acceptance of sugars by human infants. *Journal of Comparative & Physiological Psychology, 84*(3), 496-501.

Desor, J., & Beauchamp, G. K. (1987). Longitudinal changes in sweet preferences in humans. *Physiology & Behavior, 39*(5), 639-641.

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin- United Nations University, 24*(1), 5-28.

Dewey, K. G., Finley, D. A., & Lönnerdal, B. (1984). Breast milk volume and composition during late lactation (7-20 months). *Journal of Pediatric Gastroenterology &Nutrition, 3*(5), 713-720.

Dias, A. G., Rousseau, D., Duizer, L., Cockburn, M., Chiu, W., Nielsen, D., & El-Sohemy, A. (2013). Genetic variation in putative salt taste receptors and salt taste perception in humans. *Chemical Senses, 38*(2), 137-145.

Ding, D., Sallis, J. F., Norman, G. J., Saelens, B. E., Harris, S. K., Kerr, J., . . . Glanz, K. (2012). Community food environment, home food environment, and fruit and vegetable intake of children and adolescents. *Journal of Nutrition Education and Behavior, 44*(6), 634-638.

Donovan, S. M. (2009). Human milk nutritional properties. In C. Duggan, J. B. Watkins & W. A. Walker (Eds.), *Nutrition in Pediatrics* (pp. 341-353).

Doran, L., & Evers, S. (1997). Energy and nutrient inadequacies in the diets of low-income women who breast-feed. *Journal of the American Dietetic Association, 97*(11), 1283-1287.

Drewnowski, A. (2004). Obesity and the food environment: Dietary energy density and diet costs. *American Journal of Preventive Medicine, 27*(3), 154-162.

Drewnowski, A., & Rock, C. L. (1995). The influence of genetic taste markers on food acceptance. *The American Journal of Clinical Nutrition, 62*(3), 506-511.

Dubois, L., & Girard, M. (2003). Social determinants of initiation, duration and exclusivity of breastfeeding at the population level: The results of the longitudinal study of child development in quebec (ELDEQ 1998-2002). *Canadian Journal of Public Health = Revue Canadienne De Sante Publique, 94*(4), 300-305.

Duffy, V., Bartoshuk, L., Lucchina, L., Snyder, D., & Tym, A. (1996). Supertasters of PROP (6-n-propylthiouracil) rate the highest creaminess to high-fat milk products. *Chemical Senses, 21*(5), 598.

Duhn, L. J., & Medves, J. M. (2004). A systematic integrative review of infant pain assessment tools. *Advances in Neonatal Care, 4*(3), 126-140.

Duijts, L., Jaddoe, V. W., Hofman, A., & Moll, H. A. (2010). Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. *Pediatrics, 126*(1), e18-e25.

Duijts, L., Ramadhani, M. K., & Moll, H. A. (2009). Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review. *Maternal & Child Nutrition, 5*(3), 199-210.

Dunne, T., Farrell, P., & Kelly, V. (2011). *Feed your Child Well* (3rd ed.). Dublin, Ireland: A& A Farmar.

Dyson, L., McCormick, F., & Renfrew, M. (2005). Interventions for promoting the initiation of breastfeeding. *Cochrane Database Syst Rev, 2*

Ek, J. & Magnus, EM. (1979). Plasma and red blood cell folate in breastfed infants. *Acta Paediatr. Scand, 68*, 239-243.

Ekman, P., & Friesen, W. (Eds.). (1978). *Facial action coding system: A technique for the measurement of facial movement. ,* Palo. Alto: Consulting Psychologists Press.

Ekman, P., & Oster, H. (1979). Facial expressions of emotion. *Annual Review of Psychology, 30*(1), 527-554.

European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (2009). Scientific opinion on the appropriate age for introduction of complementary feeding of infants. *European Food Safety Authority Journal, 7(1)*, 1423-1461.

European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies (2010). Scientific opinion on dietary reference values for carbohydrates and dietary fibre. *European Food Safety Authority Journal, 8*(3), 1462-1539.

European Food Safety Authority Panel on Dietetic Products, Nutrition, and Allergies (2012). Scientific opinion on the tolerable upper intake levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *European Food Safety Authority Journal, 10*(7), 2815-2863.

European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies. (2013). Scientific opinion on dietary reference values for energy. *European Food Safety Authority Journal,* 11(1), 1305-1417.

European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). (2008). Complementary feeding, a commentary by the ESPGHAN committee on nutrition: Medical position paper. *Journal of Pediatrics' Gastroenterology & Nutrition, 46*, 99-110.

Fagot-Campagna, A., Pettitt, D. J., Engelgau, M. M., Burrows, N. R., Geiss, L. S., Valdez, R., . . . Venkat Narayan, K. M. (2000). Type 2 diabetes among north adolescents: An epidemiologic health perspective. *The Journal of Pediatrics, 136*(5), 664-672. DOI: 10.1067/mpd.2000.105141

FAO/WHO/UNU. (2004). *Human energy requirements: Report of a joint FAO/WHO/UNU expert consultation. FAO food and nutrition technical report series 1. Rome, FAO,*

FAO/WHO/UNU. (1985). *Energy and protein requirements: Report of a joint FAO/WHO/UNU expert consultation*. Geneva: World Health Organization.

Fattah, C., Farah, N., O'Toole, F., Barry, S., Stuart, B., & Turner, M. J. (2009). Body mass index (BMI) in women booking for antenatal care: Comparison between self-reported and digital measurements. *European Journal of Obstetrics & Gynaecology and Reproductive Biology, 144*(1), 32-34.

Fereidoun, H., Ahmad, R., Ahmad, S., & Pouria, H. (2011). Effect of feeding with different salt concentrations in prenatal period on the vascular response to epinephrine and acetylcholine. *Pakistan Journal of Physiology, 7*(1), 8-12.

Fewtrell, M., Wilson, D. C., Booth, I., & Lucas, A. (2011). Six months of exclusive breast feeding: How good is the evidence? *BMJ: British Medical Journal, 342* doi: http://dx.doi.org/10.1136/bmj.c5955

Fewtrell, M. (2004). The long-term benefits of having been breast-fed. *Current Paediatrics, 14*(2), 97-103.

Field, A. (2009). *Discovering statistics using SPSS: And sex and drugs and rock 'n' roll* (3rd ed.). London: Sage.

Figueroa-Colon, R., Franklin, F. A., Lee, J. Y., Aldridge, R., & Alexander, L. (1997). Prevalence of obesity with increased blood pressure in elementary school-aged children. *Southern Medical Journal, 90*(8), 806-813.

Fiorito, L. M., Marini, M., Mitchell, D. C., Smiciklas-Wright, H., & Birch, L. L. (2010). Girls' early sweetened carbonated beverage intake predicts different patterns of beverage and nutrient intake across childhood and adolescence. *Journal of the American Dietetic Association, 110*(4), 543-550.

Fitzpatrick, C., Hughes, P., White, M., D'Arcy, T., Carey, M., O'Leary, J., & Ryan, J. (2012). *Coombe Women & Infants University Hospital Annual Clinical Report 2010*. Dublin: Coombe Women & Infants University Hospital.

Fitzsimon, N., Fallon, U., O'Mahony, D., Loftus, B., Bury, G., Murphy, A., & Kelleher, C. (2007). Mothers' dietary patterns during pregnancy and risk of asthma symptoms in children at 3 years. *Irish Medical Journal, 100*(8), 27–32.

Flaxman, S. M., & Sherman, P. W. (2000). Morning sickness: A mechanism for protecting mother and embryo. *Quarterly Review of Biology, 75*(2), 113-148.

Fomon, S. J. (2001). Infant feeding in the 20th century: Formula and beikost. *The Journal of Nutrition, 131*(2), 409S-420S.

Food Safety Authority of Ireland (Ed.). (1999). *Recommended dietary allowances for Ireland.* Dublin: FSAI.

Food Safety Authority of Ireland. (2004). FSAI issues guidelines on consumption of shark, swordfish, marlin and tuna. Retrieved march 8th, 2012, from http://www.fsai.ie/details.aspx?id=7160

Food Safety Authority of Ireland (Ed.). (2005). *Salt and health: Review of the scientific evidence and recommendations for public policy in Ireland.* Dublin: FSAI.

Food Safety Authority of Ireland. (2007). *Recommendations for a national policy on vitamin D supplementation for infants in Ireland.* Dublin, Ireland: Food Safety Authority of Ireland.

Food Safety Authority of Ireland (Ed.). (2009). *Report of the implementation group on folic acid food fortification to the department of health and children*. Dublin, Ireland: Department of Health and Children.

Food Safety Authority of Ireland. (2011). *Scientific recommendations for a national infant feeding policy, 2nd edition.* Dublin: FSAI.

Food Safety Authority of Ireland (Ed.). (2012). *Healthy eating and active living for adults, teenagers and children over 5 years*. Dublin Ireland: Food Safety Authority of Ireland.

Food Standards Agency (Ed.). (2002a). *Food portion sizes* (3rd ed.). London: Food Standards Agency.

Food Standards Agency. (2002b). *McCance and Widdowson's the Composition of Foods* (Sixth Summary Edition ed.). Cambridge: Royal Society of Chemistry.

Foote, K., & Marriott, L. (2003). Weaning of infants. *Archives of Disease in Childhood, 88*(6), 488-492.

Ford, E. S., & Mokdad, A. H. (2001). Fruit and vegetable consumption and diabetes mellitus incidence among U.S. adults. *Preventive Medicine, 32*(1), 33-39. doi:http://dx.doi.org/10.1006/pmed.2000.0772

Forestell, C. A., & Mennella, J. A. (2012). More than just a pretty face. the relationship between infant's temperament, food acceptance, and mothers' perceptions of their enjoyment of food. *Appetite, 58*(3), 1136-1142.

Forestell, C. A., & Mennella, J. A. (2007). Early determinants of fruit and vegetable acceptance. *Pediatrics, 120*(6), 1247-1254. doi:10.1542/peds.2007-0858

Forestfield Software Ltd. (2011). *Dietplan 6.4*. Nottingham: UK:

Forsyth, J. S., Ogston, S. A., Clark, A., & Florey, C.D. & Howie, P.W. (1993). Relation between early introduction of solid food to infants and their weight and illness during the first two years of life. . *BMJ: British Medical Journal, 306*, 1572-1576.

Fox, M. K., Reidy, K., Novak, T., & Ziegler, P. (2006). Sources of energy and nutrients in the diets of infants and toddlers. *Journal of the American Dietetic Association, 106*(1S), 28-28.

Freeman, V. E. (1996). *A longitudinal study of growth, feeding practices and iron status in healthy children from birth until age two years.* PhD thesis. University of Dublin.

Freisling, H., Elmadfa, I., & Gall, I. (2006). The effect of socioeconomic status on dietary intake, physical activity and body mass index in Austrian pregnant women. *Journal of Human Nutrition and Dietetics, 19*(6), 437-445.

Galtier-Dereure, F., Boegner, C., & Bringer, J. (2000). Obesity and pregnancy: Complications and cost. *The American Journal of Clinical Nutrition, 71*(5), 1242S-8S.

Ganchrow, J. R., & Mennella, J. A. (2003). The ontogeny of human flavor perception. . In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (pp. 823-846). New York: Dekker.

Ganchrow, J. R., Steiner, J. E., & Daher, M. (1983). Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli. *Infant Behavior and Development, 6*, 473-484.

Garcia-Bailo, B., Toguri, C., Eny, K. M., & El-Sohemy, A. (2009). Genetic variation in taste and its influence on food selection. *OMICS A Journal of Integrative Biology, 13*(1), 69-80.

Gardner, D. S., Hosking, J., Metcalf, B. S., Jeffery, A. N., Voss, L. D., & Wilkin, T. J. (2009). Contribution of early weight gain to childhood overweight and metabolic health: A longitudinal study (EarlyBird 36). *Pediatrics, 123*(1), e67-e73.

Garza, C. & Rasmussen, K.M. (2000). Pregnancy and lactation. In J. S. Garrow, W. P. T. James & A. Ralph (Eds.), *Human Nutrition and Dietetics* (pp. 437-448). Edinburgh, Scotland: Churchill-Livingstone.

Geleijnse, J. M., Hofman, A., Witteman, J. C. M., Hazebroek, A. A. J. M., Valkenburg, H. A., & Grobbee, D. E. (1997). Long-term effects of neonatal sodium restriction on blood pressure. *Hypertension, 29*(4), 913-917.

George, G. C., Hanss-Nuss, H., Milani, T. J., & Freeland-Graves, J. H. (2005). Food choices of low-income women during pregnancy and postpartum. *Journal of the American Dietetic Association, 105*(6), 899-907. DOI: 10.1016/j.jada.2005.03.028

George, G. C., Milani, T. J., Hanss-Nuss, H., & Freeland-Graves, J. H. (2005). Compliance with dietary guidelines and relationship to psychosocial factors in low-income women in late postpartum. *Journal of the American Dietetic Association, 105*(6), 916-926. DOI: 10.1016/j.jada.2005.03.009

Gerrish, C. J., & Mennella, J. A. (2001). Flavor variety enhances food acceptance in formula-fed infants. *American Journal of Clinical Nutrition, 73*(6), 1080-1085.

Gilbert, G. L. (2002). 1: Infections in pregnant women. *Medical Journal of Australia, 176*(5), 229-236.

Giltinan, M., Walton, J., McNulty, B., Nugent, A., Gibney, M., & Flynn, A. (2011). Sodium (na) intakes in Irish adults. *Proceedings of the Nutrition Society, 70*(OCE3)

Giovannini, M., Riva, E., Banderali, G., Scaglioni, S., Veehof, S., Sala, M., . . . Agostoni, C. (2004). Feeding practices of infants through the first year of life in Italy. *Acta Paediatrica, 93*(4), 492-497.

Gluckman, P. D., Hanson, M. A., Buklijas, T., Low, F. M., & Beedle, A. S. (2009). Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nature Reviews Endocrinology, 5*(7), 401-408.

Gluckman, P. D., Hanson, M. A., Cooper, C., & Thornburg, K. L. (2008). Effect of in utero and early-life conditions on adult health and disease. *The New England Journal of Medicine, 359*(1), 61-73. doi:10.1056/NEJMra0708473

Glynn, L. M., Davis, E. P., Schetter, C. D., Chicz-DeMet, A., Hobel, C. J., & Sandman, C. A. (2007). Postnatal maternal cortisol levels predict temperament in healthy breastfed infants. *Early Human Development, 83*(10), 675-681. doi:http://dx.doi.org/10.1016/j.earlhumdev.2007.01.003

Godfrey, K., Robinson, S., Barker, D. J. P., Osmond, C., & Cox, V. (1996). Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ: British Medical Journal, 312*, 410.

Godfrey, K. M., & Barker, D. J. P. (2001). Fetal programming and adult health. *Public Health Nutrition, 4*(2b), 611-624.

Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., Gluckman, P. D., & Hanson, M. A. (2007). Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatric Research, 61*, 5R-10R.

Goldberg, G., Black, A., Jebb, S., Cole, T., Murgatroyd, P., Coward, W., & Prentice, A. (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. derivation of cut-off limits to identify under-recording. *European Journal of Clinical Nutrition, 45*(12), 569-581.

Golden, B. E. (2000). Infnacy, childhood and adolescence. In J. S. Garrow, W. P. T. James & A. Palph (Eds.), *Human Nutrition and Dietetics* (pp. 449-469) Edinburgh, Scotland: Churchill-Livingstone.

Golding, J., Emmett, P. M., & Rogers, I. S. (1997a). Does breast feeding have any impact on non-infectious, non-allergic disorders? *Early Human Development, 49*, S131-S142.

Golding, J., Emmett, P. M., & Rogers, I. S. (1997b). Gastroenteritis, diarrhoea and breast feeding. *Early Human Development, 49*, S83-S103.

Gordon, C. M., Feldman, H. A., Sinclair, L., Williams, A. L. B., Kleinman, P. K., Perez-Rossello, J., & Cox, J. E. (2008). Prevalence of vitamin D deficiency among healthy infants and toddlers. *Archives of Pediatrics and Adolescent Medicine, 162*(6), 505-512.

Greenberg, J. A., Bell, S. J., & Van Ausdal, W. (2008). Omega-3 fatty acid supplementation during pregnancy. *Reviews in Obstetrics and Gynecology, 1*(4), 162-169.

Grieger, J. A., Scott, J., & Cobiac, L. (2011). Dietary patterns and breast-feeding in Australian children. *Public Health Nutrition, 14*(11), 1939-1947.

Grote, V., Schiess, S. A., Closa-Monasterolo, R., Escribano, J., Giovannini, M., Scaglioni, S., . . . Poncelet, P. (2011). The introduction of solid food and growth in the first 2 y of life in formula-fed children: Analysis of data from a European cohort study. *The American Journal of Clinical Nutrition, 94*(6 Suppl), 1785S-1793S.

Grunau, R. E., Oberlander, T., Holsti, L., & Whitfield, M. F. (1998). Bedside application of the neonatal facial coding system in pain assessment of premature infants. *Pain, 76*(3), 277-286.

Grunau, R. V. E., Johnston, C. C., & Craig, K. D. (1990). Neonatal facial and cry responses to invasive and non-invasive procedures. *Pain, 42*(3), 295-305.

Gudnadottir, M., Sigurdur Gunnarsson, B., & Thorsdottir, I. (2006). Effects of sociodemographic factors on adherence to breastfeeding and other important infant dietary recommendations. *Acta Paediatrica, 95*(4), 419-424.

Guinard, J. X. (2000). Sensory and consumer testing with children. *Trends in Food Science & Technology, 11*(8), 273-283.

Günther, A. L., Buyken, A. E., & Kroke, A. (2007). Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. *The American Journal of Clinical Nutrition, 85*(6), 1626-1633.

Günther, A. L., Remer, T., Kroke, A., & Buyken, A. E. (2007). Early protein intake and later obesity risk: Which protein sources at which time points throughout infancy and childhood are important for body mass index and body fat percentage at 7 y of age? *The American Journal of Clinical Nutrition, 86*(6), 1765-1772.

Guthrie, J. F., Lin, B., & Frazao, E. (2002). Role of food prepared away from home in the american diet, 1977-78 versus 1994-96: Changes and consequences. *Journal of Nutrition Education and Behavior, 34*(3), 140-150.

Hall, M. J., Bartoshuk, L. M., Cain, W. S., & Stevens, J. C. (1975). PTC taste blindness and the taste of caffeine. *Nature, 253*(5491), 442-443.

Hamosh, M. (2001). Bioactive factors in human milk. *Pediatric Clinics of North America, 48*(1), 69-86.

Hanson, M., Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., & Gluckman, P. D. (2011). Developmental plasticity and developmental origins of non-communicable disease: Theoretical considerations and epigenetic mechanisms. *Progress in Biophysics and Molecular Biology, 106*(1), 272-280.

Hariri, N., Gougeon, R., & Thibault, L. (2010). A highly saturated fat-rich diet is more obesogenic than diets with lower saturated fat content. *Nutrition Research, 30*(9), 632-643.

Harrington, J., Perry, I., Lutomski, J., Morgan, K., McGee, H., Shelley, E., . . . Barry, M. (2008). *Survey of lifestyle, attitudes and nutrition in Ireland. dietary habits of the Irish population,* *Department of health and children.* Dublin: The Stationery Office. Retrieved from http://www.dohc.ie/publications/pdf/slan_summary.pdf?direct=1

Harris, G. (2008). Development of taste and food preferences in children. *Current Opinion in Clinical Nutrition & Metabolic Care, 11*(3), 315-319.

Harris, G., Thomas, A., & Booth, D. A. (1990a). Development of salt taste in infancy. *Developmental Psychology, 26*(4), 534.

Harris, G., Thomas, A., & Booth, D. A. (1990b). Development of salt taste in infancy. *Developmental Psychology, 26*(4), 534.

Hart, K., Bishop, J., & Truby, H. (2002). An investigation into school children's knowledge and awareness of food and nutrition. *Journal of Human Nutrition and Dietetics, 15*(2), 129-140.

Hauser, G. J., Chitayat, D., Berns, L., Braver, D., & Muhlbauer, B. (1985). Peculiar odours in newborns and maternal prenatal ingestion of spicy food. *European Journal of Pediatrics, 144*(4), 403-403.

Hausner, H., Nicklaus, S., Issanchou, S., Mølgaard, C., & Møller, P. (2009). Breastfeeding facilitates acceptance of a novel dietary flavour compound. *E-SPEN, the European E-Journal of Clinical Nutrition and Metabolism, 4*(5), e231-e238.

Hausner, H., Bredie, W. L. P., Mølgaard, C., Petersen, M. A., & Møller, P. (2008). Differential transfer of dietary flavour compounds into human breast milk. *Physiology & Behavior, 95*(1-2), 118-124.

Havas, S., Treiman, K., Langenberg, P., Ballesteros, M., Anliker, J., Damron, D., & Feldman, R. (1998). Factors associated with fruit and vegetable consumption among women participating in WIC. *Journal of the American Dietetic Association, 98*(10), 1141-1148. doi:DOI: 10.1016/S0002-8223(98)00264-8

Hay, G., Sandstad, B., Whitelaw, A., & Borch-Iohnsen, B. (2004). Iron status in a group of norwegian children aged 6-24 months. *Acta Paediatrica, 93*(5), 592-598. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=12916970&site=ehost-live

He, F. J., Nowson, C. A., & MacGregor, G. A. (2006). Fruit and vegetable consumption and stroke: Meta-analysis of cohort studies. *The Lancet, 367*(9507), 320-326.

He, F., Nowson, C., Lucas, M., & MacGregor, G. (2007). Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: Meta-analysis of cohort studies. *Journal of Human Hypertension, 21*(9), 717-728.

He, J., Ogden, L. G., Vupputuri, S., Bazzano, L. A., Loria, C., & Whelton, P. K. (1999). Dietary sodium intake and subsequent risk of cardiovascular disease in overweight adults. *JAMA: The Journal of the American Medical Association, 282*(21), 2027-2034.

Health Promotion Unit (Ed.). (2012). *How to spoon feed your child*. Dublin: Health Promotion Unit.

Health Promotion Unit (Ed.). (2006). *Healthy eating for pregnancy*. Dublin: Health Promotion Unit.

Health Service Executive. (2013). Breastfeeding.ie- Every breastfeed makes a difference. Retrieved August /23, 2013, from http://www.breastfeeding.ie/

Health Service Executive. (2010). HSE website warns of risks of toxoplasmosis . Retrieved March/4, 2011, from http://www.hse.ie/eng/services/newscentre/2010archive/feb2010/toxoplasmosis.html

Health Service Executive (HSE). (2010). *Policy on vitamin D supplementation for infants in Ireland*. Dublin Ireland: HSE.

Hearn, M. D., Baranowski, T., Baranowski, J., Doyle, C., Smith, M., Lin, L. S., & Resnicow, K. (1998). Environmental influences on dietary behavior among children: Availability and accessibility of fruits and vegetables enable consumption. *Journal of Health Education, 29*(1), 26-32.

Heikkilä, K., Sacker, A., Kelly, Y., Renfrew, M. J., & Quigley, M. A. (2011). Breast feeding and child behaviour in the millennium cohort study. *Archives of Disease in Childhood, 96*(7), 635-642.

Heinig, M. J. (1997). Health effects of breast feeding for mothers: A critical. *Nutrition Research Reviews, 10*, 35-56.

Henry, C. J. K. (2005). Basal metabolic rate studies in humans: Measurement and development of new equations. *Public Health Nutrition, 8*(7a), 1133-1152.

Hepper, P. (1988). Adaptive fetal learning: Prenatal exposure to garlic affects postnatal preferences. *Animal Behaviour, 36*, 935-936.

Herrera, E. (2000). Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *European Journal of Clinical Nutrition, 54*(3), S47-S51. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db =a9h&AN=9380416&site=ehost-live

Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *Journal of Clinical Investigation, 116*(8), 2062-2072.

Hollis, B. W., & Wagner, C. L. (2004). Assessment of dietary vitamin D requirements during pregnancy and lactation. *The American Journal of Clinical Nutrition, 79*(5), 717-726.

Hoppu, U., Kalliomäki, M., & Isolauri, E. (2000). Maternal diet rich in saturated fat during breastfeeding is associated with atopic sensitization of the infant. *European Journal of Clinical Nutrition, 54*(9), 702-705.

Hoppu, U., Rinne, M., Lampi, A. M., & Isolauri, E. (2005). Breast milk fatty acid composition is associated with development of atopic dermatitis in the infant. *Journal of Pediatric Gastroenterology and Nutrition, 41*(3), 335.

Horne, P., Hardman, C., Lowe, C., Tapper, K., Le Noury, J., Madden, P., . . . Doody, M. (2008). Increasing parental provision and children's consumption of lunchbox fruit and vegetables in Ireland: The food dudes intervention. *European Journal of Clinical Nutrition, 63*(5), 613-618.

Horta, B. L., Bahl, R., Martines, J. C., & Victora, C. G. (2007). *Evidence on the long-term effects of breastfeeding. systematic reviews and meta-analyses.* Geneva: World Health Organization.

Howie, P. W., Forsyth, J. S., Ogston, S. A., Clark, A., & Florey, C. (1990). Protective effect of breast feeding against infection. *BMJ: British Medical Journal, 300*(6716), 11-16.

Huang, A. L., Chen, X., Hoon, M. A., Chandrashekar, J., Guo, W., Tränkner, D., . . . Zuker, C. S. (2006). The cells and logic for mammalian sour taste detection. *Nature, 442*(7105), 934-938.

Hughes, L. A. E., Arts, I. C. W., Ambergen, T., Brants, H. A. M., Dagnelie, P. C., Goldbohm, R. A., . . . Weijenberg, M. P. (2008). Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: A longitudinal analysis from the Netherlands cohort study. *The American Journal of Clinical Nutrition, 88*(5), 1341-1352.

Huh, S. Y., Rifas-Shiman, S. L., Taveras, E. M., Oken, E., & Gillman, M. W. (2011). Timing of solid food introduction and risk of obesity in preschool-aged children. *Pediatrics, 127*(3), e544-e551.

IBM SPSS. (2010). *SPSS for windows 19.0.* Chicago: SPSS Inc.

Ikeda, K. (2002). New seasonings. *Chemical Senses, 27*(9), 847.

Ikehara, S., Iso, H., Date, C., Kikuchi, S., Watanabe, Y., Inaba, Y., & Tamakoshi, A. (2012). Salt preference and mortality from stroke and coronary heart disease for Japanese men and women: The JACC study. *Preventive Medicine, 54*(1), 32-37.

Innis, S. M. (2005). Essential fatty acid transfer and fetal development. *Placenta, 26, Supplement*(0), S70-S75.

Institute of Medicine (IMO) (Ed.). (1991). *Nutrition during lactation*. Washington D.C.: National Academy Press.

Institute of Medicine (IMO). (2009). *Weight gain during pregnancy: Re-examining the guidelines* The National Academies Press. http://www.nap.edu/openbook.php?record_id=1258 [Retrieved 21st July, 2012]

International Obesity Taskforce. (2012). World obesity Map. http://www.iaso.org/resources/world-map-obesity/?map=children. [Retrieved 21st July, 2012]

Ip, S., Chung, M., Raman, G., Chew, P., Magula, N., DeVine, D., . . . Lau, J. (2007). Breastfeeding and maternal and infant health outcomes in developed countries. *Evidence Report/Technology Assessment,* (153), 1-186.

Irish Universities Nutrition Alliance (Ed.). (2011). *National adult nutrition survey summary report on food and nutrient intakes, physical measurements, physical activity patterns and food choice motives*. Dublin: IUNA.

Ishimaru, Y., Inada, H., Kubota, M., Zhuang, H., Tominaga, M., & Matsunami, H. (2006). Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. *Proceedings of the National Academy of Sciences, 103*(33), 12569-12574.

Ivarsson, A., Myléus, A., Norström, F., van der Pals, M., Rosén, A., Högberg, L., . . . Hernell, O. (2013). Prevalence of childhood celiac disease and changes in infant feeding. *Pediatrics, 131*(3), e687-e694.

Jacob, J. The physiology of taste. Retrieved from http://www.cf.ac.uk/biosi/staffinfo/jacob/teaching/sensory/taste.html [Retrieved 13th July 2012].

James, P. (2006). Marabou 2005: Nutrition and human development. *Nutrition Reviews, 64*, 1-11.

Jensen, R. G. (1999). Lipids in human milk. *Lipids, 34*(12), 1243-1271.

Kafatos, A., Vlachonikolis, I., & Codrington, C. (1989). Nutrition during pregnancy: The effects of an educational intervention program in Greece. *The American Journal of Clinical Nutrition, 50*(5), 970-979.

Kajiura, H., Cowart, B. J., & Beauchamp, G. K. (1992). Early developmental change in bitter taste responses in human infants. *Developmental Psychobiology, 25*(5), 375-386.

Kalapesi Z, K. J. (1974). Infant feeding practices in Dublin. *Journal of Irish Medical Association, 67*, 256-258.

Kaplan, A. R., Fischer, R., Karras, A., Griffin, F., Powell, W., Marsters, R. W., & Glanville, E. V. (1967). Taste thresholds in twins and siblings. *Acta Geneticae Medicae Et Gemellologiae, 16*(3), 229-243.

Kelder, S. H., Perry, C. L., Klepp, K. I., & Lytle, L. L. (1994). Longitudinal tracking of adolescent smoking, physical activity, and food choice behaviors. *American Journal of Public Health, 84*(7), 1121-1126.

Kelleher, C. C., Lotya, J., O'Hara, M. C., & Murrin, C. (2008). Session 1: Public health nutrition and social disadvantage in Ireland. *Proceedings of the Nutrition Society, 67*(04), 363-370.

Keller, K. L., Steinmann, L., Nurse, R. J., & Tepper, B. J. (2002). Genetic taste sensitivity to 6-n-propylthiouracil influences food preference and reported intake in preschool children. *Appetite, 38*(1), 3-12.

Kelly, C., Gavin, A., Molcho, M., & Nic Gabhainn, S. (2012). *The Irish health behaviour in school-aged children (HBSC) study 2010*. Department of Health and National University of Ireland, Galway.

Kelly, Y., & Watt, R. (2005). Breast-feeding initiation and exclusive duration at 6 months by social class–results from the millennium cohort study. *Public Health Nutrition, 8*(04), 417-421.

Kent, J. C., Mitoulas, L., Cox, D. B., Owens, R. A., & Hartmann, P. E. (1999). Breast volume and milk production during extended lactation in women. *Experimental Physiology, 84*(02), 435-447.

Kien, C. L., Bunn, J. Y., & Ugrasbul, F. (2005). Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. *American Journal of Clinical Nutrition, 82*(2), 320-326. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-24044501294&partnerID=40&md5=04fd59b7a841ccf98b9b4ee5122e1752

Kim, U. K., Breslin, P., Reed, D., & Drayna, D. (2004). Genetics of human taste perception. *Journal of Dental Research, 83*(6), 448-453.

Kinugasa, A., Tsunamoto, K., Furukawa, N., Sawada, T., Kusunoki, T., & Shimada, N. (1984). Fatty liver and its fibrous changes found in simple obesity of children. *Journal of Pediatric Gastroenterology and Nutrition, 3*(3), 408-414.

Koleganova, N., Piecha, G., Ritz, E., Becker, L. E., Müller, A., Weckbach, M., . . . Gross-Weissmann, M. L. (2011). Both high and low maternal salt intake in pregnancy alter kidney development in the offspring. *American Journal of Physiology-Renal Physiology, 301*(2), F344-F354.

Koletzko, B., Cetin, I., & Brenna, J. e. a. (2007). Dietary fat intakes for pregnant and lactating women. *British Journal of Nutrition, 98*, 873-877.

Koletzko, B., Baker, S., Cleghorn, G., Neto, U. F., Gopalan, S., Hernell, O., . . . Pencharz, P. (2005). Global standard for the composition of infant formula: Recommendations of an ESPGHAN coordinated international expert group. *Journal of Pediatric Gastroenterology and Nutrition, 41*(5), 584-599.

Koletzko, B., Lien, E., Agostoni, C., Böhles, H., Campoy, C., Cetin, I., . . . Forsyth, S. (2008). The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: Review of current knowledge and consensus recommendations. *Journal of Perinatal Medicine, 36*(1), 5-14.

Koletzko, B., von Kries, R., Closa, R., Escribano, J., Scaglioni, S., Giovannini, M., . . . Dobrzanska, A. (2009). Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. *The American Journal of Clinical Nutrition, 89*(6), 1836-1845.

Koletzko, B., von Kries, R., Monasterolo, R. C., Subías, J. E., Scaglioni, S., Giovannini, M., . . . Gruszfeld, D. (2009). Can infant feeding choices modulate later obesity risk? *The American Journal of Clinical Nutrition, 89*(5), 1502S-1508S.

Kopp-Hoolihan, L. E., van Loan, M. D., Wong, W. W., & King, J. C. (1999). Longitudinal assessment of energy balance in well-nourished, pregnant women. *The American Journal of Clinical Nutrition, 69*(4), 697-704.

Kovacs, C. S. (2005). Calcium and bone metabolism during pregnancy and lactation. *Journal of Mammary Gland Biology and Neoplasia, 10*(2), 105-118.

Kramer, M. S., Séguin, L., Lydon, J., & Goulet, L. (2000). Socio-economic disparities in pregnancy outcome: Why do the poor fare so poorly? *Paediatric and Perinatal Epidemiology, 14*(3), 194-210.

Kramer, M.S. & Kakuma, R. (Ed.). (2002). *The optimal duration of exclusive breastfeeding: A systematic review.* Geneva: WHO: Department of Child and Adolescent Health and Development.

Kramer, M. S., Aboud, F., Mironova, E., Vanilovich, I., Platt, R. W., Matush, L., . . . Ducruet, T. (2008). Breastfeeding and child cognitive development: New evidence from a large randomized trial. *Archives of General Psychiatry, 65*(5), 578-584.

Kramer, M. S., Matush, L., Vanilovich, I., Platt, R. W., Bogdanovich, N., Sevkovskaya, Z., . . . Martin, R. M. (2009). A randomized breast-feeding promotion intervention did not reduce child obesity in Belarus. *The Journal of Nutrition, 139*(2), 417S-421S.

Krishnaveni, G. V., Veena, S. R., Winder, N. R., Hill, J. C., Noonan, K., Boucher, B. J., . . . Fall, C. H. (2011). Maternal vitamin D status during pregnancy and body composition and cardiovascular risk markers in Indian children: The mysore parthenon study. *The American Journal of Clinical Nutrition, 93*(3), 628-635.

Kuan, L. W., Britto, M., Decolongon, J., Schoettker, P. J., Atherton, H. D., & Kotagal, U. R. (1999). Health system factors contributing to breastfeeding success. *Pediatrics, 104*(3), e28-e28.

Lachat, C., Nago, E., Verstraeten, R., Roberfroid, D., Van Camp, J., & Kolsteren, P. (2012). Eating out of home and its association with dietary intake: A systematic review of the evidence. *Obesity Reviews, 13*(4), 329-346.

Lanigan, J., Wells, J., Lawson, M., Cole, T., & Lucas, A. (2004). Number of days needed to assess energy and nutrient intake in infants and young children between 6 months and 2 years of age. *European Journal of Clinical Nutrition, 58*(5), 745-750.

Latva-Pukkila, U., Isolauri, E., & Laitinen, K. (2010). Dietary and clinical impacts of nausea and vomiting during pregnancy. *Journal of Human Nutrition and Dietetics, 23*(1), 69-77.

Lawless, H. (1985). Sensory development in children: Research in taste and olfaction. *Journal of the American Dietetic Association, 85*(5), 577-82, 585.

Lawrence, M., Coward, W., Lawrence, F., Cole, T. J., & Whitehead, R. G. (1987). Energy requirements of pregnancy in the Gambia. *The Lancet, 330*(8567), 1072-1076.

Lawrence, P. B. (1994). Breast milk. best source of nutrition for term and preterm infants. *Pediatric Clinics of North America, 41*(5), 925-941.

Lawson, M., Thomas, M., & Hardiman, A. (1998). Iron status of Asian children aged 2 years living in England. *Archives of Disease in Childhood, 78*(5), 420-426.

Leahy-Warren, P., Mulcahy, H. & Phelan, A. (2009).Review of breastfeeding support services provided by public health nurses. Dublin, HSE.

LeCanuet, J. P., & Schaal, B. (2002). Sensory performances in the human foetus: A brief summary of research. *Intellectica, 1*(34), 29-56.

Lennox, A., Sommerville, J., Ong, K., Henderson H., & Allen, R. (Eds.). (2013). *Diet and nutrition survey of infants and young children, 2011*. London: Department of Health.

Leshem, M., Maroun, M., & Weintraub, Z. (1998). Neonatal diuretic therapy may not alter children's preference for salt taste. *Appetite, 30*(1), 53-64.

Lewallen, L. P. (2004). Healthy behaviors and sources of health information among Low-Income pregnant women. *Public Health Nursing, 21*(3), 200-206.

Li, F. (2013). Taste perception: From the tongue to the testis. *Molecular Human Reproduction, 19*(6), 349-360.

Li, Y. C., Qiao, G., Uskokovic, M., Xiang, W., Zheng, W., & Kong, J. (2004). Vitamin D: A negative endocrine regulator of the renin–angiotensin system and blood pressure. *The Journal of Steroid Biochemistry and Molecular Biology, 89–90(0)*, 387-392.

Liem, D. G., & Mennella, J. A. (2002). Sweet and sour preferences during childhood: Role of early experiences. *Developmental Psychobiology, 41*(4), 388-395.

Liem, D. G., Miremadi, F., & Keast, R. S. (2011). Reducing sodium in foods: The effect on flavor. *Nutrients, 3*(6), 694-711.

Liem, D. G., Bogers, R. P., Dagnelie, P. C., & de Graaf, C. (2006). Fruit consumption of boys (8–11 years) is related to preferences for sour taste. *Appetite, 46*(1), 93-96.

Liem, D. G., & de Graaf, C. (2004). Sweet and sour preferences in young children and adults: Role of repeated exposure. *Physiology & Behavior, 83*(3), 421-429.

Liem, D. G., & Mennella, J. A. (2003). Heightened sour preferences during childhood. *Chemical Senses, 28*(2), 173-180. doi:10.1093/chemse/28.2.173

Liem, D. G., Westerbeek, A., Wolterink, S., Kok, F. J., & de Graaf, C. (2004). Sour taste preferences of children relate to preference for novel and intense stimuli. *Chemical Senses, 29*(8), 713-720. doi:10.1093/chemse/bjh077

Lilley, A. W. (1972). The fetus as a personality. *Austral New Zealand J. Psychiatr, 6*, 99-105.

Lin, B. H., & Morrison, R. M. (2002). Higher fruit consumption linked with lower body mass index. *Food Review, 25*(3), 28-32.

Lindemann, B. (2001). Receptors and transduction in taste. *Nature, 413*(6852), 219-225.

Lioret, S., Touvier, M., Balin, M., Huybrechts, I., Dubuisson, C., Dufour, A., . . . Lafay, L. (2011). Characteristics of energy under-reporting in children and adolescents. *British Journal of Nutrition, 105*(11), 1671.

Lipchock, S. V., Reed, D. R., & Mennella, J. A. (2011). The gustatory and olfactory systems during infancy: Implications for development of feeding behaviors in the high-risk neonate. *Clinics in Perinatology, 38*(4), 627-641.

Liu, P., Kieckhefer, G. M., & Gau, B. (2013). A systematic review of the association between obesity and asthma in children. *Journal of Advanced Nursing, 69*(7), 1446-1465.

Lobstein, T., Baur, L., & Uauy, R. (2004). Obesity in children and young people: A crisis in public health. *Obesity Reviews, 5*(s1), 4-85.

Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Bretscher, A., Ploegh, H., . . . Scott, M. P. (2013). *Molecular cell biology* (7th ed.). New York: W.H. Freeman and Company.

Ludwig, D. S., & Ebbeling, C. B. (2001). Type 2 diabetes mellitus in children. *The Journal of the American Medical Association, 286*(12), 1427-1430.

Luoto, R., Kinnunen, T. I., Aittasalo, M., Kolu, P., Raitanen, J., Ojala, K., . . . Komulainen, T. (2011). Primary prevention of gestational diabetes mellitus and large-for-gestational-age newborns by lifestyle counseling: A cluster-randomized controlled trial. *PLoS Medicine, 8*(5), e1001036.

Lussana, F., Painter, R. C., Ocke, M. C., Buller, H. R., Bossuyt, P. M., & Roseboom, T. J. (2008). Prenatal exposure to the dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. *The American Journal of Clinical Nutrition, 88*(6), 1648-1652.

Lytle, L. A., Seifert, S., Greenstein, J., & McGovern, P. (2000). How do children's eating patterns and food choices change over time? results from a cohort study. *American Journal of Health Promotion, 14*(4), 222-228.

Mackey, A. D., Picciano, M., Mitchell, D. C., & Smiciklas-Wright, H. (1998). Self-selected diets of lactating women often fail to meet dietary recommendations. *Journal of the American Dietetic Association, 98*(3), 297-302.

Mackey, A. D., & Picciano, M. F. (1999). Maternal folate status during extended lactation and the effect of supplemental folic acid. *The American Journal of Clinical Nutrition, 69*(2), 285-292.

Maffeis, C. (2000). Aetiology of overweight and obesity in children and adolescents. *European Journal of Pediatrics, 159*(1), S35-S44.

Maghbooli, Z., Hossein-Nezhad, A., Karimi, F., Shafaei, A. R., & Larijani, B. (2007). Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. *Diabetes/Metabolism Research and Reviews, 24*(1), 27-32.

Maier, A. S., Chabanet, C., Schaal, B., Leathwood, P. D., & Issanchou, S. N. (2008). Breastfeeding and experience with variety early in weaning increase infants' acceptance of new foods for up to two months. *Clinical Nutrition (Edinburgh, Scotland), 27*(6), 849-857. doi:10.1016/j.clnu.2008.08.002

Málaga, I., Arguelles, J., Díaz, J. J., Perillán, C., Vijande, M., & Málaga, S. (2005). Maternal pregnancy vomiting and offspring salt taste sensitivity and blood pressure. *Pediatric Nephrology, 20*(7), 956-960.

Male, C., Persson, L., Freeman, V., Guerra, A., & Hof, M. (2001). Prevalence of iron deficiency in 12 month old infants from 11 European areas and influence of dietary factors on iron status (Euro-Growth study). *Acta Paediatrica, 90*(5), 492-498.

Malik, V. S., Pan, A., Willett, W. C., & Hu, F. B. (2013). Sugar-sweetened beverages and weight gain in children and adults: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition, 98*(4), 1084-1102.

Malik, V. S., Schulze, M. B., & Hu, F. B. (2006). Intake of sugar-sweetened beverages and weight gain: A systematic review. *The American Journal of Clinical Nutrition, 84*(2), 274-288.

Malik, V. S., Willett, W. C., & Hu, F. B. (2009). Sugar-sweetened beverages and BMI in children and adolescents: Reanalyses of a meta-analysis. *The American Journal of Clinical Nutrition, 89*(1), 438-439.

Mandel, D., Lubetzky, R., Dollberg, S., Barak, S., & Mimouni, F. B. (2005). Fat and energy contents of expressed human breast milk in prolonged lactation. *Pediatrics, 116*(3), e432-e435.

Martin, R. M., Gunnell, D., & Smith, G. D. (2005). Breastfeeding in infancy and blood pressure in later life: Systematic review and meta-analysis. *American Journal of Epidemiology, 161*(1), 15-26.

McAndrew, F., Thompson, J., Fellows, L., Large, A., Speed, M., & Renfrew, M. J. (2012). Infant feeding survey 2010. *London: Health and Social Care Information Centre,*

McCance, R., & Widdowson, E. (2002). *The composition of foods* (6th ed. ed.). London: FSA.

McDonald, S. D., Han, Z., Mulla, S., & Beyene, J. (2010). Overweight and obesity in mothers and risk of preterm birth and low birth weight infants: Systematic review and meta-analyses. *BMJ: British Medical Journal, 341, c3428.*

McGinnis, J. M., Gootman, J. A., & Kraak, V. I. (2006). *Food marketing to children and youth: Threat or opportunity?* Washington D.C.: Institute of Medicine -National Academies Press.

McGowan, C., Byrne, J., Walsh, J., & McAuliffe, F. (2011). Insufficient vitamin D intakes among pregnant women. *European Journal of Clinical Nutrition, 65*(9), 1076-1078.

McGowan, C., & McAuliffe, F. (2012). Maternal nutrient intakes and levels of energy underreporting during early pregnancy. *European Journal of Clinical Nutrition, 66*(8), 906-913.

MCR Vitamin Study Research Group. (1991). Prevention of neural tube defects: Results of the medical research council vitamin study. *Lancet, 338*, 131-137.

Mennella, J. A., & Beauchamp, G. K. (1991a). Maternal diet alters the sensory qualities of human milk and the nursling's behavior. *Pediatrics, 88*(4), 737-744.

Mennella, J. A., & Beauchamp, G. K. (1991b). The transfer of alcohol to human milk: Effects on flavor and the infant's behavior. *New England Journal of Medicine, 325*(14), 981-985.

Mennella, J. A., & Beauchamp, G. K. (1999). Experience with a flavor in mother's milk modifies the infant's acceptance of flavored cereal. *Developmental Psychobiology, 35*(3), 197-203.

Mennella, J. A., Jagnow, C. P., & Beauchamp, G. K. (2001). Prenatal and postnatal flavor learning by human infants. *Pediatrics, 107*(6), e88

Mennella, J. A., Johnson, A., & Beauchamp, G. K. (1995). Garlic ingestion by pregnant women alters the odor of amniotic fluid. *Chemical Senses, 20*(2), 207-209.

Mennella, J. (2009). Flavour programming during breast-feeding. *Breast-Feeding: Early Influences on Later Health,* pp113-120.

Mennella, J. A., & Beauchamp, G. K. (2002). Flavor experiences during formula feeding are related to preferences during childhood. *Early Human Development, 68*(2), 71-82.

Mennella, J. A., & Beauchamp, G. K. (2009). Early flavor experiences: Research update. *Nutrition Reviews, 56*(7), 205-211.

Mennella, J. A., Forestell, C. A., Morgan, L. K., & Beauchamp, G. K. (2009). Early milk feeding influences taste acceptance and liking during infancy. *The American Journal of Clinical Nutrition, 90*(3), 780S-788S.

Mennella, J. A., & Trabulsi, J. C. (2012). Complementary foods and flavor experiences: Setting the foundation. *Annals of Nutrition and Metabolism, 60*(2), 40-50.

Mennella, J. A., & Ventura, A. K. (2010). Understanding the basic biology underlying the flavor world of children. *Current Zoology, 56*(6), 834-841.

Mennella, J. A., & Beauchamp, G. K. (1996). The human infants' response to vanilla flavors in mother's milk and formula. *Infant Behavior and Development, 19*(1), 13-19.

Mennella, J. A., & Beauchamp, G. K. (1997). Mothers' milk enhances the acceptance of cereal during weaning. *Pediatric Research, 41*(2), 188-192.

Mennella, J. A., & Beauchamp, G. K. (1999). Experience with a flavor in mother's milk modifies the infant's acceptance of flavored cereal. *Developmental Psychobiology, 35*(3), 197-203.

Mennella, J. A., Nicklaus, S., Jagolino, A. L., & Yourshaw, L. M. (2008). Variety is the spice of life: Strategies for promoting fruit and vegetable acceptance during infancy. *Physiology & Behavior, 94*(1), 29-38.

Mennella, J. A., Turnbull, B., Ziegler, P. J., & Martinez, H. (2005). Infant feeding practices and early flavor experiences in mexican infants: An intra-cultural study. *Journal of the American Dietetic Association, 105*(6), 908-915.

Mikiel-Kostyra, K., Mazur, J., & Boltruszko, I. (2002). Effect of early skin-to-skin contact after delivery on duration of breastfeeding: A prospective cohort study. *Acta Paediatrica, 91*(12), 1301-1306.

Mikkelsen, T. B., Osler, M., Orozova-Bekkevold, I., Knudsen, V. K., & Olsen, S. F. (2006). Association between fruit and vegetable consumption and birth weight: A prospective study among 43,585 danish women. *Scandinavian Journal of Public Health, 34*(6), 616-622.

Mikkilä, V., Räsänen, L., Raitakari, O., Pietinen, P., & Viikari, J. (2005). Consistent dietary patterns identified from childhood to adulthood: The cardiovascular risk in young finns study. *British Journal of Nutrition, 93*(06), 923-931.

Ministry of Health (Ed.). (2008). *Food and nutrition guidelines for healthy pregnant and breastfeeding women: A background paper*. Wellington, New Zealand: Ministry of Health.

Mølgaard, C., & Fleischer Michaelsen, K. (2003). Vitamin D and bone health in early life. *Proceedings of the Nutrition Society, 62*(04), 823-828.

Möller, T., Olsson, H., Ranstam, J., & Bergkvist, L. (2002). Breast cancer and breastfeeding: Collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet, 360*(9328), 187-195.

Morisset, A. S., St-Yves, A., Veillette, J., Weisnagel, S. J., Tchernof, A., & Robitaille, J. (2010). Prevention of gestational diabetes mellitus: A review of studies on weight management. *Diabetes/Metabolism Research and Reviews, 26*(1), 17-25.

Moses, R. G., Shand, J. L., & Tapsell, L. C. (1997). The recurrence of gestational diabetes: Could dietary differences in fat intake be an explanation? *Diabetes Care, 20*(11), 1647-1650.

Mughal, M., Salama, H., Greenaway, T., Laing, I., & Mawer, E. (1999). Florid rickets associated with prolonged breast feeding without vitamin D supplementation. *British Medical Journal, 318*(7175), 39-40.

Muldowney, S. (2010). *Vitamin D status and its association with metabolic risk factors throughout the lifecycle.* PhD thesis. National University of Ireland

Mulligan, M. L., Felton, S. K., Riek, A. E., & Bernal-Mizrachi, C. (2010). Implications of vitamin D deficiency in pregnancy and lactation. *American Journal of Obstetrics and Gynecology, 202*(5), 429. e1-429. e9.

Murrin, C., Fallon, U. B., Hannon, F., Nolan, G., O'Mahony, D., Bury, G., . . . Kelleher, C. C. (2007). Dietary habits of pregnant women in Ireland. *Irish Medical Journal, 100*(Suppl), S12-S15.

Murrin, C., Shrivistava, A., & Kelleher, C. (2010). Maternal nutrient intake during pregnancy and childhood weight status aged five years. *Proceedings of the Nutrition Society, 69*(OCE5) *e*345.

Nader, P. R., O'Brien, M., Houts, R., Bradley, R., Belsky, J., Crosnoe, R., . . . Susman, E. J. (2006). Identifying risk for obesity in early childhood. *Pediatrics, 118*(3), e594-e601.

Naylor, A.J. & Morrow, A.L. (2001). *Developmental readiness of normal full term infants to progress from exclusive breastfeeding to the introduction of complementary foods: Reviews of the relevant literature concerning infant immunologic, gastrointestinal, oral motor and maternal reproductive and lactational development.* Washington, D.C.: Wellstart International and the LINKAGES Project/Academy for Educational Development.

Nelson, G., Chandrashekar, J., Hoon, M. A., Feng, L., Zhao, G., Ryba, N. J. P., & Zuker, C. S. (2002). An amino-acid taste receptor. *Nature, 416*(6877), 199-202.

Nelson, T. M., LopezJimenez, N. D., Tessarollo, L., Inoue, M., Bachmanov, A. A., & Sullivan, S. L. (2010). Taste function in mice with a targeted mutation of the pkd1l3 gene. *Chemical Senses, 35*(7), 565-577.

Ness, A. R., & Powles, J. W. (1997). Fruit and vegetables, and cardiovascular disease: A review. *International Journal of Epidemiology, 26*(1), 1-13.

National Health Service. (2011). 5 a day. Retrieved from http://www.nhs.uk/LiveWell/5ADAY/Pages/5ADAYhome.aspx

Nic Gabhainn, S., Kelly, C., & Molcho, M. (Eds.). (2007). *HBSC Ireland 2006:National report of the 2006 health behaviour in school-aged children in Ireland.* Dublin: Department of Health and Children.

Nicklas, T., Farris, R., Smoak, C., Frank, G., Srinivasan, S., Webber, L., & Berenson, G. (1988). Dietary factors relate to cardiovascular risk factors in early life. Bogalusa heart study. *Arteriosclerosis, Thrombosis, and Vascular Biology, 8*(2), 193-199.

Nicklaus, S., Boggio, V., & Issanchou, S. (2005). Food choices at lunch during the third year of life: High selection of animal and starchy foods but avoidance of vegetables. *Acta Paediatrica, 94*(7), 943-951.

Nicklaus, S., & Remy, E. (2013). Early origins of overeating: Tracking between early food habits and later eating patterns. *Current Obesity Reports, 2*(2), 179-184.

Nicklaus, S., Boggio, V., Chabanet, C., & Issanchou, S. (2004). A prospective study of food preferences in childhood. *Food Quality and Preference, 15*(7-8), 805-818. doi:10.1016/j.foodqual.2004.02.010

Nolan, K., Schell, L. M., Stark, A. D., & Gómez, M. I. (2002). Longitudinal study of energy and nutrient intakes for infants from low-income, urban families. *Public Health Nutrition, 5*(3), 405-412.

Noldus Information Technology. (2012). *Observer XT 10.5*. Heerlen, Netherlands: Noldus Information Technology.

Northstone, K., Emmett, P., & Nethersole, F. & the ALSPAC Study Team. (2001). The effect of age of introduction to lumpy solids on foods eaten and reported feeding difficulties at 6 and 15 months. *Journal of Human Nutrition and Dietetics, 14*, 43-54.

Northstone, K., & Emmett, P. M. (2008). Are dietary patterns stable throughout early and mid-childhood? A birth cohort study. *British Journal of Nutrition, 100*(5), 1069.

O'Riordan, M., Kiely, M., Higgins, J., & Cashman, K. (2008). Prevalence of suboptimal vitamin D status during pregnancy *Irish Medical Journal, 101*(8), 240-242.

O'Brien, M., Nader, P. R., Houts, R. M., Bradley, R., Friedman, S. L., Belsky, J., & Susman, E. (2007). The ecology of childhood overweight: A 12-year longitudinal analysis. *International Journal of Obesity, 31*(9), 1469-1478.

Oddy, W., Sly, P., De Klerk, N., Landau, L., Kendall, G., Holt, P., & Stanley, F. (2003). Breast feeding and respiratory morbidity in infancy: A birth cohort study. *Archives of Disease in Childhood, 88*(3), 224-228.

Oken, E., Taveras, E. M., Kleinman, K. P., Rich-Edwards, J. W., & Gillman, M. W. (2007). Gestational weight gain and child adiposity at age 3 years. *American Journal of Obstetrics and Gynecology, 196*(4), 322.e1-322.e8. doi:DOI: 10.1016/j.ajog.2006.11.027

Olden, K. (2006). Toxicogenomics—a new systems toxicology approach to understanding of gene–environment interactions. *Annals of the New York Academy of Sciences*, *1076*(1), 703-706.

Olsen, N., & Heitmann, B. (2009). Intake of calorically sweetened beverages and obesity. *Obesity Reviews, 10*(1), 68-75.

Olson, C. M., Strawderman, M. S., & Dennison, B. A. (2009). Maternal weight gain during pregnancy and child weight at age 3 years. *Maternal and Child Health Journal, 13*(6), 839-846.

O'Neill, J. L., Keaveney, E. M., O'Connor, N., Cox, M., Regan, A., Shannon, E., & Turner, M. J. (2011). Are women in early pregnancy following the national pyramid recommendations? *Irish Medical Journal, 104*(9), 270-272.

Ong, K. K., & Loos, R. J. (2006). Rapid infancy weight gain and subsequent obesity: Systematic reviews and hopeful suggestions. *Acta Paediatrica, 95*(8), 904-908.

Ortega, R. M. (2001). Dietary guidelines for pregnant women. *Public Health Nutrition, 4*(6a), 1343-1346.

Owen, C. G., Martin, R. M., Whincup, P. H., Davey-Smith, G., Gillman, M. W., & Cook, D. G. (2005). The effect of breastfeeding on mean body mass index throughout life: A quantitative review of published and unpublished observational evidence. *The American Journal of Clinical Nutrition, 82*(6), 1298-1307.

Owen, C. G., Martin, R. M., Whincup, P. H., Smith, G. D., & Cook, D. G. (2006). Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *The American Journal of Clinical Nutrition, 84*(5), 1043-1054.

Owen, C. G., Whincup, P. H., Gilg, J. A., & Cook, D. G. (2003). Effect of breast feeding in infancy on blood pressure in later life: Systematic review and meta-analysis. *BMJ: British Medical Journal, 327*(7425), 1189-1195.

Owen, C. G., Whincup, P. H., Kaye, S. J., Martin, R. M., Smith, G. D., Cook, D. G., . . . Fall, C. H. (2008). Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. *The American Journal of Clinical Nutrition, 88*(2), 305-314.

Owen, C. G., Martin, R. M., Whincup, P. H., Smith, G. D., & Cook, D. G. (2005). Effect of infant feeding on the risk of obesity across the life course: A quantitative review of published evidence. *Pediatrics, 115*(5), 1367-1377. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=rzh&AN=2009011492&site=ehost-live

Pelchat, M. L., & Pliner, P. (1986). Antecedents and correlates of feeding problems in young children. *Journal of Nutrition Education, 18*(1), 23-29.

Peña-Rosas, J., & Viteri, F. (2009a). Effects and safety of preventive oral iron or iron folic acid supplementation for women during pregnancy (review). *Cochrane Database Syst.Rev., 4*

Peña-Rosas, J., & Viteri, F. (2009b). Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. *Cochrane Database of Systematic Reviews,* (Issue 4. Art.), No.: CD004736. DOI: 10.1002/14651858.CD004736.pub3.

Perälä, M., Männistö, S., Kaartinen, N. E., Kajantie, E., Osmond, C., Barker, D. J., . . . Eriksson, J. G. (2012). Body size at birth is associated with food and nutrient intake in adulthood. *PloS One, 7*(9), e46139.

Pérez-Rodrigo, C., Ribas, L., Serra-Majem, L., & Aranceta, J. (2003). Food preferences of spanish children and young people: The enKid study. *European Journal of Clinical Nutrition, 57*, S45-S48.

Philipsen, N. M., & Philipsen, N. C. (2008). Childhood overweight: Prevention strategies for parents. *Journal of Perinatal Education, 17*(1), 44-47. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=rzh&AN=2009881183&site=ehost-live

Picciano, M. F. (2003). Pregnancy and lactation: Physiological adjustments, nutritional requirements and the role of dietary supplements. *The Journal of Nutrition, 133*(6), 1997S-2002S.

Pinhas-Hamiel, O., Standiford, D., Hamiel, D., Dolan, L. M., Cohen, R., & Zeitler, P. S. (1999). The type 2 family: A setting for development and treatment of adolescent type 2 diabetes mellitus. *Archives of Pediatrics and Adolescent Medicine, 153*(10), 1063.

Porter, N., & Ispa, J. M. (2012). Mothers' online message board questions about parenting infants and toddlers. *Journal of Advanced Nursing, 69(3), 559-568.*

Poti, J. M., & Popkin, B. M. (2011). Trends in energy intake among US children by eating location and food source, 1977-2006. *Journal of the American Dietetic Association, 111*(8), 1156-1164.

Prentice, A. (2003). Micronutrients and the bone mineral content of the mother, fetus and newborn. *The Journal of Nutrition, 133*(5), 1693S-1699S.

Prentice, A., Goldberg, G., Davies, H., Murgatroyd, P., & Scott, W. (1989). Energy-sparing adaptations in human pregnancy assessed by whole-body calorimetry. *British Journal of Nutrition, 62*(01), 5-22.

Prescott, S. L., Smith, P., Tang, M., Palmer, D. J., Sinn, J., Huntley, S. J., . . . Makrides, M. (2008). The importance of early complementary feeding in the development of oral tolerance: Concerns and controversies. *Pediatric Allergy and Immunology, 19*(5), 375-380.

Quigley, M. A., Hockley, C., Carson, C., Kelly, Y., Renfrew, M. J., & Sacker, A. (2012). Breastfeeding is associated with improved child cognitive development: A population-based cohort study. *The Journal of Pediatrics, 160*(1), 25-32.

Quigley, M. A., Kelly, Y. J., & Sacker, A. (2007). Breastfeeding and hospitalization for diarrheal and respiratory infection in the United Kingdom millennium cohort study. *Pediatrics, 119*(4), e837-e842.

Qvist, I., Abdulla, M., Jägerstad, M., & Svensson, S. (1986). Iron, zinc and folate status during pregnancy and two months after delivery. *Acta Obstetricia Et Gynecologica Scandinavica, 65*(1), 15-22.

Ramón, R., Ballester, F., Iñiguez, C., Rebagliato, M., Murcia, M., Esplugues, A., . . . Vioque, J. (2009). Vegetable but not fruit intake during pregnancy is associated with newborn anthropometric measures. *The Journal of Nutrition, 139*(3), 561-567.

Rao, S., Yajnik, C. S., Kanade, A., Fall, C. H. D., Margetts, B. M., Jackson, A. A., . . . Lubree, H. (2001). Intake of micronutrient-rich foods in rural indian mothers is associated with the size of their babies at birth: Pune maternal nutrition study 1. *The Journal of Nutrition, 131*(4), 1217-1224.

Rasmussen, S. A., Chu, S. Y., Kim, S. Y., Schmid, C. H., & Lau, J. (2008). Maternal obesity and risk of neural tube defects: A metaanalysis. *American Journal of Obstetrics and Gynecology, 198*(6), 611-619.

Ravelli, A. C. J., van der Meulen, J. H. P., Michels, R., Osmond, C., Barker, D. J. P., Hales, C., & Bleker, O. P. (1998). Glucose tolerance in adults after prenatal exposure to famine. *The Lancet, 351*(9097), 173-177.

Reilly, J., & Kelly, J. (2010). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: Systematic review. *International Journal of Obesity, 35*(7), 891-898.

Remy, E., Issanchou, S., Chabanet, C., & Nicklaus, S. (2013). Repeated exposure of infants at complementary feeding to a vegetable purée increases acceptance as effectively as flavor-flavor learning and more effectively than flavor-nutrient learning. *The Journal of Nutrition, 143*(7), 1194-1200.

Resnicow, K., Davis-Hearn, M., Smith, M., Baranowski, T., Lin, L. S., Baranowski, J., . . . Wang, D. T. (1997). Social-cognitive predictors of fruit and vegetable intake in children. *Health Psychology, 16*, 272-276.

Resnicow, K., Smith, M., Baranowski, T., Baranowski, J., Vaughan, R., & Davis, M. (1998). 2-year tracking of children's fruit and vegetable intake. *Journal of the American Dietetic Association, 98*(7), 785-789.

Ricketts, C. D. (1997). Fat preferences, dietary fat intake and body composition in children. *European Journal of Clinical Nutrition, 51*(11), 778-781.

Rogers, I., Emmett, P., & Study Team, A. L. S. P. A. C. (1998). Diet during pregnancy in a population of pregnant women in south west England. *European Journal of Clinical Nutrition, 52*(4), 246-250. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=hch&AN=471158&site=ehost-live

Rolland-Cachera, M., Deheeger, M., Akrout, M., & Bellisle, F. (1995). Influence of macronutrients on adiposity development: A follow up study of nutrition and growth from 10 months to 8 years of age. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 19*(8), 573-578.

Rolls, B. J., Ello-Martin, J. A., & Tohill, B. C. (2004). What can intervention studies tell us about the relationship between fruit and vegetable consumption and weight management? *Nutrition Reviews, 62*(1), 1-17.

Roseboom, T. J., Van der Meulen, J., Osmond, C., Barker, D. J. P., Ravelli, A., Schroeder-Tanka, J., . . . Bleker, O. (2000). Coronary heart disease after prenatal exposure to the dutch famine, 1944–45. *Heart, 84*(6), 595.

Rosenstein, D., & Oster, H. (1988). Differential facial responses to four basic tastes in newborns. *Child Development, 59*, 1555-1568. doi:10.1111/1467-8624.ep8591101

Rössner, S., & Ohlin, A. (1995). Pregnancy as a risk factor for obesity: Lessons from the Stockholm pregnancy and weight development study. *Obesity Research, 3*, 267s.

Royal College of Paediatrics and Child Health /World Health Organisation/Department of Health. (2013). *UK-WHO growth charts 0-4 years* (2nd ed.). UK: RCPCH.

Sagawa, N. (2010). Maternal nutrition and long-term consequences of the offspring. *Endocrine Journal, 57*(6), 465-466.

Saldana, T. M., Siega-Riz, A. M., & Adair, L. S. (2004). Effect of macronutrient intake on the development of glucose intolerance during pregnancy. *The American Journal of Clinical Nutrition, 79*(3), 479-486.

Saliba, A. J., Wragg, K., & Richardson, P. (2009). Sweet taste preference and personality traits using a white wine. *Food Quality and Preference, 20*(8), 572-575.

Salmenperä, L., Perheentupa, J., & Siimes, M. A. (1986). Folate nutrition is optimal in exclusively breast-fed infants but inadequate in some of their mothers and in formula-fed infants. *Journal of Pediatric Gastroenterology and Nutrition, 5*(2), 283.

San Gabriel, A., & Uneyama, H. (2012). Amino acid sensing in the gastrointestinal tract. *Amino Acids,* , 1-11.

Sausenthaler, S., Koletzko, S., Koletzko, B., Reinhardt, D., Krämer, U., von Berg, A., . . . Wichmann, H. (2010). Effect of hydrolysed formula feeding on taste preferences at 10 years. data from the german infant nutritional intervention program plus study. *Clinical Nutrition, 29*(3), 304-306.

Schaal, B., Marlier, L., & Soussignan, R. (2000). Human foetuses learn odours from their pregnant mother's diet. *Chemical Senses, 25*(6), 729-737.

Scholl, T. O., Chen, X., Khoo, C. S., & Lenders, C. (2004). The dietary glycemic index during pregnancy: Influence on infant birth weight, fetal growth, and biomarkers of carbohydrate metabolism. *American Journal of Epidemiology, 159*(5), 467-474.

Scholl, T. O., Hediger, M. L., Schall, J. I., Ances, I. G., & Smith, W. K. (1995). Gestational weight gain, pregnancy outcome, and postpartum weight retention. *Obstetrics & Gynecology, 86*(3), 423-427.

Scholl, T. O., & Johnson, W. G. (2000). Folic acid: Influence on the outcome of pregnancy. *The American Journal of Clinical Nutrition, 71*(5), 1295S-1303S.

Scientific Advisory Committee on Nutrition (2011). *Paper for information: Terms of reference and scope for a review of complementary and young child feeding* (SACN/SMCN/10/04 ed.) http://www.sacn.gov.uk/pdfs/smcn1105_tor_and_scope_for_review_of_complementary_and_y_.pdf (last accessed December 2012)

Scientific Advisory Committee on Nutrition (2012). *Dietary Reference values for energy- Scientific advisory committee on nutrition 2011.* UK: The Stationery Office Limited.

Schwartz, C., Issanchou, S., & Nicklaus, S. (2009). Developmental changes in the acceptance of the five basic tastes in the first year of life. *British Journal of Nutrition, 102*(09), 1375-1385.

Schwartz, C., Chabanet, C., Laval, C., Issanchou, S., & Nicklaus, S. (2012). Breast-feeding duration: Influence on taste acceptance over the first year of life. *British Journal of Nutrition, 1*(1), 1-8.

Scott, J. A., Chih, T. Y., & Oddy, W. H. (2012). Food variety at 2 years of age is related to duration of breastfeeding. *Nutrients, 4*(10), 1464-1474.

Scott, J. A., Binns, C. W., Graham, K. I., & Oddy, W. H. (2009). Predictors of the early introduction of solid foods in infants: Results of a cohort study. *BMC Pediatrics, 9*, 60. doi:10.1186/1471-2431-9-60

Senn, K., McGuinness, B., Buck, G., Vena, J., Anderson, S., & Rogers, B. (2005). Longitudinal study of babies born to mothers enrolled in a preconception prospective pregnancy study: Study design and methodology, new york state angler cohort study. *Environmental Research, 97*(2), 163-169.

Shaker, I., Scott, J. A., & Reid, M. (2004). Infant feeding attitudes of expectant parents: Breastfeeding and formula feeding. *Journal of Advanced Nursing, 45*(3), 260-268.

Shimoda, M., Yoshimura, T., Ishikawa, H., Hayakawa, I., & Osajima, Y. (2000). Volatile compounds of human milk. *Journal- Faculty of Agriculture Kyushu University* 45:199–206

Shirazki, A., Weintraub, Z., Reich, D., Gershon, E., & Leshem, M. (2007). Lowest neonatal serum sodium predicts sodium intake in low birth weight children. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology, 292*(4), R1683-R1689.

Sibai, B. M. (2011). Calcium supplementation during pregnancy reduces risk of high blood pressure, pre-eclampsia and premature birth compared with placebo? *Evidence Based Medicine, 16*(2), 40-41.

Singhal, A., & Lucas, A. (2004). Early origins of cardiovascular disease: Is there a unifying hypothesis? *The Lancet, 363*(9421), 1642-1645.

Skinner, J. D., & Carruth, B. R. (2001). A longitudinal study of children's juice intake and growth: The juice controversy revisited. *Journal of the American Dietetic Association, 101*(4), 432-437.

Skinner, J. D., Carruth, B. R., Bounds, W., & Ziegler, P. J. (2002a). Children's food preferences: A longitudinal analysis. *Journal of the American Dietetic Association, 102*(11), 1638-1647.

Skinner, J. D., Carruth, B. R., Bounds, W., Ziegler, P., & Reidy, K. (2002b). Do food-related experiences in the first 2 years of life predict dietary variety in school-aged children? *Journal of Nutrition Education and Behavior, 34*(6), 310-315.

Skinner, J. D., Ziegler, P., Pac, S., & Devaney, B. (2004). Meal and snack patterns of infants and toddlers. *Journal of the American Dietetic Association, 104*(Supplement 1), 65-70.

Sloan, S., Sneddon, H., Stewart, M., & Iwaniec, D. (2006). Breast is best? reasons why mothers decide to breastfeed or bottlefeed their babies and factors influencing the duration of breastfeeding. *Child Care in Practice, 12*(3), 283-297.

SMA. (2012). SMA first infant milk powders nutritional information & feeding guide. Retrieved from http://www.smahcp.co.uk/sma-products/first-infant-milk/first-infant-milk/nutritional-data-926.aspx?catid=21&accepted=true

Smith, A. M., Picciano, M. F., & Deering, R. H. (1985). Folate intake and blood concentrations of term infants. *The American Journal of Clinical Nutrition, 41*(3), 590-598.

Sörgel, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauer, M., Skott, A., & Landersdorfer, C. (2003). Acrylamide: Increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy, 48*(6), 267-274.

Soussignan, R., Schaal, B., Marlier, L., & Jiang, T. (1997). Facial and autonomic responses to biological and artificial olfactory stimuli in human neonates: Re-examining early hedonic discrimination of odors. *Physiology and Behavior, 62*(4), 745-758.

Sowers, M. (1996). Pregnancy and lactation as risk factors for subsequent bone loss and osteoporosis. *Journal of Bone and Mineral Research, 11*(8), 1052-1060.

Stein, A. D., Rundle, A., Wada, N., Goldbohm, R., & Lumey, L. (2009). Associations of gestational exposure to famine with energy balance and macronutrient density of the diet at age 58 years differ according to the reference population used. *The Journal of Nutrition, 139*(8), 1555-1561.

Stein, A. D., Shea, S., Basch, C. E., Contento, I. R., & Zybert, P. (1991). Variability and tracking of nutrient intakes of preschool children based on multiple administrations of the 24-hour dietary recall. *American Journal of Epidemiology, 134*(12), 1427-1437.

Stein, L. J., Cowart, B. J., & Beauchamp, G. K. (2006). Salty taste acceptance by infants and young children is related to birth weight: Longitudinal analysis of infants within the normal birth weight range. *European Journal of Clinical Nutrition, 60*, 272-279.

Steiner, J. E. (1977). Facial expressions of the neonate infant indicating the hedonics of food-related chemical stimuli. In J. M. Weiffenbach (Ed.), *Taste and development: The gensis of sweet preference* (pp. 173-188). Washington DC: U.S. Government Printing Office.

Steiner, J. E., Glaser, D., Hawilo, M. E., & Berridge, K. C. (2001). Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. *Neuroscience & Biobehavioral Reviews, 25*(1), 53-74.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: A review. *Journal of the American Dietetic Association, 96*(10), 1027-1039.

Stevens, B., Johnston, C., Petryshen, P., & Taddio, A. (1996). Premature infant pain profile: Development and initial validation. *The Clinical Journal of Pain, 12*(1), 13-22.

Stevens, E. E., Patrick, T. E., & Pickler, R. (2009). A history of infant feeding. *The Journal of Perinatal Education, 18*(2), 32-39.

Stevens, J. P. (2002). *Applied multivariate statistics for the social sciences* (4th ed ed.). Mahwah, NJ: L. Erlbaum Associates, Inc.

Storlien, L. H., Huang, X. F., Lin, S., Xin, X., Wang, H. Q., & Else, P. L. (2001). Dietary fat subtypes and obesity. *World Review of Nutrition and Dietetics, 88*, 148-154.

Strazzullo, P., D'Elia, L., Kandala, N. B., & Cappuccio, F. P. (2009). Salt intake, stroke, and cardiovascular disease: Meta-analysis of prospective studies. *BMJ: British Medical Journal, 339*, b4567

Strazzullo, P., Campanozzi, A., & Avallone, S. (2012). Does salt intake in the first two years of life affect the development of cardiovascular disorders in adulthood? *Nutrition, Metabolism and Cardiovascular Diseases, 22*(10), 787-792.

Sullivan, S. A., & Birch, L. L. (1994). Infant dietary experience and acceptance of solid foods. *Pediatrics, 93*(2), 271-277.

Sullivan, S. A. (1994). Infant dietary experience and acceptance of solid foods. *Pediatrics, 93*(2), 271. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=hch&AN=9610172422&site=ehost-live

Sullivan, S. A., & Birch, L. L. (1990). Pass the sugar, pass the salt: Experience dictates preference. *Developmental Psychology, 26*(4), 546-551. doi:10.1037/0012-1649.26.4.546

Susser, M., & Stein, Z. (1994). Timing in prenatal nutrition: A reprise of the dutch famine study. *Nutrition Reviews, 52*(3), 84-94.

Sweet, S. D., & McGrath, P. J. (1998). Physiological measures of pain
. In G. A. Finley, & P. J. McGrath (Eds.), *Measurement of pain in infants and children. progress in pain research and management.* (pp. 59-81). Seattle: IASP.

Szajewska, H., Horvath, A., & Koletzko, B. (2006). Effect of n–3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: A meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition, 83*(6), 1337-1344.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics* (5th ed. ed.). Boston, MA: Allyn and Bacon.

Taddio, A., Katz, J., Ilersich, A. L., & Koren, G. (1997). Effect of neonatal circumcision on pain response during subsequent routine vaccination. *The Lancet, 349*(9052), 599-603.

Tamura, T., Yoshimura, Y., & Arakawa, T. (1980). Human milk folate and folate status in lactating mothers and their infants. *The American Journal of Clinical Nutrition, 33*(2), 193-197.

Tarrant, R. (2008). *An investigation of the diets of infants born in Ireland during the first six months of life.* PhD Thesis. Dublin Institute of Technology.

Tarrant, R. C., Younger, K. M., Sheridan-Pereira, M., White, M. J., & Kearney, J. M. (2009). The prevalence and determinants of breast-feeding initiation and duration in a sample of women in Ireland. *Public Health Nutrition, 13*(6), 760.

Tarrant, R. C., Younger, K. M., Sheridan-Pereira, M., White, M. J., & Kearney, J. M. (2010). Factors associated with weaning practices in term infants: A prospective observational study in Ireland. *British Journal of Nutrition, 104*(10), 1544-1554.

Tarrant, R. C., Younger, K. M., Sheridan-Pereira, M., & Kearney, J. M. (2011). Factors associated with duration of breastfeeding in Ireland: Potential areas for improvement. *Journal of Human Lactation, 27*(3), 262-271.

Tarrant, R. C., Sheridan-Pereira, M., Younger, K. M., & Kearney, J. M. (2012). The positive role of breastfeeding on infant health during the first 6 weeks: Findings from a prospective observational study based on maternal reports. *Irish Medical Journal, 105*(3), 75-78.

The Economic and Social Research Institute - Health Research and Information Division. (2012). *Perinatal statistics report 2011: ESRI survey and statistical report series.* (No. 46). Dublin: The Economic and Social Research Institute.

Thomas, B., & Bishop, J. (Eds.). (2007). *Manual of dietetics* (4th ed ed.). Oxford: Blackwell Publishing.

Thulier, D., & Mercer, J. (2009). Variables associated with breastfeeding duration. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 38*(3), 259-268.

Thurnham, D. I., Bender, D. A., Scott, J., & Halsted, C. H. (2000). Water soluble vitamins. In J. S. Garrow, & James, W.P.T. & Ralph, A. (Eds.), *Human nutrition and dietetics* (pp. 249-287)

Todd, J. M., & Parnell, W. R. (1994). Nutrient intakes of women who are breastfeeding. *European Journal of Clinical Nutrition, 48*(8), 567-574.

Trabulsi, J. C., & Mennella, J. A. (2012). Diet, sensitive periods in flavour learning, and growth. *International Review of Psychiatry, 24*(3), 219-230.

Treiman, K., Freimuth, V., Damron, D., Lasswell, A., Anliker, J., Havas, S., . . . Feldman, R. (1996). Attitudes and behaviors related to fruits and vegetables among low-income women in the WIC program. *Journal of Nutrition Education, 28*(3), 149-156.

Trivedi, B. P. (2012). Neuroscience: Hardwired for taste. *Nature, 486*(7403), S7-S9.

Turnbull, B., & Matisoo-Smith, E. (2002). Taste sensitivity to 6-n-propylthiouracil predicts acceptance of bitter-tasting spinach in 3–6-y-old children. *The American Journal of Clinical Nutrition, 76*(5), 1101-1105.

Twomey, A., Kiberd, B., Matthews, T., & O'Regan, M. (2000). Feeding infants: An investment in the future. *Irish Medical Journal, 93*, 248-250.

Umesawa, M., Iso, H., Date, C., Yamamoto, A., Toyoshima, H., Watanabe, Y., . . . Inaba, Y. (2008). Relations between dietary sodium and potassium intakes and mortality from cardiovascular disease: The Japan collaborative cohort study for evaluation of cancer risks. *The American Journal of Clinical Nutrition, 88*(1), 195-202.

Van Teijlingen, E. R., Wilson, B., Barry, N., Ralph, A., McNeill, G., Graham, W., . . . Campbell, D. (1998). *Effectiveness of interventions to promote healthy eating in pregnant women and women of childbearing age: A review.* (Vol. 11). Health Education Authority London.

Vartanian, L. R., Schwartz, M. B., & Brownell, K. D. (2007). Effects of soft drink consumption on nutrition and health: A systematic review and meta-analysis. *American Journal of Public Health, 97*(4), 667-675.

Verbeke, W., & De Bourdeaudhuij, I. (2007). Dietary behaviour of pregnant versus non-pregnant women. *Appetite, 48*(1), 78-86.

Wagner, C. L., & Greer, F. R. (2008). Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics, 122*(5), 1142-1152.

Wallace, J. P., Inbar, G., & Ernsthausen, K. (1992). Infant acceptance of postexercise breast milk. *Pediatrics, 89*(6), 1245-1247. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=4749439&site=ehost-live

Walton, J. (Ed.). (2012). *National preschool nutrition survey- summary report on: Food and nutrient intakes, physical measurements and barriers to healthy eating*. Dublin: Irish Universities Nutrition Alliance.

Wang, Y., Bentley, M. E., Zhai, F., & Popkin, B. M. (2002). Tracking of dietary intake patterns of Chinese from childhood to adolescence over a six-year follow-up period. *The Journal of Nutrition, 132*(3), 430-438.

Ward, L. M. (2005). Vitamin D deficiency in the 21st century: A persistent problem among Canadian infants and mothers. *Canadian Medical Association Journal, 172*(6), 769-770.

Wardle, J., & Cooke, L. (2008). Genetic and environmental determinants of children's food preferences. *British Journal of Nutrition, 99*(S1), 15-21.

Watson, P. E., & McDonald, B. W. (2009). Major influences on nutrient intake in pregnant New Zealand women. *Maternal and Child Health Journal, 13*(5), 695-706.

Wen, L. M., Flood, V. M., Simpson, J. M., Rissel, C., & Baur, L. A. (2010). Dietary behaviours during pregnancy: Findings from first-time mothers in southwest sydney, Australia. *The International Journal of Behavioral Nutrition and Physical Activity, 7*, 13-19. doi:10.1186/1479-5868-7-13

Wharton, B., & Bishop, N. (2003). Rickets. *The Lancet, 362*(9393), 1389-1400.

Whelton, H., Harrington, J., Crowley, E., Kelleher, V., Cronin, M., & Perry, I. J. (2007). Prevalence of overweight and obesity on the island of Ireland: Results from the north south survey of children's height, weight and body mass index, 2002. *BMC Public Health, 7*, 187.

Wilkinson, S. A., Miller, Y. D., & Watson, B. (2009). Prevalence of health behaviours in pregnancy at service entry in a Queensland health service district. *Australian and New Zealand Journal of Public Health, 33*(3), 228-233.

Williams, J., Greene, S., McNally, S., Murray, A., & Quail, A. (Eds.). (2010). *Growing up in Ireland national longitudinal study of children- the infants and their families*. Dublin: The Stationery Office.

Williams, J., Murray, A., & McCrory, C. & McNally, S. (2013). *Growing up in Ireland. National longitudinal study of children- development from birth to three years.* (No. 5). Dublin: The Stationery Office.

Wilson, A. C., Forsyth, J. S., Greene, S. A., Irvine, L., Hau, C., & Howie, P. W. (1998). Relation of infant diet to childhood health: Seven year follow up of cohort of children in Dundee infant feeding study. *BMJ: British Medical Journal, 316*(7124), 21-25.

Wise, P. M., Hansen, J. L., Reed, D. R., & Breslin, P. A. S. (2007). Twin study of the heritability of recognition thresholds for sour and salty taste. *Chemical Senses, 32*(8), 749.

World Cancer Research Fund, & American Institute for Cancer Research. (2007). *Food, nutrition, physical activity, and the prevention of cancer: A global perspective* Washington D.C.: American Institute for Cancer Research.

World Health Organization (Ed.). (1991). *Indicators for assessing breastfeeding practices, division of child health and development (WHO/CDD/SER/91).* Geneva.: WHO.

World Health Organisation (Ed.). (1995). The world health organization's infant-feeding recommendations. *Weekly Epidemiological Record, 70*, 119-120.

World Health Organisation (Ed.). (1998). *Complementary feeding of young children in developing countries: A review of current scientific knowledge.* No. WHO/NUT/98.1. Geneva: WHO.

World Health Organisation (Ed.). (2001). *54th world health assembly: Global strategy for infant and young child feeding: The optimal duration of exclusive breast feeding.* Geneva: WHO Department of Child and Adolescent Health and Development.

World Health Organization (Ed.). (2004). *Promoting proper feeding for infants and young children*. Geneva: WHO.

World Health Organisation (Ed.). (2007).*The challenge of obesity in the WHO European region and the strategies for response*. Copenhagen: WHO Regional Office for Europe.

World Health Organisation (Ed.). (2009). *Population-based prevention strategies for childhood obesity: Report of a WHO forum and technical meeting*. Geneva: WHO Regional Office for Europe.

World Health Organisation (2013a). Obesity and overweight fact sheet N°311. Retrieved July 5th, 2013, from http://www.who.int/mediacentre/factsheets/fs311/en/index.html

World Health Organisation (2013b). Ten facts on breastfeeding. Retrieved from July 5th, 2013, http://www.who.int/features/factfiles/breastfeeding/en/index.html

World Health Organisation /Food & Agricultural Organisation. (2005). *Fruit and vegetables for health : Report of a joint FAO/WHO workshop*. Geneva: WHO/FAO.

World Health Organisation Multicentre Growth Reference Study Group. (2006). *WHO child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development.* Geneva: WHO.

Yang, Z., Lönnerdal, B., Adu-Afarwuah, S., Brown, K. H., Chaparro, C. M., Cohen, R. J., . . . Dewey, K. G. (2009). Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: Comparison of data from 6 studies. *The American Journal of Clinical Nutrition, 89*(5), 1433-1440.

Yasumatsu, K., Horio, N., Murata, Y., Shirosaki, S., Ohkuri, T., Yoshida, R., & Ninomiya, Y. (2009). Multiple receptors underlie glutamate taste responses in mice. *The American Journal of Clinical Nutrition, 90*(3), 747S-752S.

Zempsky, W. T., & Schechter, N. L. (2003). What's new in the management of pain in children. *Pediatrics in Review, 24*(10), 337-348.

Zhang, Y., Hoon, M. A., Chandrashekar, J., Mueller, K. L., Cook, B., Wu, D., . . . Ryba, N. J. P. (2003). Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways. *Cell, 112*(3), 293-301.

Ziegler, E. E., Fomon, S. J., Nelson, S. E., Rebouche, C. J., Edwards, B. B., Rogers, R. R., & Lehman, L. J. (1990). Cow milk feeding in infancy: Further observations on blood loss from the gastrointestinal tract. *The Journal of Pediatrics, 116*(1), 11-18.

Appendix A

Summary of Weaning Recommendations

Summary of the weaning recommendation for healthy infants (Source: Thomas & Bishop, 2007; Dunne *et al.,* 2011).

Food	Stage 1	Stage 2	Stage 3	Stage 4
Skills to learn /develop	• Taking foods from a spoon • Moving food from the front of the mouth to the back for swallowing • Managing thicker purees & mashed	• Moving soft lumps around the mouth • Chewing soft lumps • Self feeding using hands & fingers	• Chewing minced & chopped foods • Self feeding attempts with spoon	• Hard finger foods • Minced & chopped family foods
Milk Feed	Breastfeed on demand or continue to give usual volume of formula milk. Don't reduce volume of milk too quickly.	Breastfeed on demand / minimum 500-600ml formula milk per day		
No of meals/day	1-2 meals Approx size of each meal 5-10 teaspoons	2-3 meals Approx size of each meal 5-10 tsp	3meals. Approx size of each meal 2-4tbsp	3 meals plus 1-2 snacks. Approx size of each meal 4-6 tbsp
Textures	**S**mooth runny puree, no lumps	Smooth slightly thicker puree (add less liquid) no lumps	Minced & mashed with soft lumps. Introduce soft finger foods	Minced & finely chopped
Cereals	Baby rice, ground white rice,	Baby rice, ground white rice, baby porridge, Ready Brek	Pasta, Bread ,Weetabix, Ready Brek, Porridge or other breakfast cereal, wholemeal bread, rusk; limit foods made with white refined flour e.g. biscuits cakes	

Vegetables	Root vegetables: carrots, turnip, parsnip, butternut squash,	Potato and stronger flavoured vegetables: broccoli, spinach, cauliflower, peas, courgettes.	Stage 1+ 2 Vegetables plus leek, onion, cabbage, spinach, sweet-corn, tomato, peppers, mushroom.	Wide range
Fruit	Ripe fruit uncooked banana, stewed apple and pear	Peach plum kiwi melon avocado	Citrus fruit: remove pith and seed berry fruits put through a metal sieve to remove pips mango grapes: peel and deseed (avoid whole grapes-risk of choking).	Wide range
Meat & meat alternatives (e.g. eggs fish & pulses	Once the baby is accepting food from a spoon: soft well cooked meat poultry fish remove all bones peas beans and lentils can be pureed then mixed with vegetable		Soft cooked minced or pureed meat/poultry fish (bones removed) or pulses; hard boiled or scrambled eggs (avoid lightly cooked eggs)	Increase variety introduce stronger flavoured fish e.g. oily fish mackerel tuna salmon
Cow's Milk	Small amounts of full fat cow's milk can be mixed into solids but it's best to use expressed breast milk or formula milk at this stage		**S**mall amounts of full fat cow's milk can be mixed into solids or cereal; not to be given as a drink until 12 months old	
Dairy foods:	Natural yogurt -full fat		full fat yogurts; mild hard cheeses; cream	

Appendix B

PARTICIPANT INFORMATION SHEET

Title of research project: An examination of factors associated with the development of sour taste preference (e.g. citrus fruits) in infants.

Introduction:

You are invited to take part in a research study. Before you decide to take part or not, it is important that you understand why the study is being undertaken and what it will involve. Please read the following information carefully and discuss it with others, if you wish. If you require further information, or would like to ask any questions, please contact Aileen Kennedy from the School of Nursing in DCU on 01-700-7797.

In this study, we will be examining a number of factors that may play a role in determining what foods an infant will like or dislike. There is a growing body of evidence suggesting that the food choices a mother makes during her pregnancy may affect her infant's later acceptance of solid foods. This research is funded by the School of Nursing, Dublin City University. It was devised by a research team consisting of Dr. Tracey Harrington and Ms Aileen Kennedy, the School of Nursing, Dublin City University.

Procedures:

This study will be conducted over 15 months. Initially we will focus on your diet during pregnancy. Foods that a woman eats during this time may have an influence on infants' food preferences later on. During the $8^{th}/9^{th}$ month of pregnancy, we will ask you to complete a food frequency questionnaire and a food record, where you will record over seven days whatever you eat and drink. We will also ask you to fill out a questionnaire at the start of the study giving basic information about you e.g. ethnicity, weight, height and educational level attained as these may have an influence on your baby's preference for foods.

After the birth of your baby, we will focus on their diet. We will obtain food diaries the eating/feeding habits of your baby at different intervals: when you first introduce solid foods to your baby, if that is before 6 months then at 6 months & then finally at 12 months. Each time we will ask you to record over three days whatever your child eats/drinks. You will also ask you to complete another food diary after your baby if born.

For the final stage of the project, we will arrange to meet with you and your baby when your baby is first weaned if that is before six months then at 6 & finally at 12 months. During these visits, you will be asked to give your baby a 4 drinks to try and rate their preference for each drink. These drinks will consist of diluted fruit squash and citric acid. Citric acid is found naturally in many fruits especially citrus fruit and is added to many foods by manufacturers. During these visits, your infant's facial expressions will be videotaped in order to analysis these preferences further. We will also measure the growth of your infant at these times.

Benefits:

There are no direct benefits to taking part in this study. However, the information that will be collected will be used to gain an understanding of why infants eat what they do, and why they don't like the things they don't. This may help to develop better information for parents and health professionals. Research that helps to develop information in this way, will in turn, benefit parents and infants and may improve their overall nutrition. We would hope that it is for this reason that you would agree to participate. However if you agree to participate in this study we can give you feedback to you about your own diet and that of your baby.

Risks:

This project is deemed as having no more than minimal risk. The study team does not foresee or anticipate any direct risk to you or your infant by taking part in this study.

Exclusion from participation:

If you are not pregnant, or if you or your infant has any dietary or medical problems, which you are receiving treatment for, or you are under 18 years of age, you will be unable to take part in the study. The study will also exclude multiple birth pregnancies e.g. twins.

Confidentiality:

If you agree to take part, all information collected will be kept strictly confidential within the limitations of the law. All information will have your name and address removed so as to preserve confidentiality. Any information that will identify you in any way will also be removed. The video tapes will only be used to analyse the infant's facial expressions. Only members of the research team will view these tapes. Upon completion of the project, all videotapes will be destroyed by the principal investigators.

The study findings will form the basis for preparation of reports, academic publications, conference papers and other scientific publications.

Voluntary participation:

If you decide not to participate, or if you leave, there will be no penalty for withdrawing before all stages of the research study have been completed.

Permission:

This research project has been approved by Dublin City University Research Ethics Committee.

Further Information:

If you need more information about your participation in the study, your rights, or answers to your questions about the study, contact Aileen Kennedy from the School of Nursing in DCU on 01 7007797 or by email aileen.kennedy35@mail.dcu.ie. You can also write to Aileen at the School of Nursing, DCU, Dublin 9.

Please Note: If you have concerns about this study and wish to contact an independent person, please contact:

The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-700-8000

Appendix C

Informed Consent Form

Title of Research Project: An examination of factors associated with the development of sour taste (e.g. citrus fruits) preference in infants.

This Research Study is being conducted by a research team consisting of Dr. Tracey Harrington (Lecturer) and Ms Aileen Kennedy (Research Dietitian), the School of Nursing, Dublin City University.

In this study, we will be examining a number of factors that may play a role in determining food preference in infants. There is a growing body of evidence suggesting that the food choices a mother makes during her pregnancy may affect her infant's later acceptance of solid foods.

Participant – please complete the following (Circle Yes or No)

- Have you read or had read to you the Participant Information Leaflet? Yes/No
- Do you understand the information provided? Yes/No
- Have you had an opportunity to ask questions and discuss this study? Yes/No
- Have you received satisfactory answers to all your questions? Yes/No
- Are you aware that you will be asked to complete food diary for both you and your infants? Yes/No
- Are you aware that your infant will be videotaped? Yes/No
- Are you aware that your infant will be given several drinks to taste? Yes/No
- Do you consent to the researchers contacting you by phone during this study? Yes/No

Please be aware the you and your infant may withdraw at any time from the Research Study, without giving reason, and without this decision affecting you or your infant's future treatment or medical care. There will be no penalty for withdrawing before all stages of the Research Study have been completed.

If you agree to take part, all information collected will be kept strictly confidential within the limitations of the law. All information will have your name and address removed so as to preserve confidentiality. Any information that will identify you in any way will also be removed. The video tapes will only be used to analyse your infant's facial expressions. Only members of the research team will view these tapes. Upon completion of the study these videotapes will be destroyed by Dr. Tracey Harrington.

I have read, or had read to me, this Consent Form. I have also read, or had read to me the Information Sheet. I have had the opportunity to ask questions about the Consent Form as well as the study and all my questions have been answered by the researchers to my satisfaction and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: ____________________________________

Name in Block Capitals: ________________________________

Witness: ________________________________

Date: ________________________________

Due Date: ______________________

Home address ______________________

____

Contact Number:______________________

Please return this completed form to Aileen Kennedy, School of Nursing, DCU, Dublin 9

Appendix D

Recruitment Poster

Are you Pregnant? Would you like to help Infant Health?

If you answered YES to these questions, you maybe eligible to participate in a nutrition research study.

The purpose of this research study is to investigate factors which may influence the development of sour tastes in infancy.

We are currently looking for pregnant women who would like to participate in a study with the School of Nursing in Dublin City University

Your involvement will be invaluable and may help to better understand infants' taste development.

DCU
School of Nursing
Dublin City University

If you would like to discuss this study further or would like more information on what is involved, please contact:

Aileen Kennedy
School of Nursing, DCU, Dublin 9.
Tel: 01-700-7797
Email: aileen.kennedy35@mail.dcu.ie

Appendix E

Text used in Recruitment Leaflet

A Study on Pregnancy and Early Feeding Practices and Taste Preference.

We are currently recruiting pregnant women, who would like to participate in a study to investigate whether a mother's diet during pregnancy and early infant feeding practices may play a role in determining what foods a child will like or dislike.

QU: "What is the purpose of the Study??"

ANS: In this study, we will be examining a number of factors that may play a role in determining what foods a child will like or dislike. With obesity levels in children increasing in Ireland it is believed that nutrition during pregnancy and in the first two years of life may be very important in fight against the rise of obesity.

Initially we will focus on your diet during pregnancy. Foods that a woman eats during this time may also be experienced by the unborn child and may affect preference later on. After the birth of your baby, we will focus on their diet. We will measure the growth of your infant and ask you questions about the foods that you first introduced to your baby, whether you breast fed your infant and foods that your infant likes or dislikes at four different time points.

QU: "If I agree to participate, what will be involved in participating in the study??"

ANS: Initially we will look at your diet during the 3rd trimester of your pregnancy. You will be asked to write down everything you eat or drink for 7 days as well as completing a questionnaire. If you decided to breast feed will ask you complete another food diary during this time. You will also to given a short questionnaire to complete at the start of the study, designed to gather general information about yourself e.g. ethnicity and occupation.

When your baby has started to try solid foods, we will ask you to write everything that you feed your infant over a 3-day period. The height and weight of your baby will also be recorded. We will also ask you to repeat this when the infant is 6 & 12 months old.

For the final stage of the project, you and your infant will be invited to DCU at each time interval - your baby has started to try solid foods, 6 months and 12 months. Here, we will ask you to offer your infants drinks which will vary in taste to try. The drinks will vary in sour taste and may contain citric acid. Citric acid is a weak organic acid and is found naturally in many fruits especially citrus fruit. We will videotape the facial expressions of your baby to analyse if they like the drinks or not. We will also ask you to rate your infant's enjoyment of these drinks.

QU: "Are there any benefits to completing this study??"

ANS: The information that will be collected to gain an understanding of why children eat what they do, and why they don't like the things they don't. We would hope that it is for this reason that you would agree to participate. However, Parents who submit food diaries can receive copies of the nutritional analysis if they wish.

If you are interested in participating and would like further information, please contact Aileen Kennedy from the School of Nursing in DCU on 01 7007797 or by email aileen.kennedy35@mail.dcu.ie. You can also write to Aileen at the School of Nursing, DCU, Dublin 9.

Appendix F

Adult Food Diary

FOOD DIARY

Please read through these instructions and the example carefully once or twice before you start.

We would like you to record, as accurately as possible, what you eat and drink for 7 days.

Please record ALL food and drink consumed, including alcohol. Please try to record this information at the time of eating and NOT from memory at the end of the day. Keep this record sheet with you throughout the day.

You should include all meals and snacks, plus sweets, drinks etc. When recording food eaten at meals, please include any sauces, dressings or extras e.g. gravy, salad dressings, pickles as well as the main food.

If you do not eat a particular meal or snack simply draw a line across the space at this point.

Guidelines for describing food and drink:

1. Please give details of method of cooking e.g. grilled, boiled, roasted etc. and if you added anything to the cooking e.g. vegetable oil (1 tsp)

2. Give as many details as possible about the type of food you eat:
    a. State brand names where application: John West tuna in tomato sauce OR Tesco's half-fat Edam cheese.
    b. Name the type of biscuit, cake or cereal
    c. Name the type of cheese, fish or meat

GENERAL QUESTIONS

Which type of bread do you usually eat?

- ☐ White
- ☐ Brown/Hovis
- ☐ Granary
- ☐ Wholemeal
- ☐ None

Do you usually buy large or small loaves, sliced or unsliced?

- ☐ Large
- ☐ Small
- ☐ Sliced
- ☐ Unsliced

Which type of milk do you usually use?

- ☐ Full cream milk
- ☐ Semi-skimmed milk
- ☐ Skimmed milk
- ☐ Super Milk
- ☐ None
- ☐ Other   Please specify what ____________________________

How much milk do you usually use?

- ☐ 1-2 pints
- ☐ ½ - 1 pint
- ☐ ¼ - ½ pint
- ☐ None

FOOD RECORD SHEET

Day: EXAMPLE		**DATE: 1st march 2009**
Approx Time	**Quantity Eaten**	**Details of Foods and Drinks Taken**
Meal 1	1 cup 1 tbsp	Tea with semi-skimmed milk
Meal 2	3 heaped tbsp 1/4 pint 1 med slice 1 tsp 1 mug	Branflakes (Kellog's) Semi- Skimmed milk for cereal and drinks Wholemeal bread (large loaf) Flora margarine Coffee
Meal 3	1 mug 1 tsp 1 medium	Coffee with Semi-skimmed milk Apple
Meal 4	4 slices 4 level tsp 2 thin slices 1 large 1 can (330ml)	Sandwiches: white bread (Brennans) large loaf sliced with flora margarine Deli ham - no fat Banana Coca Cola
Meal 5	1 medium 3 egg sized 2 tbsp 2 tbsp 1 pot (125g)	Pork chop fat removed - grilled Boiled potatoes Frozen peas (Bird's eye) Boiled carrots Strawberry yogurt (Yoplait)
Meal 6	1 mug 2	Hot chocolate (Options -Belgian chocolate) Chocolate digestive biscuits – McVities
Any comments		

FOOD RECORD SHEET

Day:	DATE:	
Approx Time	**Quantity Eaten**	**Details of Foods and Drinks Taken**
Meal 1		
Meal 2		
Meal 3		
Meal 4		
Meal 5		
Meal 6		
Any comments		

Appendix G

Text of Baseline Questionnaire

Please complete the following questions using BLOCK CAPITALS.

1. Mother's Name: ____________________________________

2. Address: ____

____

____

3. Mother's date of birth? (DD/MM/YYYY) ______________

4. Telephone Contact numbers

a) Mobile Number: ____________________

b) Home Number: ____________________

c) Work Number: ____________________

5. Email address: ________________________________

6. Mother's Ethnicity: _____________________________

7. Father's Ethnicity: _____________________________

8. (a) Current Gestational age of Infant: _____________________

(b) Estimated Delivery Date: _________________________

9. Height: ____ **ft** _____**ins** **OR** ______ **cm**

10. Pre-pregnancy weight: ___ stone ____ lbs OR _____ kg

11. What is your present Occupation: ___________________________

Full-time ☐

Part- time ☐

12. What level of education did you complete? Please tick

Primary school ☐
Secondary school ☐
training course (e.g. hairdresser, FAS, VEC, apprentice) ☐

Primary Degree ☐

Post-graduate ☐

Other, please mention _________________________ ☐

13. Do you have a partner?

Yes ☐ => Please go to Q15

No ☐ => Please go to Q18

14. Date of Birth of your partner? (DD/MM/YYYY) ________________

15. What is your partner's present occupation: ______________________

Full-time ☐

Part-time ☐

16. What level of education did your partner complete? Please tick
Primary school ☐
Secondary school ☐

Training course (e.g. hairdresser, FAS, VEC, apprentice) ☐

Primary Degree ☐

Post-graduate ☐

Other, please mention ______________________ ☐

17. What is your current living arrangement/ situation?

Married, living with husband ☐

Married, not living with husband ☐

Not married, living with partner ☐

Partnered, but not living together ☐

Single, living with parents ☐

Single, living alone ☐

Divorced ☐

Widowed ☐

18. Do you live?

In your own home ☐

With your parents ☐

With your parents-in-law ☐

Rented accommodation ☐

Apartment ☐

Mother's shelter ☐

Other, please mention ___________________________

19. Do you plan to return to work after the birth?

Yes ☐

No ☐

20. If so how long after the birth do you intend to return to work? ____________

21. GP contact details:

Name: ______________________________

Surgery Address: ______________________________

____

____

Contact Number: ______________________________

22. Consultant Details:

Name: ______________________________

Address: ______________________________

____

____

Contact Number: ______________________________

Appendix H

6-month Data Collection Sheet

Participant Number ☐☐☐☐

1. Baby's Name: ______________________

2. Today's Date: ______________________

3. Height: _____ins **OR** ______ cm

4. Weight: ____ lbs **OR** _____ kg

5. Head Circumference _______________cm

6. Date when baby started solids: ______________________

7. Has your baby tried any fruit? If Yes please list.

______________________ ______________________

______________________ ______________________

_____________________ ______________________

8. What other foods has your baby tried?

______________________ ______________________

______________________ ______________________

______________________ ______________________

9. Where did you information come from?

Appendix I

Likert Scale of Infant's Preference as assessed by Mothers

Baby's Baseline

Participant Number ☐☐☐☐

Baby's Name: ______________________

Today's Date: ______________________

Solution :

1 2 3 4 5 6 7 8 9 10

Strongly Dislike Strongly Like

Solution :

1 2 3 4 5 6 7 8 9 10

Strongly Dislike Strongly Like

Solution :

1 2 3 4 5 6 7 8 9 10

Strongly Dislike Strongly Like

Solution :

1 2 3 4 5 6 7 8 9 10

Strongly Dislike Strongly Like

OFFICE USE:

Bottle 1: Amt 1 __________ Amt 2_______________ == Total

Bottle 2 Amt 1 __________ Amt 2_______________ == Total

Bottle 3 Amt 1 __________ Amt 2_______________ == Total

Bottle 4 Amt 1 __________ Amt 2_______________ == Total

Appendix J

Explanation of RDA, AR and LTI

Mean micronutrient intake levels are assessed using current Irish recommended dietary allowances (RDA: point c on the graph below), average requirements (AR; point b on the graph below) and lowest threshold intakes (LTI; point a on the graph below). These 3 dietary reference points (RDA, AR and LTI) define individual nutrient requirements based on a population distribution see figure below. The single value RDA is often misinterpreted as the lowest acceptable intake of a nutrient when, in fact, it is substantially more than an individual requires.

Point 'a' represents the LTI and is the equivalent of the population mean minus two standard deviations. The LTI represents the intake below which nearly all individuals would be deficient. Therefore, if a substantial proportion of a population consumes less than the LTI, the population may be at risk of nutrient deficiency.

Point 'b' represents the AR and is the mean requirement of the population. On a population level, as the proportion of the population with a nutrient intake less than the AR increases, the likelihood of a deficiency for that nutrient increases.

Point 'c' represents the RDA and is the equivalent of the population mean plus two standard deviations. The RDA should meet the nutritional requirement of nearly all healthy people in the group (approximately 97.5%).

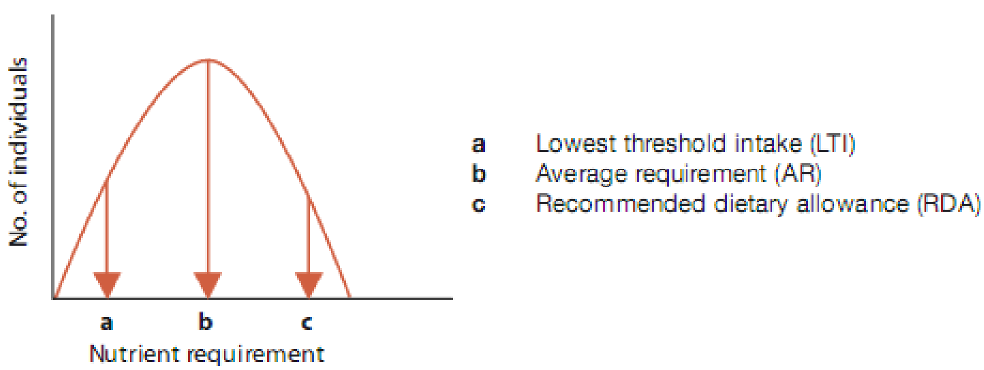
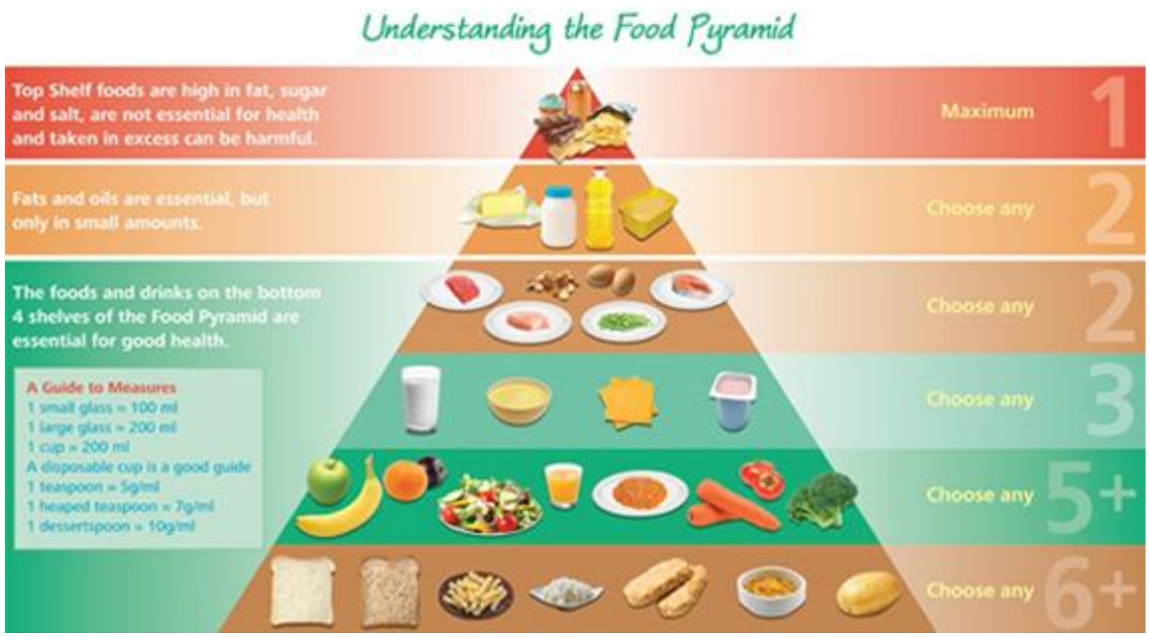


Figure 1 Distribution of individual requirements for a nutrient

Appendix K

Irish Food Pyramid



- Fruit and vegetables: Choose at least 5 or more of servings each day
- Milk, cheese and yogurt: Choose any 3 servings each day
- Note: Choose low fat choices frequently Prior to 2012 Pregnant & Breastfeeding women should choose at least 5 servings (HSE, 2006)
- Since 2012 Pregnant & Breastfeeding women should choose at least 3servings
- Meat, fish and alternatives: Choose any 2 servings each day
- Note: Choose 3 servings during pregnancy
- Oils and fats, Sugars, confectionary, cakes, biscuits and high fat snack foods: choose sparingly -less than 3 portions per day

http://www.safefood.eu/Blog/Blog/July-2012/New-dietary-guidelines-in-the-Republic-of-Ireland.aspx

Appendix L

Henry's Equation For BMR

Henry Equation formula used in the estimation of basal metabolic rate (BMR) in calories of adult women (Henry, 2005).

Age: 18 - 29 years

BMR = 13.1 x wt+ 558 SEE = 0.564

Age: 30 - 60 years

BMR = 9.74 x wt + 694 SEE=0.581

wt = Body weight in kilograms

SEE = Standard error of estimation

Appendix M

Descriptive Statistics for Predictor and Outcome Variables at 6 months

Variable	*Valid N*	*Minimum*	*Maximum*	*Mean*	*SD*
Predictor variables					
No. wks eating solid food	64	1	9	4.3	2.3
No. Fruit Tried	64	0	10	4.6	2.6
Age starting solids (wks)	64	16	27	21.3	2.8
Length EBF (wks)	64	0	26	16	25
No of Fruit eaten during preg	64	0	7.4	2.3	1.4
Outcome variables @ 6 months					
R-LR 0.013M	64	0.04	0.89	0.42	0.17
R-LR 0.029M	64	0.01	0.74	0.33	0.17
R-LR 0.065M	62	0.01	0.63	0.26	0.16
IR 0.013M	62	0.09	0.67	0.36	0.12
IR 0.029M	62	0.07	0.56	0.25	0.12
IR 0.065M	64	0.00	0.50	0.18	0.12
M-LR 0.013M	64	0.09	0.57	0.37	0.11
M-LR 0.029M	64	0.09	0.59	0.26	0.12
M-LR 0.065M	64	0.09	0.47	0.18	0.10

Note: R-LR= Rater's Liking Ratio IR= Ingestion Ratio M-LR= Mother's Liking Ratio EBF= Exclusive breastfeeding

Appendix N

Descriptive Statistics for Predictor and Outcome Variables at 12 months

Variable	*Valid N*	*Minimum*	*Maximum*	*Mean*	*SD*
Predictor variables					
No of fruit tried at 6 months	50	1	10	4.9	2.5
Fruit intake 12 months (g)	50	0	290	127.2	67.8
Vitamin C 12 months (mg)	50	22	204	70	46
Length EBF (wks)	50	0	26	18.5	25
Outcome variables @ 12 months					
R-LR 0.013M	50	0.12	0.74	0.39	0.15
R-LR 0.029M	50	0.01	0.69	0.33	0.15
R-LR 0.065M	50	0.01	0.48	0.26	0.13
IR 0.013M	50	0.16	0.67	0.39	0.12
IR 0.029M	50	0.06	0.58	0.29	0.14
IR 0.065M	50	0.00	0.44	0.18	0.10
M-LR 0.013M	50	0.13	0.56	0.41	0.09
M-LR 0.029M	50	0.09	0.54	0.32	0.14
M-LR 0.065M	50	0.09	0.47	0.20	0.12

Note: R-LR= Rater's Liking Ratio IR= Ingestion ratio M-LR= mother's Liking Ratio EBF= Exclusive Breastfeeding

Appendix O- Graphs showing the relationships between the methods at 6 months

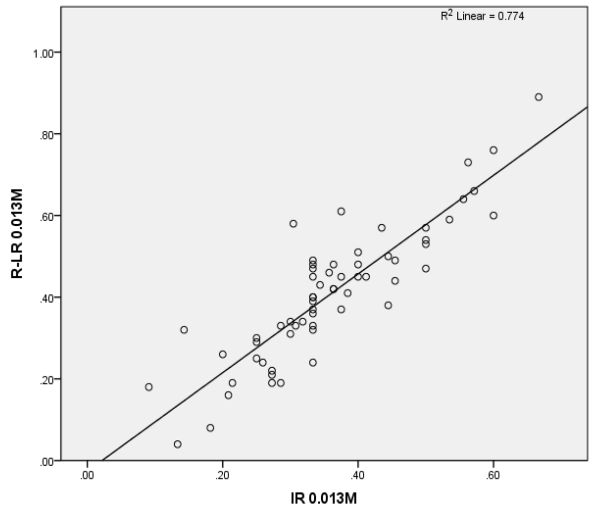


Fig 1: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Ingestion Ratio (IR) at 0.013M citric acid in infants at 6 months

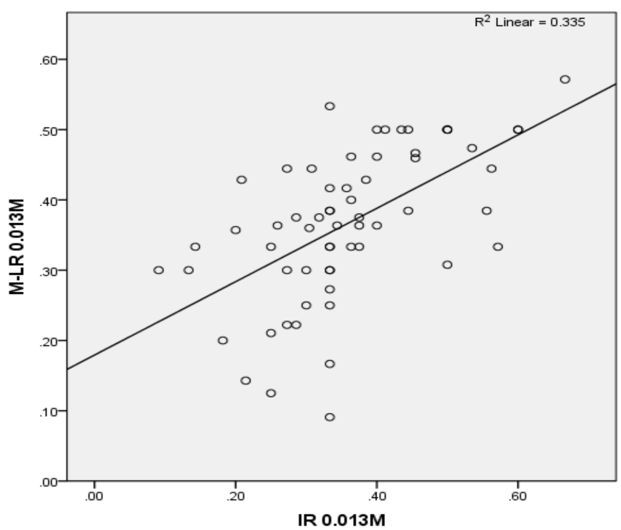


Fig 2. The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.013M citric acid in infants at 6 months

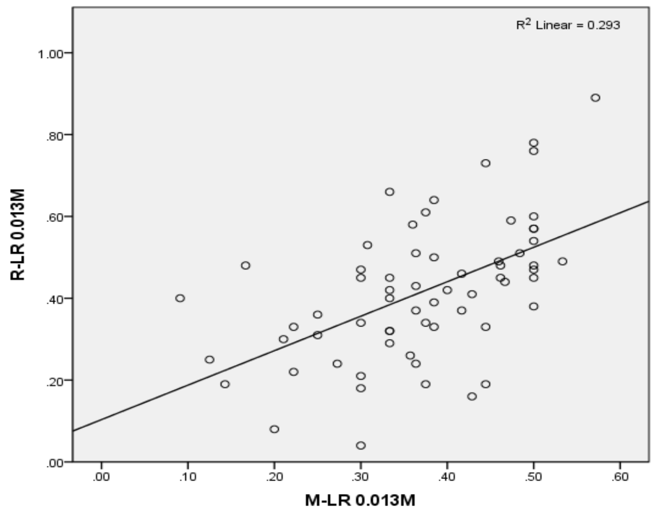


Fig 3: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.013M citric acid in infants at 6 months

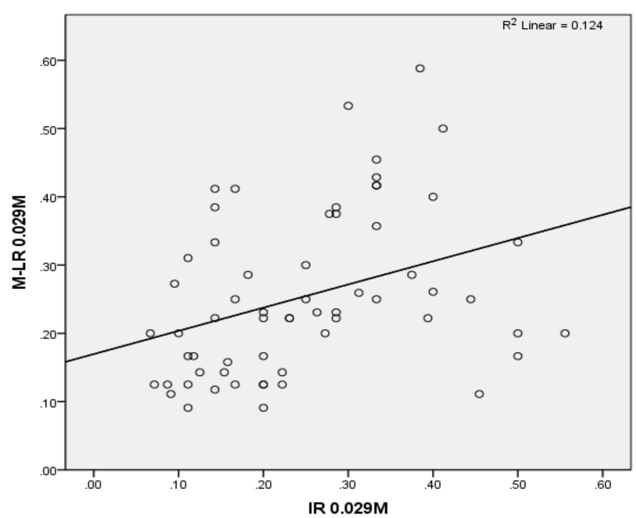


Fig 4: The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.029M citric acid in infants at 6 months

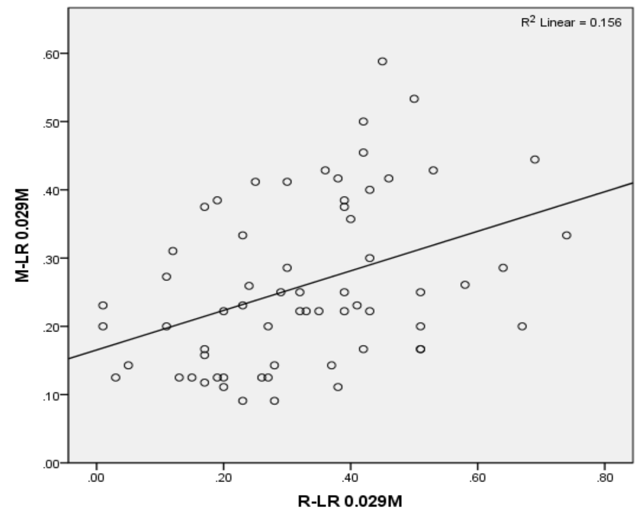


Fig 5: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.029M citric acid in infants at 6 months

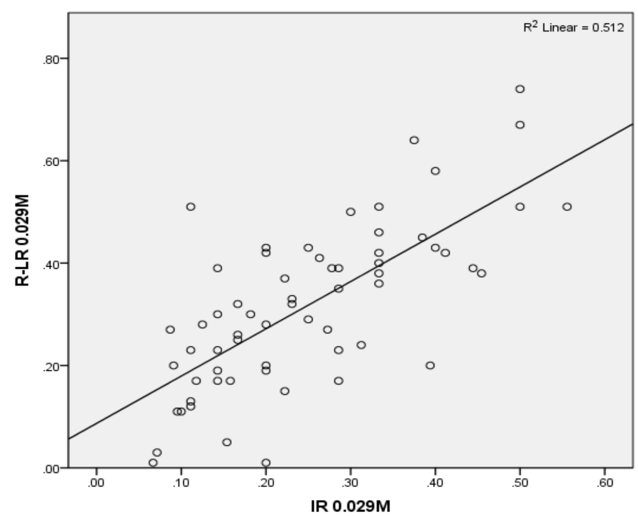


Fig 6: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.029M citric acid in infants at 6 months

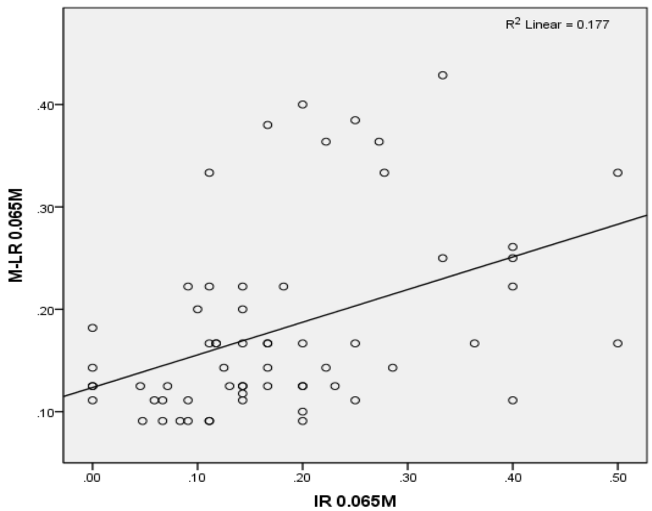


Fig 7: The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.065M citric acid in infants at 6 months

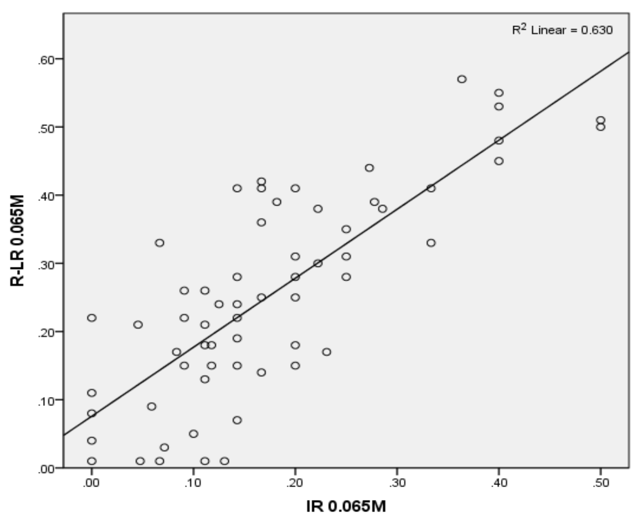


Fig 8: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.065M citric acid in infants at 6 months

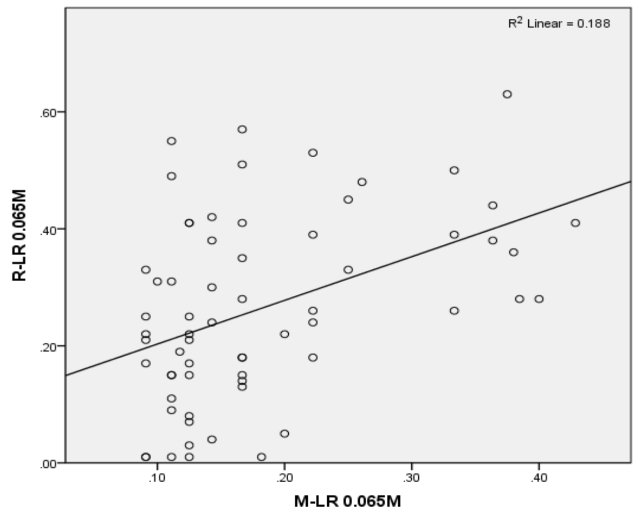


Fig 9: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.065M citric acid in infants at 6 months

Appendix P- Graphs showing the relationships between the methods at 12 months

---

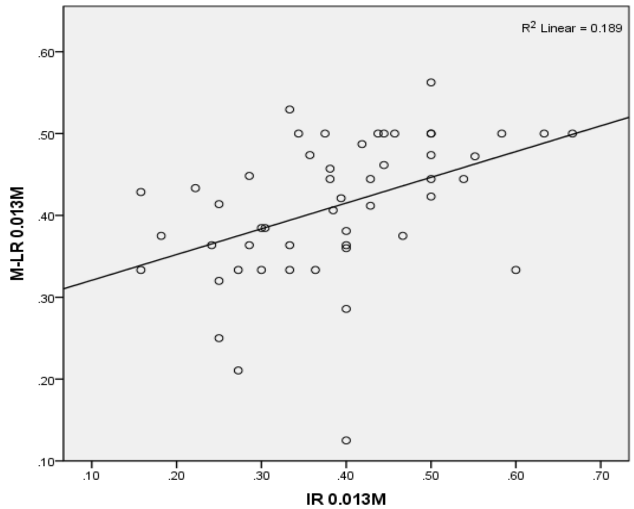


Fig 1: The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.013M citric acid in infants at 12 months

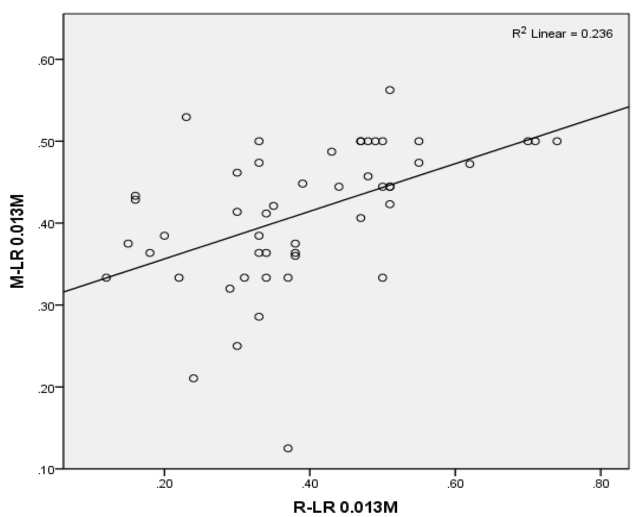


Fig 2: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.013M citric acid in infants at 12 months

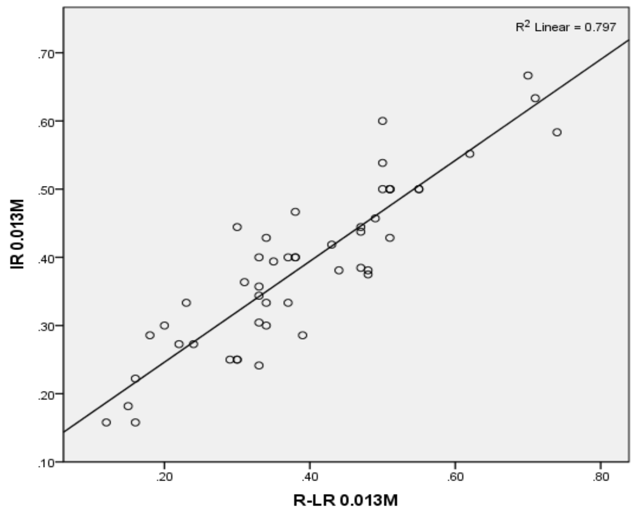


Fig 3: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.013M citric acid in infants at 12 months

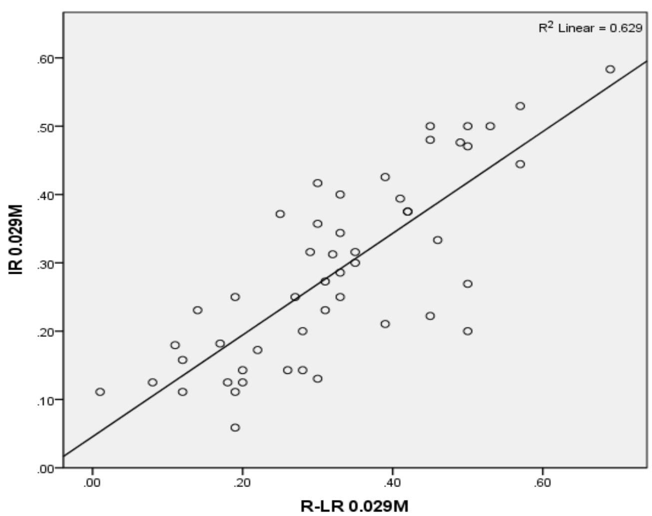


Fig 4: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.029M citric acid in infants at 12 months

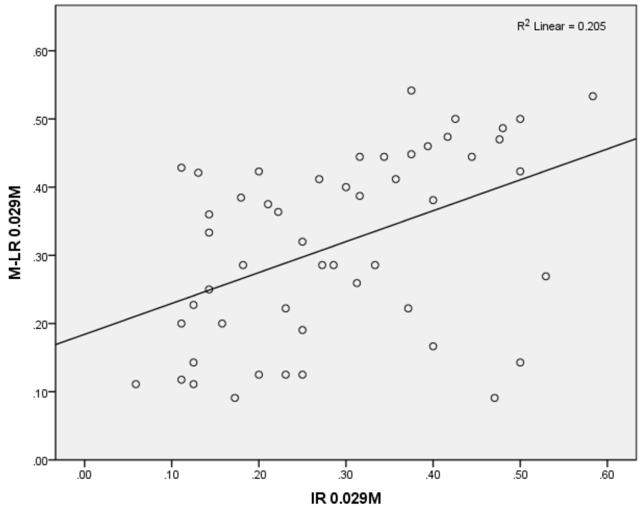


Fig 5: The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.029M citric acid in infants at 12 months

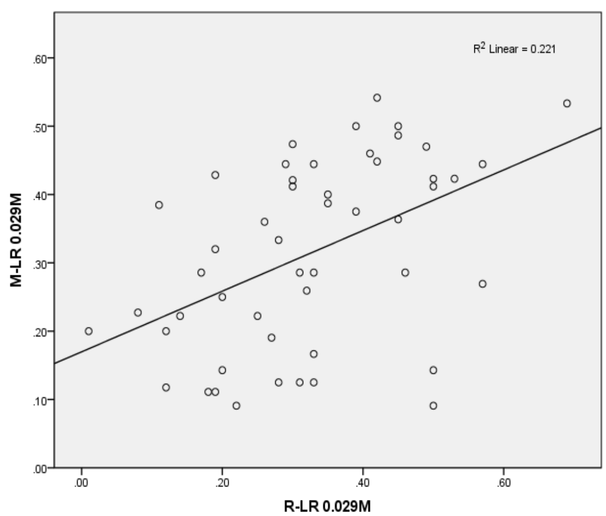


Fig 6; The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.029M citric acid in infants at 12 months

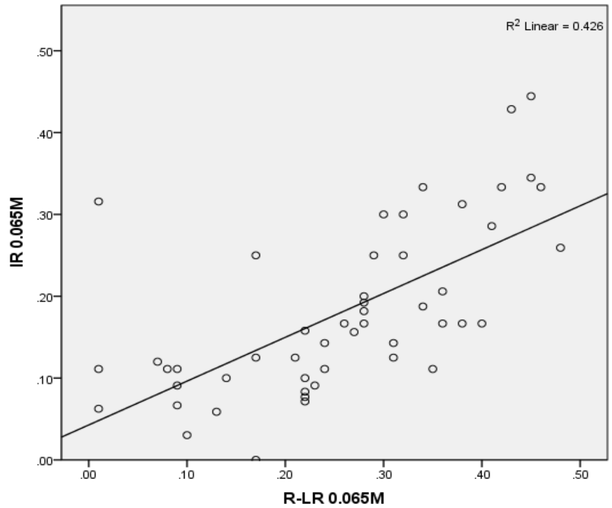


Fig 7: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.065M citric acid in infants at 12 months

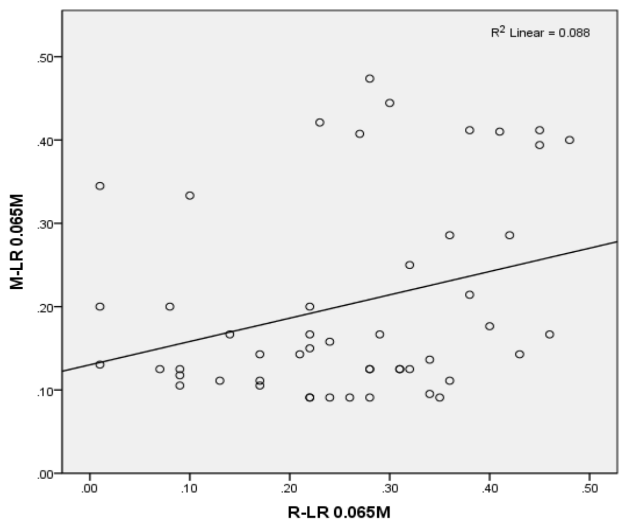


Fig 8: The relationship between sour taste acceptance using Rater's Liking Ratio (R-LR) and Mother's Liking Ratio (M-LR) at 0.065M citric acid in infants at 12 months

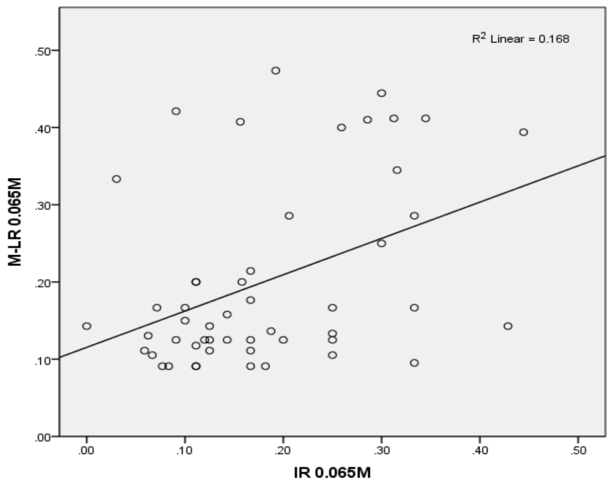


Fig 9: The relationship between sour taste acceptance using Mother's Liking Ratio (M-LR) and Ingestion Ratio (IR) at 0.065M citric acid in infants at 12 months