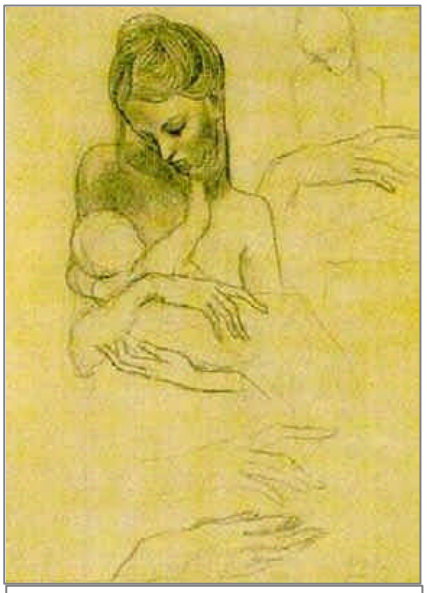
Nutritional Influences in Pregnancy and Postpartum for Women and their Children



Pablo Picasso - Mother and child
and four sketches of the right hand

Alexis J Hure, BND (Hons I)

A thesis submitted to the University of Newcastle, Australia in fulfilment of the requirements of the degree of Doctor of Philosophy (PhD)

December 2008

Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library**, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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Declaration of Collaboration

The work embodied in Chapter 4 (Diet and Pregnancy Status in Australian Women) has been published in Public Health Nutrition, an international peer-reviewed journal.

This work was completed in collaboration with Dr Anne Young from Women's Health Australia. Dr Anne Young performed the statistical analyses for this paper and provided intellectual input for the development of the manuscript.

Associate Professor Clare Collins (primary PhD supervisor) and I attest to the significant and independent contribution I have made to this paper. This has been formally recognised with leading authorship on this publication.

All other work contained within this thesis was completed with appropriate input and guidance from my PhD supervisors, Associate Professor Clare Collins and Professor Roger Smith.

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Clare E Collins

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Alexis J Hure

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Contents

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List of Abbreviations

ABCD Obesity	Assessment Before Children Develop Obesity
AC	Abdominal circumference
ACCVFFQ	Anti Cancer Council of Victoria Food Frequency Questionnaire
AGA	Appropriate for gestational age
AI	Adequate intake
ALSPAC	Avon Longitudinal Study of Parents and Children
ALSWH	Australian Longitudinal Study on Women's Health
ANOVA	Analysis of variance
ARFS	Australian Recommended Food Score
BMI	Body mass index
BMR	Basal metabolic rate
BPD	Biparietal diameter
CE	Coefficient
CHD	Coronary heart disease
CI	Confidence interval
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEXA	Dual energy x-ray absorptiometry
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
DQES	Dietary Questionnaire for Epidemiological Studies
DQI-P	Diet Quality Index for Pregnancy
EAR	Estimated average requirement
FFQ	Food frequency questionnaire
FL	Femur length
FOAD	Fetal Origins of Adult Disease
Folbp1	Folate-binding protein one
HAPS	Hunter Area Pathology Service
HC	Head circumference
HDL-C	High density lipoprotein-cholesterol
HHS	Hordaland Homocysteine Study
holoTC	holotranscobalamin
IF	Intrinsic factor
ISAK	International Society for the Advancement of Kinanthropometry
IUGR	Intrauterine growth restriction
kcal	Kilocalorie
kg	Kilograms
LBW	Low birthweight
LDL-C	Low density lipoprotein-cholesterol
LMP	Last menstrual period
Math Model	Mathematical Model of Pregnancy
MJ	Megajoule

MMA	Methylmalonic acid
MMA-CoA	Methylmalonyl coenzyme A
MRI	Magnetic resonance imaging
mTHF	Methyltetrahydrofolate
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NRVs	Nutrient Reference Values
NSW	New South Wales
NTDs	Neural tube defects
NUTTAB	Nutrient tables for use in Australia
OR	Odds ratio
PAR	Predictive adaptive response
pB12	Plasma vitamin B12
pHcy	Plasma homocysteine
PhD	Doctor of Philosophy
PPAQ	Pregnancy Physical Activity Questionnaire
PPAR-a	Peroxisome proliferator-activated receptor-alpha
PTD	Preterm delivery
rcFol	Red cell folate
RDI	Recommended dietary intake
RNA	Ribonucleic acid
RR	Relative risk
SAS	Statistical Analysis Systems
SD	Standard deviation
SE	Standard error of the mean
SGA	Small for gestational age
SIDS	Sudden infant death syndrome
Suc-CoA	Succinyl-CoA
TC	Transcobalamin
THF	Tetrahydrofolate
TIHS	Tasmanian Infant Health Survey
TNF-a	Tumour necrosis factor-alpha
UK	United Kingdom
UL	Upper level
USA	United States of America
WATCH	Women and their Children's Health
WFR	Weighed food record
WHO	World Health Organization

Thesis Publications and Presentations

AWARDS

1) Nutrition Society of Australia, Early Career International Conference Scholarship, awarded December 1, 2008.

2) The University of Newcastle, Faculty of Health, 10 of the Best, Research Higher Degrees Showcase Finalist, awarded September 26, 2008.

PUBLISHED ARTICLES

1) Hure A, Young A, Smith R, Collins C. Diet and pregnancy status in Australian women. Public Health Nutrition 2008: 1-9.

2) Hure AJ, Smith R, Collins CE. A recruiting failure turned success. BMC Health Services Research 2008; 8:64.

ORAL PRESENTATIONS WITH PUBLISHED ABSTRACTS

1) Hure AJ, Collins CE, Smith R. Maternal and infant vitamin B12, folate and homocysteine in pregnancy and postpartum. Proceedings of the Nutrition Society of Australia 32nd Annual Scientific Meeting, Asia Pacific Journal of Clinical Nutrition 2008; 17 (Suppl 3): S96.

2) Hure AJ, Giles WB, Smith R, Collins CE. Maternal weight change in pregnancy predicts fetal size but not adiposity. Proceedings of the 4th Australian Health and Medical Research Congress 2008: p.284.

3) Hure AJ, Young AF, Smith R, Collins CE. Is diet quality higher during pregnancy? Proceedings of the Perinatal Society of Australia and New Zealand 11th Annual Congress, Journal of Paediatrics and Child Health 2007; 43 (Suppl 1): A43-44.

4) Hure AJ, Young AF, Smith R, Collins CE. A comparison of diet quality in young Australian women according to pregnancy status. Proceedings of the Nutrition Society of Australia 30th Annual Scientific Meeting, Asia Pacific Journal of Clinical Nutrition 2006; 15 (Suppl 3): S53.

POSTER PRESENTATIONS WITH PUBLISHED ABSTRACTS

1) Hure A, Wright I, Smith R, Collins C. Nutrient supplementation in pregnancy: development of evidence-based best-practice guidelines. Proceedings of the Perinatal Society of Australia and New Zealand 13th Annual Congress, Journal of Paediatrics and Child Health 2009; 4(Supp.1): A124.

2) Hure AJ, Collins CE, Smith R. Maternal weight change in pregnancy predicts fetal size but not adiposity. Proceedings of the 56th Annual Meeting of the Society for Gynecologic Investigation, Reproductive Sciences 2009;16(3): 555.

3) Hure AJ, Collins CE, Smith R. Maternal pregnancy folate predicts homocysteine in the six month old infant. Proceedings of the 56th Annual Meeting of the Society for Gynecologic Investigation, Reproductive Sciences 2009;16(3): 556.

4) Hure AJ, Smith R, Giles W, Somerset D, Collins CE. Fetal fatness is not associated with maternal adiposity in pregnancy. Proceedings of the 5th International Congress on the Developmental Origins of Health and Disease, Early Human Development 2007; 83 (Suppl 1): S161.

5) Hure AJ, Smith R, Collins CE. Methodological barriers to studying the predictive adaptive response in humans. Proceedings of the 10th International Congress on Obesity, Obesity Reviews 2006; 7 (Suppl 2): 152.

6) Hure AJ, Smith R, Collins CE. Energy intake versus expenditure in breastfed infants: Aren't we missing something? Proceedings of the Dietitians Association of Australia 24th National Conference, Nutrition and Dietetics 2006; 63 (Suppl 1): A40.

INVITED SPEAKER PRESENTATIONS

1) Hure AJ. A nutritional journey. Proceedings of the John Hunter Children's Hospital Annual Neonatal Seminar, Hunter Valley, Australia, April 11, 2008.

2) Hure AJ, Smith R, Collins CE. Nutritional genomics concerning mothers and babies. Meeting of the Newcastle Branch of the Nutrition Society of Australia, Newcastle, Australia, August 25, 2006.

Abstract

Maternal factors prior to conception and during pregnancy may influence the development of the metabolic, cardiovascular and endocrine systems of the offspring and subsequent disease pathogenesis. This thesis explores the concept of the developmental origins of health and disease.

Human observational research studies were undertaken to test the relationships amongst maternal dietary intake, weight gain during pregnancy and changes in biochemical markers between pregnancy and postpartum for the mother and infant. This thesis presents three chapters of original research related to maternal and fetal nutrition, and one chapter of empirical data concerning the methodological challenges faced when recruiting for research purposes.

An analysis of dietary intake data from the young cohort of the Australian Longitudinal Study on Women's Health was used to determine the overall diet quality in a contemporary cohort, and to assess whether those who are pregnant eat differently to those who are not. Only small differences in diet quality and nutrient intakes were detected between pregnancy groups, and diet quality scores were consistently low. When the intake data were compared to Australian recommendations it appears that many young women fail to reach key nutrient targets, including those set for folate, fibre, calcium, iron, potassium and vitamin E.

The research focus then shifted to prospective longitudinal data collection for women and their children during pregnancy and after birth. Low recruitment to this component of the studies threatened the potential to achieve the research aims. Rather than jeopardising the power of the investigations efforts were made to understand what had gone wrong and how the situation could be rectified.

An investigation of the relationship between fetal adiposity and maternal weight changes in pregnancy was performed. Pre-pregnancy body mass index (BMI) and weight changes during pregnancy were taken as broad markers of maternal nutritional status and energy regulation. Intrauterine growth, including the accumulation of adipose tissue, was assessed using serial ultrasounds. Fetal size was positively related to maternal pre-pregnancy weight (and BMI) and weight gain (change in BMI) during

pregnancy. Maternal pre-pregnancy weight was positively associated with adiposity at the fetal abdomen, but not the thigh. However, overall maternal weight gain was not associated with fetal adiposity.

To then determine whether maternal vitamin B12 and folate (methyl donors) in pregnancy could influence the offspring's homocysteine metabolism at birth, changes in plasma vitamin B12, plasma folate and red cell folate were characterised for the cohort of more than 100 women during pregnancy and up to six months after birth. A small sub-sample of infants also had blood collected at six months postpartum. Average maternal plasma folate during pregnancy was significantly predictive of infant plasma homocysteine.

In conclusion, the research outlined herein demonstrates important interactions between the mother and her offspring during the critical windows of early development. The research is multidisciplinary in its application and contributes to our understanding of some of the nutritional influences in pregnancy and postpartum for women and their children.

Chapter 1

General Introduction

1.1 PREGNANCY AND PROGRAMMING

Human pregnancy begins with conception, the fusion of an oocyte and spermatozoon to form a zygote. In the subsequent eight weeks the embryo develops. The fertilised ovum starts as a single cell, and is responsible for forming the infant delivered at the end of the pregnancy. However, only 40% of conceptions produce embryos that survive the first trimester[1]. Fetal development begins from the ninth week and continues until birth. The prenatal development period is referred to as 'gestation'. A normal human pregnancy spans 40 completed weeks of gestation. Though it can vary from 37 to 42 weeks, counting from the first day of the last menstrual period (LMP)[2], and it is broken up into trimesters. The first trimester is the embryological and early fetal development stage which is complete at the end of the twelfth week. The second trimester is characterised by organ development including their supporting systems, and is complete at the end of the twenty-sixth week. The third trimester involves rapid fetal growth and spans the remainder of the pregnancy.

The fetal circulation develops separately from the maternal circulation. The placenta functions as the conduit for exchanges between the two[3]. It carries oxygen and nutrients from the maternal system to the fetus, and removes wastes from the fetal blood supply. These are returned to the mother's blood for disposal via her kidneys. The placenta forms within the first 10 weeks of pregnancy from the same cell as the embryo, and attaches to the wall of the uterus[4]. It confers some protection to the fetus, preventing the passage of most bacteria[1]. However, smaller microorganisms including viruses and most drugs are able to pass through[4]. The placenta itself can consume between 30% and 50% of the nutrients derived from the utero-placental circulation[5].

Historically the fetus has been considered a superior parasite, able to extort maternal nutrient intakes and stores throughout periods of adversity[6]. The argument for this was based on the assumption that the feto-placental unit had a higher metabolic rate than any other maternal organ except for the brain[7]. However, a parsimonious expansion of the cardiac output and placental blood flow has been reported in undernourished mothers, compared to well-fed controls[8]. This study was conducted using radioactive microspheres, in anesthetised control and food-restricted rats, measuring organ blood flow and cardiac output[8].

Both animal and human studies show that if the maternal nutrient supply is compromised, the balance between maternal and fetal needs is replaced by a state of biological competition[9]. The maternal nutritional status at the time of conception influences nutrient partitioning between the maternal-fetal dyad[9]. A significantly undernourished mother does not adapt to sustain fetal growth but rather to maintain her own body stores[7]. Whereas if she is in a marginally deficient state the fetus takes, or is given precedence[9]. Animal models have also demonstrated that when nutrients become available after a period of food-shortage the mother is preferentially nourished over the fetus[7].

Fetal growth is primarily determined by fetal nutrition[10], which is regulated by genetic, maternal and placental factors. Historic studies of cross-breeding horses and ponies provided early evidence for the 'maternal constraint' imposed on fetal growth[11]. A mother may limit the supply of nutrients to the fetus to prevent it outgrowing the dimensions of the pelvis, which increases the risk of difficulties at birth[12, 13]. When smaller and larger breeds of animals are crossed the size of the offspring is consistently appropriate for the size of the mother[11]. Evidence exists that this phenomenon also occurs in humans where an infant's birthweight has been found to correlate with maternal, but not paternal size[14]. However, postnatal height is best predicted by mid-parental height[10].

Disease pathogenesis is currently being investigated with the knowledge that maternal factors in the lead up to conception, and during the pregnancy, may influence the development of the metabolic, cardiovascular and endocrine systems of the offspring[15]. This programming occurs because of the phenotypic or developmental plasticity of the fetus, which allows a single genotype to manifest different phenotypes depending on the environmental cues received[16]. These can affect gene expression, by inducing epigenetic or heritable changes in gene function that occur without a change in the deoxyribonucleic acid (DNA) sequence[17]. Phenotypic plasticity is common throughout nature with many examples of metabolic and morphological adaptations observed across a diverse range of species[18]. For instance, tadpoles can accelerate their metamorphosis to a toad in response to habitat desiccation, thus escaping the need for an aquatic environment[19].

In humans this concept of programming, more broadly known as the developmental origins of health and disease (DOHaD), is a burgeoning field of research[15]. Its genesis stems from geographical[20] and retrospective studies conducted in the UK from the late 1980s[21]. Barker *et al.* (1989) followed-up men born in Hertfordshire, who had birth records available from early in the century[21]. Those with the lowest weights at birth and at one year of age were found to have the highest rates of death from ischaemic heart disease[21]. Hence the prenatal and early postnatal periods were identified as potentially critical windows, whereby the propensity for adult disease could be biologically programmed. These findings have since been replicated in many studies, involving a variety of populations[22, 23] including women[24]. While altered size at birth was the statistical predictor of later disease risk, it is understood now that this is because it acts as a proxy for a disturbed fetal environment[25]. The pattern of fetal growth is thought to offer greater insight into an individual's disease propensity, given that various trajectories may result in the same weight at birth[26]. Animal models are currently helping to elucidate the mechanistic pathways by which the effects are mediated.

The developmental or 'plastic' window can be viewed as a continuum, encompassing preconception, pregnancy, birth and the postnatal environment, including lactation and infancy[25]. Observational and experimental advances have determined the importance of consistency in these phases, with the degree of mismatch between the projected and encountered environments considered fundamental to the overall disease risk[18, 27]. The 'predictive adaptive response' (PAR) is a term used to describe how the developmental trajectory may be established according to environmental signals which are interpreted as indicative of the adult milieu[25]. Slow fetal and infant growth is often followed by rapid weight gain during childhood[28]. This may be interpreted as a mismatch between the predicted nutrient-sparse environments encountered in the pre- and early postnatal periods, followed by a liberalisation of dietary intake. Individuals exposed to this scenario are known to be at high risk of adult coronary heart disease (CHD) and type two diabetes[29].

To date, several maternal influences have been coupled with the subsequent adult health of the offspring. These include: (i) the mother's own birthweight, (ii) maternal body composition, including fat and lean mass, (iii) maternal dietary intake, including macro-

and micronutrient profiles, and (iv) maternal endocrine factors[29]. Furthermore, there is increasing evidence that fetal development can be affected by variations considered to be within the normal range of Western diets[30]. Many women eat unbalanced diets, whether unwittingly, through restrictive dieting practices[30], or because of poverty, poor knowledge or food insecurity.

1.2 THESIS SUMMARY

This thesis supports the concept of developmental programming and the importance of early-life nutritional influences. Human observational research studies have been undertaken to better understand the relevance of maternal dietary intake, weight gain during pregnancy and changes in biochemical markers between pregnancy and postpartum. More specifically, a secondary data analysis from a large, national cross-sectional survey of dietary intake in women of childbearing age was conducted. The aim was to determine the overall diet quality of young Australian women, and to assess whether those who are pregnant eat differently to those who are not[31]. Concurrently, a small longitudinal cohort of pregnant women and their offspring was established in the Hunter Region of New South Wales, Australia, to intensively monitor the early-life nutritional and environmental exposures that require further discussion within the DOHaD literature[32].

Following on from this general introduction, Chapter 2 aims to synthesise the current knowledge and work which is being conducted on the nutritional influences in pregnancy and postpartum for women and children. A very substantial and broad literature is considered within this review, which concludes with a 'big picture' public health and life-course perspective. The materials and methods for this thesis are then described in Chapter 3, the majority of which are concerned with data collection for the prospective longitudinal cohort that was established for hypothesis testing within and beyond this dissertation.

The major original research chapters (Chapter 4, Chapter 6 and Chapter 7) are presented in a logical sequence, which descend in a stepwise manner to an increasing level of detail. Chapter 4 starts at the population level with a large descriptive study of diet quality in women of childbearing age. Chapter 5 then gives a 'behind-the-scenes' evaluation of the methodological barriers faced when recruiting to research and the strategies used to correct the recruitment issues. Chapter 6 and Chapter 7 utilise original data that were collected as part of the newly established prospective cohort study: Women and Their Children's Health or WATCH Study. In Chapter 6 novel anthropometric data on developing fetuses, as collected via ultrasound scans, are evaluated as the potential outcomes of maternal weight changes during the pregnancy.

Physical measurements (a broad marker of nutritional status) then transition to the level of biochemical measurement in Chapter 7, with a specific focus on the nutritional biomarkers vitamin B12, folate and homocysteine. These nutritional biomarkers have specifically been identified as potentially mechanistic in the developmental programming pathways, and hence are of great interest.

1.2.1 Thesis aims and hypotheses

1.2.1.1 Aims for Chapter 4: Diet and pregnancy status in Australian women

To describe the dietary intakes and overall diet quality of young Australian women, and to determine whether there are any differences according to pregnancy status.

Null Hypotheses:

- There is no difference in the energy-adjusted nutrient intakes of women who are pregnant, trying to conceive, have had a baby in the previous 12 months or are otherwise not pregnant.
- There is no difference in the diet quality scores of women who are pregnant, trying to conceive, have had a baby in the previous 12 months or are otherwise not pregnant.

1.2.1.2 Aims for Chapter 5: A recruiting failure turned success

To describe the problems faced with recruitment to the original planned research project and to highlight the strategies that were adopted which resulted in a greatly improved rate of response.

1.2.1.3 Aims for Chapter 6: Maternal weight change in pregnancy predicts fetal size but not adiposity

To test whether maternal prepregnancy weight and subsequent changes throughout pregnancy predict absolute fetal size and adiposity, as well as change in this state.

Null Hypotheses:

- Maternal pre-pregnancy weight and BMI do not predict fetal adiposity and size.
- Maternal weight and BMI change between 0 and 20 weeks' gestation do not predict fetal adiposity and size at, and from, 20 weeks' gestation.

- Maternal weight and BMI at 20 weeks' gestation are not predictive of fetal adiposity and size at, and from, 20 weeks' gestation.
- Maternal weight and BMI during pregnancy is not predictive of fetal adiposity and size.
- Maternal weight and BMI change over several weeks is not predictive of fetal adiposity and growth for the same period.
- Maternal weight and BMI change from prepregnancy to late pregnancy is not predictive of fetal adiposity and growth for the same period.

1.2.1.4 Aims for Chapter 7: Maternal and infant vitamin B12, folate and homocysteine in pregnancy and postpartum

To longitudinally characterise plasma vitamin B12, plasma folate and red cell folate in a cohort of Australian women in pregnancy and after birth, and to characterise the mother-infant relationship for plasma homocysteine in a subset of the cohort with paired data at six moths after birth.

Null Hypotheses:

- There are no differences in the longitudinal measurements of maternal plasma vitamin B12, plasma folate and red cell folate during pregnancy and after birth.
- Maternal levels of plasma vitamin B12, plasma folate and red cell folate during pregnancy are not correlated with corresponding plasma homocysteine measurements.
- Maternal levels of plasma vitamin B12, plasma folate and red cell folate during pregnancy do not predict the respective markers in their infants six months after birth.
- Maternal levels of plasma vitamin B12, plasma and red cell folate, and plasma homocysteine at three and six months postpartum do not predict the respective markers in their six month old infants.

Chapter 2

Literature Review

2.1 INTRODUCTION

This chapter provides an overview of the nutritional influences in pregnancy and after birth for women and their children. The important concepts and evidence which apply to this thesis are summarised by five central themes. Section 2.2 presents the chronology of the research in the developmental origins field. Both human and animal data are considered, in addition to the controversies and limitations that accompany this work. Section 2.3 focuses on the physiologic and metabolic adjustments which occur at, or around the time of, pregnancy. Section 2.4 considers maternal nutrition, and the demands of human pregnancy. Reports of maternal dietary practices in relation to birth outcomes are also reviewed. Section 2.5 describes the nutritional factors associated with the growth and development of the offspring during the pre- and postnatal periods. Later life outcomes are addressed according to the patterns of growth observed during these developmental phases. Finally, Section 2.6 presents the life-course perspective and discusses implications for public health interventions, as a means of contextualising the merits of advancements in this field.

2.2 THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

2.2.1 An Introduction to the Barker hypothesis

Professor David Barker is frequently credited as the first to start publishing hypotheses on the relationship between intrauterine and childhood nutrition exposures, and health in later life: "Ischaemic heart disease is strongly correlated with both neonatal and postneonatal mortality. It is suggested that poor nutrition in early life increases susceptibility to the effects of an affluent diet"[20]. In fact, almost a decade earlier Anders Forsdahl from Norway had proposed something very similar, owing to the results of his own epidemiological research[33]. He found a marked correlation between a poor standard of living during childhood and adolescence, followed by prosperity, and an increased risk of arteriosclerotic heart disease[33]. A study by Notkola *et al.* (1985) of East and West Finland came to a similar conclusion, when poor living conditions in childhood were associated with an increased risk of CHD in later life[34].

Neither Forsdahl nor Notkola did anything more to advance these curious findings. Barker and his colleagues, on the other hand, published a series of studies undertaken in Sheffield, Hertfordshire, and Preston, England, linking low birthweight (LBW) to later life diseases. He thus became known as the founder of the Fetal Origins of Adult Disease (FOAD), or 'Barker Hypothesis'[21, 35-42]. Their participants were followed-up retrospectively during adulthood. Birth records were available from a time when fetal exposures to undernutrition and famine, as well as other adverse events like infection and smoking, were common[43]. This led to the discovery that people who were born growth restricted, rather than simply premature, were at risk of adult disease[42].

The Barker Hypothesis suggests that intrauterine undernutrition, which leads to disproportionate fetal growth, can result in permanent adjustments to the offspring's physiology and metabolism, thus predisposing the individual to chronic conditions such as CHD later in life[44]. This programming occurs because of the developmental or phenotypic plasticity of the fetus, which allows a single genotype to manifest different phenotypes depending on the combination of environmental signals received[16].

Studies in other countries which replicated this hypothesis, have reported on various clinical diseases or their intermediate markers. In The Netherlands, obesity and CHD data were collected for those who had been subjected to the Dutch famine or 'hunger winter' of 1944 to 1945[45, 46]. Diabetes and insulin resistance were studied prospectively in India[47, 48] and retrospectively in Sweden[49]. Epidemiological data for both men and women in the USA which linked birthweight to adult hypertension were published[50, 51].

While highly controversial and met with scepticism early on[52], the proliferation in experimental, mechanistic and prospective longitudinal research branching from this original work has paved the way for broad acceptance of this aetiology. The scope of investigations has diversified from the FOAD to become the DOHaD, which encompasses factors extending beyond the intrauterine experience, and considers the potential for health promotion rather than simply disease prevention[27]. At present, evidence is mounting to support a developmental programming role in the occurrence of cardiovascular disease, diabetes, asthma, cancers, osteoporosis, and neuropsychiatric disorders[53].

2.2.1.1 The thrifty phenotype

The 'thrifty phenotype' was a term coined by Hales and Barker to specifically describe the ontogenic effects of intrauterine undernutrition on the development of insulin resistance and type two diabetes[16, 38]. It suggests that an undernourished fetus becomes thrifty with the available nutrients, adjusting glucose metabolism to prioritise brain development at the expense of muscle growth[16]. Once adopted, this metabolic shift becomes permanent and, in combination with excess adiposity, results in an increased risk of developing type two diabetes in adulthood[16].

2.2.2 Epidemiological evidence

2.2.2.1 Coronary heart disease

The incidence of CHD in Western countries rose sharply at the beginning of the twentieth century, making it the leading cause of death[29]. Consequently, strategies for its treatment and prevention have received considerable scientific attention, with the majority of work focusing on the traditional risk factors of cigarette smoking, excess

body weight, elevated blood lipids (cholesterol), hypertension, impaired glucose tolerance, and physical inactivity[52]. However, a large degree of individual disease propensity is not accounted for by these risk factors. To illustrate, a man who falls into the lowest category for coronary risk is still most likely to die from CHD, at least in Western countries[54].

Many of the Western countries have shown a decline in the incidence of CHD over recent decades[52], a phenomenon which is not accounted for by changes in adult lifestyle. Developing regions undergoing the 'nutrition transition'[55] towards a more typically Western diet, such as China, South Asia and Eastern Europe, are now seeing an escalation in CHD occurrence[29]. With limited headway made in the areas of the traditional risk factors it is not surprising that a new set of antecedents have been welcomed[52]. Further, when the funding and infrastructure dedicated to tertiary healthcare in these developing nations is considered, it becomes apparent that primary prevention strategies will likely offer a more economically viable and sustainable outcome.

The first direct evidence of an inverse association between fetal growth and CHD were the results of Barker and colleagues[24, 56] (refer to Chapter 1 and section 2.2.1). Replication of the UK studies linking increasing birthweight with a progressive decline in adult CHD has confirmed these findings[22, 57]. Follow-up studies that have used birth data beyond weight, such as length, head circumference (HC), ponderal index (birthweight/length^3), and/or placental size, as measures of fetal growth have shown stronger associations with later health outcomes[26, 58]. In a Finish study of 3302 men born in Helsinki between 1924 and 1933 placental weight, length and HC were used with birthweight to more accurately describe the proportions of fetal growth[26]. Men who were thin at birth (in the lowest quarter) as assessed using ponderal index were twice as likely to die from CHD as those with high ponderal indexes[26].

2.2.2.2 Diabetes and the U-shaped curve

While progressively higher birthweights are associated with lower CHD risk it is different for diabetes. In this case, birthweights at both the low and high ends of the spectrum are associated with a higher risk of adult disease. However, the strength of the association between birthweight and diabetes can vary according to the mean

birthweight, maternal size and the incidence of gestational diabetes within the population of interest[59].

McCance *et al.* (1994)[60] and Rich-Edwards *et al.* (1999)[61] were among the first studies to have demonstrated the U-shaped risk profile for birthweight and subsequent type two diabetes. Maternal diabetes during pregnancy specifically contributes to the increased risk observed for the heavier offspring[62]. The main driver for this mother-to-offspring transmission of diabetes is thought to be the hyperglycaemia and hyperinsulinaemia experienced during fetal development[63, 64], although genetic factors also play a role[62]. Since 1980 the phenomenon of a 'fuel-mediated teratogenesis' has been described within the diabetes literature[65]. It suggests that the alteration of fuels during a diabetic pregnancy lead to long-term functional changes in the offspring[65].

Other studies have investigated maternal glycaemia as a potential 'programmer' of diabetes in the offspring, with a shorter interval between birth and follow-up[66, 67]. In a recent study of 785 contemporary pregnancies in Mysore, India, the prevalence of gestational diabetes was high (6.2%) despite low averages for maternal age and BMI[67]. The babies born to the diabetic mothers were, unsurprisingly, heavier (mean birthweight 3339 grams) and more adipose than babies born to mothers with normal glucose tolerance (mean birthweight 2956 grams). This data suggests that while gestational diabetes is common in some populations within developing countries, the macrosomic effects on the fetus may be masked by the lower mean birthweights[62]. Furthermore, maternal glucose concentrations were positively associated with the size of the newborn, even in the non-diabetic cohort, although glucose and insulin concentrations were strongly confounded by both maternal BMI and fat mass[67].

2.2.2.3 Obesity and birthweight

Worldwide there are now as many individuals who are overnourished as there are undernourished[68]. The rapid rise in obesity prevalence means that genetic factors are unable to account for the changes we have seen over just one or two generations[68, 69]. Rather, there is a growing body of evidence to suggest that the origins of obesity may be programmed *in utero* in response to environmental factors via epigenetic mechanisms[68, 69].

Epidemiological studies which have assessed the relationship between weight at birth and adult obesity have produced some conflicting evidence. A large number of studies support an association between higher birthweight and an increased BMI in childhood[70, 71], adolescence[72] and adulthood[73-75]. Conversely, other studies show a high birthweight to be protective of subsequent obesity, and that it is the babies born small who later become overweight[76]. Law *et al.* (1992) specifically assessed the waist-to-hip ratio of adult men as a measure of abdominal fatness and found that a higher ratio was associated with reduced growth during fetal development and infancy[77]. Cuhan *et al.* (1996) demonstrated a shallow U-shaped association for birthweight and adult BMI in 92,940 women participating in the Nurses' Health Study II, in the USA[50]. However, the study of men carried out by the same research team, only showed high birthweights to be associated with an increased risk of obesity[51]. In response to the criticism that BMI may be a relatively poor indicator of fatness, Kensara *et al.* (2005) have recently shown that adult adiposity, as assessed by dual energy x-ray absorptiometry (DEXA), is associated with LBW[78].

When considered altogether it becomes apparent that being born either small or large is associated with later obesity[68, 69]. However, the relationship is complex. Higher birthweight is associated with greater subsequent lean mass, rather than fat mass[75]. In contrast, lower birthweight is associated with a subsequent higher ratio of fat-to-lean mass, greater central adiposity and insulin resistance[75]. The exact nature of the association between size at birth and risk of adult obesity is also influenced by sex, ethnicity, and length of gestation, in addition to the nutritional exposures directly following birth (refer to section 2.5).

2.2.3 The 'thin-fat' Indian baby

The Pune Maternal Nutrition Study is a longitudinal population-based study of rural Indian women and their children, which has prospectively observed maternal nutrition in relation to the outcomes for 762 live offspring[79-82]. This is a landmark study within the developmental origins field, in that the participants were recruited prior to conception (*n* 2466), with only those women who became pregnant during the enrolment period eligible for follow-up (*n* 797)[80]. Recently the research team have published data for the 698, six year old children who were born into this cohort[82]. This

was 97% of the children available for follow-up at age six years (note there were 39 deaths between birth and six years)[82].

Among the significant finding from this study, Yajnik *et al.* (2003) have recognised that while Indian babies are born smaller and lighter compared to white Caucasian babies born in the UK, their body fat is somewhat spared[81]. The Indian babies were almost two standard deviations (SD) lighter in reference to the UK babies (SD -1.74, 95% confidence interval (CI) -1.81, -1.68), with a similar discrepancy evident for measurements of the head (SD -1.68, 95% CI -1.76, -1.61) and mid-upper arm circumference (SD -1.82 95% CI -1.89, -1.75), and close to three standard deviations difference for the abdominal circumference (AC) (SD -2.99, 95% CI -3.09, -2.89)[81]. Yet the subscapular skinfold thickness (a measurement of truncal fat) was only half a standard deviation smaller (SD -0.53, 95% CI -0.61, -0.46), suggesting a disproportionate sparing of adiposity[81]. Thinness at birth has been shown in other studies to increase the risk of subsequent insulin resistance later in life[83].

Figure 2.1 illustrates the 'thin-fat' phenotype in an adult Indian compared to white Caucasian male. It shows how body composition can vary considerably, despite no difference in BMI.

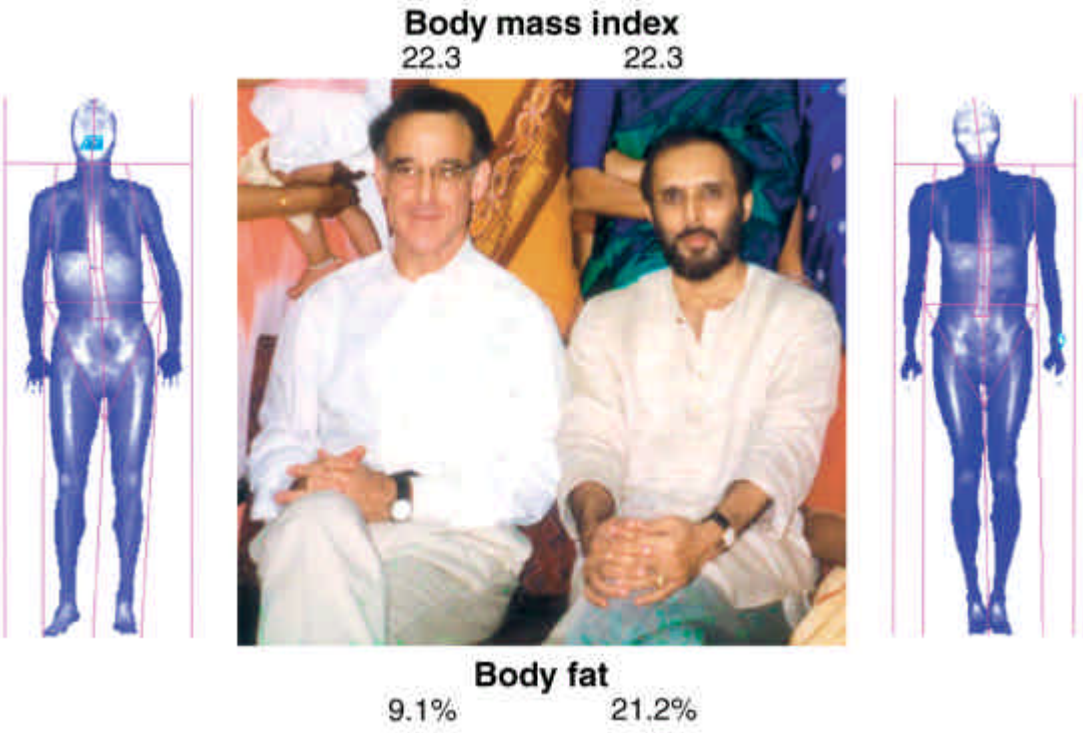

Figure 2.1 Variation in body fat measured by dual energy x-ray absorptiometry (DEXA) despite no difference in body mass index (BMI)

John Yudkin (left) and Chittaranjan Yajnik (right) share the same BMI, however DEXA reveals significant variations in body fat: 21.2% for Yajnik, compared with Yudkin's 9.1%.

Reprinted from the Lancet, 363(9403), CS Yajnik and JS Yudkin, The Y-Y paradox, p.163, 2004, with permission from Elsevier and Chittaranjan Yajnik[84].

In an earlier study conducted by the same research team, cardiovascular disease risk factors, including the insulin resistance syndrome, were assessed at four[85], and eight years of age[86]. Two hundred and one children had data collected at birth and were studied at age four[85]. Of these, 190 (95%) participated in the follow-up at eight years of age, and a further 287 children with birth records available were also included (total *n* 477)[86]. Lower birthweight was associated with higher plasma glucose and insulin concentrations at age four, following an oral glucose challenge[85]. This remained significant after adjustment for current weight, which was independently predictive[85]. Follow-up at eight years then showed lower birthweight (less than 2000 grams) to also be associated with higher mean total and low density lipoprotein-cholesterol (LDL-C) concentrations (3.5 *vs.* 3.2 mmol/L and 2.1 *vs.* 1.8 mmol/L respectively), increased truncal adiposity, defined by the subscapular to triceps skinfold ratio (88.3 *vs.* 77.7), higher systolic blood pressure (115.7 *vs.* 111.8 mmHg), and greater calculated insulin resistance (1.3 *vs.* 0.9 homeostasis model assessment) compared to those weighing 3000

to 3250 grams at birth[86]. Interestingly it was the children who were born small but became large by eight years (defined by weight, height and adiposity) who displayed the most adverse cardiovascular risk profiles[86] (refer to section 2.2.4).

2.2.4 Catch-up growth

Catch-up growth can generally be defined by growth velocity (cm/years) greater than the median for chronological age and gender[87]. Most children who are born small for gestational age (SGA) experience catch-up growth, to achieve a height greater than two standard deviations below the mean[87]. This catch-up process is usually completed by two years of age[87]. Whilst potentially beneficial in the short term, catch-up growth may be detrimental to long-term survival[88].

The paradoxical effect of lower birthweight and higher adult BMI is at least partly explained by the observation that infants who have been growth restricted *in utero* tend to 'catch-up' with more rapid weight gain during the early postnatal period, which leads to an increase in central fat deposition[75]. Stettler *et al.* (2003) have shown a link between rapid weight gain during the first four months after birth and obesity, described by both BMI and skinfold thicknesses, in young adulthood[89].

Similarly, Singhal *et al.* (2003) have conducted quite a number of studies which focus on the early postnatal nutritional environment as a potential modifier of health in later life. In one of their clinical studies, 32-33 split proinsulin (a marker of insulin resistance) was measured in adolescents born preterm who had participated in randomised intervention trials of neonatal nutrition (*n* 216), and in adolescent controls born at term (*n* 61)[90]. In the 1980s at the time of randomisation, the diets commonly used to feed preterm infants included unsupplemented donor breast milk and standard infant term formula[90]. We now recognise that these diets did not meet the nutritional requirements of preterm infants. They resulted in reduced rates of growth, poor bone mineralisation, and specific nutrient deficiencies[90].

The authors found that the adolescents born preterm who were randomised to the lower-nutrient diets had better insulin sensitivity, compared to those given a nutrient-enriched diet[90]. These diets were administered while in hospital and were ceased upon discharge at a median age of four weeks[90, 91]. Other outcome measures used in their studies include flow-mediated endothelium-dependent dilation[92], blood pressure[93, 94], lipid

profiles[95, 96], and leptin concentrations[97]. Their findings consistently show an adverse association between more rapid postnatal growth and long-term cardiovascular health, which have led the authors to their 'growth acceleration hypothesis'[98]. This proposes that the long-term benefits of breastfeeding for obesity prevention may result from the slower pattern of growth seen in breast- compared to formula-fed infants[98].

Hoffman *et al.* (2000) have undertaken a series of investigations of good methodological quality, in a small cohort of previously undernourished (stunted) (*n* 58) and nonstunted (*n* 30) children, aged eight to eleven years, from Sao Paulo, Brazil[99-101]. In one study they sought to determine whether the increased rates of obesity seen in stunted children may result from a lowered metabolic rate and impaired fat oxidation. During a three-day resident study, in which all food was provided, indirect calorimetry was used to measure fasting and postprandial energy expenditure, respiratory quotient, and substrate oxidation[100]. They showed that while resting energy expenditure and postprandial thermogenesis did not differ between groups, the fasting respiratory quotient for the stunted children was significantly higher, and consequently fat was metabolised at a lower rate, compared their nonstunted counterparts[100]. Additionally the stunted children also demonstrated a higher energy intake per kilogram of body weight and higher energy intake to resting energy expenditure ratio[101]. However, there was no difference in energy expenditure between the two groups when the variations in body size and composition (measured by DEXA) were accounted for[99]. These findings may help explain the recent increases in adiposity and the prevalence of obesity among stunted adults and adolescents seen in developing countries.

2.2.5 The predictive adaptive response and theory of mismatch

Animal models have more recently shown that the most marked programming effects occur where there is a mismatch between the intra- and extrauterine nutritional environments[102]. The PAR suggests that if pre- and postnatal environments match, the physiological settings achieved through the processes of developmental plasticity will leave the organism well prepared for the later life environment[18]. However, a mismatch between the two environments may be pathogenic[18].

In a study of male mice, growth restriction in the offspring was induced via a low-protein diet in the mother during pregnancy[102]. The growth restricted pups who were

then cross-fostered at birth to a normally fed dam experienced rapid catch-up growth, and subsequently died at a younger age than the controls[102]. In direct contrast, the offspring that had normal intrauterine growth but were nursed by dams receiving a low-protein diet experienced an extended longevity[102]. In this study, the largest differences observed were when the catch-up mice were fed a 'cafeteria' or high-fat diet post-weaning[102]. Similarly Vickers *et al.* (2000) have shown that dams undernourished in pregnancy deliver rats who go on to develop insulin resistance and hypertension[103]. Interestingly, the insulin resistance, hypertension and visceral obesity was exacerbated if the rats were put on a 'cafeteria' diet[103]. Both studies lend support to the PAR and the theory of environmental mismatch. They also provide an experimental perspective on the interaction between the intrauterine and postnatal nutritional exposures. This may help us to better understand the factors underpinning the long-term health adversity that is associated with human catch-up growth.

2.2.6 Critical windows

Certain stages of human development are thought to be more significant than others with respect to the adjustments that occur in response to nutritional disturbances, hypoxia or stress. Typically these sensitive periods coincide with a phase of rapid growth, which is fastest in the first few weeks after birth[96]. More than a decade ago Dietz recognised three critical periods for the development of obesity. These are gestation and early infancy, the period of adiposity rebound between five (or three[104]) and seven years, and adolescence[105, 106]. He argued that an accumulation of excess adiposity during these periods increases the risk of persistent obesity and its complications.

Zafon (2007) concisely synthesises an evolutionary perspective on the changes in body fat over the life-course in a recent literature review[107]. He argues that the increases in adiposity seen at key stages are controlled by regulatory mechanisms that favour fat storage when energy is readily available, and that high fat mass is a survival currency in the face of stressors likely to be encountered during developmental periods[107].

At birth humans are enormously fat compared to other mammals[108] (refer to Figure 2.2), and this is especially true relative to our fellow primates[109, 110]. Human infants then grow rapidly postnatally, and attain a peak in adiposity at about 25% of body fat

after four to six months[111]. Intrauterine fat storage is thought to protect brain development during temporary nutritional deficits immediately after birth[108]. Additionally fat storage in early infancy provides a buffer for the nutritional inadequacies that often accompany weaning[108].

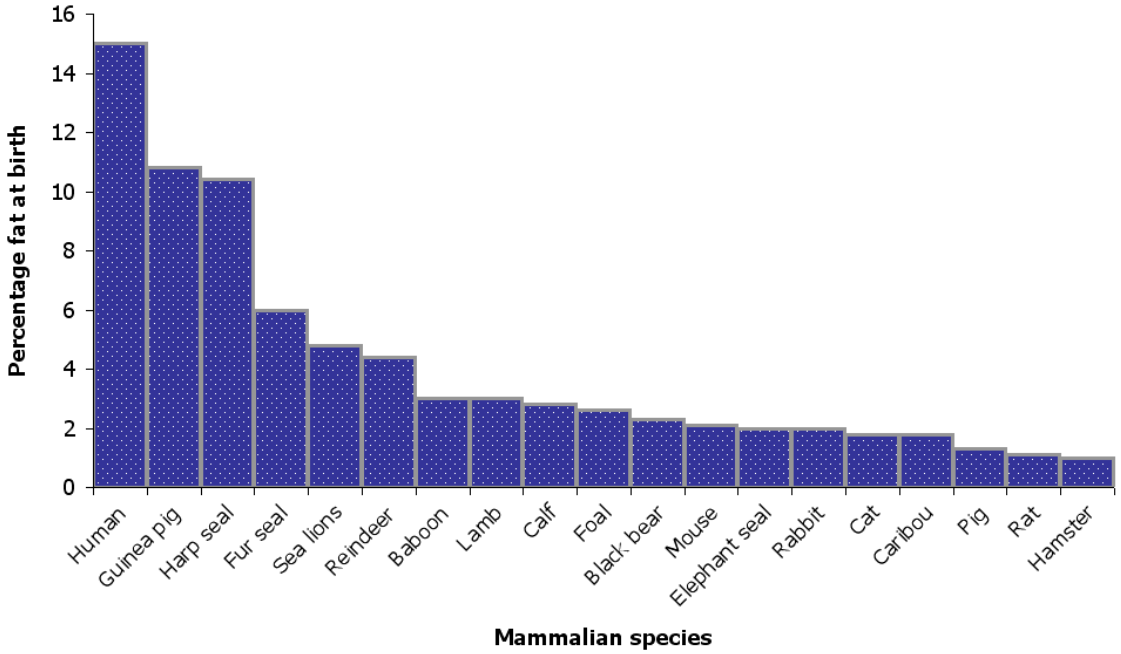

Figure 2.2 Percentage body fat at birth comparing 19 mammalian species

Reprinted from the American Journal of Physical Anthropology, Suppl 27, CW Kuzawa, Adipose tissue in human infancy and childhood: an evolutionary perspective, p.182, 1998, with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.[108].

The adiposity rebound is the second rise in BMI that occurs during childhood[104] and it corresponds to an increase in the number of adipocytes (fat cells)[112]. An early adiposity rebound is suggested to be a key risk factor for later adiposity and type two diabetes[112-114]. Children who have an early adiposity rebound have higher BMIs in later childhood[114]. Figure 2.3 shows the changes is in BMI between birth and 12 years according to the age of the adiposity rebound.

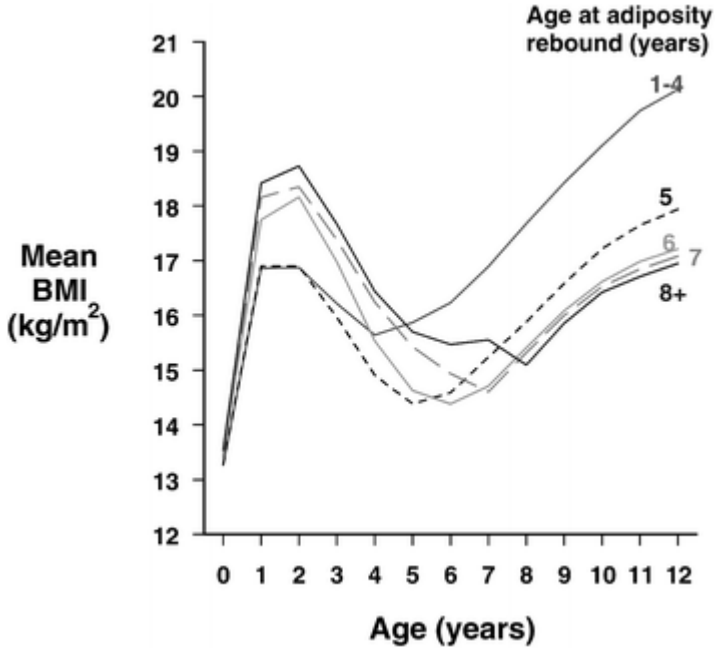

Figure 2.3 Mean body mass index (BMI) between birth and 12 years in 6060 Finish children born in Helsinki between 1934 and 1944, according to their age of adiposity rebound

Reprinted from Diabetologia, 46(2), JG Eriksson, T Forsen, J Tuomilehto, C Osmond, and DJ Barker, Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life, p.192, Figure 2, 2003, with permission of Springer Science and Johan Eriksson[114].

Adolescence is further characterised by large changes in body size and composition and phenotypic changes may track into adulthood. Higher adiposity in childhood is associated with earlier sexual maturation in girls[115] but not boys[116]. Earlier maturation in boys, however, is associated with lower a BMI and adiposity[116]. In a study of 579 Swedish males, Kindblom *et al.* (2006) showed that the age at peak height velocity (an indicator of maturational timing) was inversely associated with young adult BMI and fat mass (as measured by DEXA)[117]. Early puberty predicted a more central fat distribution, while more subcutaneous fat was more strongly predicted by BMI at age 10[117]. Bratberg *et al.* (2007) found no association between pubertal status and likelihood of overweight at 18 years of age in 697 Norwegian boys[118]. However, in the cohort of 864 girls, a higher risk of overweight in late adolescence was associated with earlier menarche, though the effect was restricted to those girls who had a higher waist circumference in early childhood[118]. Whether sexual maturation in girls is caused by, and/or the consequence of, a higher pre-pubertal body mass remains an issue of debate throughout the literature[119].

2.2.7 Experimental evidence

2.2.7.1 Animal models

Using animal models to investigate the developmental origins poses several advantages to human studies; namely reduced time and costs, liberalised experimental techniques, and convenience[27]. Different species have been used to investigate the details of various aetiologies. While this poses somewhat of a challenge in the interpretation of the work, one commonality is the ease with which phenotypic changes are induced in the offspring as a result of the insults presented in early life[27].

Animal nutrition programming work has been pioneered since the 1960s when McCance (1962) showed that rats raised in smaller litters, who were fed more during the suckling period compared to those raised with a greater litter size, were larger as adults[120]. Since then, the programming effects of overfeeding during infancy have been demonstrated in a number of animal models, including baboons, where increased adiposity in adulthood was observed[110]. In mice, an increase in adiposity was also seen, but furthermore a reduction in lifespan was reported[102]. Maternal dietary protein restriction in rat dams is a common model described throughout the developmental origins literature, and is used to induce fetal growth restriction in the offspring[88]. As Hales and Ozanne describe: *"In vivo* and *in vitro* studies of the growth-restricted offspring of such pregnancies have provided findings showing remarkable parallels with the human conditions"[88].

2.2.8 Underlying mechanisms

2.2.8.1 Epigenetics

Epigenetics is the study of mechanisms which modify DNA structure, and thus gene function, without a change in the DNA sequence[17]. Environmental cues can affect gene expression, particularly by inducing epigenetic changes in the DNA. Epigenetic mechanisms include DNA methylation, histone acetylation, and ribonucleic acid (RNA) interference, which influence gene activation and inactivation[17]. The programming effects of epigenetic mechanisms are considerably easier to investigate in animal models, where the confounding of genetic and environmental factors can be carefully controlled and where the burden of longitudinal timeframes is heavily reduced.

Epigenetics is a relatively recent and exciting area of research within the DOHaD. It offers both plausible and testable opportunities for understanding the relationship between genetic potential, the environment, and the development of the phenotype. Because these epigenetic processes depend on the availability of essential macro- and micronutrients it is logical to suspect epigenetics as candidate mechanisms for developmental origins[27]. While this largely remains theoretical a great deal of work is underway to test this current hypothesis.

The *agouti* mouse is one experimental model used to demonstrate epigenetic changes in the DNA. In genetically identical mice, the coat colour pattern displays epigenetic variation in the expression of the *agouti* viable yellow allele[121]. Aside from coat colour, a high level of *agouti* expression causes multiple downstream metabolic and endocrine changes, which result in obesity and reduced longevity[121]. Maternal diets supplemented with methyl donors and cofactors to enhance DNA methylation have produced phenotypic changes in the offspring, with improved morbidity and mortality outcomes[121]. The supplemental nutrients acting as methyl donors included folic acid, vitamin B12, zinc, choline, betaine, and L-methionine[121].

Similarly, Sinclair *et al.* (2007) reported dramatic intrauterine epigenetic programming for the metabolic syndrome in offspring born to ewes[122]. These were periconceptionally restricted (within normal physiological ranges) of dietary folate, vitamin B12 and methionine[122]. Early, but modest periconceptional dietary restriction resulted in adult offspring who were heavier and more adipose, more insulin resistant, and had elevated blood pressure, with the effects most pronounced in the male offspring[122]. There were no differences between the dietary restricted and control mothers in terms of pregnancy establishment and birthweight of the offspring[122]. The altered methylation status of a significant proportion (4%) of the genomic regions studied in the fetal liver tissue provided compelling evidence of a widespread epigenetic response mediated by this nutritional deficit[122]. These animal data suggest that specific dietary factors which feed into the methionine/homocysteine metabolic pathway are important mediators of the adult health-related phenotypes expressed and warrant further investigation in human studies (refer to Chapter 6).

2.2.8.2 Transgenerational responses

In addition to the association seen between the current maternal nutritional environment and the risk of disease in the offspring, there is evidence to suggest that the nutritional experience of the mother during her own childhood can influence the programming outcomes for the offspring. Martyn *et al*. (1996) have suggested that stroke may originate from poor nutrition during the mother's childhood, which impairs the development of the bony pelvis and subsequently reduces her ability to sustain placental and fetal growth late in pregnancy[58]. In this retrospective analysis of the men born in Sheffield and Hertfordshire, UK (refer to section 2.2.1) the standardised mortality ratios for stroke and CHD were highest in those with the lowest birthweight. However, mortality from stroke was most strongly associated with LBW and low placental weight, in relations to head size; a pattern of growth which occurred in the offspring of mothers' with flat bony pelvises. This was compared to a small HC, thinness or reduced length at birth, and an altered placental ratio (placental weight to birthweight) in the adult offspring dying from CHD. The authors suggest that CHD risk may be programmed as a result of adaptations made by the fetus to the inadequate supply of nutrients, when it occurs for reasons other than failed placental growth[58].

Furthermore, the nutrition of the grandmother during pregnancy appears to influence not only the intrauterine nutritional exposures of her daughter (who inturn becomes the mother), but also the grandchild's birthweight[123]. Apart from transgenerational responses from the maternal side, Kaati *et al.* (2007) have demonstrated some evidence to support a sex-specific, male-line transgenerational response system in humans, which captures nutritional information from the previous generation(s)[124]. Data from three random samples in Sweden, comprising 271 probands and their 1626 parents and grandparents were analysed[124]. A proband is the family member who triggers the study of other members of the family, to identify the possible genetic factors involved in a given disease, condition, or characteristic[125]. Food availability, based on regional statistics, during the ancestors' slow growth period (before the pubertal peak) and the probands' childhood circumstances were tested as predictors of mortality[124]. The male probands had an increased risk of mortality (hazard ratio 1.7, *P* 0.01) if their fathers had good nutrition during their slow growth period[124].

2.2.8.3 Other mechanistic pathways

Although it is beyond the scope of this literature review, quite a number of other mechanistic pathways have been suggested in recent publications[126-128], including those which relate to specific disease pathogenesis for conditions such as cardiovascular disease[129, 130], diabetes[131, 132], hypertension[133, 134], and obesity[69, 135].

2.2.9 Controversy and limitations

It is understandable that early fetal programming work was met with reservation and criticism. After all, none of the major retrospective studies provided an actual measure of nutritional intake in mothers or their offspring. Early nutrition was inferred from fetal and infant growth, and fetal growth was itself inferred by the surrogate measure, birthweight. Thus even if the findings were taken as valid, questions were raised as to whether nutrition or some other modifier was being measured[52]. The strength of the relationships observed between developmental factors and later health outcome, while reproducible, varied considerably according to the population studied and the age of the participants[88]. There was also some epidemiological evidence which did not support the hypothesis[136].

Yet several key factors are likely to account for much of the variation seen between study findings in the developmental origins field. Birthweight is commonly reported on, largely because of its ease of use and accessibility from current and historic records. However, fetal growth and birthweight are not synonymous; size at birth, adjusted for the length of gestation, is but a snapshot of the intrauterine growth trajectory[137]. Even large fetuses can be growth restricted, according to their genetic potential[137]. It should also be noted that some prenatal exposures that 'program' disease propensity may not disrupt intrauterine weight gain. The recent association between maternal paracetamol use in late pregnancy and the subsequent risk of asthma in the preschool aged offspring may be an example of this[138].

The low sensitivity (i.e. the proportion of people who have the condition who have positive tests that correctly identify the condition; the true positive rate) of BMI to detect true adiposity is another one of these factors. A cross-sectional study of almost 1000 children aged eight to 12 years was undertaken to determine the sensitivity and specificity (the proportion of people who do not have the condition who are correctly

identified as being free of the condition; the true negative rate) of BMI in detecting true fatness, as measured in a sub-sample by DEXA[139]. At the ninety-fifth percentile the sensitivity of BMI was low (0.39), while the specificity was high (0.99) [139]. This indicates that many individuals with excess body fat are not detected by simply applying BMI calculations to height and weight. An example of this has previously been demonstrated in this thesis in Figure 2.1 comparing the white-Caucasian to Indian adult male.

Other discrepancies between studies may be the result of inconsistent loss to follow-up, changes in infant growth from one era to another, and population-based differences[53]. The work conducted within animal models has also been subject to criticism. Primarily the applicability to humans is questioned. Although experiments in animals illustrate the principle that adult health outcomes can be, in-part, causally related to early development, the extent to which similar developmental processes explain variations in human health outcomes remains unclear[53].

Hence human research is yet to unravel the complexities between the intrauterine and postnatal nutritional environments. To do this, serial measures over the life-course, with care taken to include the potential 'critical windows' starting with fetal development, are needed. Several major studies which are attempting to fill this niche are currently underway[140-144].

2.3 PHYSIOLOGIC AND METABOLIC ADJUSTMENTS IN PREGNANCY

2.3.1 The placenta

As well as acting as the conduit for the exchange between the maternal and fetal circulation, the placenta also functions as an endocrine organ. It produces a large number and quantity of hormones responsible for regulating fetal growth, the development of maternal support tissues[145] and the timing of birth[146, 147]. The two primary steroid hormones, which are synthesised by the placenta from maternal cholesterol, are progesterone and oestrogen[148, 149]. Progesterone initiates the relaxation of smooth muscles in the uterus, facilitating its expansion as the fetus grows[149]. Relaxation occurs at other smooth muscle sites throughout the body, including along the gastrointestinal tract. This reduces gut motility, providing more time for nutrients to be absorbed[150]. Additionally, progesterone favours maternal fat deposition and increases renal sodium excretion[149, 150]. Oestrogen on the other hand, causes increased fluid retention and assists in regulating the production of thyroid hormone[150]. Other placental peptide hormones include human placental lactogen, responsible for elevating blood glucose from the breakdown of glycogen, and human chorionic thyrotropin which, like oestrogen, stimulates the production of thyroid hormones[149].

2.3.2 Blood volume and composition

During pregnancy, maternal blood volume expands to meet the greater circulatory needs of the placenta and mother's organs[151]. Blood volume expansion results in an increase in both plasma (the fluid component of blood) and erythrocytes (red blood cells). Usually, a greater proportion of plasma is added[3] and this has begun by about the tenth week of gestation[152]. By 30 to 34 weeks the mean increase in plasma volume is approximately 50%[152]. However, the change in plasma volume can vary greatly between women, with a range from minimal to doubling reported in the literature[3, 151, 153]. While much of the variation in plasma volume expansion is unexplained, some factors are known to be positively associated, including higher maternal pre-pregnancy BMI[154], higher birthweight of the offspring[155, 156], multiple births[157], and parity[158]. Conditions of compromised placental development (such as preeclampsia) and

intrauterine growth restriction (IUGR) are inversely associated[153], and ethnic variations also exist[158].

2.3.2.1 Hemodilution

Plasma volume expansion causes hemodilution, where the concentrations of some blood constituents are diminished in response to the increased fluid content. It is generally regarded that the biochemical decline in isolation is not an indicator of nutrient deficiency, since the total quantity of the available nutrient is frequently the same or higher. However, this makes detecting true deficiencies somewhat problematic[145]. Biochemical markers that are known to decrease during pregnancy include haemoglobin, blood glucose, serum albumin and other serum proteins, and water-soluble vitamins[145]. Interestingly, the serum concentrations of fat-soluble vitamins and other lipid derivatives including triglycerides, cholesterol and free fatty acids are known to increase[145]. The mechanisms that support high lipid concentrations during pregnancy require elucidation, although the steroid hormones have been implicated[149].

2.3.3 Weight gain

Most of the weight gained during pregnancy is accounted for by the uterus and its contents, although other maternal tissues, blood and extravascular fluid also contribute to total maternal weight gain. Table 2.1 details compartmental weight gain over gestation for a normal pregnancy[159].

Table 2.1 Compartmental and cumulative weight gain during pregnancy

Tissue and fluids	Cumulative increase in weight (grams) up to:			
	10 weeks	20 weeks	30 weeks	40 weeks
Fetus	5	300	1500	3400
Placenta	20	170	430	650
Amniotic fluid	30	350	750	800
Uterus	140	320	600	970
Breasts	45	180	360	405
Blood	100	600	1300	1450
Extravascular fluid	0	30	80	1480
Maternal fat	310	2050	3480	3345
Total	650	4000	8500	12500

Reprinted from Weight gain in pregnancy, FE Hytten, in Clinical physiology in obstetrics, G Chamberlain and FE Hytten (editors), 1991, with permission from Frank Hytten[159].

Nevertheless, controversy exists as to what constitutes 'optimal' weight gain in pregnancy and it varies considerably according to the outcome of interest. Internationally the most widely quoted guidelines for pregnancy weight gain are from the Institute of Medicine's *Nutrition During Pregnancy* publication[160]. This report was written in the late 1980s, at a time when the obstetric priority was the prevention of LBW infants, as they are subject to high rates of morbidity and mortality[161]. Since then the focus for LBW has broadened to consider the potential long-term programming effects for the offspring of excess maternal weight gain[161].

Recently, optimal gestational weight gain in women by pre-pregnancy BMI was explored in a large population-based study[162]. This involved close to 300,000 singleton pregnancies, recorded in the Swedish Medical Birth Registry from 1994 to 2004[162]. Table 2.2 compares the recommended maternal weight gain for pregnancy, according to the Institute of Medicine guidelines and this contemporary study. The findings of the Swedish study were based on a large number of adverse maternal and perinatal outcomes[162]. The optimal pregnancy weight gain for the lowest risk of adverse outcomes was significantly lower than previous Institute of Medicine recommendations, especially among overweight and obese women where no lower limit for weight gain was deemed appropriate[162].

Table 2.2 Optimal total pregnancy weight gain by pre-pregnancy body mass index (BMI) based on the odds ratio for adverse maternal and perinatal outcome*

BMI (kg/m²)	Optimal gestational weight gain		Recommended gestational weight gain (lb)†
	Pounds (lb)	Kilograms	
Less than 20	9-22	4-10	28-40
20-24.9	5-22	2-10	25-35
25-29.9	Less than 20	Less than 9	15-25
30 or more	Less than 13	Less than 6	More than 15

*Adjustments were made for maternal age and parity.
†Institute of Medicine[160].
Reprinted from Obstetrics & Gynecology, 110(4), MI Cedergren, Optimal gestational weight gain for body mass index categories, p.762, Table 3, 2007, with permission of Wolters Kluwer Health and Marie Cedergren[162].

The merits of routinely measuring maternal weight as part of standard antenatal care continue to be debated[163, 164]. In a national survey of 672 midwives in the UK, 61.8% thought that the pattern of maternal weight gain was 'not important' in antenatal

care[164]. Only 51.5% of those who were currently practicing weighed women at every antenatal visit[164]. In Australia, a survey conducted during the mid 1990s, aimed to express the views of midwives (*n* 196) and obstetricians (*n* 114) about the important components of antenatal care[165]. It included 77 items from the 1988 National Health and Medical Research Council (NHMRC) guidelines for antenatal care[166]. Pre-pregnancy weight and gestational weight gain were even not mentioned as components for consideration[165].

From the literature it appears that a single weight gain recommendation is not appropriate for all women. Viswanathan *et al.* (2008) highlight some factors that are needed for a more comprehensive understanding of the impact of gestational weight gain on the short- and long-term outcomes for women and their offspring[167]. These include the use of consistent definitions of weight gain during pregnancy, careful consideration of potential confounders, improved study designs with longer periods of follow-up and appropriate statistical modelling[167].

2.3.4 Energy

Apart from the energy needs of the fetus and accompanying maternal tissues, pregnant women may expend larger amounts of energy simply in response to gaining weight. Some women may compensate for this by reducing energy demanding tasks, such as physical activity and work[168].

2.3.4.1 Basal metabolic rate

The elevation in basal metabolic rate (BMR) that accompanies normal pregnancies results from the oxygen demands of the placental-fetal unit and the additional maternal cardiac output[149]. This elevation is usually apparent by the fourth month of pregnancy[149]. Although adaptive changes in BMR and maternal fat storage may profoundly influence this[169]. The additional energy demands of a pregnancy have been estimated to be approximately 335 MJ in total (or 1.25 MJ per day)[170]. Prentice and Goldberg (2000) have recorded mean population energy needs ranging from as high as 520 MJ per pregnancy, to as low as -30 MJ per pregnancy[169]. Figure 2.4 illustrates this variation, showing the mean change in BMR during pregnancy for women from an affluent country (England), compared to a poor country (The Gambia). In addition two

individuals (subject 1 and 2) from the same population (Cambridge, England) are juxtaposed to highlight the potential within-population disparities that exist.

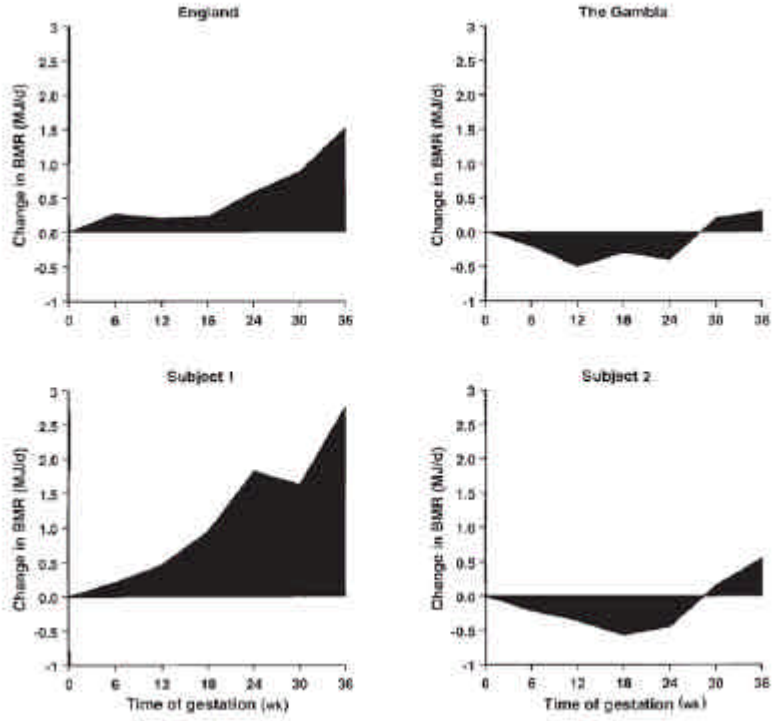

Figure 2.4 Changes in basal metabolic rate (BMR) during pregnancy in women from Cambridge, England (mean values); in women from Keneba, The Gambia (mean values); and in two individuals from Cambridge (subjects 1 and 2)

Reprinted from The American Journal of Clinical Nutrition, 71(5), AM Prentice and GR Goldberg, Energy adaptations in human pregnancy: limits and long-term consequences, p.1227S, 2000, with permission of The American Journal of Clinical Nutrition, American Society for Nutrition, and Andrew Prentice[169].

Pre-pregnancy maternal nutritional status appears to be an important determinant of an individual's metabolic response to pregnancy and lactation. Underweight or undernourished women appear better equipped to conserve energy during pregnancy compared to their well-nourished counterparts[150]. The cumulative increase in BMR over pregnancy is also shown to correlate well with maternal weight gain[171]. While this biological plasticity makes it possible to reproduce during periods of nutritional adversity (traditionally famine) it is now understood to be a situation of compromise with limits and consequences for the mother and offspring[169].

2.3.5 Macronutrient metabolism

Changes to carbohydrate, protein and fat metabolism occur during pregnancy to maintain a continuous supply of fuel to meet the varying needs of the fetus. Nutrients are stored early in pregnancy during an anabolic period, which involves the enhancement of maternal adipose tissue stores and modest improvements in insulin sensitivity. Later in pregnancy maternal catabolism occurs to support the increased feto-placental demands and to prepare for lactation.

2.3.5.1 Carbohydrate metabolism

Early in pregnancy glucose tolerance is maintained or slightly improved. Insulin sensitivity varies according to maternal pregravid sensitivity and potentially other mechanisms[172-174]. Longitudinal studies of glucose tolerance during pregnancy show a progressive increase in the insulin response to glucose with only minor deteriorations in glucose tolerance[175]. This profile is consistent with insulin resistance. Insulin resistance serves to increase substrate availability for fetal growth, by providing higher concentrations of glucose and free fatty acids.

Ordinarily hepatic glucose production, whether from gluconeogenesis or glycogenolysis, would be suppressed by insulin. In pregnancy however, hepatic glucose production is increased at the same time as a rise in fasting insulin, indicating a reduction in maternal hepatic insulin sensitivity[174]. Hepatic insulin sensitivity is further reduced in the presence of obesity[176]. Despite this, maternal blood glucose levels progressively decline with advancing gestation[172]. This may be in response to plasma volume expansion, increased utilisation and/or inadequate production by the liver[174]. Insulin resistance shunts diet-derived nutrients to the fetus and, if excessive, can result in large adipose stores in the fetus[177]. Macrosomic fetuses born to mothers with poorly controlled gestational diabetes exhibit this trait. Figure 2.5 illustrates how the interaction between the maternal hormonal and nutritional milieu can influence the phenotype of the offspring, in the presence of gestational diabetes. The precise mechanisms for this reduced insulin sensitivity are unknown, although variations in the hormonal milieu (human placental lactogen, progesterone, prolactin and/or cortisol) are likely to be involved[178].

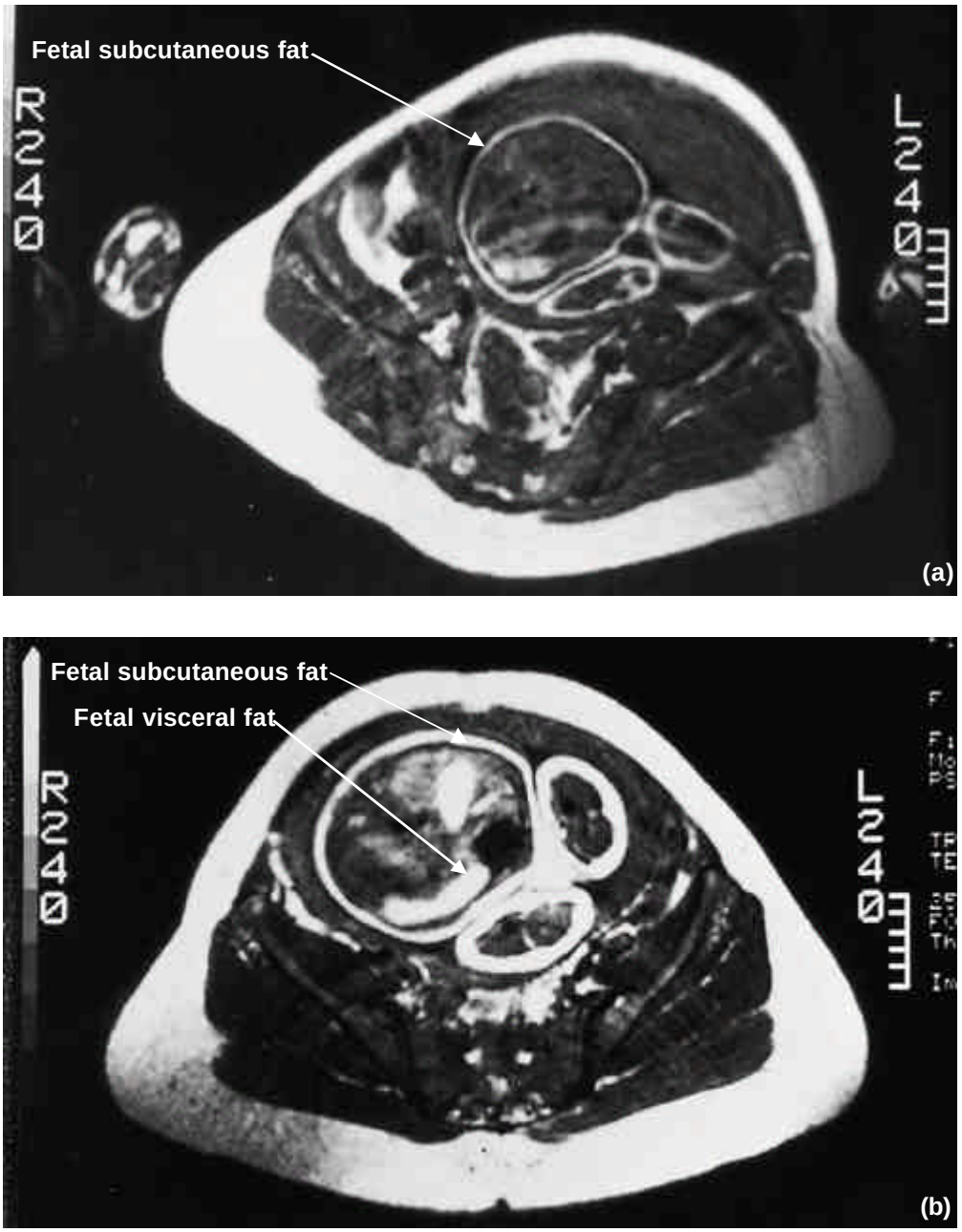

Figure 2.5 Magnetic resonance images at 38 weeks' gestation, showing fetal adiposity in two mothers with gestational diabetes (diet controlled only)

Fetus (a) weighed 3900 grams at birth and had a normal proportion (15%) of body fat. Fetus (b) weighed 3940 grams at birth and was 50% body fat.

Reprinted from the American Journal of Perinatology, 10(6), L Jovanovic-Peterson, J Crues, E Durak, and CM Peterson, Magnetic resonance imaging in pregnancies complicated by gestational diabetes predicts infant birthweight ratio and neonatal morbidity, p.434-5, 1993, with permission of Thieme Medical Publishers, Inc.[179].

2.3.5.2 Protein metabolism

In an average pregnancy, just less than one kilogram of protein, or approximately five grams per day averaged over the second and third trimester, is deposited in the fetus and supportive maternal tissues[150]. There is no evidence that protein is stored early in pregnancy to assist with these later fetal demands[180]. Rather, late in gestation nitrogen retention is promoted via a reduction in urinary nitrogen excretion, primarily in the form of urea[180]. Furthermore, longitudinal changes in 3-methylhistidine from pre-pregnancy to 34 weeks' gestation have not been found in healthy Dutch women, indicating that maternal muscle stores are not necessarily mobilised to meet the protein demands of pregnancy[181]. If dietary protein intake is low, more drastic physiological adjustments may be required. Pre-pregnancy protein status may also influence the level of physical adjustment made to nitrogen metabolism[180].

2.3.5.3 Lipid metabolism

The net effect of maternal fuel adaptation is for the mother to increase the use of fats to preserve glucose for the fetus[149]. Lipid metabolism differs between lean and obese women with normal glucose metabolism[174]. In lean women, net lipogenesis occurs early in pregnancy, with a switch to lipolysis occurring late in the pregnancy[182]. In obese women, lipogenesis occurs preconceptionally and lipolysis predominates in both early and late gestation[183].

Biochemical markers of lipid metabolism also show dramatic changes in pregnancy. Total triglyceride levels increase between two- and four-fold and total cholesterol concentrations increase by 25 to 50% during normal pregnancy[174, 184]. By mid-gestation, LDL-C has increased by approximately 50%[174]. High density lipoprotein-cholesterol (HDL-C) concurrently increases by 30%, although HDL-C then declines slightly to term[174].

Adipocytes may also play an important role in the lipid metabolism of pregnancy. Adipose tissue is now recognised not only for its energy storage capacity but also as a producer of cytokines and inflammatory mediators which influence insulin resistance (for example, adiponectin or tumour necrosis factor-alpha (TNF-α))[174]. Challier *et al.* (2008) recently reported the chronic inflammation state of pre-pregnancy obesity is extending into intrauterine life with an accumulation of a heterogeneous macrophage

population and pro-inflammatory mediators in the placenta[185]. The authors speculate that the resulting inflammatory milieu in which the fetus develops may play a role in the short- and long-term programming of obesity in the offspring[185].

Additionally, adipocytes and some other tissues, including the placenta and several fetal tissues, secrete leptin[186]. Leptin is an appetite-suppressing peptide hormone involved in the regulation of energy balance[186]. It exerts its effects upon the central nervous system[186]. Maternal leptin levels progressively increase from early pregnancy to peak during the second trimester. They plateau at term, with concentrations three- to four-fold higher than in non-pregnant women[3]. The elevated leptin secretion may be partly related to increased prolactin and placental lactogen (chorionic somatomammotrophin) secretion in pregnancy[187]. Leptin secretion continues to decrease early in lactation, as the mother enters negative energy balance[186].

2.3.6 Micronutrient metabolism

2.3.6.1 Vitamin B12, folate and homocysteine

Vitamin B12 (cobalamin) and folate are water-soluble micronutrients which are essential for normal DNA and RNA biosynthesis[188]. During embryogenesis and fetal growth, nucleic acid and protein synthesis is maintained by the maternal supply of folate[189] and vitamin B12. A deficiency in the intake of folate or a genetic error in its metabolism may impair normal cell formation and tissue growth[190]. Maternal-to-fetal folate transfer is mediated by placental folate receptors[191]. These are found on the maternal-facing chorionic surface and capture 5-methyltetrahydrofolate from the mother's circulation[192]. Mouse-models have shown the folate receptor analogous to that of humans (folate-binding protein one (Folbp1)) is highly expressed in the yolk sac, the neural folds, and the neural tubes of the developing embryo[193, 194].

Folate deficiency or impaired folate metabolism is widely accepted now as the major cause of neural tube defects (NTDs) (refer to section 2.4.1.2). Meta-analysis data also suggest it may play a role in the development of oral clefts[195], congenital heart defects[195], urinary tract anomalies[195], limb defects[195], and paediatric cancers, including leukaemia, brain tumours, and neuroblastoma[196]. Vitamin B12 deficiency has historically received little attention with respect to adverse pregnancy outcomes,

however, recently it has been independently associated with NTDs[197], preterm delivery[198], IUGR[199, 200], and recurrent pregnancy loss[201].

Vitamin B12 and folate are integral components of homocysteine metabolism. Homocysteine is a thiol-containing amino acid which originates from the demethylation of methionine, an essential amino acid, via the methionine cycle[202] (refer to Figure 2.6). Homocysteine is regulated via two discrete pathways: by trans-sulphuration to cysteine, or by remethylation to methionine[203, 204]. The trans-sulphuration pathway requires vitamin B6 (pyridoxine) as a cofactor for the enzyme cystathionine β-synthase[204]. Methionine synthase catalyses the remethylation of homocysteine to methionine, and requires folate (5-methyltetrahydrofolate) as a co-substrate and vitamin B12 (methylcobalamin) as a cofactor[205]. The methionine cycle occurs in every mammalian cell[205]. Homocysteine can replace methionine under experimental conditions[206]. However, homocysteine does not occur naturally in human diets, hence methionine plays a unique nutritional role in this cyclical product-precursor relationship[206].

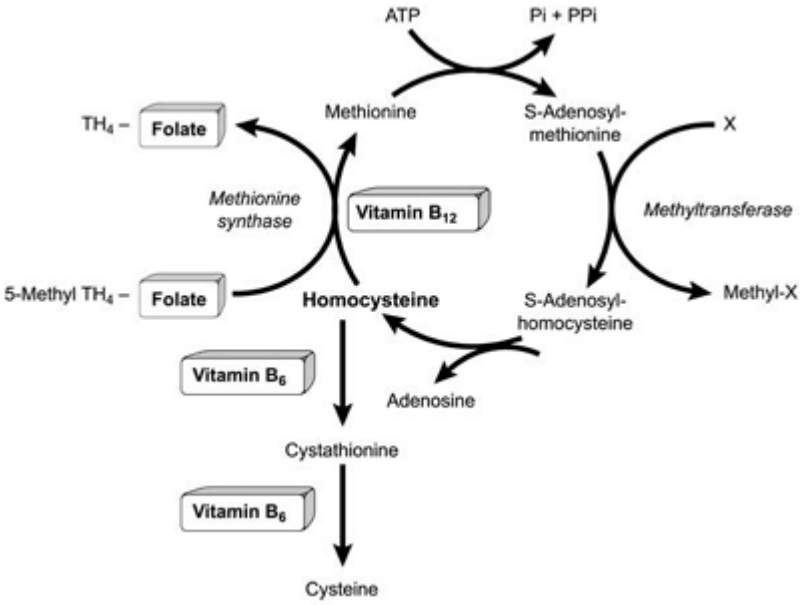

Figure 2.6 The methionine-homocysteine metabolic cycle

S-adenosylhomocysteine is formed during S-adenosylmethionine-dependent methylation reactions, and the hydrolysis of S-adenosylhomocysteine results in homocysteine. Homocysteine may be remethylated to form methionine by a folate-dependent reaction that is catalysed by methionine synthase, a vitamin B12-dependent enzyme. Alternately, homocysteine may be metabolised to cysteine in reactions catalysed by vitamin B6-dependent enzymes[207].

2.3.7 Homocysteine

Age, sex, folate intake, smoking status and coffee intake are known to be the strongest determinants of homocysteine levels in the general population[208]. Men have higher levels than women and concentrations increase with age[208]. For the lifestyle factors, there are strong positive dose-dependent relationships between the number of cigarettes smoked per day, as well as the quantity of coffee consumed, and plasma homocysteine (pHcy) levels[208]. Plasma folate, which is affected by folate intake from both diet and supplementation, is inversely associated with pHcy levels[208].

2.3.7.1 Homocysteine during pregnancy

During uncomplicated pregnancy, pHcy is known to decline[209-212]. Holmes (2003) has extensively reviewed the literature and suggests that this is most likely the physiological result of several contributing factors. These include: hormonal changes, most notably oestrogen; an uptake of homocysteine by the fetus; plasma volume expansion; a reduction in plasma albumin, which binds 70% of the pHcy in humans; and folic acid supplementation[204].

2.3.7.2 Homocysteine and pregnancy complications

High pHcy is a responsive marker of impaired folate status[213, 214]. Although recent studies have shown that even in the presence of normal circulating folate, mothers of children born with NTDs have elevated homocysteine, suggesting an effect which is independent of folate[215, 216]. Other studies have produced similar findings with respect to placenta-mediated diseases. High maternal circulating homocysteine has been linked to placental abruption, pre-eclampsia, spontaneous and recurrent pregnancy loss, preterm delivery, LBW, stillbirth, and various congenital malformations[217-219].

Figure 2.7 shows the association between pHcy and the risk of various clinical outcomes including pregnancy complications[208]. These data are from the Hordaland Homocysteine Study (HHS), a population-based study of 18,044 subjects living in Western Norway[208].

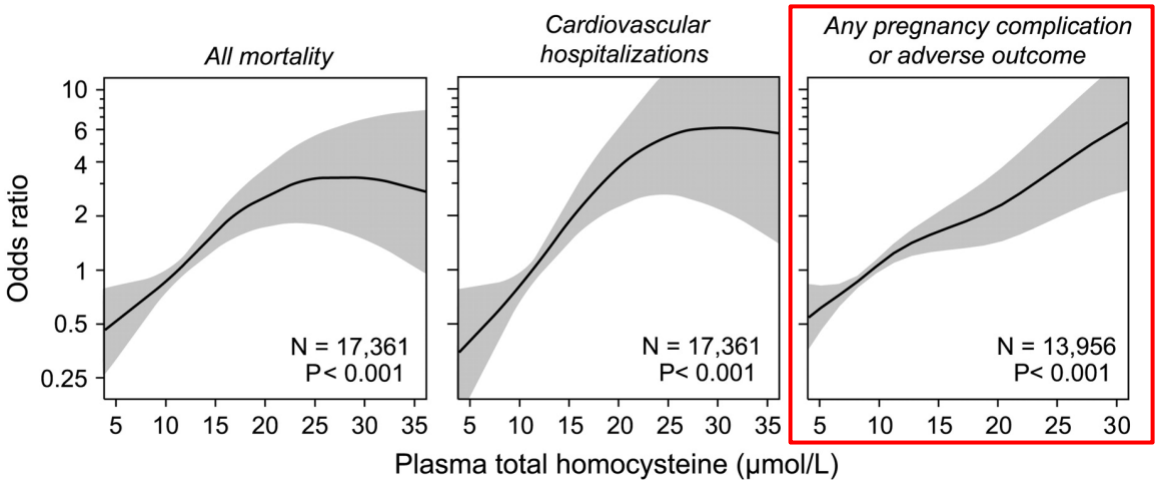

Figure 2.7 The association between plasma total homocysteine (pHcy) and various clinical outcomes, including pregnancy complications, as measured in the Hordaland Homocysteine Study (HHS), Norway

The concentration-response relation was obtained by generalised stepwise regression adjusted for age and sex, except for any complication or adverse outcomes of pregnancy, which was adjusted for maternal age and parity. Shaded areas represent 95% confidence intervals. The *P*-values have been obtained by linear regression analyses, using the same multivariate adjustments. The results are limited to the 0.5 and 99.5 percentiles of the pHcy concentration in each panel. An odds ratio of one corresponds to the mean pHcy level for all subjects. Levels of pHcy are from HHS-I.

Reprinted from the Journal of Nutrition, 136(6 Suppl), H Refsum, E Nurk, AD Smith, PM Ueland, CG Gjesdal, I Bjelland, A Tverdal, GS Tell, O Nygard, SE Vollset, The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease, p.1735S, 2006, with permission of the Journal of Nutrition, American Society for Nutrition, and Helga Refsum[208].

More specifically, analyses from the same cohort have shown associations between increasing pHcy and the risk of:

- Preeclampsia (odds ratio (OR) 1.32; 95% CI 0.98, 1.77; *P* 0.020),
- Preterm delivery (OR 1.38; 95% CI 1.09, 1.75; *P* 0.005),
- LBW, less than 2500 grams, (OR 1.48; 95% CI 1.15, 1.91; *P* 0.003),
- Very low birthweight, less than 1500 grams, (OR 2.01; 95% CI 1.23, 3.27; *P* 0.003),
- Stillbirth (OR 2.03; 95% CI 0.98, 4.21; *P* 0.020), and
- Various congenital malformations, such as NTDs (OR 3.57; 95% CI 0.78, 16.29; *P* 0.040) and clubfoot (OR 2.53; 95% CI 1.19, 5.37; *P* 0.007)[217].

The association between placental abruption and pHcy was evident only at very high concentrations (greater than 15 µmol/L)[217]. However, it must be noted that the data for

pregnancy complications was retrospectively obtained and assessed in relation to homocysteine levels which were tested in most cases more than 10 years after the event[217]. While this is a significant limitation the authors suggest that it makes the findings of the strong correlations even more remarkable and they comment on the evidence for the long-term stability of an individual's homocysteine concentration[217, 220].

Many other articles have been published to support an association between high maternal homocysteine levels and adverse pregnancy outcomes; see reviews[216, 221-223].

2.3.7.3 Homocysteine and fetal programming

At present, the mechanisms which convey information about the maternal environment to the fetus and the process of interpreting these cues into an adapted phenotype are of major research interest. Studies of maternal dietary constraint during pregnancy in animal models have suggested a causal link between the disruption of one-carbon metabolism, altered epigenetic regulation of gene expression, and phenotypic induction[224, 225] (refer to section 2.2.8.1). The activities of several important signal-transduction pathways and transcription factors are directly modified by homocysteine[225-234]. Therefore exposing an embryo or fetus to high circulating homocysteine must be considered as a potential modifier of specific tissues to intrauterine cues. For example, Lillycrop *et al.* (2005) have reported hypomethylation and increased expression of transcription factors which regulate energy homeostasis (namely the glucocorticoid receptor and PPAR-α (peroxisome proliferator-activated receptor-alpha)) in the liver of the offspring born to mothers fed a protein restricted diet during pregnancy[224]. However, supplementation of the restricted diet with folic acid prevented this hypomethylation and the associated increase in the expression of the glucocorticoid receptor and PPAR-α[224]. Very few animal models have tested the precise role of maternal homocysteine in fetal programming, and discourse over the findings continues for those that have[235, 236].

2.4 MATERNAL NUTRITION

Assessing maternal nutrition comprises a number of components including anthropometry, including pre-pregnancy weight, height, and when combined, BMI[237]. It also includes gestational weight gain, which reflects the balance between energy intake and expenditure, and increases in maternal body water[237]. Energy intake is made up of a combination of macronutrients, including carbohydrate, fat, protein, and alcohol. Micronutrients (vitamins, minerals, and phytonutrients) are provided in foods, beverages and supplements, in varying concentrations. Increasing one's intake of nutrients during pregnancy, via supplementation is common[238], with higher requirements for some nutrients during pregnancy and lactation.

2.4.1 Nutritional requirements of pregnancy

2.4.1.1 Nutrient Reference Values

The NHMRC have recently published the Nutrient Reference Values (NRVs) for Australia and New Zealand[239]. These provide guidance on the amounts of specific nutrients required, on average on a daily basis, for sustenance or avoidance of states of deficiency[239]. The requirements are based on the best available evidence and are categorised according to age and sex. Pregnancy and lactation are stages which present unique nutritional demands for women. These nutritional needs are confounded if the pregnancy (plus or minus lactation) occurs during adolescence, before the mother's own physical growth is complete. Table 2.3 provides an overview of the nutritional requirements of females, according to pregnancy and lactation status and age[239].

Table 2.3 Nutrient Reference Values for Australian and New Zealand women according to pregnancy status

Nutrient	Age (years)	Nonpregnant	Pregnant	Lactating
Protein g/day RDI (UL)	14-18	45	58	63
	19-50	46	60	67
Linoleic (n-6) g/day AI	14-50	8	10	12
α-linolenic (n-3) g/day AI	14-50	0.8	1.0	1.2
LC n-3 (DHA/EPA/DPA) mg/day AI (UL)	14-18	85 (3000)	110 (3000)	140 (3000)
	19-50	90 (3000)	115 (3000)	145 (3000)
Dietary fibre g/day AI	14-18	22	25	27
	19-50	25	28	30
Thiamin mg/day RDI	14-50	1.1	1.4	1.4
Riboflavin mg/day RDI	14-50	1.1	1.4	1.6
Niacin as niacin equivalents mg/day RDI (UL)	14-18	14 (30)	18 (30)	17 (30)
	19-50	14 (35)	18 (35)	17(35)
Vitamin B6 mg/day RDI (UL)	14-18	1.2 (40)	1.9 (40)	2.0 (40)
	19-50	1.3 (50)	1.9 (50)	2.0 (50)
Vitamin B12 μg/day RDI	14-50	2.4	2.6	2.8
Folate as dietary folate equivalents μg/day RDI (UL)	14-18	400 (800)	600 (800)	500 (800)
	19-50	400 (1000)	600 (1000)	500 (1000)
Pantothenic acid mg/day AI	14-50	4.0	5.0	6.0
Biotin μg/day AI	14-50	25	30	35
Vitamin A (retinol equivalents) μg/day RDI (UL)	14-18	700 (2800)	700 (2800)	1100 (2800)
	19-50	700 (3000)	800 (3000)	1100 (3000)
Vitamin C mg/day RDI	14-18	40	55	80
	19-50	45	60	85
Vitamin D μg/day AI (UL)	14-50	5 (80)	5 (80)	5 (80)
Vitamin E (α-tocopherol equivalents) mg/day AI (UL)	14-18	8 (250)	8 (300)	12 (300)
	19-50	7 (300)	7 (300)	11 (300)
Vitamin K μg/day AI	14-18	55	60	60
	19-50	60	60	60
Choline mg/day AI (UL)	14-18	400 (3000)	415 (3000)	525 (3000)
	19-50	425 (3500)	440 (3500)	550 (3500)
Calcium mg/day RDI (UL)	14-18	1300 (2500)	1300 (2500)	1300 (2500)
	19-50	1000 (2500)	1000 (2500)	1000 (2500)
Phosphorus mg/day RDI (UL)	14-18	1250 (4000)	1250 (3500)	1250 (4000)
	19-50	1000 (4000)	1000 (3500)	1000 (4000)
Zinc mg/day RDI (UL)	14-18	7 (35)	10 (35)	11 (35)
	19-50	8 (40)	11 (40)	12 (40)
Iron mg/day RDI (UL)	14-18	15 (45)	27 (45)	10 (45)
	19-50	18 (45)	27 (45)	9 (45)
Magnesium mg/day RDI (UL)	14-18	360 (350)	400 (350)	360 (350)
	19-30	310 (350)	350 (350)	310 (350)

Nutrient	Age (years)	Nonpregnant	Pregnant	Lactating
	31-50	320 (350)	360 (350)	320 (350)
Iodine μg/day RDI (UL)	14-18	150 (900)	220 (900)	270 (900)
	19-50	150 (1100)	220 (1100)	270 (1100)
Selenium μg/day RDI (UL)	14-50	60 (400)	65 (400)	75 (400)
Molybdenum μg/day RDI (UL)	14-18	42 (1700)	50 (1700)	50 (1700)
	19-50	45 (2000)	50 (2000)	50 (2000)
Copper mg/day AI (UL)	14-18	1.1 (8)	1.2 (8)	1.4 (8)
	19-50	1.2 (10)	1.3 (10)	1.5 (10)
Chromium μg/day AI	14-18	24	30	45
	19-50	25	30	45
Manganese mg/day AI	14-18	3	5	5
	19-50	5	5	5
Fluoride mg/day AI (UL)	14-50	3 (10)	3 (10)	3 (10)
Sodium mg/day AI (UL)	14-50	460-920 (2300)	460-920 (2300)	460-920 (2300)
Potassium mg/day AI	14-18	2600	2800	3200
	19-50	2800	2800	3200

AI, adequate intake; RDI, recommended dietary intake; UL, upper level of intake, which includes nutrients from all sources .

Data tabulated from the Nutrient Reference Values for Australia and New Zealand, National Health and Medical Research Council, Commonwealth of Australia, 2006[239].

An estimated average requirement (EAR) is defined as 'a daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group'[239]. It is used to estimate the prevalence of inadequate intakes within a group. Recommended dietary intakes are calculated from the EARs. However, RDIs are more appropriate when assessing an individual's dietary intake, as they are the levels defined to meet the known nutritional needs of practically all (97.5%) healthy people[239]. When EARs and hence RDIs are not available, adequate intakes (AIs) are shown instead. Adequate intakes are 'the average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate'[239]. The upper level (UL) of intake is the 'highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population'[239]. The potential risk of adverse effects increases at an intake above the UL[239].

Most nutrient requirements increase in response to pregnancy, and further still with lactation. Niacin (vitamin B3), folate, iron and magnesium requirements are exceptions to this, given their requirements are highest during pregnancy.

2.4.1.2 Folic acid and neural tube defects

The best available evidence supports the use of folic acid supplements in the lead up to and during the early stages of pregnancy[240]. Research has shown this nutrient to have the capacity to prevent 72% of all cases of NTDs[241]. Spina bifida, encephalocoele, and anencephaly are examples of NTDs, which result from a failure of the fetal brain and spinal cord to develop normally[240]. Less than 40% of those affected by NTDs *in utero* survive to birth, and those that do survive may experience life-long disability[240]. In 2001, the total prevalence of NTDs in Australia was estimated at 1.4 per 1000 births, which includes terminations of pregnancy[242]. For the same period, the estimated birth prevalence of NTDs was 0.6 per 1000 births[242]. The prevalence in Indigenous Australians is estimated to be almost double the non-Indigenous rates[243].

Current guidelines recommend a 500 µg per day folic acid supplement to be taken at least one month prior to conception and for the first three months of the pregnancy[240]. Women who have had a previous pregnancy affected by a NTD, who have a family history including on the father's side, or have been treated for epilepsy, are considered to be at high risk and are recommended to take 10 times the minimal dose (i.e. 5 mg folic acid per day during the periconception period)[244]. Surveys assessing the public awareness of the link between folate and NTDs show large interstate variation, with figures of between 30% and 63% awareness reported[245-248]. Furthermore, knowledge about pre-pregnancy supplementation is only useful when pregnancies are planned. In excess of 40%, or an estimated 200,000 Australian pregnancies each year are unplanned[249]. Hence strategies to increase incidental folate intakes at the population level have been initiated[240]. The primary strategy is the fortification of breads and cereals within the Australian food supply, which was trialled on a voluntary basis for 10 years in lead up to the Mandatory Folic Acid Fortification Standard being accepted in June 2007[250]. The mechanisms by which folic acid supplementation prevents NTDs are not well understood, although most studies suggest that it is correcting a pre-existing nutritional deficiency. Others suggests that it compensates for a common genetic trait

which causes those affected to ineffectively utilise the folate available with their diet[251] (refer to section 7.1).

2.4.1.3 Sources and endogenous production of vitamin B12

In nature all vitamin B12 is synthesised by bacteria, fungi and algae[252]. Animal food products contain vitamin B12, because animals ingest the microorganisms containing the vitamin B12, or because they absorb some of the vitamin produced by their intestinal bacteria[252]. Some plant foods, mushrooms for example, may be contaminated with vitamin B12 synthesising bacteria[252]. However, these foods cannot be considered a reliable source of the vitamin[252]. It remains unclear whether this source is biologically active vitamin B12 or an analogue, and they appear in quantities too small to be considered of dietary significance[252]. Therefore women who do not consume animal foods, such as vegans and vegetarians, are at risk of vitamin B12 deficiency. If sustained, this deficiency can manifest as megaloblastic anaemia[253]. Pernicious anaemia specifically refers to the gastric causes of vitamin B12 deficiency[253]. It results from atrophic gastritis and parietal cell loss, causing impaired absorption due to an inadequate production of intrinsic factor[253].

Like other animals, the bacteria of the human digestive tract also produce considerable amounts of active vitamin B12[252]. Yet our ability to absorb this source is questionable[252]. Small amounts may be synthesised within the small intestine, and evidence suggests that some of this can be absorbed[254, 255]. Most is produced too far down in the colon to be absorbed and ends up being excreted[256, 257]. Interestingly, it is reported that Indian vegetarians have larger colonies of intestinal microflora than their nonvegetarian counterparts[258]. In a study of 441 rural, slum and urban Indian men by Yajnik *et al.* (2006), there was an inverse association between higher education and income, and reduced plasma vitamin B12 (pB12)[259]. The results reported pB12 levels which were 39% lower for urban middle-class men compared to those from slum regions (median values 89 and 145 pmol/L respectively)[259]. The authors suggest that this association could reflect a lack of microbial vitamin B12 from ingestion of contaminated food and water, as well as recycled colonic bacteria[259].

2.4.1.4 Decline of vitamin B12 in pregnancy

The reference values for nonpregnant women are often applied in the assessment of vitamin B12 status in pregnancy because, until recently, reference values specific to this population had not been established. Plasma vitamin B12 is known to decline as pregnancy progresses, with Koebnick *et al.* (2002) estimating the ratio between the third and first trimester levels to be 0.7 (95% CI 0.62-0.79)[260]. While this study was limited by the small sample size of 39 German women, Morkbak *et al.* (2007) have published similar data in a cohort of 140 Danish women[261]. Data from the same Danish group, although reported for a larger sample (*n* 437), has since been used to generate reference intervals for a range of haematological indices during pregnancy, including pB12[262].

Transcobalamin and haptocorrin are the two vitamin B12 binding-proteins present in human plasma[263] (refer to Figure 2.8). When circulating in the blood these proteins may be either partly saturated (holo) or partly unsaturated (apo)[263]. The decline in plasma B12 seen in pregnancy is thought to be a normal event caused by changes in the cobalamins attached to haptocorrin[261]. The precise function of haptocorrin is unknown[264]. The decline does not reflect a change in holotranscobalamin (holoTC), which is the biologically active vitamin B12 fraction that is delivered to all tissues in the body[264]. Morkbak *et al.* (2007) have reported that holoTC remains unchanged during normal pregnancy, and that it may be a better marker of true vitamin B12 deficiency than pB12[261]. Alternatively, elevated methylmalonic acid (MMA) and pHcy levels are highly sensitive (number of true positives) markers of vitamin B12 deficiency, although their reported specificities (number of true negatives) are lower[264].

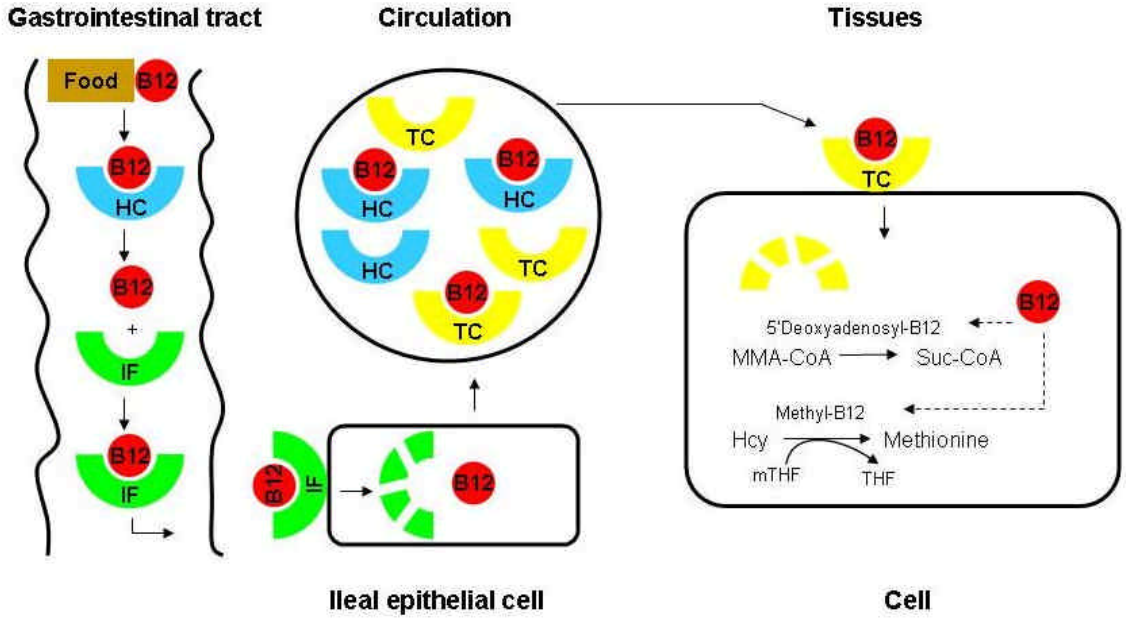

Figure 2.8 Vitamin B12 absorption from the gastrointestinal tract, transport in the circulation, and function within the cell

Vitamin B12 is supplied by animal products (food B12). After ingestion, dietary vitamin B12 enters the stomach bound to animal proteins and is released from the proteins by pepsin and hydrochloric acid. The free vitamin B12 is then bound to haptocorrin (HC, blue) released from the salivary glands. In the small intestine, haptocorrin is degraded by pancreatic enzymes, and vitamin B12 is transferred to intrinsic factor (IF, green), a protein synthesised in the gastric parietal cell and secreted into the gastric juice. The IF-vitamin B12 complex is internalised in the distal part of the small intestine by the IF-vitamin B12 receptor-complex cubilin-amnionless and thereafter IF is degraded by proteolysis.

Subsequently, only vitamin B12 enters the systemic circulation. Approximately 1% of the ingested vitamin B12 is believed to be taken up by passive diffusion in its free form, which may explain why vitamin B12 deficiency can be treated by a large dose of oral vitamin B12. In the circulation, vitamin B12 is bound to two proteins, transcobalamin (TC, yellow) and HC (blue). Vitamin B12 attached to TC is referred to as holotranscobalamin (holoTC). Holotranscobalamin represents the biologically active fraction that is delivered to all tissues of the body, whereas the function of haptocorrin is unknown. After cellular uptake of holoTC, transcobalamin is degraded, and vitamin B12 functions as a co-enzyme for two enzymatic reactions: the conversion of methylmalonyl coenzyme A (MMA-CoA) to succinyl-CoA (Suc-CoA), and the conversion of homocysteine (Hcy) to methionine, which is accompanied, in the same enzymatic reaction, by the conversion of methyltetrahydrofolate (mTHF) to tetrahydrofolate (THF). Because THF is needed for normal DNA synthesis, vitamin B12 deficiency results in impaired synthesis of DNA.

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2.4.2 Nutrient supplementation

Despite common practice[265], routine multivitamin supplementation is not recommended in pregnancy, unless the maternal diet is systematically inadequate or the mother is at an increased nutritional risk[3]. This may include cases of multiple pregnancy, substance abuse, vegan or strict vegetarianism, epilepsy, adolescent pregnancy and haemoglobinopathies[3].

The evidence for and against the use of nutrient supplements in pregnancy is summarised in Table 2.4. The Cochrane Library was searched for systematic reviews of nutrient supplementation in pregnancy, using the search terms 'pregnant' and 'supplement' in the title, abstract, or keyword, for records available on 25 June 2008. This method produced 191 available records, and of these a total of 17 systematic reviews and meta-analyses have been retrieved and summarised to give a global picture of nutrient supplementation recommendations for pregnancy. Articles were excluded if they did not specifically assess the effect of one or more nutrients supplemented before and/or during pregnancy.

A comprehensive table summarising each publication can be found in the Appendices. This preliminary review will be expanded as a component of a post-doctoral project arising from this thesis. This project aims to develop national evidence-based, best-practice guidelines for the use of nutritional supplements in pregnancy. It recently received competitive funding from the Dietitians Association of Australia. Table 2.4 presents a brief summary of these findings, indicating which nutrients have been studied and whether there is current evidence to support their recommendation.

Table 2.4 A summary of Cochrane systematic reviews and meta-analyses of nutrient supplementation for pregnancy

Records accessed 25 June 2008

Nutrient(s)	Recommended	Reason	Ref.*
Protein/energy	√/ X	Balanced protein/energy supplementation improves fetal growth; may reduce the risk of fetal and neonatal death Isocaloric-protein supplementation (i.e. without energy) may be harmful to the fetus	(266)
Long-chain polyunsaturated fatty acids	X	Not enough high quality evidence	(267)
Multiple-micronutrients	X	No advantage over combined iron and folate supplementation	(268)
Multiple (vitamin A, C, E, β-carotene, folic acid, iron, zinc, multivitamins)	X	Does not prevent miscarriage or stillbirth May: (i) increase risk of multiple births; (ii) reduce risk of pre-eclampsia	(269)
Other (carnitine, protein-free calf blood extract, amino acids, glucose)	X	Not enough high quality evidence	(270)
Antioxidants (vitamin A, C, E, β-carotene, lycopene, selenium, multivitamins)	X	Not enough high quality evidence	(271)
Vitamin A, β-carotene	X	Not enough high quality evidence	(272)
Vitamin D	X	Not enough high quality evidence	(273)
Vitamin E	X	Not enough high quality evidence	(274)
Folate	√	Strong protective effect against neural tube defects when taken periconceptionally It may increase the risk of multiple births	(275)
Vitamin B6 (pyridoxine)	X	Not enough high quality evidence	(276)
Vitamin C	X	Not enough high quality evidence	(277)
Iron	X	Not enough high quality evidence linked to clinically significant outcomes Concern about benefit versus harm	(278)
Iron with or without folic acid	X	Not enough high quality evidence related to clinical outcomes	(279)
Calcium	√	Reduces the risk of pre-eclampsia and maternal death or serious morbidity in high risk women No known adverse effects	(280)
Magnesium	X	Not enough high quality evidence	(281)
Zinc	X	Not enough high quality evidence	(282)

*Refer to List of References

Data from these meta-analyses show no benefit for either mother or child in terms of the clinical and other outcomes assessed for most nutritional supplements trialled in pregnancy, largely due to a lack of high quality research about their safety and efficacy.

Folate (refer to section 2.4.1.2), calcium and balanced protein/energy were the exceptions, and showed significant clinical advantages in the populations studied.

2.4.3 Dietary intake in pregnant women

A large number of observational studies which investigate the importance of one or more nutrients during pregnancy, in relation to pregnancy outcomes, have been published. However, a large degree of heterogeneity exists in the conclusions drawn by these studies, most likely as a result of methodological weaknesses. Study limitations include:

- Inaccuracies in the assessment of the nutrient intakes,
- Reporting biases,
- Measurement errors including those associated with variations in plasma volume expansion during pregnancy,
- Measurements of outcomes that are not subject to variation, and
- Confounding factors, such as drug use including cigarette smoking, the intake other nutrients and total energy, and socioeconomic status[283].

2.4.3.1 A national perspective

There are relatively few studies in Australia that have in any way reported the dietary intakes of pregnant women. An Australian National Nutrition Survey has not been conducted since 1995, making our most current data set considerably outdated. Nevertheless, 24-hour food recall and food frequency questionnaire (FFQ) data were collected as part of this survey, including from a small group of pregnant women (*n* 162 or 1% of all responders)[284]. A special article on *Food and Nutrient Consumption During Pregnancy*[285] was compiled using this data, and was released as part of the Australian Bureau of Statistics, Births Australia 1999 report[286]. Average energy and nutrient intakes from the 24-hour recalls were compared between pregnant and non-pregnant women, showing that while pregnant women were meeting or exceeding the Australian RDIs for most macro- and micronutrients, there were some key vitamins and minerals that fell short of national targets[285]. Pregnant women consumed, on average, approximately 13% less calcium than the RDI, 32% less folate, 34% less zinc, and 44% less iron[285]. However, it should be noted that the 1991 NHMRC RDIs for use in

Australia that were applied in this report have recently been updated with the release of the NRVs for Australia and New Zealand[239] (refer to section 2.4.1.1).

A recent and larger study, of high methodological quality, on diet during pregnancy has been undertaken in Adelaide, South Australia[287]. Moore *et al.* (2004) have conducted a prospective longitudinal study of 557 women aged 18 to 41 years, assessing dietary intake in early (before 16 weeks' gestation) and late pregnancy (between 30 and 34 weeks' gestation). They used a self-developed FFQ, validated against four-day weighed food records (WFRs)[287]. Seventy-seven percent of the cohort provided plausible dietary data based on the energy exclusion criterion of 1.7 times greater than, or 0.3 times less than, the individual's estimated energy requirements[287]. The macronutrient content of the mothers diet was then analysed as a potential predictor of birth outcomes, including birthweight, placental weight and ponderal index[287]. Adjustments were made for potentially confounding factors including pre-pregnancy weight, maternal weight gain during pregnancy, height, primiparity, smoking, and drug and alcohol use[287]. The authors showed a positive association between the proportion of protein in the early pregnancy maternal diet and birth outcomes: birthweight (coefficient (CE) 17.7, *P* 0.02), placental weight (CE 4.9, *P* 0.04), and ponderal index (CE 0.09, *P* 0.04), for those with reliable data[287, 288]. Micronutrient estimates have not been reported for this study.

Further macronutrient data for women during pregnancy were published in 2007 from the Tasmanian Infant Health Survey (TIHS), a cohort initiated to investigate sudden infant death syndrome (SIDS)[289]. This is despite the administration of the study's FFQ occurring almost two decades earlier (between 1988 and 1989)[289]. The FFQ used was developed for self-administration in adults, by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Division of Human Nutrition[290]. Mothers completed the FFQ soon after birth (*n* 1040), reporting on their dietary intake in their third trimester of pregnancy[289]. Energy cut-points of between 5 and 18 MJ per day were applied, with 892 records (86%) fulfilling this inclusion criteria[289]. The main finding from this study was that an increase in the absolute quantity of daily protein was inversely associated with birthweight and ponderal index, but positively associated with a relatively larger HC[289]. For each 1% increase in the proportion of energy from protein, birthweight was reduced by 12.7 grams (adjusted for infant sex, gestational age,

maternal smoking, maternal height, and total energy intake)[289]. This is in direct contrast to the previous findings of Moore *et al.* (2004). The absolute quantities of protein, carbohydrate and fat were considerably higher in the TIHS compared to that of Moore *et al.* (2004). However, the proportions of protein were very similar for the median, and lower and upper quartile values (16.3 *vs.* 16.2%, 14.7 *vs.* 14.4%, and 18.1 *vs.* 18.1% respectively)[287, 289]. Clearly further research is needed, as Andreasyan *et al.* (2007) acknowledge[289]. Additional measures of maternal diet (which would ideally include biomarkers of intake) during pregnancy and the relationship with neonatal outcomes (including body composition) are required.

Micronutrient dietary data have been reported for pregnant Australian women in two studies[291, 292]. Ash (1995) reported mean dietary intakes from three-day WFRs in 49 women from Sydney, New South Wales[291]. Jones *et al.* (2000) have utilised the same pregnancy food frequency data collected as part of the TIHS for their study of 173 mother-child pairs. They studied the relationship between maternal diet during pregnancy and the subsequent bone mineral density of the eight year old children, focusing on the micronutrients calcium, potassium, magnesium and phosphorus[292]. The likelihood of over-reporting in the TIHS again becomes evident, because of the high mean intakes of calcium: 1905 mg per day, compared to 1030 mg per day in the study of pregnant women in Sydney[291], and 962 mg per day in the National Nutrition Survey data[285]. However, some under-reporting is also likely to be inherent in both the Ash (1995) study[291] and 1995 National Nutrition Survey[293].

There are few other studies investigating diet during pregnancy in Australia. Of those that are available, there are several factors which limit their relevance, hence only brief details are provided. Two relate to single micronutrients only, including one which developed a checklist for iron (*n* 179)[294], and a case-control study of non-neural birth defects related to folate intake from both dietary and supplement sources (*n* 465 cases, *n* 578 controls)[295]. Another case-control study investigated dietary fat intake as a potential explanation of the recurrence of gestational diabetes (*n* 14 cases, *n* 21 controls)[296]. Energy intake and macronutrient contributions at 29 to 35 weeks' gestation were investigated in a study of 80 twin pregnancies, showing minimal changes in energy intakes and only a weak association between a reduced carbohydrate intake and advancing gestation[297]. The only other work on maternal energy and nutrient

intakes, and pregnancy outcomes in Australia was conducted during the 1950s[298, 299], 1960s[300] and late 1980s[301]. Since then there have been considerable changes in the Australian food supply, and the population's eating behaviours, which makes the findings from these early studies less suitable for application to a modern cohort. Nevertheless, Hankin *et al.* (1964) showed no correlation between protein and energy intakes and the baby's birthweight[300], while Woodhill *et al.* (1955) and found an association between poor maternal diet and the incidence of toxaemia and prematurity[299].

2.4.3.2 Internationally

There are a number of large studies internationally which have published data on dietary intake during pregnancy, again producing conflicting results. Two contemporary studies that have been conducted in relatively similar populations within the UK are that of Godfrey *et al.* (1996)[302] and Matthews *et al.* (1999)[303]. Godfrey *et al.* studied the dietary intakes of 538 pregnant women from Southampton in relation to placental and fetal growth[302]. Diet was assessed in early (before 17 weeks' gestation) and late (at around 32 weeks' gestation) pregnancy, using semi-quantitative FFQs, which recorded information about the previous three months of intake. The 100-item FFQ has been validated in a cohort of 569 pregnant women from Southampton[304]. During the validation study, the FFQ was issued at 15 weeks' gestation, and compared against four-day food diaries, which were recorded at 16 weeks of pregnancy[304]. The authors were satisfied that the FFQ provided meaningful estimates of nutrient intakes early in pregnancy and that it was able to rank individuals within the distribution[304]. From this dietary data, they found that placental size and birthweight were inversely associated with maternal carbohydrate intake, particularly sugars, early in pregnancy[302]. When carbohydrate intake early in pregnancy was taken into account, low intakes of protein late in pregnancy were associated with decreased birth and placental weights[302].

On the other hand, Mathews *et al.* (1999) considered the dietary intakes of 693 women in Portsmouth, again in early (at 12 weeks' gestation) and then later (at 28 weeks' gestation) pregnancy. Seven-day food diaries were completed early in pregnancy and then a FFQ was issued later in gestation[303]. This study showed no association between macronutrients at either time-point with the size of either the placenta or baby at birth[303]. The authors of this study suggest their data may be less biased by

measurement error than that of Godfrey *et al.* (1996)[303]. A comparison of the variability of the nutrient intake data, particularly energy and carbohydrates, showed considerably less variation in the Matthews *et al.* (1999) study. Furthermore, this was almost the same as the variability for women in the national dietary and nutritional survey of British adults, which used seven-day weighed food diaries[303, 305]. Only nutritional data from term pregnancies were included in both studies. However, gestational diabetes was not part of the exclusion criteria and no discussion was provided on the potential for confounding within the two cohorts.

Data collected in 1983 as part of the Special Supplementation Program for Women, Infants and Children in the United States was used to further explore the relationship between dietary protein intake during pregnancy and birthweight, in a large cohort of 2187 women[306]. Protein intake was estimated using an average of two 24-hour dietary recalls, the first of which was generally completed between four and six months gestation, the second in the eighth month of pregnancy[306]. Sloan *et al.* (2001) divided their analysis according to pre-determined groups of low (less than 50 grams per day), intermediate (50 to 84.9 grams per day) and high (greater than or equal to 85 grams per day) protein intake and found that both high and low intakes were associated with lower birthweights (-71 grams, *P* 0.009 and -77 grams, *P* 0.021, respectively)[306]. These findings are hard to interpret given the authors have not reported other macronutrient intakes (just energy). Others have also questioned the validity of the data, highlighting that women in the high protein intake group were significantly lighter than those in the low-protein intake group, yet reportedly consumed twice the daily energy[288].

These differences found even within similar populations in the UK, and in the Australian studies by Moore *et al.* (2004)[287] and Andreasyan *et al.* (2007)[289], demonstrate how observational research on nutrient intakes in pregnancy have the potential to produce mixed results, despite similar adjustments for potential confounders. The time points at which maternal diet is assessed and the methods of dietary measurement are obvious factors which may contribute to the discrepancies found throughout current literature. When the dynamics of fetal growth and development are considered, it appears biologically plausible that maternal effects on the fetus may vary according to the nutritional environment at the time of conception and with advancing gestation pregnancy[288]. Hence, studying this phenomenon at

different times, using different techniques which all have inherent measurement errors, biases and limitations, yields incompatible results.

As far as experimental human studies are concerned, a recent Cochrane systematic review on protein and energy intake in pregnancy was undertaken[266] (refer to section 2.4.2). Thirteen randomised trials involving 4665 women were available for 'balanced energy and protein supplementation', defined as less than 25% of total energy from protein. Overall, balanced energy and protein supplementation resulted in higher birthweight (random effects weighted mean difference 37.62 grams, 95% CI -0.21, 75.45) and lower relative risk (RR) of SGA (RR 0.68, 95% CI 0.56, 0.84)[266]. However, even within this meta-analysis the data concerning protein intake was somewhat incongruous, showing Isocaloric protein supplements (i.e. without energy supplementation) may be harmful to the fetus, by increasing the risk of SGA (RR 1.35, 95% CI 1.12, 1.61)[266].

Two independent follow-up studies in Scotland (Aberdeen[307] and Motherwell[308]) have reported a link between maternal intakes of protein and later life blood pressure in the offspring. The Aberdeen study reported two groups of pregnant women produced offspring with elevated blood pressure as adults at age 40 years: (i) those who consumed more than 50 grams of animal protein per day but had low carbohydrate intakes; (ii) those who consumed less than 50 grams of animal protein per day but had high carbohydrate intakes[307]. In the Motherwell study, high maternal intakes of meat and fish, and low intakes of carbohydrate during late pregnancy was positively associated with blood pressure in the male and female offspring at age 27 to 30 years[308].

Taken together it appears that, at least in Western settings, that the balance of macronutrients in a women's diet during pregnancy can influence outcomes for the offspring in both the short and longer term. While the effects on birthweight are modest at best, we now understand this to be a crude marker of predicting the child's future health[288]. Also, altering the nutritional plane of a woman during pregnancy may be detrimental to the developing child and few nutritional recommendations can be made based on the state of current evidence. The absence of association as is often reported may reflect the resilience of the fetus in expressing perturbations at birth, however we now appreciate that the effects may not be seen until later in life[169].

2.4.3.3 Quality versus quantity

Amid the confusion, questions of diet quality in terms of micronutrients, rather than absolute quantity in terms of energy, also need to be raised. Diet quality considers women who may be able to meet their energy and protein requirements, but fall short of micronutrient targets. It may include, but is not limited to, women consuming energy-dense but nutrient-poor diets. This has been described in relation to the increasing prevalence of overweight and obesity[309, 310]. In fact, Laraia *et al.* (2007) have reported that pre-pregnancy BMI is inversely associated with diet quality during pregnancy, in a cohort of 2394 women participating in the Pregnancy, Infection and Nutrition study in North Carolina, USA[311]. Diet quality was estimated from a self-reported FFQ at 26 to 28 weeks' gestation, using the Diet Quality Index for Pregnancy (DQI-P) developed by Bodnar and Siega-Riz (2002)[312].

As extensively reviewed by others, maternal micronutrient intakes during pregnancy are likely to be of importance to pregnancy outcomes, potentially including LBW and prematurity [313-316]. Micronutrients are integrally involved at the cellular level, including in protein translation, enzymatic reactions and regulation of gene expression[317], make this biologically plausible. But, evidence which elucidates the mechanistic pathways is lacking. Nonetheless it is well established that micronutrient deficits during embryonic and fetal development can induce anomalies in the offspring[318]. For example, a causal relationship exists between iodine deficiency in pregnancy and cretinism (impaired physical and mental growth) in the offspring[319]. The causal relationship between inadequate periconceptional maternal folate and NTDs has previously been described (refer to section 2.4.1.2). Yet many studies, including interventions, have found no association between either single or multiple micronutrient intakes during pregnancy and birth size[320] or other outcomes (refer to section 2.4.2).

Observational data certainly suggests that micronutrient intakes during pregnancy may be an important predictor of fetal outcomes, including growth. In the Pune Maternal Nutrition Study in India (refer to section 2.2.3) the size of the newborn infant was studied in relation to the mother's dietary intake (24-hour recall and FFQ), as well as circulating micronutrient concentrations during the pregnancy[80]. Birth size, described by weight, length, HC and placental size, were positively associated with an increased frequency of maternal consumption of micronutrient-rich foods, including green leafy

vegetables, fruits and milk products during the pregnancy[80]. Furthermore the authors showed a significant association between the biomarkers, erythrocyte folate and serum vitamin C, and the reported frequency of consumption of green leafy vegetable and fruits, which are respectively good food sources[80].

The same research team has reported follow-up data on the six-year old children born to the mothers who were studied during pregnancy[143]. Interestingly the offspring born to mothers with both high erythrocyte folate and low pB12 in pregnancy, were the most insulin resistant[143]. This lends support to the concept that studying single micronutrients in isolation from one another may be inappropriate, particularly where multiple deficiencies are likely to exist[313].

Watson and McDonald (2007) have recently published interesting data on the seasonal variation of nutrient intakes in pregnancy for a cohort of 197 women from the lower North Island, New Zealand[321]. Sixteen days of weighed diet records were collected for each participant: two lots of eight-day diet records, at four and seven months' gestation. For the micronutrients, statistically significant seasonal variations in vitamin C and D, B vitamins, β-carotene, sodium, potassium, calcium, phosphorous, sulphur, chloride, zinc and selenium were found[321]. The authors hypothesise, with support from their data, that the observed seasonal variations in maternal dietary intake may be influencing the development of conditions known to be related to season of birth, including type one schizophrenia, multiple sclerosis, type one diabetes and longevity[321].

Investigating micronutrients, like other dietary studies, is accompanied by a number of limitations that are hard to overcome. Firstly, the detection of deficiency and inadequate intake from dietary sources is constrained by the measurement tools and how they are employed. Secondary or 'conditioned' deficiencies may arise through mechanisms which are even harder to measure and account for, including genetic variations, nutrient-nutrient interactions, and drug-nutrient interactions[318].

In conclusion, there is some evidence to support the importance of maternal dietary quality and micronutrient status with later outcomes for the offspring. However, deductions from the available literature are obscured by the considerable heterogeneity that exists. The different responses may be, in part, related to the level of undernutrition present in the population studied[317]. Further, some effects of maternal micronutrient

status during pregnancy may not alter the outcomes typically measured, such as birthweight, but are nevertheless important for the subsequent health of the offspring. Finally, the timing of measuring the maternal diet and the window that is attempting to be captured (bearing in mind the dynamics of human pregnancy) may influence the ability to detect where true difference exist.

2.5 EARLY GROWTH AND DEVELOPMENT

2.5.1 Fetal growth

The fetus is thought to have an inherent growth potential that, under normal circumstances, yields a healthy newborn of appropriate size[322, 323]. Infants born preterm (before 37 completed weeks' gestation) or post-term (after 42 completed weeks' gestation), as well as those born too small or large for their gestational age and/or genetic growth potential, are at an increased risk of perinatal morbidity and mortality[323, 324]. Population standards are used in classifying fetal growth and size at birth[324].

Small for gestational age is defined by a fetal weight below the tenth percentile for gestational age[322]. It includes, but is not limited to, infants who are constitutionally small but have reached their growth potential[322]. Defining IUGR is more problematic, in that we do not know the inherent growth potential for any given fetus[324]. A fetus whose weight is at the fiftieth percentile for age but whose genetic potential was for the ninetieth percentile could be considered growth restricted[324]. However, the most widely used definition of IUGR is a fetus whose estimated weight is below the tenth percentile (i.e. SGA) and whose AC is below the second and a half percentile[322]. It is estimated that approximately 70% of SGA fetuses are constitutionally small, with the remaining 30% IUGR[322].

In Western societies the most important risk factor for reduced fetal growth is cigarette smoking, followed by low pre-pregnancy BMI and low gestational weight gain[283]. Other important risk factors for IUGR include a previous IUGR fetus, marked maternal hypertension (pregnancy induced hypertension or preeclampsia), a uterine anomaly (bicornuate uterus or large fibroids), and placental haemorrhage[324]. Mother's height is another strong predictor of the baby's size at birth[325]. Interestingly, cigarette smoking during pregnancy and maternal height have been shown to be unrelated to the blood pressure of the offspring at ages four to seven years in two studies by different research groups[326, 327]. The first was conducted in the Salisbury health district, UK, in a cohort of 405 children ages four years[326]. The second study, published one year later, included a large cohort of 3360 European children aged five to seven years[327].

The classification of IUGR has historically been based on the morphological characteristics of the fetus[324]. Symmetric IUGR describes a fetus whose entire body is proportionally small throughout the pregnancy[322]. This usually results from a first trimester insult, such as a chromosomal abnormality or infection[324]. Asymmetric IUGR describes a fetus who is undernourished, usually as a result of placental insufficiency, and is directing most of its energy to maintaining the growth of its vital organs, in particular the brain and heart, at the expense of the liver, muscle and fat[322]. A fetus with asymmetric IUGR has a normal head dimension but a small AC (due to decreased liver size), scrawny limbs (because of decreased muscle mass) and reduced skinfold thicknesses, because of decreased subcutaneous fat[322]. If the insult causing asymmetric growth restriction is sustained long enough, or is severe enough, the fetus may lose its brain-sparing ability and become more symmetrically growth restricted[322, 324]. Intrauterine growth restricted fetuses are at risk long-term of neurological and intellectual impairment when delivered at term, however early delivery (at 34 to 35 weeks' gestation) can largely correct for this[324].

Given that major methodological limitations were detected in the earlier studies which established fetal growth charts[328], Altman and Chitty (1993 and 1994) published guidelines on the key design and analytical components required to appropriately construct fetal reference growth charts[328, 329]. There are now numerous reference data available for the assessment of fetal biometric measurements, including for the head[330-332], abdomen[330, 332, 333], femur[330, 332, 334] and other limb bones[335], and kidneys and renal pelvis[336]. The Australasian Society for the Study of Ultrasound in Medicine has produced a statement on normal ultrasonic fetal measurements, which is designed to ensure uniform reporting of obstetric measurements across Australia and New Zealand[337]. Their recommended charts are based on an Australian cohort of 3800 pregnancies, for which 11,600 measurements of fetal parameters were collected at 26 different practices, over a three year period[337].

2.5.2 Estimating gestational age

Gestational age throughout the pregnancy and at birth is an important covariate in maternal-fetal research, acting as an exposure, effect modifier, predictor and determinant of appropriate intrauterine growth[338]. Although not easily measured, there

are three primary methods of estimating the gestational age of a fetus: (i) dating based on LMP, (ii) ultrasound-based dating and (iii) neonatal estimates[339]. Each method offers strengths and limitations, which need to be carefully considered.

2.5.2.1 Last menstrual period dating

Last menstrual period estimation assumes that the average menstrual cycle is 28 days in length, with ovulation (a proxy for conception) occurring on day 14. The due date is based on the assumption of an average pregnancy lasting 40 weeks from the first day of the LMP. Several factors may influence the accuracy of this estimation including: the regularity of menstrual periods, the usual length of the menstrual cycle, any hormonal disturbances prior to conception (including oral contraceptive use or a prior pregnancy), break-through bleeding between menstrual periods, and reporting errors[338, 339].

2.5.2.2 Ultrasound dating

Fetal ultrasound measurements can be compared with gestational age-specific references, such as crown-to-rump length or biparietal diameter (BPD), to determine gestational age[332]. Early ultrasound assessment, ideally between eight and 13 weeks' gestation, is more precise for estimating gestational age than ultrasound later in pregnancy[322]. Ultrasound estimations should be performed no later than the estimated twentieth gestational week, when the margin of error based on the LMP is seven to 10 days[322]. Given the routine use of ultrasound scans in antenatal care within Australia, it is increasingly common for LMP estimates to be verified using this technique. The main limitation is that estimations for symmetrically large or small fetuses will be biased. The formula used to calculate gestational age from fetal growth parameters have been developed using reliable LMP dates from assisted pregnancies as the gold standard. However, delayed ovulation may bias these estimations in the same direction as the dates calculated using LMP[339].

2.5.2.3 Neonatal estimates

Where little or no antenatal care is administered, gestational age can be retrospectively determined using either the Dubowitz[340, 341] or Ballard[342, 343] neonatal examination technique. Clinicians are able to estimate the gestational age at birth by assessing the physical and neuromuscular maturity of the infant using a standardised scoring system.

Of the three methods of estimating gestational age, this technique is the least precise. While it may hold some clinical value, it is not ideal for research purposes[339].

2.5.3 Fetal nutrition

Although it is now evident that fetal growth is also dependent upon fetal nutrition, the extent to which maternal dietary composition contributes to this is widely debated. The interaction between the complex chain of supply from both existing maternal stores and the current nutritional environment, and the variation in utilisation must be carefully regulated to result in optimal fetal growth and development. Fetal nutrition is affected by: (i) maternal oxygenated blood supply to the uterine circulation; (ii) the quality of placentation and the placental transfer function; and (iii) the ability of the fetus to derive the nutrients from the placenta for accretion within its own tissues[344].

The size of the placenta, an indirect measure of its ability to convey nutrients to the fetus, is strongly associated with the offspring's size at birth[29]. Periconceptional nutritional status appears to modify the effect of the maternal diet on placental size. Robinson *et al.* (1994) have reported experimental data in sheep, showing poorly nourished ewes around the time of conception, who subsequently consumed a nutrient-dense diet early in pregnancy, produced larger placentas and hence increased fetal growth[345]. Conversely, smaller placentas were seen in the ewes who were well-nourished before conception, who also consumed a nutrient-dense diet early in pregnancy[345]. While paradoxical, it has been common practice within sheep farming to liberalise ewe feeding prior to mating, and then in early pregnancy move them to a poor pasture for a period[325]. These experimental findings add strength to the observational data in humans linking a high carbohydrate diet in early pregnancy with a reduced placental size, particularly when in combination with low dairy protein intakes late in pregnancy (refer to section 2.4.3.2)[302]. This effect was independent of the mother's body size, social class and smoking, and resulted in a low ponderal index at birth[346].

Lumley (1998) has also published human data, using records for infants born prior to (pre-famine controls) and during (famine-exposed) the war-induced Dutch famine (refer to Chapter 1), linking maternal undernutrition early in pregnancy to increased placental size[347]. However, a corresponding increase in birthweight was not detected for these infants[347]. The placental ratio (the placental weight to birthweight) has been described

as an important predictor of future health[58]. Martyn *et al.* (1996) reported a U-shaped curve relating the placental ratio to later CHD, from their follow-up data of men born in Hertfordshire and Sheffield, UK[58], suggesting both high and low placental ratios are potentially adverse.

2.5.4 Sex-specific differences

Thinness at birth and reduced length at birth are different manifestations of reduced fetal growth. It is therefore not surprising that each is associated with different biological risk factors for CHD[348]. In their recent review of the sex differences in the fetal programming of hypertension, Grigore *et al.* (2008) suggest that in both men and women CHD is associated with LBW[348]. Yet in women it is more strongly linked to reduced length at birth, whereas in men it is more strongly associated with thinness at birth, as measured by ponderal index[348].

2.5.4.1 Body composition

It is now well documented that body composition differences between males and females are evident from birth[349, 350]. Females have a greater fat mass and lower lean mass than their male counterparts[349-352]. Rigo *et al.* (1998) have estimated this to be approximately 50 grams more fat in female newborns, compared to males, in their study of healthy term (n 53), and preterm infants (n 53) [352]. Females are also shorter and weigh less than males at birth and throughout infancy[350, 353]. In contrast, there is no difference in infant bone mass according to sex[354, 355].

2.5.4.2 Morbidity and mortality

Sexually dimorphic differences are further evident at birth when morbidity and mortality are considered. A Norwegian study of 1.69 million singleton births between 1967 and 1998 has shown higher rates of perinatal mortality and preterm delivery in male offspring[356]. Engel *et al.* (2008) published concordant results in their secondary data analysis of 16445 Australian births[357]. They showed that male fetuses were 1.5 times more likely to die of stillbirth, independent of other known risk factors (IUGR, birth defects, gestational age, Aboriginal ethnicity, previous stillbirth, parity greater than three, and placental abruption)[357].

Clifton and colleagues have studied the sexually dimorphic effects of maternal asthma as an intrauterine stressor, on placental glucocorticoid metabolism and fetal growth[358]. It is suggested that male fetuses are more likely to maintain intrauterine growth via a degree of glucocorticoid insensitivity, which may be advantageous for *ex utero* survival, although risky for birth outcomes[358]. Female fetuses appear more responsive to the maternal stressor, and will compromise fetal growth to promote the chance of survival[358]. From an evolutionary perspective, larger male size may be relevant to male–male competition, whereas reduced female size may be advantageous to the high energy demands of future reproduction[359].

2.5.5 Infant growth

Despite the drastic change in environment, infant growth may be viewed as a continuation of the fetal growth trajectory. Consequently birthweight and weight at one year are closely related[28]. However, this relationship creates difficulties in separating the effects of the intrauterine and early postnatal nutritional environments[28].

Section 2.2.4 describes the phenomenon of catch-up growth, as often seen in infants born preterm and/or SGA. It is important to note here that the findings relating rapid growth in infancy to adverse health outcomes later in life cannot be applied in the same way to full term, appropriately sized infants[28].

In the Netherlands, Veening *et al.* (2003) have conducted a small, contemporary study of β-cell capacity and insulin sensitivity in prepubertal children born either SGA (*n* 28, mean age 9.1 ± 1.1 years) or appropriate for gestational age (AGA) (*n* 22, mean age 9.0 ± 1.1 years)[360]. Consistent with other data[361], they showed that SGA children with the highest BMI were the most insulin resistant and therefore were at greater risk of developing type two diabetes[360]. There was no difference in the insulin resistance of the AGA children, based on a higher (modestly overweight) current BMI[360].

2.5.5.1 Overweight and obesity

There is strong evidence for the tracking of obesity[362]. High birthweight is a risk factor for becoming overweight as an adult[51, 73, 74, 363-365] (refer to section 2.2.2.3). High BMI in childhood is also associated with an increased risk of obesity[74, 366-368]. An early adiposity rebound is also a predictor of later obesity (refer to section 2.2.6)[113, 114]. The

risk of adult obesity is at least twice as high for obese children as compared to non-obese, and it increases with higher BMI and with the advancing age of the child[366].

Recently Ong *et al.* (2000) demonstrated in a cohort of 848 full term singletons that catch-up growth between birth and two years was associated with greater BMI, fat mass and central fat distribution at five years of age[369] (refer to section 2.2.4). The infants who gained greater than 0.67 standard deviations between birth and two years were lighter, shorter, and thinner at birth. This was compared to the infants who tracked within their birth percentiles, or crossed down to a lower percentile (catch-down growth)[369].

2.6 PUBLIC HEALTH AND THE LIFE-COURSE PERSPECTIVE

The heart of public health lies in a comprehensive understanding of the ways in which lifestyles and living conditions determine health status[370]. There is a recognised need to mobilise resources and invest wisely in policies, programmes and services which create, maintain and protect health[370]. The developmental origins field offers great potential for globally reducing the burden of disease via primary prevention. In fact, the World Health Organization (WHO) has recognised the importance of early life environments and advocates the adoption of a life-course perspective for chronic disease prevention[371]. Yet an evidence gap related to early intervention continues to endorse inaction, which is compounded by the rule of *first do no harm*[372].

There is no question as to whether maternal nutritional status and diet can influence the outcomes of pregnancy[373]. The causal relationships that exist for folate and NTDs (refer to 2.4.1.2), or alcohol intake during pregnancy and fetal alcohol spectrum disorders[374] provide direct evidence for this. Though there are many gaps in our knowledge which require thorough investigation prior to intervention. Law and Baird (2006) highlight some aspects for consideration, including: whether to act for the short or long term; what is the likelihood of benefit and/or risk of harm; and how strong is the evidence given the limitations of what can be done scientifically[372]. With the recent advances and growing interests in this research field, it is possible that in the future, primary prevention during pregnancy may extend to optimising the physiology and phenotype of the fetus for the postnatal nutritional environment to which they will be exposed[375]. However, as a recent review of human data from randomised controlled trials concluded, there is a real need for further research on the outcomes of nutritional interventions during pregnancy which factors in an evaluation of their public health relevance[376].

Part of the reason for the delays in intervention is that much of the experimental work has been conducted in animals. This is necessary to ensure that no adverse effects are experienced in human trials. But animal data somewhat complicates the issue, given that different species have different feeding behaviours and nutritional requirements. For example, sheep which are often studied as a developmental model have much

higher growth rates and much lower proportions of body fat compared to humans, as well as significant differences in the placentation process[377].

Basic science suggests that the greatest potential for programming the offspring occurs very early in life. This is highly appealing when you consider that interventions applied to the mother in the periconceptional period have the potential to influence outcomes for the resulting child, and potentially future generations[372]. One should note however, that functional changes *in utero* may increase or decrease the risk of disease, but over the life-course this programming is but one factor amongst many. The environmental, social and/or economic factors that exist for one generation may not be those encountered by the next. This creates the possibility of eliciting deleterious effects in the offspring, as seen in global regions undergoing rapid nutritional transitions (refer to section 2.2.2.1), but also within certain populations; for example those exposed to the Dutch famine (refer to Chapter 1). Therefore effective health strategies need to take this 'life-course perspective' rather than purely focusing on improving perinatal or pregnancy environments[378].

Taken together, the evidence for the DOHaD is convincing. But one needs to be careful when making a case for acting on this knowledge[378]. As Osler (2006) explains, the challenge of modern public health research is to disentangle the complex interplay between biological, behavioural, genetic and social factors which extend across the entire life-course[379]. Fathalla (1994) concluded in a world report that improvements in women's health require not only advances in science and health care, but also social justice for women and the removal of cultural barriers to equal opportunities[380]. A nutritional 'fix' without addressing the underlying causes of poverty and poor nutritional choices made within the context would be irresponsible[378]. To illustrate, Yajnik (2004) has cautioned that 'improvements' in maternal nutrition that simply increase maternal adiposity, and hence insulin resistance, could exacerbate the 'thin-fat' phenotype[59] (refer to section 2.2.3).

Chapter 3

Materials and Methods

3.1 WOMEN'S HEALTH AUSTRALIA

3.1.1 Ethics approval

The Australian Longitudinal Study on Women's Health (ALSWH), also known as Women's Health Australia, received ethics approval from the Human Research Ethics Committees for the University of Newcastle and the University of Queensland, prior to commencing in 1996. Researchers who are granted permission to utilise the data collected during this study are covered under the original ethics application.

3.1.2 Data acquisition

An expression of interest was lodged and accepted by Women's Health Australia in April 2006, with the aim of comparing the dietary intakes of Australian women during pregnancy and non-pregnancy. Permission was granted to analyse the dietary data and information pertaining to pregnancy status that were collected during Survey 3 in March 2003, from the young-aged cohort. Participants were aged 25 to 30 years at the issue of this survey.

3.1.3 Statistical analyses

Data manipulation and statistical analyses were performed using Statistical Analysis Systems (SAS) software package version 8 (SAS Institute, Cary, NC, USA)[381]. Descriptive statistics were undertaken and 95% confidence intervals were calculated on all proportions. General linear regressions, which included potential confounders as covariates in the model, were used. Given the large sample size and the number of comparisons that were performed, only *P*-values <0.001 were considered statistically significant. Tests of association were performed using c^2 analyses.

3.2 THE ABCD OBESITY STUDY

3.2.1 Ethics approval

The original ethics application for the Assessment Before Children Develop Obesity (ABCD Obesity) Study was made to the Hunter Area Research Ethics Committee (now the Hunter New England Human Research Ethics Committee) and the Human Research Ethics Committee of the University of Newcastle in May 2005. Approval from both committees was obtained in September 2005 (approval numbers: 05/06/08/3.14 and H-107-0905, respectively). Prior to commencing recruitment, a variation to the original application was submitted and approval was received in October 2005. Recruitment then commenced immediately and continued for the duration of six weeks. After this time it became evident that we were not going to be successful in meeting our sample size targets and subsequently our research objectives, due to the very poor rate of response (10%). Further methodological details are presented in Chapter 5 A Recruiting Failure Turned Success.

3.3 THE WATCH STUDY

3.3.1 Ethics approval

The original ethics application for the Women and Their Children's Health (WATCH) Study was submitted to the Hunter New England Human Research Ethics Committee in May 2006 and approval was received in June 2006 (approval number: 06/05/24/5.06). Note that the ethics application process had recently changed so that the same application no longer needed to be lodged with two separate committees (i.e. the University and the Area Health Service). Recruitment commenced immediately, and a very minor amendment to a questionnaire was accepted in an application of variance in July 2006, prior to the commencement of data collection.

3.3.2 Study design

The WATCH Study is a prospective longitudinal research project designed to span pregnancy and early childhood. It is conducted at the John Hunter Hospital, a large public health tertiary referral centre and major obstetric facility within northern New South Wales, Australia. The first study visit was usually between 18 and 20 weeks' gestation, to coincide with the fetal morphology scan. The follow-up visits and overall timeframe of the project are shown in Figure 3.1.

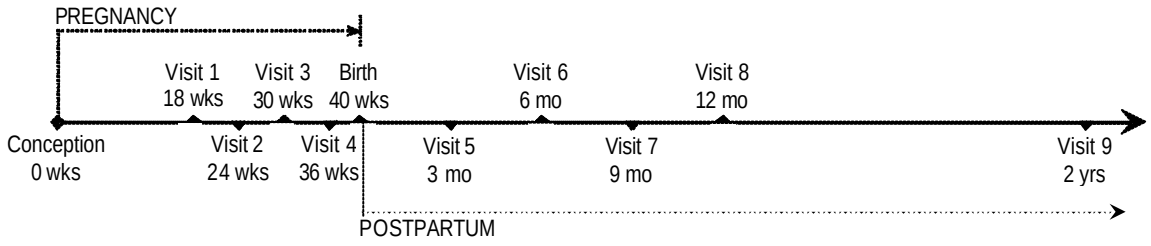
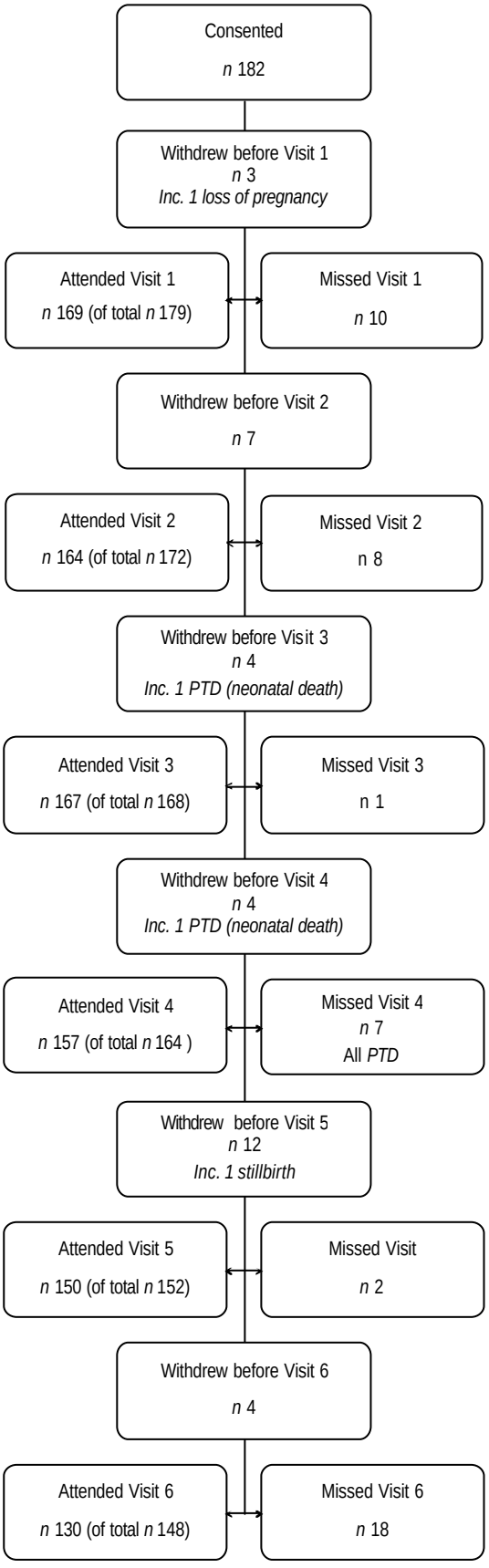


Figure 3.1 Timeline of the WATCH Study visits, from approximately 18 weeks' gestation to two years after birth

3.3.3 Subjects

Recruitment occurred primarily as a result of research midwives approaching pregnant women, who were usually attending their booking-in visit at the Antenatal Clinic, about the study. A number of women were recruited as a result of local media coverage, and additionally, word-of-mouth. All women who were less than 18 weeks pregnant were

considered eligible to participate, including women receiving antenatal care from alternative (private) antenatal facilities. Only those who gave informed consent were enrolled in the study. Figure 3.2 shows the recruitment outcomes for the WATCH Study and the attrition up to six months postpartum (visit six). Recruitment spanned the 18 month period between June 2006 and December 2007.



PTD, preterm delivery

Figure 3.2 Attrition for the WATCH Study up to six months after birth (visit 6) for the 182 women who gave their consent to participate

3.3.4 Data collection

Table 3.1 provides a chronological overview of the data that has been, and is currently being, collected as part of the WATCH Study.

Table 3.1 Data collection for the WATCH Study: when, what and who?

PREGNANCY	**=18 weeks' gestation**	
	Recruitment	Research Midwives, Word of mouth, Media
	Study visit 1: 20 weeks (± 2 weeks)	
	Maternal blood test 1 (fasting sample 1)	HAPS
	Ultrasound 1 (including fetal anomaly scan)	Obstetrician/Sonographer
	Maternal anthropometry, including own birthweight, pre-pregnancy weight, current weight, height, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Questionnaires issued: 4d-WFR no. 1; FFQ no. 1 (average of previous 6 months intake); physical activity; weight-related behaviours; medical; socio-economic	Dietitian
	Study visit 2: 24 weeks (± 2 weeks)	
	Maternal blood test 2 (non-fasting sample 1)	HAPS
	Ultrasound 2	Obstetrician/Sonographer
	Maternal anthropometry, including current weight, second height, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Study visit 3: 30 weeks (± 2 weeks)	
	Maternal blood test 3 (non-fasting sample 2)	HAPS
	Ultrasound 3	Obstetrician/Sonographer
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Study visit 4: 36 weeks (± 2 weeks)	
	Maternal blood test 4 (fasting sample 2)	HAPS
	Ultrasound 4	Obstetrician/Sonographer
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Questionnaires issued: 4d-WFR no. 2; FFQ no. 2 (average of previous 6 months intake)	Dietitian
	Birth	
	Maternal blood test 5 (non-fasting sample 3)	Delivery suite midwives
	Fetal (cord) blood sample	Delivery suite midwives
	Newborn anthropometry, including birthweight, length, head circumference	Neonatal nurses
	Study visit 5: 3 months postpartum	
	Maternal blood test 6 (fasting sample 3)	HAPS
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Infant anthropometry, including current weight, length, head circumference, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Blood pressure for mother and child	Dietitian
	Infant feeding history	Dietitian
	Questionnaires issued: 4d-WFR no. 3; FFQ no. 3 (average of previous 3 months intake); paternal anthropometry	Dietitian

POSTPARTUM	**Study visit 6: 6 months**	
	Maternal blood test 7 (fasting sample 4)	HAPS
	Optional child blood test 1 (non-fasting sample 1)	HAPS
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Infant anthropometry, including current weight, length, head circumference, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Blood pressure for mother and child	Dietitian
	Infant feeding history	Dietitian
	Questionnaires issued: 4d-WFR no. 4; FFQ no. 4 (average of previous 3 months intake); physical activity; child 4d-WFR no. 1	Dietitian
	Study visit 7: 9 months	
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Infant anthropometry, including current weight, length, head circumference, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Blood pressure for mother and child	Dietitian
	Infant feeding history	Dietitian
	24-hour recall of infant intake	Dietitian
	Study visit 8: 12 months	
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Infant anthropometry, including current weight, length, head circumference, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Infant feeding history	Dietitian
	24-hour recall of infant intake	Dietitian
	Study visit 9: 2 years	
	Maternal blood test 8 (fasting sample 5)	HAPS
	Optional child blood test 2 (non-fasting sample 2)	HAPS
	Maternal anthropometry, including current weight, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Child anthropometry, including current weight, length, head circumference, skinfold thicknesses, girths	Dietitian/Anthropometrist
	Blood pressure for mother and child	Dietitian
	Child feeding history	Dietitian
	Questionnaires issued: 4d-WFR no. 5; FFQ no. 5 (average of previous 12 months intake); physical activity; child 4d-WFR no. 2; ages and stages	Dietitian
	Ongoing follow-up is under consideration	

FFQ, food frequency questionnaire; HAPS, Hunter Area Pathology Service; 4d-WFR, Four day weighed food record.

The length of follow-up, number of study visits, and sheer quantity of data that has been, and is currently being, collected as part of this study means that it is unfeasible to present comprehensive results for all data components outlined in Table 3.1. The data that has been analysed and presented relates directly to the aims and hypotheses of the chapters presented in this thesis.

3.3.4.1 Blood tests and biochemistry

Study blood samples were collected and assayed by Hunter Area Pathology Service (HAPS), with the exception of the maternal sample during labour and fetal cord blood, which were collected by the delivery suite midwives.

Fasting maternal blood samples have been tested for the following:

- Lipids (total cholesterol, triglycerides, HDL-C, LDL-C)
- C-reactive protein
- Insulin
- Glucose
- Glycosylated haemoglobin (HbA1c)
- Red cell folate (rcFol) and plasma folate (pFol)
- Plasma vitamin B12
- Plasma homocysteine

Remaining blood has been separated into red cells and plasma which been stored for future analysis.

Cord blood samples have been had a full blood count performed. Remaining red cells and plasma have been stored for future analysis.

At the six month infant follow-up, mothers had the option of consenting to the collection of a five millilitre infant blood sample, collected by a paediatric phlebotomist. Infant blood samples have undergone a full blood count and been tested for pB12, pFol and/or rcFol, and pHcy. Any remaining red cells and plasma have been stored for future analysis.

3.3.4.2 Ultrasound scans

All consenting women were booked to have four ultrasound examinations, at approximately 20, 24, 30, and 36 weeks' gestation (plus or minus two weeks). The study ultrasound scans were performed by a team of three clinical obstetricians and three accredited sonographers. Efforts were made to book each participant with the same obstetrician or sonographer for all four ultrasound scans, in an attempt to reduce potential inter-observer variation in the scan measurements.

The first ultrasound was generally performed between 18 and 20 weeks' gestation when confirmation of the gestation and the fetal anomaly scanning were undertaken. Where the menstrual dating of the pregnancy was within one week of the ultrasound dating the menstrual dates were used to determine gestation. However, if the menstrual dates were greater than one week outside the ultrasound dating the ultrasound dating was used to determine gestation. Ultrasound was performed using an Acuson Aspen® (AspenUltrasound, Oceanside, California, USA) ultrasound with a curvilinear array transducer.

Each ultrasound scan was carried out in accordance with a preset study protocol, which included standard fetal biometry. The BPD was measured as the standard axial view of the fetal head including the thalami and third ventricle (including only the calvarial diameter) and excluding the soft tissues of the scalp. The HC was measured at the same level as the BPD and included the normal soft tissues of the scalp. The AC was obtained from the transverse ultrasound of the fetal abdomen at the level of the fetal stomach and the portal vein and measured the perimeter of the abdomen including the soft tissues. The femur length (FL) was measured excluding the cartilaginous epiphyses[382].

The fetal fat estimation was performed at the level of the standard measurement of AC and at the fetal mid-thigh region. For the abdominal fat and lean mass estimation, the total area of the fetal abdomen (A_1) was calculated. Then the abdominal area excluding the hyperechoic subcutaneous fat layer was calculated (A_2). The abdominal fetal fat area was then the difference of the two areas (i.e. $A_1 - A_2$ cm^2). The measurements of fetal mid-thigh were taken following the standard measurement of the fetal femoral length and over the mid point of the femur the transducer was rotated 90^o to obtain a cross-sectional view of the fetal mid-thigh[383]. The total cross-sectional area of the fetal mid-thigh (T_1) was calculated as was the total cross-sectional area of the hypoechoic fetal mid-thigh muscle tissues (T_2). The calculated fetal mid-thigh fat tissue area was the difference of the two areas (i.e. T_1-T_2 cm^2)[383].

3.3.4.3 Anthropometry

With the exception of the stipulated self report data (i.e. pre-pregnancy maternal weight and paternal height, weight and waist circumference), physical measurements obtained during the study were collected at the John Hunter Hospital. Maternal anthropometry

has been collected by a team of three dietitians, each with level one anthropometrist certification from the International Society for the Advancement of Kinanthropometry (ISAK). Infant anthropometry has been collected by only two of the three dietitians performing the maternal anthropometry. Measurements have been conducted in accordance with the ISAK[384]. One exception to this was the mid-thigh circumference, which was measured directly in line with the front thigh® skinfold site[384], at the mid-point between the inguinal fold and the superior border of the patella (while the leg is bent at 90°)[385].

Following the ISAK protocol, the right side of the body was used irrespective of the preferred side. Where possible, two measurements were taken at each of the specified sites and then averaged. If the second measurement was not within 7.5% of the first measure for skinfolds, and 1.5% for girths, a third measure was taken and the median value was used. Note that these ranges are slightly higher than the standards set by ISAK of 5% variation for skinfolds and 1% variation for girths[384]. This was to accommodate potential inter-observer variability and the difficulties in measuring young children. Sites were measured in succession to avoid experimenter bias in addition to residual fat compression.

3.3.4.3.1 Skinfolds

Where possible, maternal skinfold thicknesses were measured at the triceps®, subscapular®, biceps®, supraspinale®, front thigh® and medial calf®[384], using the same set of Harpenden skinfold calipers (Holtain Ltd, Crosswell, UK). These skinfold sites are shown in Figure 3.3.

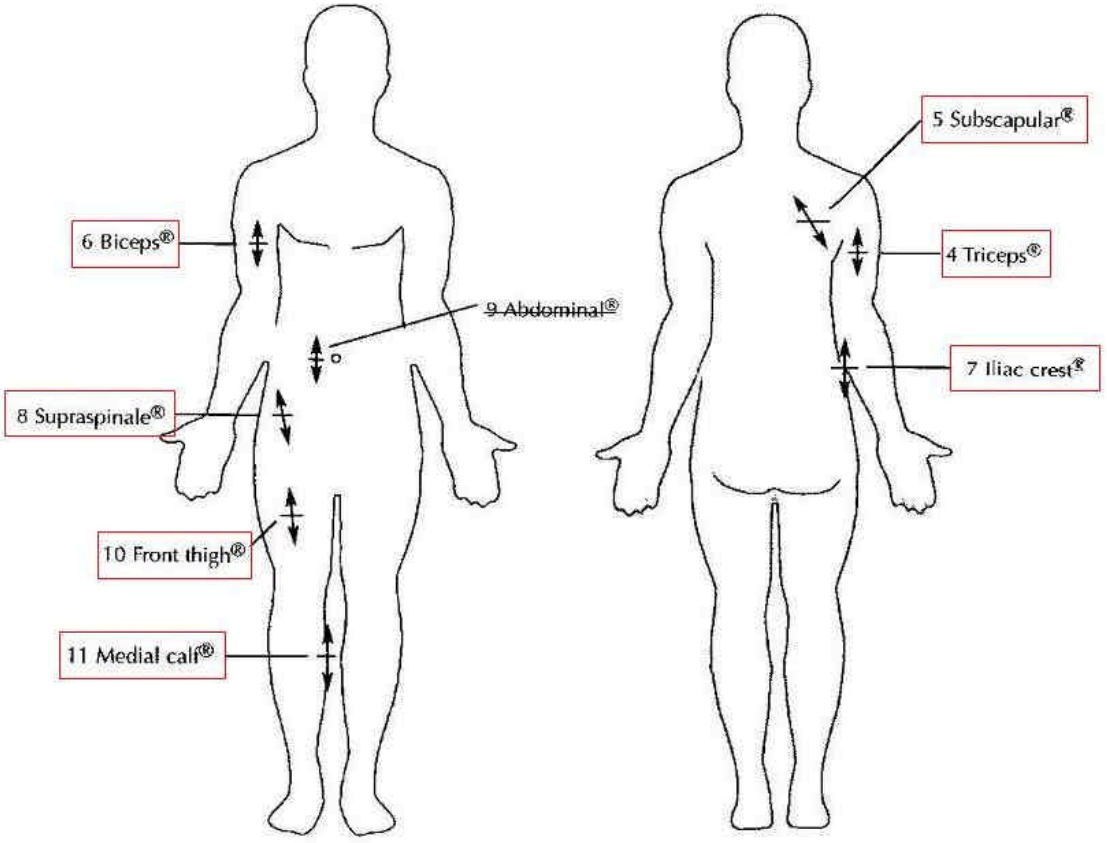


Figure 3.3 Location of skinfold sites, as defined by the International Society for the Advancement of Kinanthropometry

Anterior view (left), posterior view (right). WATCH measurements highlighted by boxes.

Reprinted from the International Standards for Anthropometric Assessment, M Marfell-Jones, T Olds, A Stewart, and L Carter, Anatomical landmarks, p.27, 2006, with permission from Timothy Olds[384].

Where possible, infant skinfold thicknesses were measured at the subscapular®, biceps®, iliac crest®, and medial calf®[384], using the same newly calibrated set of Harpenden skinfold calipers (Holtain Ltd, Crosswell, UK).

3.3.4.3.2 Girths

Maternal girths were measured at the mid-upper arm (arm relaxed®), wrist, waist®, gluteal (hip)®, mid-thigh and calf®[384] using a Lufkin Executive Thinline flexible steel measuring tape (W606PM, Cooper Hand Tools, North Carolina, USA). This anthropometric tape measures up to two metres in length and does not stretch with repeated use. Infant girths were measured at the mid-upper arm (arm relaxed®), wrist,

abdomen (at the level of the umbilicus), mid-thigh and calf®. The locations of the girth measures are shown in Figure 3.4.

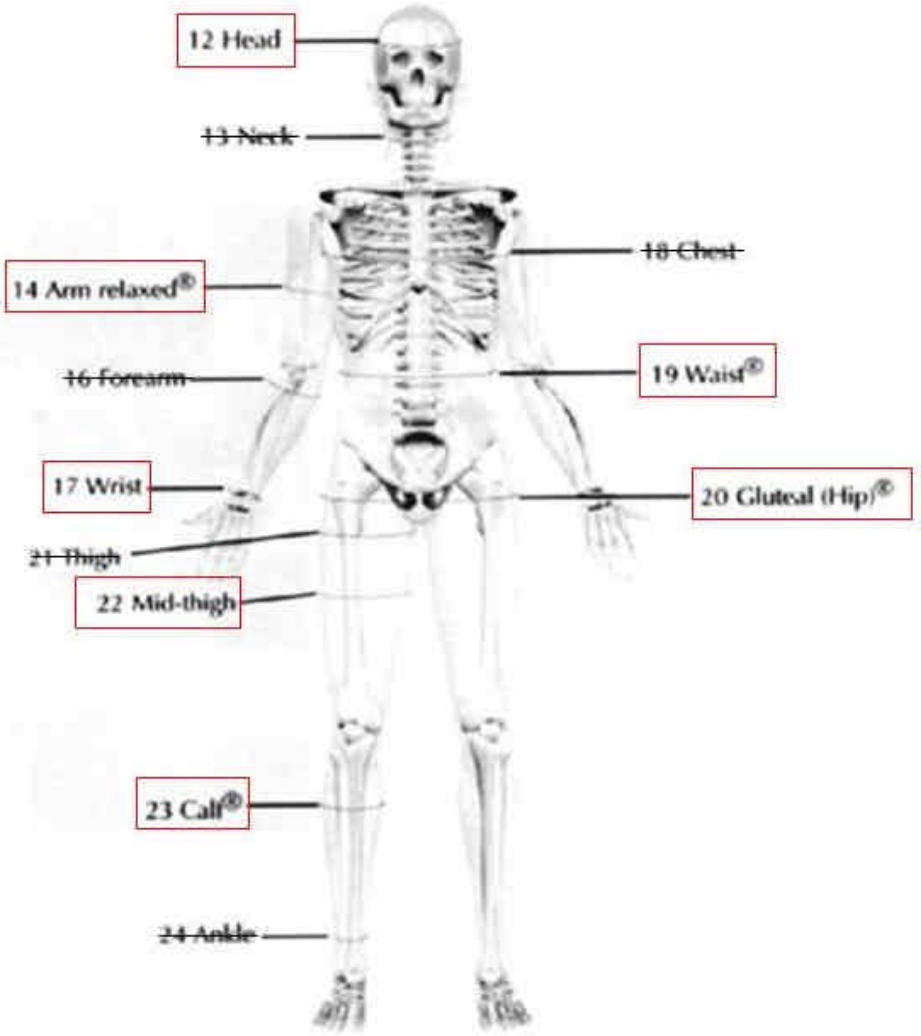


Figure 3.4 Location of the girth measurements, as defined by the International Society for the Advancement of Kinanthropometry

WATCH measurements highlighted by boxes.

Reprinted from the International Standards for Anthropometric Assessment, M Marfell-Jones, T Olds, A Stewart, and L Carter, Girths, p.78, 2006, with permission from Timothy Olds[384].

3.3.4.3.3 Height

Standing height without shoes was measured to a precision of one millimetre, at two study visits, using the same wall-mounted Seca stadiometer (Seca Deutschland, Hamburg, Germany). The two readings were averaged, unless the measures differed by more than 1.5%, where a third measure was taken and the median value then used.

3.3.4.3.4 Length

The crown-to-heel length was measured at each study visit to a precision of one millimetre using a Harpenden Infant Measuring Table (Holtain Ltd, Crosswell, UK). Each measure was taken in duplicate and length calculated as the mean of the two measures. If two measures differed by more than 1.5%, length was measured a third time and the median value then used.

3.3.4.3.5 Weight

Maternal weight was measured at each study visit using the same set of annually calibrated AND^{TM} FV-150K electronic weighing scales (A&D Mercury Pty Ltd, Thebarton, South Australia). These scales weigh up to 150 kilograms and are accurate to 50 grams. Participants were asked to remove their shoes, excess clothing, and any items from their pockets prior to being weighed.

Maternal pre-pregnancy weight was self reported at the first study visit.

Infants were weighed naked at each study visit using the same set of electronic baby scale (Nuweigh LOG 244, Newcastle Weighing Services, Newcastle, Australia) with a precision of 10 grams.

3.3.4.3.6 Paternal anthropometry

Participants were provided with father's packs at the three month infant follow, to invite the father of the child to participate. This invitation was left to the discretion of the mother as to whether or not it was handed on. Included in the father's pack was a WATCH Study information pamphlet, a consent form, a self-report anthropometry recording sheet and a self-addressed, reply-paid envelope. Instructions were provided on how to report the data (refer to Appendices).

3.3.4.4 Dietary data

A triangulation (or mixed) method of dietary data collection was employed to facilitate completeness in dietary representation and eventually a validation of the information that is collected as part of the WATCH Study. Dietary intake was assessed using self-completed FFQs and four-day WFRs. Fasting blood samples were collected and assayed

for some markers of dietary intake, providing a marker of intake that is independent of human reporting and recording bias. Unused red cell and plasma samples have been stored for further biomarker analysis. Figure 3.5 provides a simple diagram of this dietary data collection technique.

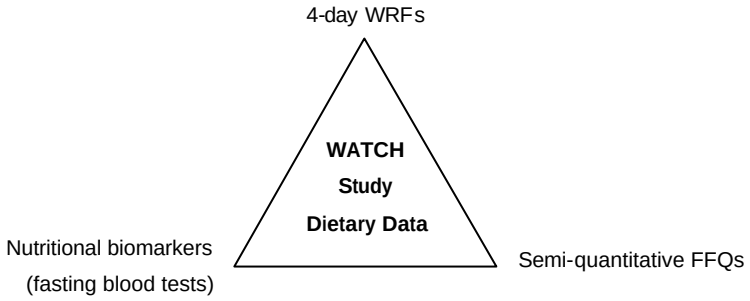


Figure 3.5 Triangulation method of the WATCH Study dietary data collection

3.3.4.4.1 Four-day weighed food records

Each participant was provided with a new set of SOEHNLE Venezia electronic kitchen scales (Soehnle-Waagen GmbH & Co, Murrhardt, Germany) for the recording of their WFRs. The Venezia scales weigh up to two kilograms and are precise to one gram in their measurements. The study dietitian demonstrated to each participant how to use these scales to quantify the amount of each food and beverage items consumed at the first study visit. Food diaries which included detailed instructions on how to complete the WFR, an example of 24 hours of dietary recording and blank recording sheets were issued with the scales, along with a self-addressed, reply-paid envelope for return of the records (refer to Appendices).

Briefly, participants were asked to record four days of dietary intake within two weeks of the study visit. The four selected days need not be consecutive, and one weekend day was to be included. Each day of recorded data was supposed to be representative of current daily intake and questions relating specifically to vitamin and mineral supplementation, and physical activity were included in the data recording sheets.

Weighed food records are being analysed using FoodWorks 2007[386], which includes the Nutrient Tables for use in Australia (NUTTAB) 1995, the most recent national government food composition database of Australian foods[387]. The database was supplemented with the direct analysis of recipes provided by participants and

manufacturers' information for products not found in the database. Dietary data is being entered by Accredited Practising Dietitians and an audit of approximately 5% of the entered records will be undertaken to ensure consistency in the entry method. Less than or equal to 10% variation in the daily energy intake will be considered acceptable between observers. Energy cut-points will be applied to reduce the biases associated with misreporting.

3.3.4.4.2 Anti Cancer Council of Victoria food frequency questionnaire

Usual dietary intake was assessed using a FFQ known as the Anti Cancer Council of Victoria Food Frequency Questionnaire (ACCVFFQ) or alternatively, the Dietary Questionnaire for Epidemiological Studies (DQES) version 2. The questionnaire was developed by the Cancer Council Victoria and the items included in the FFQ are based on those found in a pilot study to make important contributions to nutrient intakes from Australian-, Greek- and Italian-born cohort members[388]. Both the development of the questionnaire[388] and its validation in a cohort of young Australian women have been previously reported[389].

The ACCVFFQ are computer-scannable questionnaires which are purchased at a price that includes their translation into a spreadsheet of micro- and macronutrients. This data analysis is carried out by the distributors and results are returned in a Microsoft Excel format to facilitate easy importation of data into an appropriate statistical software package.

The questionnaire asks respondents to report their usual consumption of 74 foods and six alcoholic beverages over the preceding 12 months using a 10-point frequency scale. For the purposes of the WATCH Study these questionnaires were modified to ask respondents to report their usual consumption of the food and beverage items over the preceding three or six month periods at different time intervals during the study (refer to Table 3.1 for details).

The categories for reporting are: never, less than once per month, once per week, twice per week, three to four times per week, five to six times per week, once per day, twice per day, and three or more times per day. Questions on the total intakes of fruit and vegetables are used to adjust the intakes of individual fruits and vegetables, which tend to be over-estimated. Portion photographs of vegetables, potatoes, meat, and casserole

dishes are used to calculate a portion factor that is applied to scale up or down the standard portions of foods that showed variation by gender or ethnicity in the WFRs from which the FFQ was derived. Additional questions are asked about the number of serves or type of fruit, vegetables, bread, dairy products, eggs, fat spreads and sugar. Nutrient intakes are computed from NUTTAB 1995[387], using software developed by the Cancer Council of Victoria.

3.3.4.5 Physical activity

The Pregnancy Physical Activity Questionnaire (PPAQ) has previously been designed and validated against seven days of actigraph motion sensor data, in a small group of pregnant women in the United States[390]. It is self-administered and takes approximately 10 minutes to complete[390]. Subjects were asked to report their average time spent participating in 32 activities, classed as household, care-providing, occupational, sports or exercise, transportation, and inactive (sleeping not included) tasks. For each, respondents were asked to select the category that best approximated the amount of time spent, either per day or week during the current trimester. Durations ranged from zero to six or more hours per day, and from zero to three or more hours per week. There is an open-ended section at the end of the PPAQ that allows respondents to add activities which have not been listed.

This questionnaire was selected as there are very few physical activity questionnaires which have been developed specifically for women, let alone those who are pregnant[390]. The same questionnaire was re-issued to participants at the six month infant follow-up, with the title changed to 'Mothers Physical Activity Questionnaire' and the timeframe specified as 'during the last three months'.

3.3.4.6 Medical history and socioeconomic indicators

Medical history, medications, and vitamin and mineral supplementation were self-recorded using a single-page questionnaire issued at the first study visit. A similar questionnaire which asked about educational attainment, level of income (self and household), and marital status was also issued. The questions included in both were modelled on some of those found in the Women's Health Australia surveys.

Additionally, medical and socioeconomic data recorded in the ObstetriX database was extracted. ObstetriX is the major repository in New South Wales for recording antenatal information, patient and family history, and birth outcomes [391]. It was developed by the NSW Department of Health Obstet Consortium, in conjunction with Microsoft and Meridian Health Informatics [391].

3.3.4.7 Psychosocial constructs

The psychosocial constructs: locus of control; self-efficacy; body image; feelings about motherhood; and career orientation, were assessed using the USA 'Weight-Related Behaviors Questionnaire' developed by Kendall *et al.* (2001) in the United States[392]. This was a self-administered questionnaire with Likert scale responses ranging from 'strongly agree' to 'strongly disagree', 'too heavy' to 'too light', and 'very satisfied' to 'not at all satisfied'. Prior to analysis, the coding for some items needs to be reversed so that higher scale scores denote higher levels of the construct being measured[392].

3.3.4.8 Birth records

Birth outcomes, including neonatal anthropometry, were extracted from the ObstetriX database.

3.3.5 Statistical analyses

Data manipulation and statistical analyses were performed using Intercooled Stata 9 software (StataCorp LP, College Station, Texas, USA)[386]. Data has been tested for normality prior to analysis using histograms, normal probability plots and a comparison of the mean and median values. Where necessary, log transformations have been applied to achieve a Gaussian distribution. Other descriptive statistics such as scatter- and box plots are presented, along with inductive statistics, which include tests of associations: Pearson's c^2 test, Mann-Whitney U and Wilcoxon signed-rank test.

Linear regression, including analysis of variance (ANOVA), and linear mixed-models were also utilised. Linear mixed-models were particularly well suited to the longitudinal data, as they are able to handle observations which are not independent, as is the case with repeated measures on an individual over time. Linear mixed-models consider both between- and within- individual variation and are able to handle pieces of missing data

without excluding the dataset for that individual (unlike an ANOVA where the dataset for that individual would be excluded if one value was missing). In some analyses a summary statistic, rate of change, has been calculated using the standard mathematical formula $(Y_2-Y_1)/(X_2-X_1)$.

Given the relatively small sample size for the WATCH cohort, *P*-values <0.05 were considered statistically significant. Statistical support has been provided for the analyses presented in this thesis and the individuals who have assisted have been recognised in the Acknowledgements section of each chapter.

Chapter 4
Diet and Pregnancy Status in Australian Women

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4.1 INTRODUCTION

Maternal nutrition during pregnancy and in the periconception period is a key modifier of health outcomes for both mother and child, not only in the prenatal period but much longer-term[317, 393, 394]. There is now considerable evidence that variations in fetal nourishment can elicit permanent adjustments in a wide range of systems, including endocrine, organ, and metabolic, and that this intrauterine 'programming' may persist after birth[395]. It is therefore of interest to know at a population level about the dietary practices and nutritional adequacy of intakes in women of childbearing age. Additionally, there is a need to understand whether these are altered in the lead up to, and over the course of, pregnancy and whether changes persist postnatally.

There are very few large studies in Western populations that have looked at the dietary quality of pregnant women, whether in isolation or in reference to a non-pregnant control group. The Avon Longitudinal Study of Parents and Children (ALSPAC) is among the largest worldwide and has dietary data recorded for 11,923 women in the south-west of England at 32 weeks' gestation. The ALSPAC research team have assessed the adequacy of the diets of their pregnant participants and found that while most nutrients intakes met the national recommendations, there were others of importance, including folate, iron, magnesium and potassium, that did not[396]. However, this study did not include a control, non-pregnant cohort. Similar finding have been reported in a number of smaller studies in both the UK[397] and USA[398]. The Norwegian Mother and Child Cohort Study is currently underway with some dietary data published for 40,108 participants[399].

In Australia limited information is available about what pregnant women are consuming and their patterns of intake. There are a number of studies during pregnancy that include some form of dietary assessment[287, 294, 400]. Yet there are no nationally representative data reporting on total diet. The 1995 Australian National Nutrition Survey, which is the most current data, included food and nutrient intake data obtained using the 24-hour recall method and a complementary FFQ, completed by both pregnant and non-pregnant participants[284]. However, only 162 pregnant women (1.2% of all responders) were included in this survey and analysis. Like most other dietary studies in pregnancy, it has focused primarily on nutrient consumption[285]. While useful, nutrient consumption data

does not take into account dietary patterns or the actual foods and food groups from which they were derived.

Various studies have combined multiple dietary components into a single diet quality exposure or score, trading detailed nutrient information for a summative value[401-404]. Those that do so in accordance with evidence-based national dietary guidelines have been able to demonstrate correlations with indicators of morbidity and mortality[405]. This holds true for the very few studies assessing diet quality in pregnancy[404, 406]. Favourable outcomes, including reduced risk of NTDs[404] and increased birthweight[406], have been associated with better diet quality. It is within this context that we have used extensive dietary data on a large cohort of Australian women, to determine the association between pregnancy status and diet quality.

4.2 SUBJECTS AND METHODS

4.2.1 Data collection

The study used cross-sectional, self-reported data collected prospectively as part of the ALSWH. The ALSWH recruited 40,000 'young', 'mid-aged', and 'older' women with the issue of the baseline surveys in 1996. The research was designed to explore factors that relate to health promotion or diminution in women who are broadly representative of the Australian population. Ethical approval for the study was obtained from the Human Research Ethics Committee of the University of Newcastle. Further details of the ALSWH have been published elsewhere[407-409].

These analyses of diet quality in pregnancy and other states include data from the young cohort, who were aged 25 to 30 years at the time of completing Survey 3 in March 2003. The dietary data for the young cohort have not previously been published and this is, at present, the largest study in Australia to have investigated diet quality in women of childbearing age.

The DQES (version 2), a 200-item FFQ, was included in this survey. This FFQ has previously been validated in a cohort of young Australian women[389]. Collins *et al.* 2007 have reported a method for summarising diet quality using the DQES, and have measured the association between their Australian Recommended Food Score (ARFS) and indices of morbidity, including health service utilisation and self-perceived health[410, 411]. The ALSWH survey also included a range of measures of demographic characteristics, health behaviours and psychosocial measures including area of residence, country of birth, marital status, height, weight, smoking, frequency and intensity of physical activity, highest educational qualification, ability to manage on income, self-reported health and doctor-diagnosed medical conditions.

4.2.2 Sample

Of the total ALSWH cohort, 14,247 women aged 18 to 23 years participated in the baseline survey (Survey 1) of young women in 1996. This was estimated to be a 41 to 42% response rate for this aged cohort[408]. From the initial young cohort, 9076 women aged 25 to 30 years in 2003 completed Survey 3, with attrition being mainly due to young women having changed address and not being located[412]. Four groups were used

to define pregnancy status: (i) pregnant (*n* 606); (ii) trying to conceive (*n* 454); (iii) had a baby in the last 12 months (*n* 829); and (iv) other (*n* 5597). Subjects were excluded from these analyses if: (i) their pregnancy status could not be determined from their survey responses (*n* 111); (ii) they could be grouped into more than one pregnancy category (*n* 61) or (iii) their calculated energy intake was less than 4.5 MJ or greater than 20 MJ per day (*n* 1418). Energy values outside of this range were considered biologically improbable and indicative of misreporting. A total of 7486 women were included in the present analysis.

4.2.3 Australian Recommended Food Score

The development of the ARFS has been described in detail elsewhere[410, 411]. Briefly, it was modelled on the Recommended Food Score developed by Kant and Thompson in the USA[413]. The ARFS is calculated based on regular consumption of items listed in the DQES FFQ that are consistent with national recommendations including the Dietary Guidelines for Australian Adults[414] and the core foods as outlined in the Australian Guide to Healthy Eating[415]. Scoring is mostly independent of the reported quantities of foods consumed, and frequencies have been dichotomised, reducing the measurement error typically associated with FFQs. Foods that are not considered to make a beneficial contribution to dietary intake have not been scored, with the exception of ice-cream and cheese.

One point was allocated for each food or beverage item contained within the DQES that met the above criteria and was usually consumed once a week or more in the previous year. An additional point was available for specific types and amounts of core foods consumed including: at least two fruit serves daily; at least four vegetable serves daily; using high fibre, wholemeal, rye or multigrain breads; having at least four slices of bread daily; using polyunsaturated or monounsaturated spreads or no fat spread; having one or two eggs weekly; using reduced fat or skimmed milk; using soy milk; consuming at least 500mL of milk daily; using ricotta or cottage cheese; using low fat cheese; consuming ice-cream and cheese each less than once weekly; and consuming yoghurt more than once weekly. The two points that were available in the original ARFS for questions related to alcohol frequency and quantity of intake were removed from these analyses as the guidelines for safe alcohol consumption during pregnancy differ from

those for the non pregnant population[416]. Consequently the maximum ARFS that could be achieved was 72.

4.2.4 Nutrient Reference Values

The NHMRC of Australia has recently produced a set of NRVs which describe the amount of specific nutrients required on average, on a daily basis, for sustenance or avoidance of nutritional deficiency[239]. These have been described in section 2.4.1.1. Recommended dietary intakes are commonly cited in other studies, but are more appropriate when assessing an individual's dietary intake. Our study reports on group means, hence EARs have been presented whenever available. When they are not, AIs have been used instead.

4.2.5 Statistical analysis

To improve the validity of the dietary analyses, women with daily energy intakes less than 4.5 MJ or greater than 20 MJ per day were excluded. Often, BMR is calculated for each individual and the ratio of reported energy intake to BMR is used to help reduce erroneous data[417]. However, the weights were not recorded for pregnant participants in this study, and therefore BMR could not be calculated. Meltzer *et al.* (2008) have presented an alternative for handling uncertainties in reported dietary intake estimates for pregnant women, suggesting these energy values as appropriate cut-offs[399]. The same cut-offs have been applied across all pregnancy groups.

The characteristics of women in each of the pregnancy groups were compared and standard deviations were calculated for means. The relationship between pregnancy status and ARFS was assessed using a general linear model, with area of residence and educational attainment included as covariates, to adjust for the sampling frame and for socioeconomic status. Given the large sample size and the number of comparisons that were performed, *P*-values <0.001 were considered statistically significant, unless otherwise specified. Tests of association were performed using c^2 analyses. Comparisons of the food component scores that make up the ARFS were made between pregnancy groups. Mean energy and nutrient intakes by ARFS quintile (energy standardised per 1000 kcal) were calculated to ensure that the ARFS was indeed a measure of nutritional adequacy or diet quality in this cohort. All data manipulation and

statistical analyses were performed using the SAS software package version 8 (SAS Institute, Cary, NC, USA).

4.3 RESULTS

The women participating in the young cohort had a mean age of 27.2 years (SD 1.5). At Survey 3, 8.1% of women reported that they were currently pregnant, 6.1% reported that they were trying to conceive, and 11.1% reported having given birth in the previous 12 months. All women who were not in one of the above categories were classed as 'other' (74.8%). The participant demographics according to each pregnancy group are reported in Table 4.1.

Table 4.1 Participant demographics for the young cohort of the Australian Longitudinal Study on Women's Health according to pregnancy status

	Pregnant	Trying	Birth <12mo ago	Other
n (%)	606 (8.1)	454 (6.1)	829 (11.1)	5597 (74.8)
Mean age (yrs) (SD)	27.4* (1.4)	27.5* (1.4)	27.5* (1.3)	27.1 (1.5)
Mean height (cm) (SD)	165.7 (7.0)	166.7 (6.9)	166.3 (6.9)	166.2 (7.2)
Mean weight (kg) (SD)	†	70.4 (16.4)	69.4* (14.0)	67.1 (14.9)
% Born in Australia	94.8*	94.3*	94.2*	91.0
% Post school qualifications	70.1*	68.2*	65.6*	75.4
% Urban resident	65.1*	67.7*	60.0*	75.0
% Married/de facto relationship	96.2*	96.7*	93.9*	51.4
% Current smoker	9.2*	22.7	17.1*	25.9
% Depressive symptoms (‡CESD-10 score =10)	20.3*	22.4*	25.0	26.1
% Inactive/low level physical activity	68.9*	45.6*	62.3*	40.9
% Poor/fair self-reported health	3.9*	12.5*	6.7*	9.9
% Difficulty managing on available income	39.9	38.0	54.4*	38.4
n (%) excluded based on energy <4.5 MJ or >20 MJ per day	47 (7.2*)	81 (15.1)	58 (6.5*)	1212 (17.8)

*Statistically significant difference (P <0.05) compared to the 'other' group.
†Data on weight during pregnancy was not available.
‡CESD, Center for Epidemiologic Studies Depression Scale[418].

Women who reported being pregnant, trying to conceive or having given birth in the previous 12 months were more likely to be married or in a de facto relationship, and to live in a rural location, when compared to 'other' women. Women in the 'other' category were more likely to have been born outside Australia, to have post-school education, and to do more physical activity. A significantly lower proportion of women who were either pregnant or had given birth in the previous 12 months reported being a current smoker. Interestingly, women who were trying to conceive had a higher proportion of poor self-reported health compared to all other categories. Those who had

given birth in the previous 12 months were most likely to report having difficulty managing on their available income (54.4% compared to 38.4% for 'other' women).

Pregnancy status was not significantly predictive of the ARFS, even after adjusting for area of residence and education (Table 4.2). Women who were pregnant or had given birth in the previous 12 months had slightly higher ARFSs than the women classed as 'other' (*P* 0.006), although this was only a mean difference of 1.1 points.

Table 4.2 Unadjusted and adjusted* mean Australian Recommended Food Scores (ARFS) for the young cohort of the Australian Longitudinal Study on Women's Health according to pregnancy status

Pregnancy status	Unadjusted mean ARFS	SE	*P*-value	*Adjusted mean ARFS	SE	*P*-value
Pregnant (*n* 606, 8.1%)	30.2	0.4	0.007	30.2	0.4	0.006
Trying (*n* 454, 6.1%)	29.4	0.4	0.597	29.5	0.4	0.346
Birth <12mo ago (*n* 829, 11.1%)	30.0	0.3	0.023	30.2	0.3	0.002
Other (*n* 5597, 74.8%)	29.2	0.1	†	29.1	0.1	†

SE, standard error of the mean.
*Adjusted for level of education and area of residence.
†Reference to which all groups have been compared.

Examination of the component scores that make up the ARFS between pregnancy groups reveal very small absolute differences (Table 4.3). The vegetables component was overall the most highly scored group relative to the total number of points available in each component. Within the protein foods component (includes nut/bean/soy/egg, meat and fish) and overall, the nut/bean/soy/egg grouping was the most poorly scored, with on average less than two points obtained out of the available seven for all groups. Fish and grain component scores were also low relative to the other food groups.

Pregnant women performed better on their intake of fruit, grain, and meat compared to 'other' women. Similarly, women who had given birth in the previous 12 months gained more points from the grain and meat components. In all instances of statistical significance, there was less than one mean point of difference between pregnancy groups.

Table 4.3 Mean and standard deviation (SD) component scores and total Australian Recommended Food Scores (ARFS) for the young cohort of the Australian Longitudinal Study on Women's Health according to pregnancy status

Component scores (maximum)	Pregnant		Trying		Birth <12mo ago		Other	
	(*n* 606, 8.1%)		(*n* 454, 6.1%)		(*n* 829, 11.1%)		(*n* 5597, 74.8%)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Vegetables (22)	11.7	4.3	11.8	4.4	12.4	4.2	11.8	4.7
Fruit (14)	6.0*	3.2	5.1	3.2	5.4	3.2	5.1	3.3
Grain (14)	4.8*	1.8	4.4	1.8	4.8*	1.8	4.4	1.8
Dairy (7)	2.6	1.2	2.7	1.2	2.6	1.2	2.8	1.2
†Nut/beans/soy (7)	1.7	1.1	1.6	1.0	1.7	1.0	1.9	1.3
†Meat (5)	2.4*	1.2	2.5*	1.2	2.5*	1.2	2.2	1.3
†Fish (2)	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.8
Fat (1)	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.5
Total ARFS (72)	30.2	8.4	29.4	8.6	30.5	8.4	29.4	9.0

*Statistically significant (P <0.001) differences between groups.
†Subcomponents of the protein foods category.

Table 4.4 reports selected macro- and micronutrient mean intakes (±SD) by quintiles of ARFS, energy standardised per 1000 kcal. Quintile one reflects the lowest ARFS and quintile five the highest. Carbohydrate, fibre, sugars, protein, polyunsaturated fat, calcium, iron, zinc, β-carotene, folate, thiamin, riboflavin, niacin, vitamin C, vitamin E and potassium all increased as ARFS quintile increased. Conversely, total fat, saturated fat, monounsaturated fat and retinol decreased with increasing AFRS quintile. While the trend was less consistent for sodium, intake was highest in ARFS quintile two.

Table 4.4 Energy-standardised (per 1000 kcal) daily macro- and micronutrient intakes for the young cohort of the Australian Longitudinal Study on Women's Health by quintiles of Australian Recommended Food Score

Nutrients	Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5	
n (%)	1471 (19.7)		1448 (19.3)		1573 (21.0)		1574 (21.0)		1420 (19.0)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Carbohydrate (g)	108.1	15.4	110.1	15.3	111.1	15.2	112.3	14.6	112.9	15.2
Fibre (g)	9.7	2.3	10.7	2.6	11.4	2.9	12.2	3.0	13.0	3.2
Sugars (g)	46.1	14.2	48.0	14.1	49.4	13.4	51.0	12.8	52.0	12.6
Protein (g)	46.9	8.0	48.3	7.2	49.5	7.5	50.7	7.7	51.1	8.1
Total fat (g)	42.8	5.9	41.2	6.1	40.1	6.0	39.0	5.9	38.5	6.1
Saturated (g)	18.4	3.7	17.4	3.5	16.7	3.6	15.9	3.4	15.3	3.4
Polyunsaturated (g)	5.7	1.9	5.7	2.0	5.7	1.9	5.8	2.0	6.0	2.0
Monounsaturated (g)	14.9	2.4	14.4	2.4	14.1	2.4	13.8	2.4	13.6	2.5
Calcium (mg)	493.5	149.4	512.3	151.8	522.0	149.3	538.7	156.8	537.7	150.2
Iron (mg)	6.2	1.3	6.6	1.5	6.9	1.4	7.3	1.5	7.5	1.5
Zinc (mg)	6.0	1.2	6.2	1.1	6.3	1.1	6.5	1.1	6.5	1.1
Retinol equivalents (μg)	429.0	138.3	442.0	129.5	430.4	119.4	428.6	118.2	428.7	106.1
Retinol (μg)	219.0	73.8	204.9	74.2	190.5	69.3	178.5	67.3	168.9	65.7
β-carotene (μg)	1258.8	760.0	1420.1	702.8	1436.1	670.4	1497.1	660.6	1554.5	605.1
Folate (μg)	130.6	33.3	144.4	37.1	151.4	35.6	159.3	36.1	167.3	37.8
Thiamin (mg)	0.8	0.2	0.8	0.2	0.9	0.2	0.9	0.2	0.9	0.2
Riboflavin (mg)	1.2	0.4	1.3	0.4	1.4	0.3	1.4	0.4	1.5	0.4
Niacin equivalents (mg)	19.8	3.4	20.8	3.3	21.5	3.2	22.3	3.3	22.8	3.5
Niacin (mg)	10.7	2.3	11.5	2.4	11.9	2.2	12.4	2.3	12.8	2.4
Vitamin C (mg)	64.0	36.7	73.9	40.9	76.6	38.7	79.1	35.3	83.2	35.8

Nutrients	Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5	
n (%)	1471 (19.7)		1448 (19.3)		1573 (21.0)		1574 (21.0)		1420 (19.0)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Vitamin E (mg)	3.1	0.7	3.2	0.7	3.3	0.8	3.4	0.8	3.6	0.9
Sodium (mg)	1366.5	196.4	1399.3	194.4	1392.3	194.2	1393.1	187.5	1389.2	183.6
Potassium (mg)	1445.3	261.2	1511.1	262.5	1554.3	261.8	1610.6	266.4	1660.1	273.0
ARFS	17.1	3.5	24.1	1.4	29.0	1.4	34.3	1.7	42.6	4.1

All mean group intakes (Table 4.5) were above the EARs or AIs as outlined in Table 4.6 for protein, calcium, zinc, retinol equivalents, thiamin, riboflavin, niacin, and vitamin C. Nutrients that were consistently below the EARs or AIs included dietary fibre, folate and vitamin E. In order to meet the EAR, iron would need to have been markedly higher in the pregnant group. Mean sodium intakes were higher than the recommended UL of 2300 mg per day across all groups[239]. Potassium intake was lower than the recommended AIs for women trying to conceive and 'others'. As lactation status could not be confirmed, all groups have been compared to either the pregnant or non-pregnant references, however the NRVs for women aged 19-30 years who are lactating have been provided to show the differences in nutritional requirements for this group.

Table 4.5 Daily macro- and micronutrient intakes for the young cohort of the Australian Longitudinal Study on Women's Health according to pregnancy status

Nutrients	Pregnant		Trying		Birth <12mo ago		Other	
n (%)	606 (8.1)		454 (6.1)		829 (11.1)		5597 (74.8)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Energy (kJ)	7795.1	2326.6	7400.5	2282.0	8228.0	2396.0	7310.6	2272.1
Carbohydrate (g)	208.5	62.1	193.2	60.4	214.8	64.4	192.7	62.1
Fibre (g)	20.7	7.1	19.6	6.5	21.4	7.2	19.8	7.2
Sugars (g)	95.9	33.7	84.4	30.0	94.0	31.8	84.6	31.4
Protein (g)	89.0	29.8	88.8	30.1	95.2	30.0	86.2	30.1
Total fat (g)	75.6	26.6	71.9	27.7	81.6	27.7	70.9	27.0
Saturated (g)	32.3	13.0	30.3	13.0	34.5	12.9	29.3	12.4
Polyunsaturated (g)	10.6	4.3	9.9	4.3	11.4	4.8	10.1	4.7
Monounsaturated (g)	26.1	9.5	25.2	10.0	28.6	10.1	25.0	10.0
Calcium (mg)	1007.0	316.5	912.8	277.3	971.6	289.5	875.6	285.9
Iron (mg)	12.8	5.1	12.3	4.6	13.8	5.0	12.0	4.6
Zinc (mg)	11.5	4.2	11.4	4.1	12.5	4.3	11.0	4.1
Retinol equivalents (μg)	840.5	294.1	779.9	283.6	864.7	290.9	733.6	278.9
Retinol (μg)	411.4	163.7	338.5	156.5	404.9	158.2	325.0	156.7
β-carotene (μg)	2570.8	1286.3	2644.2	1324.0	2754.5	1290.9	2446.7	1294.3
Folate (μg)	284.4	106.2	265.9	92.0	288.9	97.4	259.7	94.4
Thiamine (mg)	1.7	0.7	1.6	0.6	1.7	0.6	1.5	0.6
Riboflavin (mg)	2.7	1.0	2.4	0.9	2.6	0.9	2.3	0.9
Niacin equivalents (mg)	39.4	14.4	38.6	13.6	42.0	13.8	37.6	13.6
Niacin (mg)	22.2	9.0	21.4	8.2	23.4	8.5	20.9	8.3
Vitamin C (mg)	152.1	86.3	135.5	81.9	140.1	78.5	129.0	74.5
Vitamin E (mg)	6.0	2.1	5.6	1.9	6.4	2.2	5.8	2.2
Sodium (mg)	2521.6	819.9	2474.3	885.3	2732.4	866.3	2430.3	834.6
Potassium (mg)	2950.6	888.4	2752.4	789.8	2946.6	823.5	2674.0	796.6

Table 4.6 Daily Nutrient Reference Values for Australia and New Zealand[239]: selected macro- and micronutrients

Nutrients	Women 19-30 yr		Pregnancy 19-30 yr		Lactation 19-30 yr	
	EAR	AI	EAR	AI	EAR	AI
Protein (g)	37		49*		54	
Fibre (g)		25		28		30
Calcium (mg)	840		840		840	
Iron (mg)	8.0		22		6.5	
Zinc (mg)	6.5		9.0		10.0	
Retinol equivalents (μg)	500		550		800	
Folate† (μg)	320		520		450	
Thiamin (mg)	0.9		1.2		1.2	
Riboflavin (mg)	0.9		1.2		1.3	
Niacin (mg)	11		14		13	
Vitamin C (mg)	30		40		60	
Vitamin E (mg)	7		7		11	
Sodium (mg)		460-920		460-920		460-920
Potassium (mg)		2800		2800		3200

EAR, estimated average requirement; AI, adequate intake.
*During second and third trimester only.
†This is for dietary intake. For pregnant women, it does not include the additional supplemental folic acid required to prevent neural tube defects.

4.4 DISCUSSION

This is the first comprehensive report of dietary intake in a nationally representative sample of Australian women, by pregnancy status. We have investigated the diet quality of a large cohort of young Australian women aged 25 to 30 years participating in the ALSWH and have compared nutrient intakes to the nationally recommended values. The findings indicate that there is room for improvement in dietary quality within this population.

Between pregnancy categories there were no differences in mean diet quality scores as summarised using the ARFS. The diet quality and variety of the young Australian cohort that we have studied appears to be suboptimal, as evident by the discrepancies between the recommended and reported intake levels, especially for folate, fibre and iron. These key nutrients have also been found to be at suboptimal levels of intake in pregnant women in the UK and Norway, and in pregnant and non-pregnant controls in the USA[396-398].

The ARFS has previously been developed to evaluate the diet quality of mid-aged Australian women and it has performed well in analyses of nutritional and some morbidity-related indices[410, 411]. While the absolute values of the ARFS are somewhat abstract, the diet quality score provides a useful means of ranking nutrient intakes and food variety at a population level. Analyses by component sub-scores highlight where improvements in specific food groups may be needed for young women and the food frequency data further delineates the 'at risk' nutrients.

No individual component accounted for a difference of one whole point or more on average between pregnancy categories and all components had potential for significant improvement in their scores. The grains and protein foods, especially nuts/beans/soy/eggs and fish, were the food groups most poorly scored across all pregnancy groups. To achieve a higher grain score, and thereby total ARFS, one would need to consume a variety of high fibre and wholegrain bread and cereals, and include basic ingredients like pasta, rice and noodles, on average more than once per week (no quantity specified). It is likely that the poor reported intake of grain-based products is

directly related to the low folate status of the young cohort, given that folate fortification of breads and cereals is common within the Australian food supply.

Nutritional requirements are generally higher during pregnancy and lactation with a few exceptions. For example, iron requirements are lowest during lactation. The intakes of the selected nutrients included in these analyses were consistently higher in women who were pregnant or had given birth in the previous 12 months, compared to those trying to conceive or otherwise not pregnant. However, some important nutrients consistently fell short of national targets. Most notable were the low intakes of dietary folate irrespective of pregnancy status. The EAR for folate is from dietary intake and does not include the additional folic acid required pre-pregnancy and during the first trimester to prevent NTDs[239]. This dietary deficit is a major cause for concern as many pregnancies are unplanned and supplementation may not occur prior to the closure of the neural tube, if at all. An analysis of recent population-based data from Victoria (The Victorian Survey of Recent Mothers 2000) and New South Wales (The NSW Child Health Survey 2001), Australia found that only 36% and 46% of women in these respective states used periconceptional folic acid supplements[238]. This emphasises the importance of dietary folate in women of childbearing age and lends support to the perceived need for mandatory folic acid fortification.

The sociodemographic data for this cohort has been compared to Australian census data and it is deemed to be a reasonably representative national sample[408]. We have subdivided this cohort according to whether the women reported being currently pregnant, a birth in the previous 12 months, actively trying to conceive, or 'other'. While childbearing years span beyond the 25 to 30 years aged cohort included in this study, there is evidence to suggest this as an age-appropriate target group. In 2004 the median age of Australian women giving birth was 30.0 years[419]. The average age of first-time mothers was 28.0 years and this group accounted for 42.2% of all women who gave birth[419].

In light of the original ARFS analyses that were undertaken[411], it would be reasonable to hypothesise that diet quality improves with age, at least in women between their late twenties and early fifties. However, the association between diet quality, pregnancy status, and age would require further investigation.

Clearly dietary requirements and intake change in response to pregnancy[420]. This study suggests that diet variety and representation of good quality foods do not necessarily improve in lead up to or during pregnancy. Results for 40,108 study participants enrolled in the Norwegian Mother and Child Cohort Study also support this, with evidence that pregnant women do not generally change the types of foods they consume but rather the relative amounts[399]. Exceptions to this may include soft drink, coffee and alcohol, however these beverage items have not been included in our analyses and are therefore unlikely to influence our results.

4.4.1.1 Limitations

The DQES asks participants to report their usual intake for the previous 12 months. A significant limitation of this study is that women did not specify how far along in their pregnancy they were at the time of completing the survey. It is well established that foods consumed near the time of completing an FFQ prime the memory such that the responses emphasise recently consumed foods[421]. However, some potential differences between categories may have been reduced or lost as a result of the extended time interval covered by this FFQ.

Measurement error is inherent in all methods of dietary data collection. For FFQs, this is introduced because of difficulties in estimating usual frequency and relative quantities of intake over time. The ARFS reduces this measurement error by dichotomising the frequency data and by including only a few points which relate to easily quantifiable amounts (for example, two fruit serves per day). The original validation study of the DQES which compared the FFQ with seven-day WFRs reports less than 10% variation in mean nutrient intakes for most nutrients (carbohydrate-related, and vitamin-A related nutrients varied more)[389].

The DQES is a computer scannable FFQ that is purchased and processed by the Cancer Council of Victoria, Australia. In the processing of the FFQs for the young cohort, any questions that were either missed or not answered by participants were coded as never having consumed that food or beverage item. This was a change in the analysis protocol between the processing of the mid-aged and young surveys. In the dietary analyses for the mid-aged cohort, missing data was coded as such. By replicating the same coding series for the mid-aged dataset, we were able to assess whether this change may have

affected the ARFSs obtained for the young cohort. A comparison of mean ARFS scores depending on coding protocol showed no significant difference.

It is important to note that these analyses include only food and beverage data and do not report on any vitamin and/or mineral supplementation, or enteral/parenteral sources of nutrients, as this information was unavailable. These sources may increase mean nutrient intakes and may also change the nutrient profiles across the different groups, given that vitamin and mineral supplementation often commences at or around the time of a pregnancy[265].

4.5 CONCLUSION

At present this is the largest study in Australia and one of the largest international studies to have investigated diet quality in young women and to have described the differences by pregnancy sub-groups. Clearly the diets of many young Australian women do not meet the current national recommendations outlined in the dietary guidelines, core foods and NRVs and this result is likely to be mirrored in other Western countries. In these analyses, this is a consistent finding among all young women, irrespective of being pregnant, not pregnant, having recently had a baby, or trying to conceive. This is cause for concern given the prominence of current hypotheses relating maternal nutrition pre-conception and during pregnancy, to the long-term health of their children.

4.6 ACKNOWLEDGEMENTS

The ALSWH, which was conceived and developed by groups of interdisciplinary researchers at the University of Newcastle and the University of Queensland, is funded by the Australian Government Department of Health and Ageing. I thank all participants for their valuable contribution to this project. The DQES (version 2) has been used with permission from Professor Graham Giles of the Cancer Epidemiology Centre of The Cancer Council of Victoria. Background

Chapter 5

A Recruiting Failure Turned Success

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5.1 INTRODUCTION

Recruiting is typically viewed as a means to an end, rather than a potential end in itself. This phase of the research project often turns out to be more of a challenge than anticipated. Recruiting difficulties can lead to: responder bias, an insufficient sample size to adequately power statistical analyses for hypothesis testing, costly delays in achieving the research objectives, and as a worst case, early cessation of the research project. There is limited literature to guide researchers in the practical aspects of recruiting for research studies. The magnitude of this problem has previously been demonstrated by Charlson and Horwitz (1984) who conducted a study into the impact of participant losses before randomisation. Using the multicentre trials listed in the 1979 inventory compiled by the National Institute of Health, they found that only 34% of trials ever reached their projected sample size[422].

In 2005 the ABCD Obesity Study was planned and developed as a PhD project at the University of Newcastle, Australia. The aim of this study was to investigate how maternal dietary intake affects the growth and development of the child, in both the pre- and postnatal periods. To do this we had planned to utilise the established framework of another large cohort study which was already underway at the John Hunter Hospital, a tertiary referral centre and major obstetric facility for the Hunter region of New South Wales, Australia. The Mathematical Model of Pregnancy (Math Model) study had been established since 1999 to explore methods of predicting preterm delivery[423]. It was identified as an appropriate database of eligible candidates from which to seek recruits for the ABCD Obesity Study because of the significant overlap in the data that was to be collected. Further details of the Math Model study have previously been published elsewhere[423].

5.2 METHODS

5.2.1 Participants

Research midwives were employed to approach potential recruits in the antenatal clinic of the John Hunter Hospital. All pregnant women who were up to 16 weeks' gestation were eligible to participate in the Math Model Study. At this first encounter a brief verbal description of the study was provided and the recruiting materials were issues. Follow-up phone calls were usually made two or three days after first being approached, to determine willingness to participate. The same method of recruiting continued with the change over to the WATCH Study.

Just over 600 women who had previously participated in, or were currently enrolled in, the Math Model Study were to be invited to join the ABCD Obesity Study. It was estimated that with a response rate of 60 to 70%, between 360 and 430 mother-child (up to four years of age) pairs would be recruited into this study, in addition to 100 women who were currently pregnant. This estimate was thought to be conservative, given that these women had previously consented to research of a similar nature.

Due to the large sample size recruiting was to be staggered, with a sample of 50 women approached at a time. Potential participants were to be invited into the study from both ends of the Math Model timeframe, first approaching those who had been out of the study the longest (*n* 25), as well as those who had most recently joined (*n* 25). Of the 50 invitations to participate that were mailed out, a total of only five consents were received, even after a follow-up letter was distributed. Four of these consents came from women who were currently pregnant and were still actively involved in the Math Model Study.

5.2.2 Ethics approval

Prior to submitting the ethics application for committee review, advice was sought from the Professional Research Ethics Officer on the appropriateness of re-recruiting subjects that had previously participated in research. Concerns were raised regarding the manner in which potential participants would be contacted, and measures (for example the mode of contact) were written into the research protocol to avoid consent out of a sense of obligation. The original application for the ABCD Obesity Study was made to the

Hunter Area Research Ethics Committee in May 2005 and approval was obtained in September 2005. Prior to commencing recruitment, a variation to the original application was submitted and approval for this was received in October 2005. Recruitment then commenced immediately and continued for the duration of the next six weeks. After this time it was evident that we were not going to be successful in meeting our sample size targets and subsequently our research objectives, due to the very poor rate of response (10%) to the invitation to participate.

5.2.3 Participant involvement

Women who participated in the Math Model Study consented to having three study ultrasound scans, at 18 to 20, 26 and 32 weeks' gestation. At each of these study visits non-fasting blood and urine samples were collected. Umbilical cord blood was collected at birth. Participants had the option of donating one further blood sample while in labour and additionally, their placenta after delivery.

The information statement for the ABCD Obesity Study documented the requirements of participation as attending two appointments at the John Hunter Hospital, at which physical measurements would be collected and questionnaires would be interview-administered for both mother and child. Dietary data recording for mother and child was a major component of the ABCD Obesity Study, including three-day WFRs and FFQs. Maternal and child blood samples were also listed as optional components of the study.

The WATCH Study has combined the data that was planned for collection in both parent studies, using the prospective longitudinal study design that was already in place for the Math Model Study, but with ongoing follow-up in infancy and early childhood. An extra ultrasound scan is performed at 36 weeks for the women in the WATCH Study who have not already delivered. Child follow-ups take place at three, six, nine, 12 and 24 months, with physical, medical and dietary data being collected at these study visits.

5.2.4 Procedures

A review of the study design and all recruiting materials was undertaken in an effort to identify the shortcomings of the project. Evaluation and advice was sought from those with extensive expertise in recruiting, not only for research purposes but for large-scale public health interventions. Literature on recruiting participants was consulted[424-430].

All feedback was considered in the redevelopment of the project and a complete overhaul of the method of recruitment and the materials provided to potential participants took place.

5.3 RESULTS

One of the difficulties in evaluating what went wrong is that ethically we were not able to re-contact the women who did not consent to participate and ask them what factors contributed to their decision. We were able to ask those who did consent why they agreed to volunteer in further research. However, this only highlighted that these women were likely to be different to the broader population we were targeting, with an enthusiasm and extreme willingness to be involved. We therefore relied upon the feedback sought from external sources (literature and expert consultation).

In doing so a number of issues were identified as potential contributors to the poor initial response rate. These are listed in Table 5.1 and are addressed in turn in the discussion. A complete overhaul of the study design and recruiting materials was considered necessary to both improve future recruitment and avoid unnecessary delays in our attempts to recruit successfully. Due to the extremely poor rate of response from the women who had previously participated in the Math Model Study (one consent out of 25 invitations), we decided to concentrate our efforts on only women who would prospectively be recruited to the research project. The ABCD Obesity Study was consequently amalgamated with the Math Model Study to become the WATCH Study. At this time both studies underwent an upgrade in the level of detail and attention that was devoted to the way in which potential participants were approached.

Table 5.1 Potential reasons for the recruiting failure of the Assessment Before Children Develop Obesity Study

Study Design	Recruiting Materials
The study name	Poor visual appeal
The selected participant group	Length of the information statement
The method of approaching participants	Readability of the text
Inequitable benefit gained by the research team compared to participants	General approach and content
Ethics approval too highly prioritised	

In doing so our rate of response improved from 10% for the ABCD Obesity cohort, and 35% in the Math Model cohort, to 61% for the combined WATCH Study (Table 5.2). A response rate of 35% for the Math Model cohort was considered (at that time) reasonable, as this was the first invitation to participate in research that was issued, with many women simply opting to decline. Approximately 10% of women who consented

to participate in the Math Model Study withdrew prior to their study completion at the time of delivery. The absolute number of women recruited to the study also increased from four per month in the Math Model cohort to 14 per month for the WATCH cohort (figures averaged over the six months preceding and following the changes).

Table 5.2 Recruiting response rates for the ABCD Obesity, Math Model and WATCH Studies

	ABCD Obesity	Math Model	WATCH	*P*-value
Approached *n*	50	65	141	
Consented *n* (%)	5 (10)	42(35)	86 (61)	<0.0001
Rejected *n* (%)	0 (*)	23 (65)	55 (39)	

ABCD Obesity, Assessment Before Children Develop Obesity; Math Model, Mathematical Model of Pregnancy; WATCH, Women and their Children's Health.
*The women who received the letter of invitation to participate in the ABCD Obesity Study had the option of declining the invitation using a reply-paid self addressed letter

One of the logical reasons for the improvement in participation rates was the changes that were made to the recruiting materials. Table 5.3 shows the dramatic differences in the readability statistics of the recruiting materials for each research study. Despite the complexity of merging the ABCD Obesity and Math Model Studies, the materials provided for the WATCH Study were drastically simplified.

Table 5.3 Recruiting material readability statistics for the ABCD Obesity, Math Model and WATCH Studies

	ABCD Obesity	Math Model	WATCH
Number of words	2127	1439	859
Number of A4 pages	5	3	2
Words per sentence	19.1	17.9	12.8
Number of paragraphs	96	66	53
Passive sentences	26%	31%	17%
Flesch reading ease*	50.1	45	66.3
Flesch-kincaid grade level†	10.9	11.3	7.1

ABCD Obesity, Assessment Before Children Develop Obesity; Math Model, Mathematical Model of Pregnancy; WATCH, Women and their Children's Health.
*Text is rated on a 100-point scale. The higher the score, the easier it is to understand[431].
†Text is rated on a school grade level[431].

5.4 DISCUSSION

5.4.1.1 The study name

Feedback highlighted that from the outset the title ABCD Obesity may have been a deterrent. Initially there was consensus among the research team that having a strong term like 'obesity' in the study name would attract interest, in a similar fashion to its common use in public and popular press. In hindsight however, it was likely to be the opposite. The social interpretation from consumer literature tends to be pejorative in nature, and the study title was likely to be particularly discouraging to women who were above their healthy weight range[432, 433].

5.4.1.2 The participant group

There are ethical considerations that need to be assessed before commencing research involving a population or group who are often targeted or who have previously participated in research. The National Statement on Ethical Conduct in Human Research[434] provides guidance on what is appropriate and emphasises the need to respect the rights of the participant to decline. In practise, this sensitivity translated into the way that we were able to approach potential participants, as further described below. The mode of contact proved to be so unsuccessful that during the study redesign we redirected the focus to the women who were currently pregnant and had not previously been approached.

5.4.1.3 Approaching participants

Sending a letter of invitation through the mail was always going to be a less than ideal recruiting strategy for several reasons. Firstly, we could not be sure that the intended recipient actually received the invitation, unless of course we received a reply. Conversely, we could not be sure where the recipient did not receive the invitation as a result of a change of address, unless our mail-out was delivered 'return to sender' (only two were returned in this way). Registered mail would have been useful, however this option was not considered at the time of the mail out.

In our case we were working from hospital records which may or may not have contained the potential participant's most current postal address. To put this in context,

between 1996 and 2001, 40.5% of people living in the Hunter region had changed their address[435]. While 28% of these remained living in the Hunter region, 12.5% had moved either elsewhere in NSW, interstate or overseas[435]. These figures do not take into consideration multiple changes in address that may have occurred. Lack of most current data may have been a major contributor to the very poor response rate in the sample of women who had completed their involvement in the Math Model project several years ago.

For those who did receive the information, from the outset the onus was on them to establish contact with the research team whether to agree to participate or to seek more information. A reply-paid, self-addressed envelope was provided with a response form asking potential participants to either opt-in or opt-out of the study. Nil opt-out responses were received. Furthermore, the mailed-out invitation to participate in research arriving was out of context when the research was to be based in a healthcare setting like the hospital.

5.4.1.4 Participant involvement

It was important to weigh up the participant burden in relation to what they got back from their involvement in this research. The altruistic motive of helping others can be a behaviour driver, but if there are obstacles to doing so it may not be enough. Consider (if applicable): the time of available study appointments; the location of the research and ease of getting there; any cost incurred by participating including time off work, travel, and parking; the number of study visits and duration of appointments; and availability of child care. While it is unethical to provide incentives of a disproportional magnitude (financial or otherwise) that may coerce individuals to participate, especially those who are economically vulnerable, or where inducements are undue[436], it is unreasonable not to make participation as easy and rewarding as possible for those who do consent. Reimbursement for time and travel were not deemed feasible for WATCH Study participants. Parking permits were issued to cover all parking expenses associated with study visits and a light meal was provided on the occasions when fasting samples were collected.

5.4.1.5 Prioritising ethics approval

The detail in the planning and development phase of the research project is often intertwined with the writing of the ethics application. However, this can quickly lead to lapsed judgement about your priorities. In wanting to get started on the research the focus became obtaining approval by the ethics committee. While it is the ethics committee's responsibility is to represent participants and researchers alike, the nature of the application process can hamper achieving the most desirable outcomes for both. Take, for example, the recruiting materials which are described below. By focusing too heavily on the primary function of informing the participant prior to consent we produced a document that was unable to maintain the interest of the reader and may not have been well understood.

5.4.2 Recruiting materials

5.4.2.1 Visual appeal and length of the information statement

The written information provided to potential subjects should reflect the nature of the research, not only in terms of content but also visual presentation. In our case, pregnancy is generally regarded as a positive time in a woman's life; hence we wanted to reinforce this with the written materials we provided in the revised study (refer to Appendices). The use of images including a study logo, colour and using a pamphlet format has helped us to convey the information we need participants to be aware of in order for them to provide informed consent. While the ABCD recruiting material did include a study logo, it was not colour printed and was presented as formal document rather than pamphlet style.

The length of the text is another important factor that needs to be considered. With careful deliberation we were able to condense what had been two separate information statements totalling just over eight A4 pages in length, into one single double-sided A4 information pamphlet for the WATCH Study. This information pamphlet has been more favourably received by not only by potential participants but also by the other healthcare professionals who see our participants over the course of standard antenatal care.

5.4.2.2 Readability of text

The ease of readability of the information statement is too often neglected[437] despite the simplicity in considering this. Readability statistics are available as part of the Microsoft Word (Microsoft Office Word 2003) spelling and grammar functions and they provide an objective measure of how easy materials are to read. The Flesch Reading Ease score rates text on a 100-point scale; the higher the score, the easier it is to understand. Additionally you receive a grading for your text, known as the Flesch-Kincaid Grade Level, which ascribes the text a school grade (United States) level. For example, a score of 8.0 means that an eighth grader should be able to understand the document[431]. Both are calculated using formulas that consider the average sentence length and average number of syllables per word[431]. For most standard documents, the aim is for a Flesch Reading Ease score of approximately 60 to 70 and a Flesch-Kincaid Grade Level of 7.0 to 8.0[431]. We were able to reduce our Flesch-Kincaid Grade Level from 10.9 for the ABCD Obesity Study and 11.3 for the Math Model recruiting materials, to 7.1 for the combined WATCH Study information pamphlet.

5.4.2.3 General approach and content

This incorporates many of the factors previously described and will be defined by the level of detail put into developing the study design. Ultimately we had to put ourselves in the position of potential participants and write the study materials from this viewpoint. Ethics committees provide guidance on what information must be given. But it is ultimately the researchers' responsibility to ensure that we communicate effectively with potential participants in a manner which aims to encourage participation. From our experience we would recommend seeking advice from those with expertise in recruiting who understand the common mistakes that researchers make when designing the recruitment protocol and materials. Additionally using materials that have proven to be successful as a template may also be advantageous.

5.4.3 Limitations

Whist we believe that it was the multiple changes that resulted in our improved response rate, the empirical study design is such that we cannot provide direct evidence that all of the changes contributed to the observed improvement. It is possible that only

some of the strategies were instrumental in improving the response rate, or even just one. Because all of the changes were made at once we cannot quantify the relative contributions of each. It is even possible that one or more of the changes may have had a negative impact on potential respondents, but that the positive changes compensated so that overall there was still a significant net improvement in our response rate. Future research studies will be required to determine which are the most efficient strategies for ensuring recruitment protocol success.

5.5 CONCLUSIONS

This paper describes an attempt that was made to recruit participants into a nutrition-based research study of pregnancy and early childhood, and the knowledge that was gained when this attempt initially failed. The lessons learnt are applicable to those who may try to recruit participants for their own research projects. We hope that by sharing our experience we contribute to the knowledge-base for successful recruitment and help prevent others from making the same simple mistakes. Implementation of effective recruiting strategies will facilitate the achievement of the research objectives without superfluous burden to the study timeframe and resources.

5.6 ACKNOWLEDGEMENTS

Firstly I would like express sincere gratitude to Professor Rob Sanson-Fisher for the guidance and advice he provided prompting many of the changes that took place. I would like to acknowledge Trish Engel and Therese Finnegan (Research Midwives) for their great work approaching women to participate in the Math Model and WATCH Studies. Importantly I would also like to thank all of the women who have participated, or are currently participating in any of the three studies described in this paper. I am very mindful that without your ongoing support this research could not have become the success that it has.

Chapter 6

Maternal Weight Change in Pregnancy Predicts Fetal Size but Not Adiposity

6.1 INTRODUCTION

Epidemiological evidence showing an association between early life nutrition and risk of disease later in life has prompted extensive animal-model experimentation into the biological facets of programming[394, 438, 439]. These studies have clearly established the biological plausibility of nutritional programming and are currently helping to unravel complexities at the mechanistic level. Human studies are much more difficult to carry out. They present major challenges with respect to prospective data collection, statistical analysis, and interpretation, including handling variables which may simultaneously be a key outcome measure and confounding factor, such as body mass[440].

Maternal nutritional and metabolic factors which affect fetal growth and birthweight are of particular interest, as these may offer valuable intervention points in the future for preventative programming of the offspring's adult health. The Barker hypothesis and accompanying evidence supports an inverse correlation between birthweight and chronic disease[44, 114, 441]. However, a recent systematic review has shown that it is both infants at the highest end of the spectrum for weight (whether alone or adjusted for height), in addition to those who grow rapidly during infancy, who are at increased risk of later obesity[442].

It is remarkable that long-term health outcomes are, at least in part, predicted by the size of the newborn, when birthweight is only a snapshot of the trajectory of fetal growth. Recent studies have recognised the need to extend beyond this single marker and adopt a more sophisticated approach to describing fetal and infant growth[443, 444]. This includes differentiating between lean and fat mass. Intrauterine measures of adiposity are far less common than postnatal studies but can be done using magnetic resonance imaging (MRI)[177] or ultrasound technology[383, 445].

Longitudinal data monitoring concurrent changes in maternal body weight during pregnancy and the pattern of growth exhibited by the fetus have not been reported within current fetal development literature.

6.2 MATERIALS AND METHODS

Participants were recruited at the antenatal clinic of John Hunter Hospital, a tertiary referral centre in New South Wales, Australia. Data collection occurred over the 18 months July 2006 to December 2007. Analyses include data from singleton pregnancies. Participants presenting with gestational diabetes have been excluded from the analysis as this pathology can interfere with normal fetal fat deposition. While data were available for up to 173 participants, some datasets contained missing values as a result of: (i) withdrawal from the study (*n* 15); (ii) the data being collected more than two weeks outside of the planned study visits at 20, 24, 30 and 36 weeks' gestation (*n* 21); (iii) the study appointment having been missed altogether (*n* 35), including as a result of preterm delivery (*n* 9); or (iv) minor inaccuracies in the data recording. The study received ethics approval from the Hunter New England Human Research Ethics Committee and all participants gave written informed consent.

Participants were booked to have four ultrasound examinations, at 20, 24, 30, and 36 weeks (± two weeks). The study ultrasound scans were performed by a team of three clinical obstetricians and three accredited sonographers. Efforts were made to book each participant with the same obstetrician or sonographer for all ultrasound scans, in an attempt to reduce potential inter-observer variation, though this was not always possible.

The first ultrasound was generally performed between 18 and 20 weeks when confirmation of the gestational age and fetal anomaly scanning were undertaken. Where the menstrual dating of the pregnancy was within one week of the ultrasound dating, the menstrual dates were used to determine gestation. However, if the menstrual dates were more than one week outside the ultrasound dating, the ultrasound dating was used instead. Ultrasound was performed using an Acuson Aspen (AspenUltrasound, Oceanside, California, USA) and Voluson 730 Pro (GE Healthcare, Giles, Buckinghamshire, UK) with a curvilinear array transducer.

Each ultrasound scan included standard fetal biometry. The BPD was measured as the standard axial view of the fetal head including the thalami and third ventricle including only the calvarial diameter and excluded the soft tissues of the scalp[382]. The HC was measured at the same level as the BPD and included the normal soft tissues of the

scalp[382]. The AC was obtained from the transverse ultrasound of the fetal abdomen at the level of the fetal stomach and the portal vein and measured the perimeter of the abdomen including the soft tissues[382]. The FL was measured excluding the cartilaginous epiphyses[382].

The fetal fat estimation was performed at the level of the standard measurement of AC and at the fetal mid-thigh region. For the abdominal fat and lean mass estimation, the total area of the fetal abdomen (A_1) was calculated. Then the abdominal area, excluding the hyperechoic subcutaneous fat layer was calculated (A_2). The abdominal fetal fat area was then the difference of the two areas (i.e. A_1- A_2 cm^2). The measurements of fetal mid-thigh were taken following the standard measurement of the fetal femoral length. Over the mid-point of the femur the transducer was rotated 90^o to obtain a cross-sectional view of the fetal mid-thigh[383, 445]. The total cross-sectional area of the fetal mid-thigh (T_1) was calculated, as was the total cross-sectional area of the hypoechoic fetal mid-thigh muscle tissues (T_2). The calculated fetal mid-thigh fat tissue area was the difference of the two areas (i.e. T_1-T_2 cm^2)[383].

Maternal anthropometry was collected by a team of three dietitians, each with level one anthropometrist certification from the ISAK. Weight was measured at each study visit using the same set of annually calibrated AND^{TM} FV-150K electronic weighing scales (A&D Mercury Pty Ltd, Thebarton, South Australia). These scales weigh up to 150 kilograms and are accurate to 50 grams. Participants were asked to remove their shoes, over-clothing (i.e. jackets and scarves) and any items from their pockets prior to being weighed. Pre-pregnancy weight was self-reported at visit one. Standing height without shoes was measured to one millimetre on two occasions, using the same wall-mounted Seca stadiometer (Seca Deutschland, Hamburg, Germany). The readings were averaged, unless the measures differed by more than 1.5%, where a third measure was taken and the median value used.

6.2.1Statistical analyses

As these were novel hypotheses, involving a large number of tests the statistical protocol was set *a priori,* before any of the data analyses commenced. Statistical significance was set at P =0.05 because of the relatively small sample size and because any effect size was predicted to be modest, if evident. Although a large number of tests

were undertaken, consistencies in the findings were considered in the interpretation, to avoid over-emphasis of potential by-chance results.

The statistical protocol was set to answer the following questions, which follow the sequence of pregnancy:

1. Is maternal pre-pregnancy weight/BMI predictive of fetal adiposity and size?
2. Is maternal weight/BMI change between 0 and 20 weeks' gestation predictive of fetal adiposity and size at, and from, 20 weeks' gestation?
3. Is maternal weight/BMI at 20 weeks' gestation predictive of fetal adiposity and size at, and from, 20 weeks' gestation?
4. Is maternal weight/BMI during pregnancy predictive of fetal adiposity and size?
5. Is maternal weight/BMI change between visits predictive of fetal adiposity and growth between visits (i.e. change with change)?
6. Is maternal weight/BMI change during pregnancy predictive of fetal adiposity and growth during pregnancy (i.e. change with change)?

The result section (6.3 Results) is structured to follow same protocol.

Mid-thigh and abdominal fat areas were markers of fetal adiposity. Total and lean areas at the abdomen and mid-thigh, in addition to standard fetal biometry (BPD, HC, AC and FL) were markers of size. Growth referred to changes in fetal size with time.

A change score (gradient) was calculated using the standard mathematical formula: $(Y_2-Y_1)/(X_2-X_1)$, where Y represents the maternal and fetal variables of interest at two consecutive visits (the second minus the first), and X represents the gestational age of the fetus in days at the same study visits. These derived variables were used to test for simultaneous changes in the maternal weight or BMI as the predictors, and fetal size or adiposity as the response variables: in other words, whether there was any positive association between the calculated maternal and fetal change scores.

Both maternal BMI and absolute weight have been included in these analyses. The variables pre-pregnancy weight, pre-pregnancy BMI, maternal weight, maternal BMI, and gestational age, were centralised to their minimum values instead of zero. This influences the values reported in the results by changing the value of the intercept only. It does not change the value of the slope which is what relates to the overall interpretation and meaning. Normality checks were performed on the residuals after

fitting the model. Linear mixed models and linear regressions were used for hypothesis testing. *P*-values reported in text for linear mixed-models are based on the interaction term between the maternal predictor and gestation. This is because the maternal predictors, in this case weight and BMI, show significant cross-level interactions with gestation (i.e. as gestation increases weight typically also increases). Statistical analyses were carried out using Intercooled Stata 9 (StataCorp LP, College Station, Texas, USA).

6.3 RESULTS

The total numbers for each study visit are: visit one, *n* 169; visit two, *n* 163; visit three, *n* 160; and visit four, *n* 140. Fifteen participants discontinued participation between study visits one and four. Thirteen actively withdrew, with time constraints and medical complications cited as the main reasons; two neonatal deaths occurred following preterm delivery, one of which was before 30 weeks' gestation. A further seven women delivered prematurely before the fourth study visit.

At the time of these analyses not all participants had completed their pregnancies and hence the numbers differ compared to those presented for the entire cohort presented in Figure 3.2. One participant had not yet attended visit two, seven were still to attend visit three and 17 were yet to attend visit four. The final analysis for publication will be re-run to include all of the data for the cohort.

The mean age of the women included in these analyses was 28.4 years at visit one, with additional descriptive statistics provided in Table 1. The mean gestational age at the time of the study visits corresponded well to the defined data collection time-points. Visit one tended to be on average one week earlier (mean of 19 weeks) to coincide with the standard-care morphology scan that is recommended between 18 to 20 weeks' gestation. Table 6.1 also reports the minimum values used for centralising data.

Table 6.1 Maternal age, height and weight characteristics for women in the WATCH Study, according to study visit

	n	Mean	SE	Median	Min, Max
Age at Visit 1 (yrs)	167	28.4	0.4	28.0	16, 41
Height (cm)	164	164.5	0.5	164.0	140.9, 182.8
Gestational age at each study visit					
1: 140 days (20wks)	156	134.1	0.53	133.5	112, 153
2: 168 days (24wks)	143	170.6	0.45	170.0	156, 182
3: 210 days (30 wks)	141	211.5	0.43	211.0	198, 224
4: 252 days (36 wks)	126	252.9	0.44	253.0	238, 265
Weight (kg)					
Pre-pregnancy	160	68.2	1.2	65.0	45.0, 140.0
1: 20 wks	161	74.4	1.3	69.8	48.9, 144.2
2: 24 wks	148	76.5	1.2	72.9	47.8, 144.9
3: 30 wks	154	79.7	1.3	76.7	50.3, 146.6
4: 36 wks	131	82.9	1.4	79.6	54.2, 146.6
BMI (kg/m^2)					
Pre-pregnancy	160	25.6	0.4	24.2	17.1, 43.7
1: 20 wks	161	27.5	0.5	26.3	18.0, 49.5
2: 24 wks	147	28.4	0.5	27.5	18.9, 49.8
3: 30 wks	154	29.5	0.5	28.9	20.3, 50.4
4: 36 wks	131	30.7	0.5	30.2	21.0, 50.4
Weight change (kg)					
Pre-pregnancy to 20 wks	148	4.7	0.3	4.43	-7.60, 20.00
20 to 24 wks	148	3.1	0.2	2.68	-1.40, 21.55
24 to 30 wks	154	2.8	0.1	2.70	-3.55, 7.70
30 to 36 wks	131	2.9	0.2	2.85	-1.95, 11.30

The fifth, fiftieth and ninety-fifth percentiles for fetal AC, total abdominal area and fat area are presented in Figure 6.1. Abdominal circumference increases in a linear fashion between 18 and 36 weeks' gestations, while the total and fat areas are curvilinear. Here group data have been summarised to show the usual pattern of fetal growth for these selected variables. The remainder of the analyses focus on comparing fetal trajectories within individual and between the individuals in the cohort.

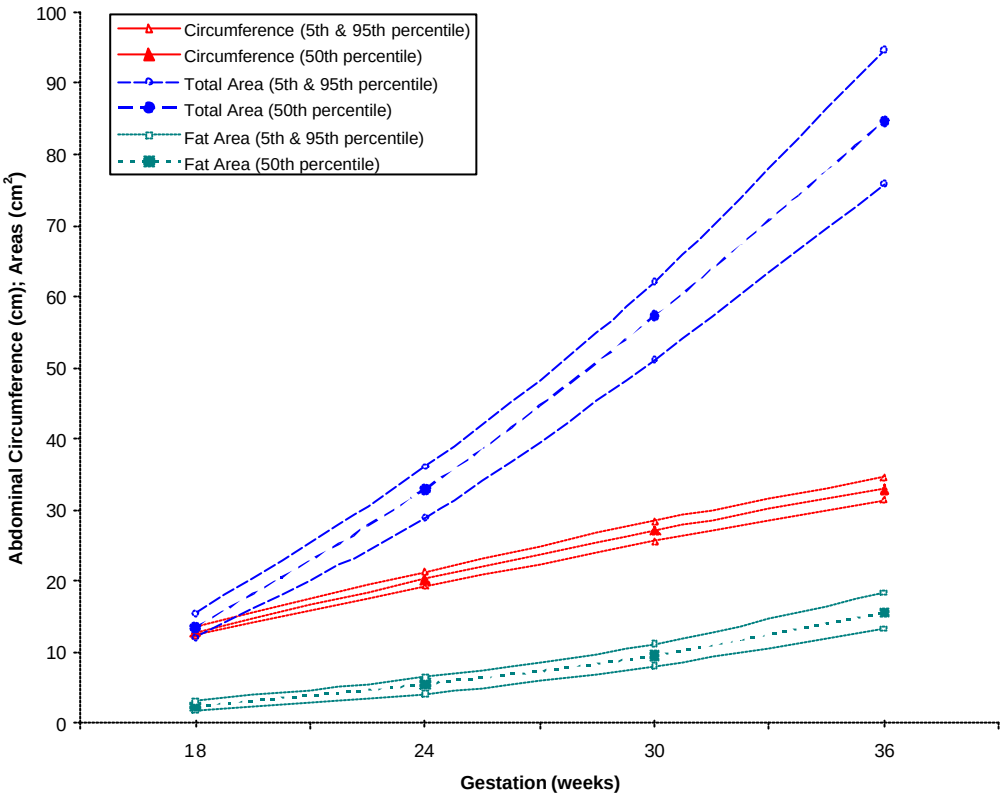

Figure 6.1 Fetal percentiles (5th, 50th and 95th) for the WATCH Study: abdominal circumference (cm), total and fat areas (cm^2), by gestation (weeks) (*n* 126)

Gestational age was included in all linear mixed-models to adjust for fetal growth with time. Table 6.2 reports pre-pregnancy weight and pre-pregnancy BMI were significantly predictive of fetal total and lean areas at both the abdomen and mid-thigh, and fat area at the abdomen, but not the mid-thigh (refer to 6.2.1, question 1). Pre-pregnancy weight predicted all fetal biometry. The *P*-value increased when BMI was used instead of weight (larger standard errors), with statistical significance lost for the HC, and borderline significance for AC. All coefficients were positive, indicating higher maternal pre-pregnancy weight is associated with larger fetal size and more adipose tissue at the abdomen, but not mid-thigh.

Table 6.2 Linear mixed-models with maternal pre-pregnancy weight and body mass index (BMI) as the predictors of fetal adiposity and size during pregnancy (*n* 160)

Fetal Variables	Maternal pre-pregnancy:	R^2			CE	SE	*P*-value
		Within	Between	Overall			
Total abdominal area	Weight (kg)	0.959	0.872	0.935	0.074	0.023	0.001*
	BMI (kg/m²)	0.959	0.873	0.935	0.209	0.062	0.001*
Lean abdominal area	Weight	0.960	0.865	0.934	0.061	0.019	0.001*
	BMI	0.960	0.867	0.934	0.173	0.053	0.001*
Fat abdominal area	Weight	0.883	0.812	0.855	0.013	0.005	0.018*
	BMI	0.883	0.809	0.854	0.035	0.015	0.021*
Total mid-thigh area	Weight	0.900	0.800	0.870	0.015	0.006	0.016*
	BMI	0.900	0.800	0.870	0.047	0.018	0.008*
Lean mid-thigh area	Weight	0.876	0.685	0.824	0.015	0.004	0.000*
	BMI	0.876	0.686	0.824	0.042	0.011	0.000*
Fat mid-thigh area	Weight	0.819	0.726	0.785	0.000	0.004	0.952
	BMI	0.819	0.727	0.785	0.004	0.012	0.741
Biparietal diameter	Weight	0.977	0.888	0.954	0.045	0.015	0.002*
	BMI	0.977	0.885	0.953	0.082	0.041	0.044*
Head circumference	Weight	0.971	0.911	0.956	0.111	0.043	0.010*
	BMI	0.971	0.909	0.955	0.151	0.121	0.211*
Abdominal circumference	Weight	0.985	0.912	0.966	0.118	0.052	0.023*
	BMI	0.985	0.912	0.966	0.281	0.143	0.050*
Femur length	Weight	0.982	0.914	0.968	0.035	0.010	0.001*
	BMI	0.982	0.912	0.967	0.063	0.029	0.030*

CE, coefficient; SE, standard error of the mean.
*Statistically significant at *P* =0.05.

Linear regression models were used to explore whether change in maternal weight and BMI from pre-pregnancy to 20 weeks' gestation predicted fetal adiposity and size at 20 weeks (refer to 6.2.1, question 2). Change in maternal weight and BMI from pre-pregnancy to 20 weeks' gestation was positively predictive of the fetal total abdominal (*n* 134; *P* 0.034, SE 0.066; *P* 0.029, SE 0.182) and lean abdominal areas (*n* 134; *P* 0.054, SE 0.057; *P* 0.043, SE 0.156), in addition to AC (*n* 139; *P* 0.036, SE 0.261; *P* 0.034, SE 0.710). All other measures were not significant. Linear mixed-models were then used to determine if this change in maternal weight and BMI between conception and 20 weeks was associated with fetal growth and adiposity from 20 to 36 weeks' gestation (*n* 159-160). The same fetal variables (total and lean abdominal areas, plus AC) were statistically significant, however the coefficients became negative, suggesting slowed growth in the second half of pregnancy (data not presented).

At 20 weeks' gestation, maternal weight and BMI were positively predictive of fetal total mid-thigh (*n* 143; *P* 0.003, SE 0.005; *P* 0.001, SE 0.013) and lean mid-thigh areas (*n* 143; *P* 0.004, SE 0.004; *P* 0.001, SE 0.012) (refer to 6.2.1, question 3). There was a trend towards significance for maternal BMI and FL (*n* 150; *P* 0.061, SE 0.043). No other fetal variables were associated at this time. Linear mixed-models testing maternal weight and BMI at 20 weeks as predictors of fetal adiposity and size from 20 to 36 weeks' gestation showed an inverse association with fetal total abdominal (*n* 147; CE -0.025, *P* 0.001, SE 0.000; CE -0.144, *P* 0.000, SE 0.001) and lean abdominal areas (*n* 147, CE -0.026, *P* 0.000, SE 0.000; *n* 160, CE -0.326, *P* 0.017, SE 0.004), in addition to AC (*n* 159, CE -0.002, *P* 0.015, SE 0.003; *n* 160, CE -0.113, *P* 0.020, SE 0.007). Abdominal fat was also significantly predicted by maternal BMI at 20 weeks but not by weight (again an inverse relationship). Mid-thigh areas and other fetal biometry were not significantly predicted by maternal weight and BMI at 20 weeks' gestation.

Overall maternal weight and BMI during pregnancy were inversely predictive of all fetal areas (Table 6.3), with the exception of mid-thigh fat which did not reach statistical significance with maternal weight (*P* 0.065) (refer to 6.2.1, question 4). Interestingly BPD, AC and FL were not predicted by maternal weight or BMI; however, BMI and HC were positively correlated. Only the statistically significant results are presented in Table 6.3.

Table 6.3 Linear mixed-models with maternal weight and body mass index (BMI) as statistically significant predictors of fetal adiposity and size during pregnancy

Fetal Variables	Maternal	*n*	R^2			CE	SE	*P*-value
			Within	Between	Overall			
Total abdominal area	[C]Weight (kg)					-0.060	0.036	0.094
	[C]Gestation	173	0.961	0.882	0.938	0.527	0.013	0.000
	Interaction					0.002	0.000	0.000*
	[C]BMI (kg/m^2)					-0.242	0.096	0.012
	[C]Gestation	172	0.963	0.885	0.939	0.515	0.012	0.000
	Interaction					0.006	0.001	0.000*
Lean abdominal area	[C]Weight					-0.048	0.029	0.095
	[C]Gestation	173	0.963	0.878	0.937	0.428	0.010	0.000
	Interaction					0.001	0.000	0.000*
	[C]BMI					-0.181	0.078	0.020
	[C]Gestation	172	0.964	0.883	0.938	0.420	0.010	0.000
	Interaction					0.005	0.001	0.000*
Fat abdominal area	[C]Weight					-0.011	0.011	0.292
	[C]Gestation	173	0.880	0.804	0.852	0.099	0.004	0.000
	Interaction					0.000	0.000	0.014*
	[C]BMI					-0.061	0.030	0.039
	[C]Gestation	172	0.882	0.803	0.854	0.094	0.004	0.000
	Interaction					0.001	0.000	0.000*
Total mid-thigh area	[C]Weight					-0.017	0.012	0.172
	[C]Gestation	173	0.905	0.817	0.876	0.120	0.005	0.000
	Interaction					0.000	0.000	0.002*
	[C]BMI					-0.058	0.033	0.075
	[C]Gestation	172	0.906	0.824	0.878	0.117	0.005	0.000
	Interaction					0.001	0.000	0.000*
Lean mid-thigh area	[C]Weight					-0.006	0.007	0.394
	[C]Gestation	173	0.883	0.729	0.833	0.060	0.003	0.000
	Interaction					0.000	0.000	0.001*
	[C]BMI					-0.015	0.020	0.445
	[C]Gestation	172	0.882	0.731	0.833	0.060	0.003	0.000
	Interaction					0.001	0.000	0.002*
Fat mid-thigh area	[C]BMI					-0.043	0.022	0.053
	[C]Gestation	172	0.819	0.740	0.787	0.057	0.003	0.000
	Interaction					0.001	0.000	0.004*
Head circumference	[C]BMI					0.506	0.188	0.007
	[C]Gestation	172	0.972	0.922	0.957	1.410	0.024	0.000
	Interaction					-0.004	0.002	0.041*

CE, coefficient; SE, standard error of the mean.
[C]Centralised to the minimum value.
*Statistically significant at *P* =0.05 for the interaction term between maternal weight and/or BMI and gestation.

For the slope data, rate of change in maternal BMI was positively predictive of the rate of fetal abdominal fat deposition between 20 and 24 weeks (*n* 116, *P* 0.007, SE 0.220), and between 24 and 30 weeks (*n* 107, *P* 0.024, SE 0.347) (refer to 6.2.1, question 5). For the rate of change in fetal mid-thigh fat area, maternal weight changes as well as change in BMI were only statistically significant between visits 20 and 24 weeks (*n* 112, *P* 0.010, SE 0.038; and *n* 113, *P* 0.010, SE 0.101 respectively). There were less consistent trends in the associations between corresponding maternal and fetal changes in total and lean areas, although those that reached statistical significance were either between 0 and 20 weeks (mid-thigh total and lean areas), or between 20 and 24 weeks (total abdominal area). All statistically significant coefficients were positively predictive indicating faster fetal growth with more rapid maternal weight gain.

Over the pregnancy (conception to 36 weeks) change with change was only statistically significant for fetal biometry (Table 6.4) (refer to 6.2.1, question 6). Higher weight gained and an increase in BMI positively predicted fetal growth according to standard biometry.

Table 6.4 Statistically significant* linear mixed-models of change in maternal weight and body mass index (BMI) as predictors of change in fetal size (between conception and 36 weeks' gestation)

Fetal Variables	Maternal change	*n*	R^2			CE	SE
			Within	Between	Overall		
Biparietal diameter	Weight (kg)	163	0.192	0.009	0.093	1.524	0.215
	BMI (kg.m^2)	162	0.201	0.006	0.100	4.407	0.599
Head circumference	Weight	163	0.185	0.009	0.088	5.529	0.803
	BMI	162	0.196	0.006	0.095	15.979	2.237
Abdominal circumference	Weight	163	0.193	0.029	0.106	4.656	0.605
	BMI	162	0.214	0.019	0.117	13.541	1.675
Femur length	Weight	162	0.191	0.006	0.092	0.894	0.126
	BMI	162	0.208	0.007	0.104	2.636	0.348

*Statistically significant at *P* =0.001.

The average amount of weight gained by participants, according to pre-pregnancy BMI category is reported in Table 6.5. Women who were underweight pre-pregnancy gained the most weight between conception and 36 weeks' gestation. However, this was a very small sub-set of women (*n* 4). Less weight was gained as BMI category increased from underweight to obese.

Table 6.5 Weight change (kg) for the WATCH Study participants from pre-pregnancy to 36 weeks' gestation according to pre-pregnancy body mass index (BMI) category

BMI category	Pre-pregnancy BMI (kg/m^2)	*n*	Mean	SE	Median	Min, Max	*IOM weight gain targets
Underweight	<18.5	4	26.9	6.1	23.0	17.0, 44.8	12.5 - 18
Normal weight	18.5 – 25.0	68	14.7	0.7	13.5	5.3, 35.1	11.5 - 16
Overweight	25.1 – 30.0	30	13.0	1.1	13.6	3.3, 23.8	7 - 11.5
Obese	>30.0	23	9.2	1.6	8.3	-4.7, 21.1	≥6.0

*IOM, US Institute of Medicine BMI categories: Underweight <19.8; Normal weight 19.6-26.0; Overweight 26.1-29.0; and Obese >29.0[160].

6.4 DISCUSSION

To the best of the authors' knowledge, this is the first study in human pregnancy to have simultaneously assessed the rate of change in maternal weight and fetal growth as a potential 'programming' pathway. Fetal body composition has been described using standard biometric measures, in addition to markers of lean and fat mass at the abdomen and mid-thigh. The findings indicate that over the course of pregnancy, the rate of rise in maternal weight is positively correlated with the rate at which the fetus grows, but not with adipose tissue accumulation. Some increases in adiposity with greater maternal weight gain are evident early in pregnancy, however a decompensatory slowing of fetal fat deposition appears in the latter stages.

Fetal biometric measures were not always consistent with their corresponding lean and fat masses. For instance, AC was not significantly predicted by maternal weight or BMI during pregnancy in the same way that the total, lean and fat abdominal areas were (Table 3). Bernstein and others (1997) have reported the relationship of the abdominal areas with gestational age follows the same linear trend as the single-dimension circumference measure[445]. This finding was not supported by our data, as Figure 6.1 clearly shows a slightly accelerated deposition of fat and lean tissue in the later weeks of gestation.

Weight is often cited as an indicator of maternal nutritional status but also as a proxy for the intrauterine nutritional environment experienced by the fetus (as long as placental function is uncompromised). It has been proposed that the intrauterine nutritional cues a fetus receives serves to forecast that of the extrauterine environment, and growth and body composition may be adapted in an effort to optimise the chance of survival[395].

The data reported here have been collected prospectively with the statistical protocol established *a priori*. Attention was given to maximising the longitudinal data that were available, while managing the missing values which are inherent in human research of this nature.

A significant limitation of this study is the lack of data collected before 20 weeks' gestation. At the time of the first study visit half of the pregnancy had lapsed and, similar to using birthweight at the end of a pregnancy, we have summarised the first

half of pregnancy with the measurements collected at 20 weeks' gestation. Additionally we have relied on self-reported data as a baseline measure of maternal weight and for the calculation of pre-pregnancy BMI. One study which actually assessed the relationship between self-reported and documented pre-pregnancy weights has shown a high correlation between the two (r 0.96)[446]. In this study women in the healthy weight range were the most accurate reporters, while women above their ideal weight were more likely to under-report[446]. If present, under-reporting pre-pregnancy weight may mute some of the effects of early maternal weight gain in relation to fetal growth.

Like the original ultrasound studies of fetal adiposity [383, 445, 447] sex has not been included. At birth males and females have similar fat masses, although males tend to be longer and carry greater lean mass[359]. Despite being a relatively small difference early in life, this dimorphism may mean that potentially important differences which exist *in utero* have not been detected. This is an area which will require investigation in future. Also data have not been separated according to whether the fetus reached term or was delivered preterm. Only a small number were affected, and most delivered after the third ultrasound.

The findings contribute to the intermediate knowledge of pregnancy and how maternal weight is associated with fetal growth and development by demonstrating:

- Pre-pregnancy BMI and pregnancy weight gain are important predictors of fetal growth, not simply assessed using birthweight alone.
- Significant variations in body composition become evident during fetal development.
- Maternal pre-pregnancy BMI was positively associated with fetal adipose tissue at the abdomen, but not the thigh.
- A positive association exists between maternal weight gain and fetal abdominal size in the first half of the pregnancy, followed by a decompensatory slowing in the second half.
- Transient increases in the rate of fetal adipose deposition may occur with larger maternal weight gain, but over the course of pregnancy fetal fat is not related to rate or total amount of weight gained.

6.5 ACKNOWLEDGEMENTS

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Chapter 7
Maternal and Infant Vitamin B12, Folate and Homocysteine in Pregnancy and Postpartum

7.1 INTRODUCTION

Vitamin B12 (cobalamin) and folate are water-soluble micronutrients which are essential for normal DNA and RNA biosynthesis[188]. During embryogenesis and fetal growth, nucleic acid and protein synthesis is maintained by the maternal supply of folate[189] and vitamin B12. A deficiency in the intake of folate or a genetic error in its metabolism may impair normal cell formation and tissue growth[190]. Maternal-to-fetal folate transfer is mediated by placental folate receptors[191]. These are found on the maternal-facing chorionic surface and capture 5-methyltetrahydrofolate from the mother's circulation[192]. Mouse-models have shown the folate receptor analogous to that of humans (Folbp1) is highly expressed in the yolk sac, the neural folds and the neural tubes of the developing embryo[193, 194]. Folate deficiency or impaired folate metabolism is widely accepted now as the major cause of NTDs. Meta-analysis data also suggests it may play a role in the development of oral clefts[195], congenital heart defects[195], urinary tract anomalies[195], limb defects[195], and paediatric cancers, including leukaemia, brain tumours, and neuroblastoma[196]. Vitamin B12 deficiency has historically received little attention with respect to adverse pregnancy outcomes, however, recently it has been independently associated with NTDs[197], preterm delivery[198], IUGR[199, 200], and recurrent pregnancy loss[201].

Vitamin B12 and folate are integral components of homocysteine metabolism. Homocysteine is a thiol-containing amino acid which originates from the demethylation of methionine, an essential amino acid, via the methionine cycle[202] (refer to Figure 2.6). Homocysteine is regulated via two discrete pathways: by trans-sulphuration to cysteine, or by remethylation to methionine[203, 204]. The trans-sulphuration pathway requires vitamin B6 (pyridoxine) as a cofactor for the enzyme cystathionine β-synthase[204]. Methionine synthase catalyses the remethylation of homocysteine to methionine, and requires folate (5-methyltetrahydrofolate) as a co-substrate and vitamin B12 (methylcobalamin) as a cofactor[205]. The methionine cycle occurs in every mammalian cell. Homocysteine can replace methionine under experimental conditions[206]. However, homocysteine does not occur naturally in human diets, hence methionine plays a unique nutritional role in this cyclical product-precursor relationship[206].

Studies of maternal dietary constraint during pregnancy in animal models have suggested a causal link between the disruption of one-carbon (methyl) metabolism, altered epigenetic regulation of gene expression, and the resulting phenotype[224, 225]. The activities of several important signal-transduction pathways and transcription factors are known to be directly modified by homocysteine[225-234] and homocysteine is directly modified by the presence or absence of vitamin B12 and folate[203].

Plasma levels of vitamin B12 and folate are known to decline during pregnancy[262, 448]. However, limited follow-up has been conducted in the postpartum period to contextualise the changes that occur. The largest study, conducted in a cohort of Danish women, has reported data at eight weeks after birth[262, 449, 450]. One study of rural Mexican subjects, which included a longitudinal subsample of women (*n* 49) during pregnancy and lactation, collected data on up to three occasions postpartum[451]. However, the results have been pooled and the data has been presented as a single value at approximately seven months (± 60 days) after birth[451]. Glorimar *et al.* (2004) monitored longitudinal changes in pB12, pFol, rcFol, and pHcy during pregnancy and postpartum in a cohort of 46 Brazilian women[452]. Although by the postpartum follow-up, which occurred 30 to 40 days after birth, blood samples were only available for 17 participants[452].

The aim of this study was to longitudinally characterise the values of pB12, pFol and rcFol, in a cohort of Australian women during pregnancy and after birth. Additionally a paired mother-infant sub-study of homocysteine in relation to vitamin B12 and folate biomarkers was undertaken, to investigate whether maternal levels during pregnancy and postpartum predict infant values at six months of age.

7.2 METHODS

7.2.1 Participants

Participants were recruited at the antenatal clinic of John Hunter Hospital, a tertiary referral centre in New South Wales, Australia. Data collection occurred over the two year period July 2006 to June 2008. Analyses include data from singleton pregnancies. The study received ethics approval from the Hunter New England Human Research Ethics Committee and all participants gave written informed consent. Maternal blood samples were collected after an overnight (10-12 hour) fast at approximately 20 and 36 weeks' gestation, and then at 13 and 26 weeks' postpartum (three and six months respectively). To be included in the homocysteine sub-study: (i) the mothers had to have provided at least three of the four fasting blood samples; and (ii) their infant had to have a non-fasting blood sample at six months of age, which had been assayed for pHcy.

A single venous blood sample for vitamin B12, folate and homocysteine detection was collected for both the mothers and infants in the study using non-gel lithium heparin tubes. All assays were performed by HAPS, a National Association of Testing Authorities (NATA) accredited laboratory. Plasma vitamin B12, pFol and rcFol were measured by paramagnetic particle, chemiluminescent immunoassay (Access® Immunoassay Systems, Beckman Coulter, Inc. CA, USA), directly following the sample's collection. Plasma homocysteine levels were also measured using a chemiluminescence immunoassay procedure (Immulite® 2000 Homocysteine, Siemens Healthcare, VIC, Australia) from the same samples, although they had been centrifuged and stored at -80^{o}C. Homocysteine assays were performed on 25 June, 2008. The accurate analytical ranges described by the manufacturers and the normal adult reference intervals reported by HAPS are presented in Table 7.1.

Table 7.1 Accurate analytical and normal reference intervals for the biomarkers assayed in the WATCH Study of vitamin B12, folate and homocysteine in pregnancy and after birth

Biomarker	Blood constituent	Abbreviation	Accurate analytical range*	Normal reference range†	Units
Vitamin B12	Plasma	pB12	37 - 1107	135 - 600	pmol/L
Folate	Plasma	pFol	1.1 - 45.3	7.0 - 34.0	nmol/L
Folate	Red cells	rcFol	1 - 2840	315 - 1420	nmol/L
Homocysteine	Plasma	pHcy	2 - 50	5.0 - 15.0	µmol/L

*From the assay manufacturers.
†Reported by Hunter Area Pathology Service for adults. No pregnancy or infant ranges were available.

Of the maternal samples, *n* 133 were collected at 20 weeks' gestation, *n* 137 at 36 weeks' gestation, *n* 112 at 13 weeks' postpartum, and *n* 100 were collected at 26 weeks' postpartum. Sixteen women and their singleton infants were included in the homocysteine sub-study. Among them, *n* 15 mothers provided a fasting blood sample at 20 weeks' gestation, *n* 16 at 36 weeks' gestation, *n* 15 at three months after birth and *n* 16 at six months after birth.

In order to analyse the data as continuous, all readings of pFol greater than 45.3 nmol/L were replaced with 45.4 nmol/L (*n* 43 changes). Similarly rcFol levels greater than 2840 nmol/L were replaced by 2841 nmol/L (*n* 1 change). No pB12 or pHcy values reached the upper detectable limits.

7.2.1.1 Statistical analysis

The distribution of each variable was assessed using a normal probability plot, mean and median comparison, and a histogram. Means and standard deviations were used to summarise normally distributed variables, whereas nonparametric data were summarised using median and tenth to ninetieth percentiles. The maternal variables pB12, and rcFol were skewed right. Log transformations for these variables were undertaken to obtain the normal (Gaussian) distribution. Repeated measures analysis of variance (ANOVA) was performed on the log-transformed variables, testing for within-individual change over time. Wilcoxon matched-pairs signed-rank tests were performed to assess the statistical significance of change between successive visits. Maternal pFol was normally distributed hence paired t-tests were used instead.

Mann-Whitney U tests were performed for infant weight, length and weight-for-length at age six months, according to sex. The maternal variables pB12, pFol, rcFol and pHcy were log transformed for the homocysteine sub-study. Folate z-scores were calculated for infants using the equation: (infant's folate value - mean folate value)/standard deviation. Forward stepwise regression was used to test for maternal predictors of infant pB12, folate z-score and pHcy. An 'average pregnancy' and 'average postpartum' value for maternal pB12, pFol, rcFol and pHcy was calculated where two values per period (pregnancy or postpartum) were available. These variables were tested as potential predictors of infant pB12, folate z-score and pHcy levels using simple linear regression. Statistical analyses were carried out using Intercooled Stata 9 (StataCorp LP, College Station, Texas, USA).

7.3 RESULTS

7.3.1 Participants

Table 7.2 presents the maternal and infant characteristics of the study participants, including those for the homocysteine sub-study. The mean age of the *n* 175 women included was 28.8 (± 5.7) years. Note that infant weight, length and weight-for-length at six months have been presented according to infant sex (Table 7.2). No statistically significant differences between the size of the male and female infants were apparent, hence all subsequent analyses combine the two groups.

Table 7.2 Maternal and infant characteristics of the 175 mothers and 16 mother-child pairs included in the WATCH Study of vitamin B12, folate and homocysteine during pregnancy and after birth

All (*n* 175)	**Mean**	**Standard deviation**		
Maternal age at conception (yr)	28.8	± 5.7		
Maternal height (cm)	164.5	± 6.7		
	Median	**10-90th percentile**		
Pre-pregnancy weight (kg)*	65.0	52.0 - 90.5		
Pre-pregnancy BMI (kg/m^2)	24.2	19.5 - 34.0		
Homocysteine sub-analysis (*n* 16)				
Maternal age at conception (yr)	30.6	20.9 - 34.8		
Maternal height (cm)	165.1	156.3 - 169.1		
Pre-pregnancy weight (kg)*	66.3	50.0 - 83.5		
Pre-pregnancy BMI (kg/m^2)	24.6	17.5 - 30.8		
Infants	**Male (*n* 10)**		**Female (*n* 6)**	
Weight at 6 months (kg)†	7.8	6.9 - 9.7	6.9	6.2 - 8.2
Length at 6 months (cm)†	70.2	63.7 - 73.4	66.6	63.5 - 70.3
Weigh-for-length percentile†	42.6	10.3 - 69.4	37.3	7.6 - 67.1

*Self-reported at 20 weeks' gestation
†No statistically significant differences between the male and female infants; Mann-Whitney U test, *P* >0.050

7.3.2 Maternal plasma and red cell folate

Plasma folate and rcFol during pregnancy and postpartum were measured in all maternal samples. The association between the corresponding measurements of maternal pFol and rcFol are shown in Figure 7.1. The pairwise correlation coefficient (*r*) was 0.64 (*r* 0.68 for log values of rcFol), *P* <0.001. The pairwise correlation

coefficient was almost identical (*r* 0.63) when the pFol values that reached the upper limit of detection were excluded.

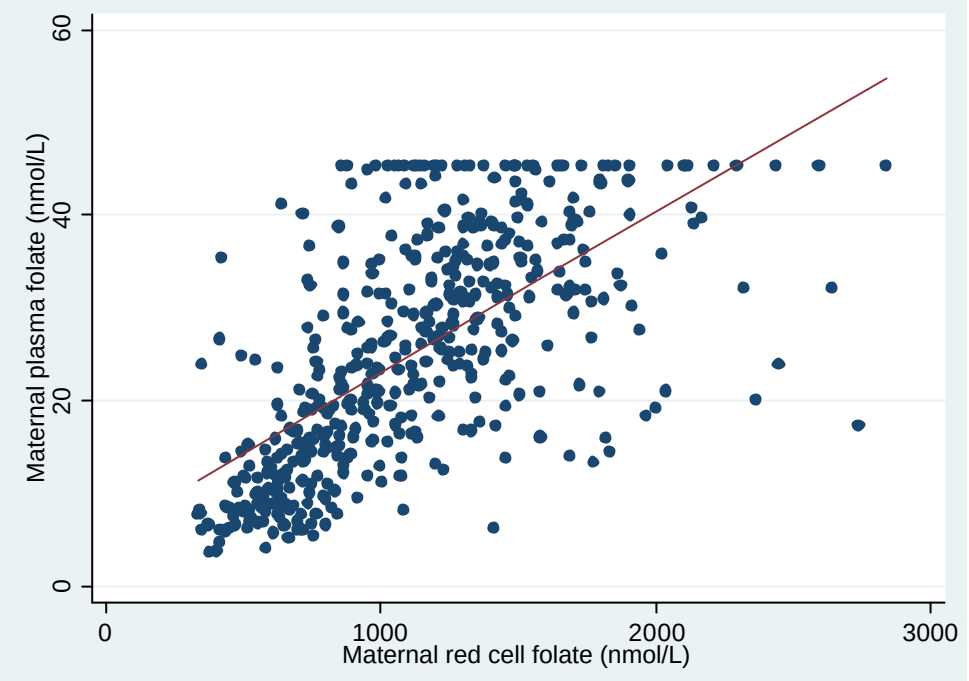


Figure 7.1 The correlation between maternal red cell folate (rcFol) and plasma folate (pFol) levels during pregnancy and postpartum; 475 observations from 166 women in the WATCH Study

The pFol assay has an upper limit of detection of >45.3 nmol/L. These values were replaced by 45.4 nmol/L (*n* 43 changes). Similarly rcFol levels >2840 nmol/L were replaced by 2841 nmol/L (*n* 1 change). The pairwise correlation coefficient (*r*) was 0.64 (*r* 0.68 for log values of rcFol), *P* <0.001.

7.3.3 Maternal vitamin B12 and folate

Maternal pB12 levels demonstrated a statistically significant decline between 20 and 36 weeks in pregnancy (*P* <0.001), followed by an increase by 13 weeks' postpartum (*P* <0.001) (refer to Figure 7.2 and Table 7.3). No significant change was observed between 13 and 26 weeks' postpartum (*P* 0.464). Maternal pFol levels showed a small but significant decline between 20 and 36 weeks during pregnancy (*P* 0.014), and a non-significant decline between 36 weeks' gestation and 13 weeks' postpartum (*P* 0.098), with no further change at 26 weeks' postpartum (*P* 0.735). Maternal rcFol levels significantly declined between each consecutive study visit (*P* 0.002, *P* <0.001, and *P* 0.003 respectively).

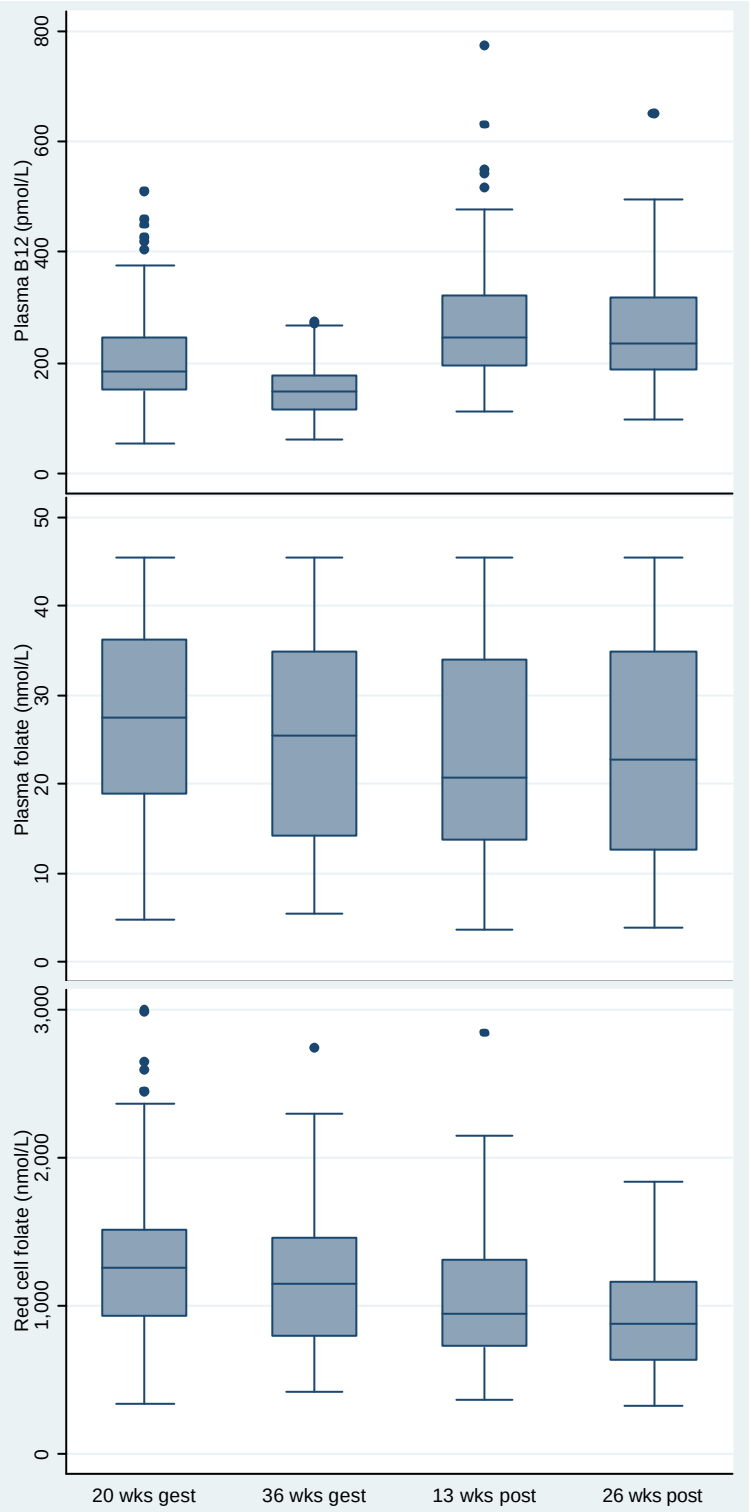


Figure 7.2 Boxplots of plasma vitamin B12, plasma folate, and red cell folate levels for 175 women in the WATCH Study at 20 and 36 weeks' gestation (gest) and at 13 and 26 weeks' postpartum (post)

Data tabulated in Table 7.3.

Table 7.3 Plasma vitamin B12, plasma folate, and red cell folate levels in the WATCH Study at 20 and 36 weeks' gestation (Gest) and at 13 and 26 weeks' postpartum (Post) for 175 women

	Weeks'				*P*-value (Adj R^2)*
	20 Gest	36 Gest	13 Post	26 Post	
Plasma vitamin B12					
Median	185.0	150.0	247.5	237.0	<0.001† (0.779)
10-90th percentile	119 - 307	95 - 221	155 - 403	149 - 410	
n <150 pmol/L‡ (*n* available)	31 (132)	68 (137)	11 (112)	10 (100)	
Plasma folate					
Mean	27.3	24.7	23.8	23.5	0.0011 (0.547)
Standard deviation (±)	11.3	12.0	12.7	12.3	
n <7 nmol/L‡ (*n* available)	4 (131)	8 (134)	7 (112)	4 (99)	
Red cell folate					
Median	1262	1151	950	872	<0.001† (0.746)
10-90th percentile	695 - 1862	606 - 1743	570 - 1642	478 - 1412	
n <400 nmol/L‡ (*n* available)	1 (133)	0 (137)	2 (111)	5 (100)	

*The adjusted (Adj) R^2 explains the proportion of overall variance (of all the data, pooling the groups) attributable to the differences among the group means for each time point. It compares the variability among the group means with the variability within the groups.

† Repeated measures analysis of variance for the log-transformed values.

‡ The 'low' cut-points have been suggested by Milman *et al.* (2006) from their pregnancy data[449]. The limit for plasma folate has been adjusted from <6 nmol/L in their cohort to <7 nmol/L in the WATCH Study.

These data have been compared to the reference intervals published for a large cohort of Danish women (*n* 441) who have had the same haematological data collected at 18, 32 and 39 weeks' gestation and 8 weeks' postpartum (Figure 7.3).

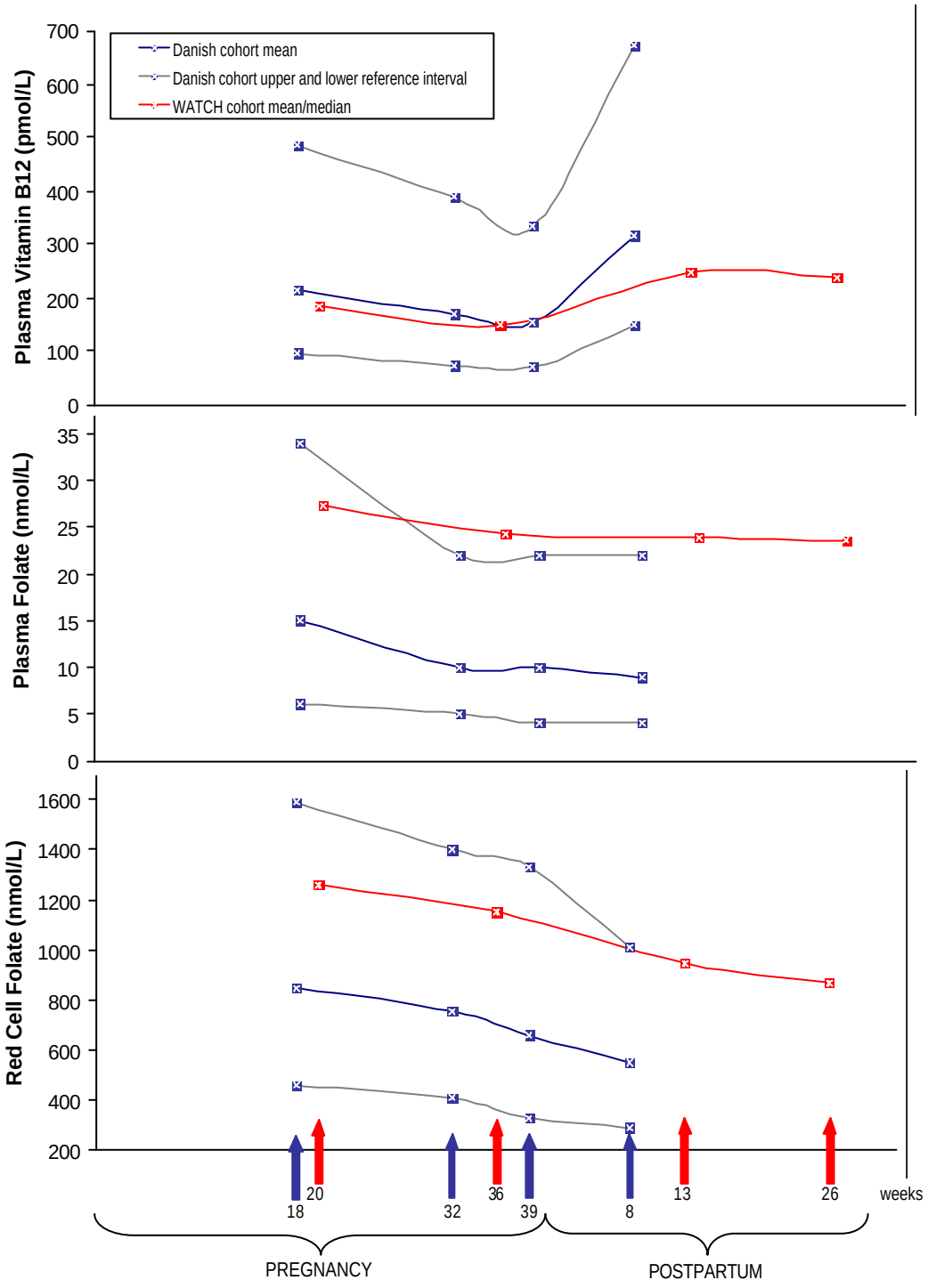


Figure 7.3 Comparison of plasma vitamin B12, plasma folate and red cell folate during pregnancy and postpartum for the WATCH cohort (*n* 175) and a reference population of healthy Danish women (*n* 441)

The Danish geometric means and reference intervals (mean ±1.96 x SD) are shown for each biomarker. The median values for plasma vitamin B12 and red cell folate, and mean values for plasma folate are shown for the WATCH cohort.

7.3.4 The homocysteine sub-study

7.3.4.1 Vitamin B12 and folate supplementation

Maternal folate supplementation pre-pregnancy and/or during pregnancy was common in this subgroup of women (Table 7.4). At the 20 week study visit, 13 of the 16 women reported taking either folate alone or in combination with vitamin B12 regularly, at least every second day and for a minimum period of two weeks. No participants supplemented vitamin B12 in isolation from folate. The majority of women who used supplements during pregnancy (9 of the 13) did so by means of a 'pregnancy-formulated' multivitamin and mineral complex.

Table 7.4 Frequency (*n*) of maternal pre-pregnancy and early pregnancy (before 20 weeks' gestation) vitamin B12 and/or folate supplementation (total *n* 16)

Vitamin supplement(s)	Pre-pregnancy	Early pregnancy
Vitamin B12	0	0
Folate	5	2
Vitamin B12 and Folate	3	11
No supplementation	8	3
Supplementation period (weeks)	4 - 52	2 - 20

Maternal pFol and rcFol levels were significantly higher at 20 weeks' gestation in women who had supplemented with folate pre-pregnancy (Table 7.5). Corresponding pHcy levels were significantly lower at 20 weeks' gestation. At 36 weeks' gestation maternal rcFol levels were significantly higher for women who had taken folate supplements with or without vitamin B12 early in their pregnancy. Plasma folate was higher at borderline statistical significance, and pHcy did not differ according to supplementation status. At 26 weeks' postpartum maternal pFol and rcFol levels were significantly higher for women who had taken folate supplements with or without vitamin B12 early in their pregnancy. Plasma homocysteine again did not differ according to supplementation status. A similar trend was observed at 13 weeks after birth however the results were not statistically significant.

Table 7.5 Maternal plasma vitamin B12 (pB12), plasma folate (pFol), red cell folate (rcFol) and plasma homocysteine (pHcy), according to pre-pregnancy and early pregnancy (before 20 weeks' gestation) vitamin B12 and/or folate supplementation

Maternal biomarkers	B12 and/or folate supplementation	*n*	Median	10 - 90th percentile	*P*-value*
20 wks gestation	**Pre-pregnancy**				
pB12	Yes	8	167.0	127 - 255	0.908
	No	7	183.0	111 - 254	
pFol	Yes	8	37.7	13.4 - 45.4	0.020†
	No	7	12.5	5.2 - 35.6	
rcFol	Yes	8	1596.5	985 - 2109	0.004†
	No	7	914.0	617 - 1300	
pHcy	Yes	5	4.3	3.8 - 5.2	0.027†
	No	4	5.55	4.9 - 7.7	
36 wks gestation	**Early pregnancy**				
pB12	Yes	12	133.5	110 - 241	0.279
	No	3	118.0	105 - 162	
pFol	Yes	11	28.7	21.0 - 37.1	0.052‡
	No	3	16.9	5.8 - 27.9	
rcFol	Yes	12	1368.0	1022 - 1578	0.009†
	No	3	734.0	606 - 767	
pHcy	Yes	11	5.1	2.9 - 6.1	0.693
	No	2	5.8	3.3 - 8.2	
13 wks postpartum	**Early pregnancy**				
pB12	Yes	11	250.0	195 - 363	0.312
	No	3	172.0	138 - 381	
pFol	Yes	12	24.4	10.1 - 38.0	0.083
	No	3	10.6	10.2 - 13.8	
rcFol	Yes	12	967.0	698 - 1203	0.061
	No	3	665.0	474 - 876	
pHcy	Yes	11	7.8	5.9 - 9.5	0.242
	No	3	8.9	7.5 - 10.0	
26 wks postpartum	**Early pregnancy**				
pB12	Yes	13	288.0	171 - 371	0.201
	No	3	218.0	107 - 280	
pFol	Yes	13	33.2	9.7 - 45.4	0.037†
	No	3	8.0	7.9 - 13.4	
rcFol	Yes	13	1040.0	538 - 1380	0.019†
	No	3	497.0	341 - 585	
pHcy	Yes	13	8.2	4.7 - 9.4	0.419
	No	3	9.4	5.9 - 13.0	

*From Mann-Whitney U-tests.
†Statistically significant at $P < 0.050$.
‡Borderline statistical significance.

No infants received nutritional supplementation in the first six months of life. There were no significant differences (P >0.050) between infant levels of pB12, pHcy or folate z-scores according to whether or not their mother had used supplements either pre-pregnancy or early in pregnancy (data not shown).

7.3.4.2 Infant feeding

At six months of age 10 of the 16 infants were continuing with breastfeeding; seven in combination with solids, and three in combination with both an infant formula and solids. Six infants were receiving no breastmilk; four were receiving a combination of formula and solids; one was exclusively formula-fed, and one was exclusively fed solids. Table 7.6 reports the infants' pB12, folate z-scores and pHcy according to breastfeeding status at six months. The infants receiving breastmilk had significantly lower levels of pB12 and lower folate z-scores, while pHcy was markedly higher compared to those not receiving any breastmilk at age six months.

Table 7.6 Infant plasma vitamin B12, folate z-scores and plasma homocysteine according to breastfeeding status at age six months

Infant Biomarkers	Breastfed at six months	*n*	Median	10 - 90th percentile	*P*-value*
Plasma vitamin B12	Yes	9	174.0	112 - 420	0.013†
	No	6	393.5	193 - 682	
Folate z-score	Yes	9	-0.431	-2.357 - 0.637	0.013†
	No	6	0.607	0.018 - 2.266	
Plasma homocysteine	Yes	10	11.1	8.6 - 13.2	0.002†
	No	6	6.4	3.9 - 8.8	

*From Mann-Whitney U-tests.
†Statistically significant at P <0.050.

7.3.4.3 Infant folate z-scores

For the infants with pFol values (n 9) the median folate value was 44.4 (tenth to ninetieth percentile: 28.9 - 45.4 nmol/L) (Table 7.7). For the infants with rcFol values (n 8) the median folate value was 879 (tenth to ninetieth percentile: 333-1988) nmol/L. Figure 7.4 shows the distribution of infant folate z-scores according to whether they were derived from plasma (0) or red cell (1) folate values. The random scatter of both above and below zero suggests that the standardised folate scores are not heavily biased by either measure of folate. Two infants had both folate assays performed. Both z-

scores for both have been presented and circled in red to highlight the similar z-scores resulting from either biomarker although only their rcFol z-scores were used in the infant analyses.

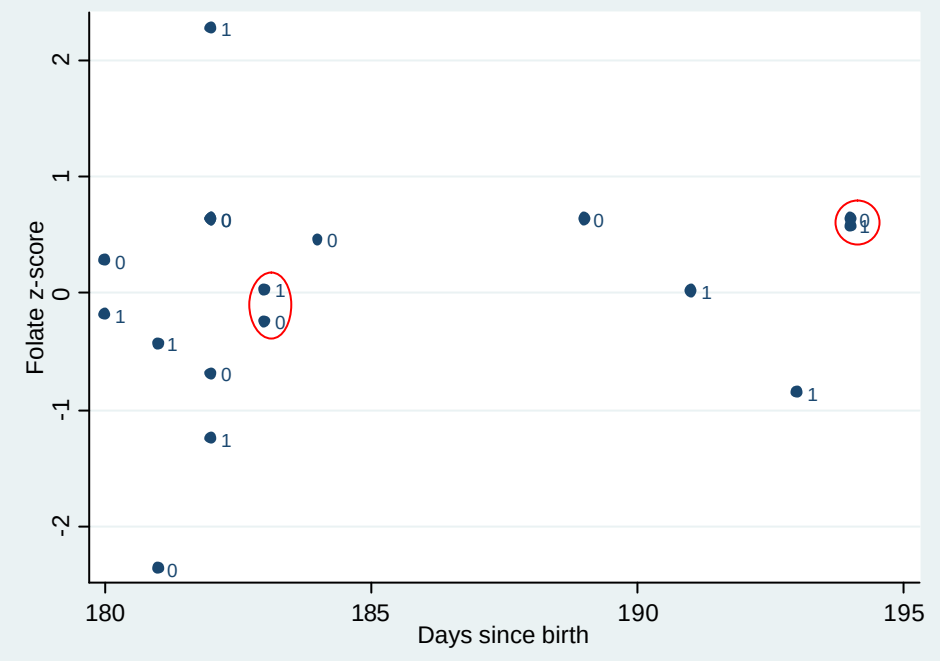
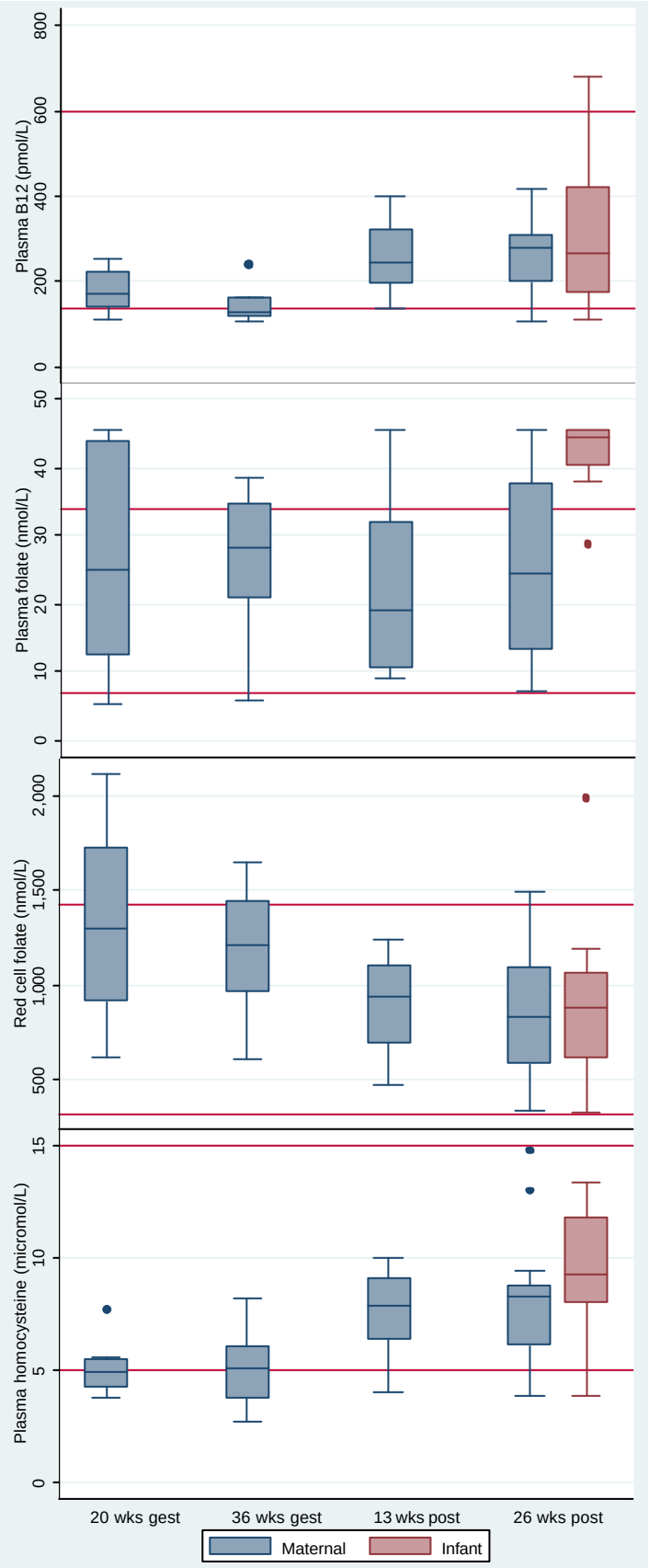


Figure 7.4 WATCH Study infant folate z-scores at age six months (*n* 16) derived from plasma (0) and red cell (1) folate values

The red circles indicate where two z-scores were available for the same infant.

7.3.4.4 Vitamin B12, folate and homocysteine

Maternal pB12 levels demonstrated a statistically significant decline between 20 and 36 weeks in pregnancy (*P* 0.003), followed by a large increase by 13 weeks' postpartum (*P* 0.001) and no significant change at 26 weeks' postpartum (*P* 0.394) (Figure 7.5 and Table 7.7). Maternal pFol levels did not change significantly between consecutive study visits (*P* 0.861, *P* 0.069, *P* 0.191). Maternal rcFol levels significantly declined between 36 weeks' gestation and 13 weeks' postpartum (*P* 0.005). Maternal homocysteine showed a corresponding rise between 36 weeks' gestation and 13 weeks' postpartum (*P* 0.004). No statistically significant differences were detected between mothers and their infants at 26 weeks' postpartum (pB12 *P* 0.408; pFol *P* 0.094 (power 0.972 at α 0.05), rcFol *P* 0.674, pHcy *P* 0.155). Two participants had pB12 levels less than 150 pmol/L and pHcy greater than 13 µmol/L during pregnancy (one at 20 weeks, one at 36 weeks' gestation) indicating true B12 deficiency. This had resolved by the subsequent visit.



Gest, gestation; Post, postpartum

Figure 7.5 Boxplots of plasma vitamin B12, plasma folate, red cell folate and plasma homocysteine during pregnancy and after birth in 16 WATCH Study women and their singleton infants at age six months

The normal adult upper and lower reference intervals for each biomarker are shown in red.

Table 7.7 Plasma vitamin B12, plasma folate, red cell folate and plasma homocysteine during pregnancy and after birth in a sample of 16 women and their singleton infants at age six months

	Weeks*				*P*-value†	Infants 26 wks post
	20 Gest	36 Gest	13 Post	26 Post		
Plasma vitamin B12						
Median	169.0	130.0	243.5	257.0		265.0
10 - 90th percentile	125 - 254	106 - 241	172 - 381	159 - 371	<0.001	126 - 530
n <150 pmol/L‡ (*n* available)	4 (15)	9 (15)	1 (14)	1 (16)		4 (16)
Plasma folate						
Median	25.1	28.2	19.0	24.4		44.4
10 - 90th percentile	8.2-45.4	16.9 - 37.1	10.1 - 38	7.9 - 45.4	0.531	28.9 - 45.4
n <7 nmol/L‡ (*n* available)	1 (15)	1 (14)	0 (15)	0 (16)		0 (9)
Red cell folate						
Median	1300	1209	940	842.5		879
10 - 90th percentile	662-1826	734 - 1578	649 - 1203	497 - 1380	<0.001	333 - 1988
n <400 nmol/L‡ (*n* available)	0 (15)	0 (15)	0 (15)	1 (16)		1 (8)
Plasma homocysteine						
Median	4.9	5.1	7.9	8.25		9.3
10 - 90th percentile	3.8-7.7	2.9 - 6.9	5.9 - 9.6	4.7 - 13	<0.001	4.6 - 12.9
n >13 µmol/L‡ (*n* available)	0 (9)	0 (13)	0 (14)	1 (16)		1 (16)

*Gest, gestation; Post, postpartum.

†Repeated measures analysis of variance for the log-transformed values.

‡The 'low' and 'high' cut-points have been suggested by Milman *et al.* (2006) from their pregnancy data[449]. The limit for plasma folate has been adjusted from <6 nmol/L in their cohort to <7 nmol/L in the WATCH Study.

7.3.4.5 Correlations between vitamin B12, folate and homocysteine

Table 7.8 shows the correlation coefficients for pHcy and pB12, pFol, rcFol, or infant folate z-scores. Maternal pFol and rcFol levels were significantly inversely correlated with maternal pHcy at 20 weeks' gestation and 26 weeks' postpartum. At 26 weeks' postpartum infant pHcy was significantly inversely correlated with infant pB12, and with infant folate z-scores.

Table 7.8 Pairwise correlation coefficients (with *P*-values) for log transformed plasma vitamin B12, plasma folate, red cell folate and plasma homocysteine during pregnancy and after birth in a sample of 16 women and their six month old infants from the WATCH Study

Correlations with plasma homocysteine	Maternal				Infant
	20 wks Gest	36 wks Gest	13 wks Post	26 wks Post	26 wks Post
Plasma vitamin B12	0.18 (0.648)	-0.43 (0.146)	-0.17 (0.588)	-0.29 (0.273)	-0.73 (0.002†)
Plasma folate	-0.88 (0.002*)	-0.55 (0.064)	-0.34 (0.240)	-0.63 (0.008*)	-0.53† (0.044*)
Red cell folate	-0.81 (0.008*)	-0.02 (0.959)	-0.43 (0.123)	-0.56 (0.024*)	

Gest, gestation; Post, postpartum.
*Statistically significant at *P* <0.050.
†Folate z-scores for infants from combined red cell and plasma data.

Table 7.9 reports the statistically significant forward stepwise regression models with maternal variables used to predict the infant outcomes. Maternal rcFol and pHcy levels were significant predictors of infant pB12 levels at 26 weeks' postpartum. Note that infant folate z-scores (CE 0.334, SE 0.044, *P* <0.001) and infant pHcy (CE -0.601, SE 0.116, *P* <0.001) were also significant predictors in the model. Maternal rcFol and pHcy were also significantly predictive of infant folate z-scores. Infant pB12 (CE 1.473, SE 0.158, *P* <0.001) and infant weight at 26 weeks' postpartum (CE 0.127, SE 0.075, *P* 0.097) were also significant predictors in the model. No maternal variables predicted infant pHcy at 26 weeks' postpartum, however infant pB12 (CE -0.470, SE 0.069, *P* <0.001) and infant length at 26 weeks' postpartum (CE 0.024, SE 0.011, *P* 0.042) were statistically predictive (*F* (2, 44) 30.81, R^2 0.583, *P* <0.001 for the model).

Table 7.9 Forward stepwise regression analyses using maternal variables during pregnancy and postpartum as predictors of infant vitamin B12, folate and homocysteine levels at age 6 months (*n* 16)

Dependent variable: infant	Predictive variable: maternal	Adj R^2	*F*	CE	SE	Predictor *P*-value	Model *P*-value
Plasma vitamin B12	Red cell folate	0.810 (0.792)	(4, 42) 44.69	0.341	0.121	0.007	<0.001
	Plasma homocysteine			0.162	0.123	0.193	
Folate z-score	Red cell folate	0.716 (0.690)	(4, 42) 26.47	-0.945	0.260	0.001	<0.001
	Plasma homocysteine			-0.543	0.269	0.049	

CE, coefficient.
Potential variables to be added to the model (*log-transformed): **Maternal:** pB12*, pFol*, rcFol*, pHcy*, age at conception, pre-pregnancy weight*, and height; **Infant:** pB12*, folate z-score, and/or pHcy* (if not being tested as the dependent variable), weight, and length at age 6 months.

Finally, linear regressions using averaged maternal variables during pregnancy and postpartum as predictors of infant B12, folate and homocysteine levels at age 6 months were undertaken. The average value of maternal pregnancy pFol was significantly predictive of infant homocysteine levels at 26 weeks' postpartum (*n* 13, *F* (1, 11) 4.77, adjusted R^2 0.239, CE -0.404, SE 0.185, *P* 0.050). All other linear regressions for the averaged pregnancy and postpartum maternal data were not statistically significant.

7.4 DISCUSSION

This is the first longitudinal study to have characterised maternal levels of vitamin B12 and folate during pregnancy and up to six months after birth, in a cohort of 175 Australian women. Additionally we have included a small (*n* 16) but detailed paired mother-infant sub-study of pHcy. Previously the largest cohort of more than 400 Danish women to follow the same haematological indices has reported data at 18, 32, and 39 weeks' gestation, in addition to 8 weeks' postpartum[262, 449, 450]. We have expanded this data range (20 and 36 weeks' gestation) to include measures at 13 and 26 weeks' postpartum, as well as the paired infant data at six months after birth.

This study of maternal and infant pB12, folate and homocysteine has produced several key findings that are consistent with other literature.

- Plasma vitamin B12 declined significantly during pregnancy but recovered within three months after birth.
- Red cell folate also declined significantly during pregnancy and continued to do so up to six months after birth.
- Plasma homocysteine was lower during pregnancy than after birth.
- Plasma folate and rcFol levels were significantly inversely correlated with pHcy in women early in pregnancy and six months after birth.
- Plasma folate and rcFol values were highly positively correlated therefore both biomarkers can be considered clinically useful.
- Pre-pregnancy and early pregnancy folate supplementation was associated with higher maternal pFol and rcFol stores.

It has also produced results which are novel to this area of research:

- Vitamin B12 and folate z-scores were inversely correlated with pHcy in six month old infants.
- There were no significant differences between mothers and their infants for levels of pB12, pFol, rcFol and pHcy at six months after birth.
- Infants who were not being breastfed at six months of age had higher pB12, higher folate z-scores, and lower pHcy than infants receiving breastmilk.

- At six months of age infants born to women who took folate supplements either before and/or during pregnancy did not have higher folate z-scores than infants born to mothers who did not take vitamin tablets.
- After multivariate adjustment, maternal rcFol and pHcy were significant predictors of infant pB12 and folate z-scores at age six months.
- The averaged value of maternal pFol in pregnancy inversely predicted the infant's level of pHcy at age six months.

These findings warrant further investigation in adequately powered, prospective studies.

The comparison of our data to the Danish cohort as a reference population shows consistent trends in the decline for pB12 and folate during pregnancy (Figure 7.3). Other studies have also observed the same declines during pregnancy[453, 454] although few studies report postpartum follow-up. The authors of the Mexican subsample showed a recovery in the mean pB12 levels between pregnancy and lactation, although interestingly low pB12 concentrations (defined as less than 103 pmol/L) were more common in lactation (30%) than in pregnancy (19%)[451].

The decline in pB12 during pregnancy is thought to be a normal event caused by alterations in the cobalamins attached to haptocorrin[261] (a protein that binds vitamin B12 in the circulation, with an unknown function) rather than alterations in holoTC, which is the biologically active vitamin B12 fraction that is delivered to all tissues in the body[264] (refer to section 2.4.1.4). Morkbak *et al.* (2007) have reported that holoTC remains unchanged during normal pregnancy, and that it may be a better marker of true vitamin B12 deficiency than pB12. Additionally, high MMA and pHcy levels are highly sensitive (number of true positives) markers of vitamin B12 deficiency, although they have debatable and low specificities (number of true negatives) respectively[264].

From our investigations, in conjunction with the Danish data, pB12 recovers after birth, while pFol appears to stabilise and rcFol continues to gradually decline. The main disparity between our Australian data and the reference intervals published from the Danish cohort[262] are the absolute levels of folate in both the red cells and plasma. They are significantly higher in our cohort, breaching the upper interval for pFol by 36 weeks' gestation and intercepting it at 8 weeks' postpartum for rcFol (Figure 7.3). This is likely to be accounted for by two factors. The first is the difference in the exclusion

criteria for each study. The Danish study excluded women who were taking nutrient supplements, whereas the Australian women in the WATCH Study were included irrespective of supplementation status. The second is the differences in the Australian and Danish fortification policies. Denmark currently has no folate fortification program[455], whereas Australia introduced a voluntary folic acid fortification program for cereal-based foods, fruit and vegetable juices, and yeast extracts over a decade ago[456]. Folic acid fortification of bread made with wheat flour will become mandatory in Australia from 2009[456].

In Table 7.3 and Table 7.7 the prevalence of low pB12, pFol, and rcFol, and high pHcy are reported. The cut-points applied have been suggested by Milman *et al.* (2006) from their own pregnancy data[449]. Compared to the normal adult reference range provided by our local pathology service, the intervals for the pregnancy data are slightly narrower. Plasma folate was the exception to this. We therefore applied the lower limit from the standard reference interval, changing the inclusion for a low value from less than 6 nmol/L to less than 7 nmol/L.

In the homocysteine sub-study, maternal pHcy levels were inversely correlated with folate status (plasma and red cell) at mid-pregnancy and six months after birth. Infant pHcy levels were inversely correlated with both pB12 and folate status. Furthermore, when maternal haematological indices during pregnancy and after birth were tested as predictors of infant outcomes at six months (adjusted for growth and other infant haematological markers) it was found that maternal rcFol and pHcy were independently associated with infant folate z-scores and pB12. Additionally the averaged value of maternal pFol during pregnancy was significantly inversely predictive of infant pHcy at six months. These findings lend support to the hypotheses that intrauterine nutrition, in particular exposure to folate, may program haematologic and metabolic endpoints for the offspring which are detectable from infancy onwards. However, theses findings require replication in studies which also include clinical outcomes to assess the true consequence.

7.4.1 Limitations

Our investigations have focused exclusively on pB12 as a biomarker of B12 status. In the general population a pB12 reading of less than 135 pmol/L would automatically

constitute B12 deficiency[264]. However, in pregnancy this can only be confirmed in the presence of perturbations in a secondary cobalamin marker, such as elevated MMA (greater than 0.28 μmol/L) or homocysteine (greater than 13 μmol/L)[450].

In order to statistically analyse the haematological data as continuous variables, several changes were needed. Differences that may exist within individuals who demonstrated high levels of folate may be better detected by the rcFol levels, which continue to differentiate between individuals with very high folate levels. The high pFol levels are likely to be the result of relatively short-term folate supplementation. Further investigation into the supplementation status of the main cohort is required.

The sample of 16 mother-infant pairs in the homocysteine sub-study is clearly inadequate to generalise the findings to the broader population or other groups. However, given the significant associations that were still detected, it does provide preliminary data and direction for further studies into the potential programming of metabolic biomarkers including homocysteine.

In conclusion, pregnancy and the postpartum period are characterised by significant changes in maternal haematological indices, including pB12, pFol and rcFol, and pHcy. These changes have now been characterised longitudinally up to 6 months after birth, including in a small sample of infants. These may be important intrauterine nutritional exposures which potentially regulate health outcomes for the offspring via epigenetic mechanisms. In our small sub-study maternal biomarkers assessed during pregnancy and after birth (folate in particular) were identified as predictors of the offspring's haematological indices at age 6 months. Further investigations are required to determine the accuracy of this finding in addition to the clinical relevance.

Chapter 8
Final Discussion

This thesis has presented three chapters of original research related to maternal and fetal nutrition, and one chapter of empirical data concerning the methodological challenges faced when recruiting for research purposes. Two of these chapters have been published as manuscripts in international peer-reviewed journals (Chapter 4 and Chapter 5), while the remaining two results chapters have been prepared for publication and each presented at national conferences (Chapter 6 and Chapter 7). Some data for this thesis have been obtained from a national resource: the ALSWH (www.alswh.org.au). Additionally, a new prospective longitudinal cohort of 182 pregnant women was recruited and followed-up postnatally, to test hypotheses which link maternal nutritional and dietary factors to outcomes for the mother and offspring during pregnancy and postpartum. Data collection continues with two-year infant follow-ups scheduled to commence for our mothers and babies in December 2008.

The concepts presented herein are of a multidisciplinary nature, offering additional insights to the fields of nutrition and dietetics, obstetrics and gynaecology, public health, haematology, and health care sciences and services. The aim of this final chapter is to provide a contextualised and integrated summary of this work, suggesting how the findings contribute to our understanding of the nutritional influences in pregnancy and postpartum for women and their children. Both the limitations and strengths of the work are acknowledged and future research directions are proposed.

An analysis of dietary data collected from the young cohort within the ALSWH was first undertaken to characterise the nutritional adequacy of the dietary intakes of young contemporary Australian women. The young cohort demographics have previously been compared with Australian census data and the sample is deemed to be reasonably representative of the general population, except for a somewhat higher inclusion of women who are married or in a *de facto* relationship, and of women with post-school education[408]. The most recent Australian National Nutrition Survey was conducted between February 1995 and March 1996 across all States and Territories[457]. It included 3178 women of child-bearing age (16 to 44 years), although only 75.7% of those (*n* 2406) reported biologically plausible energy intakes[457]. Our own study included 7486 young women after energy cut-points had been applied, making this currently the largest study of dietary intake in Australian women of child-bearing age[31]. However,

the age group included in our analysis was limited to 25 to 30 years which narrows the generalisability of the findings[31].

Beyond characterising the diets of young Australian women this data was used to test the null hypothesis, that diet quality does not differ between women who are pregnant compared to those who are not. Women who reported 'trying to conceive' and those who had given birth in the previous 12 months were separated out from the main cohorts (pregnant and nonpregnant) as these were perceived as significantly different life-stages with specific nutritional needs (for example, folate supplementation preconception for those trying to conceive, and the increased nutritional demands associated with lactation).

The null hypothesis was not refuted given that the mean diet quality scores for pregnant compared to non-pregnant women differed by only a small amount (one point or 1.4% difference). The investigation of the diet quality food group component scores also supported the null hypothesis, with very little evidence to suggest that sub-scale scores reflecting intakes of the major core food groups were scored differently between the pregnancy groups. Overall diet quality scores were consistently low, suggesting that a limited range of nutritious foods are regularly included in the diets of young Australian women.

Mean folate intakes were slightly higher for pregnant women and those who reported a birth in the previous 12 months. However, the reported intakes of folate, irrespective of pregnancy group, did not achieve the EAR for all women aged 19 to 30 years. Furthermore, the slightly higher intakes during pregnancy and after birth were not proportional to the increased demands that accompany pregnancy and lactation. Many young Australian women also failed to reach key nutrient targets, including those set for fibre, calcium, iron, potassium and vitamin E.

This cross-sectional data analysis was considered essential to the advancement of this thesis, given that there were no recent published reports on dietary intake during pregnancy, or other states, for a large, nationally representative sample of Australian women. From this work, pregnant women and those trying to conceive did not have markedly 'better' diets in terms of their micronutrient intakes, though these were assessed via the food frequency methodology. Our data serves to facilitate international

comparisons of intake during pregnancy, which may help to elucidate important associations between nutrients and disease in mothers and babies.

The diet quality scoring system (refer to section 4.2.3) implemented for this study offers several key advantages. It reduces the measurement and reporting error that is associated with FFQs by dichotomising the food and beverage items into those consumed frequently or infrequently (daily or weekly depending on the item). It also takes a 'whole of diet' approach providing a cumulative total for the represented food groups. However, not all food and beverage items consumed are represented in the 200-item FFQ, and only 72 of these 200 items have been scored using this tool (note that the original ARFS includes 74 items, with two points available for a safe consumption of alcohol)[411]. The ARFS is modelled on a scoring system previously developed and used in large population studies internationally[413]. It has been shown that intakes from the core foods (i.e. the nutrient dense items), rather than non-core (i.e. energy dense and/or nutrient poor items), are more representative of nutritional adequacy[458].

To utilise all of the dietary data that were available, nutrient intakes were included for those considered to have reported biologically plausible energy intakes (ranging from 5 MJ to 20 MJ per day). Food frequency questionnaires have the ability to rank individuals according to nutrient intakes, as does the ARFS used in this thesis. Other studies (one of which was during pregnancy[459]) have shown satisfactory agreement in validations comparing FFQs and four-day WFRs or seven-day diet diaries, with significant correlations for all major food groups and almost all nutrients[459, 460]. In fact, the Norwegian Mother and Child Cohort Study has shown a higher degree of underreporting for the four-day WFRs compared with the FFQ, when energy intakes were matched with energy expenditure data from motion sensors[461]. These studies encourage the reporting of nutrient intake data from this dietary assessment method, as we have done using the Women's Health Australia data (refer to Table 4.4 and Table 4.5).

Having addressed this gap in the literature, the research focus of this thesis then shifted to prospective longitudinal data collection for women and their children during pregnancy and after birth. The ABCD Obesity study was designed for this purpose. However, issues with recruitment were encountered early on, which threatened the potential to achieve the research aims. Rather than jeopardising the power of the

investigations or diverting attentions away from the original aims, efforts were made to understand what had gone wrong and how the situation could be rectified.

This evaluation resulted in significant changes to the study's structure and recruitment protocol, with the modified version now known as the WATCH Study. The major changes that were undertaken included: (i) modifying the recruiting materials provided to participants, in particular, improving the length and readability of the information statement and consent form and making them more visually appealing; (ii) approaching potential participants in person within the healthcare setting, rather than through written information arriving in the mail; and (iii) structuring the study to make it easier to participate, with considerations given to the time of appointments, costs that may be incurred, and the perceived benefits of participation. The process of evaluation was formalised and published, with the aim of helping others to either avoid or correct similar recruiting problems, which are now understood to be common, despite a lack of acknowledgement of such difficulties within the literature[32]. This article has now been labelled as 'highly accessed' by the journal, due to the level of interest, even though it has only been available online for a relatively short time. The remaining research chapters have utilised longitudinal data that have been collected as part of the revised study.

Within the developmental origins literature fetal growth is regularly referred to, and yet it is rarely systematically measured. As previously described (refer to section 2.2.9) birthweight, or dimensions at birth, are used as generic markers of fetal growth, despite that fact that this provides very limited information about the pattern of growth up until that point, and assumes that this is consistent between individuals. Given the availability of safe, affordable and reliable technology, such as ultrasound scanning equipment, it is surprising to learn just how limited the intrauterine growth data is. One could consider this to be an essential component of developmental origins work, given the direct insights it is able to provide. For example, in measuring fetal growth, like the plotting of infant growth charts, it becomes apparent when a child, or in this case fetus, is exhibiting either a slowing or acceleration in their growth according to their pre-established trajectory. In other words, monitoring intrauterine growth could help to identify cases of growth restriction or other anomalies, even within the normal reference

ranges. This may improve our understanding of the adaptations and outcomes that result from environmental insults experienced by the fetus.

The longitudinal measurement of fetal body composition (total area divided into lean and fat compartments) is even more novel, despite the use and validation of this ultrasound technique by others[383, 447]. To the best of the author's knowledge, we are the only research group internationally who are measuring fetal body composition to explore the relationship between the maternal nutritional environment and the concurrent growth of the offspring. This places us in the unique (future) position of being able to assess the change in an individual's growth trajectory from the prenatal to postnatal environment, with up to 10 data points available between conception and one year after birth.

The investigation of fetal adiposity in reference to maternal weight change in pregnancy reported here has produced several findings which are consistent with current literature, but has also established novel data in this area. Pre-pregnancy weight and gestational weight gain are two factors known to be positively associated with the size of the offspring at birth (refer to section 2.5.1). Intuitively, but necessary for the study's validation, these factors were also identified as significant predictors of intrauterine size and growth in the analyses presented in Chapter 6.

Our preliminary findings suggest that there are transient parallels between maternal weight changes and changes in fetal body composition, but over the entire pregnancy these variations are not significant. An increase in the fetal adipose tissue at the abdomen and thigh was predicted by greater maternal weight gain (adjusted for height) between 20 and 30 weeks' gestation. However, a compensatory slowing then seemed to negate this effect. Hence, while a woman who gains more weight, and/or commences the pregnancy at a higher BMI, is likely to have a larger baby, there is no difference in the adiposity of the offspring at term.

It is intriguing that despite huge variations in maternal pre-gravid BMI (range 26.6 kg/m^2) and weight gain throughout pregnancy (range 49.5 kg), no difference was seen in the intrauterine adiposity of the child. This fits with available literature which suggests that only small differences in the proportion of body fat in the human newborn exist. Cross-cultural studies, like the comparison of the Indian and white Caucasian

newborns (refer to section 2.2.3), show more pronounced variation in body composition, and sex-specific differences are know to exist in that girls have a slightly increased fat mass at birth relative to boys (refer to section 2.5.4.1). Nevertheless it appears that human adiposity is incredibly robust and is only compromised in extreme cases such as growth restriction and gestational diabetes. While fetal fat appears to be pre-set to a relatively constant level for birth, the subsequent adiposity of the individual remains of great interest.

The question of why humans are born with such a high proportion of fat, relative to other mammals but particularly to other primates (refer to Figure 2.2), remains largely speculative. Fat as a thermoregulatory compensation mechanism for a lack of fur and insulation have repeatedly been cited as explanations[108]. Given that the layer of adipose tissue in humans at birth is more similar to that of seals and whales, others have suggested an aquatic origin of modern man, known as the 'aquatic ape' hypothesis[462]. By and large this theory has been rejected by others[108]. Adipose tissue primarily functions as an energy reserve, providing stores for periods of nutritional adversity and infection, although these challenges are commonly encountered by other species which do not accumulated fat before birth[108]. The enlarged size of the human brain however, and its accompanying energy demands provides at least a partial explanation for the evolutionary adaptation of human intrauterine fat storage[108].

In this study, pre-pregnancy BMI and weight change during pregnancy are taken as general markers of maternal nutritional status and energy regulation. As described in section 2.3, an individual's metabolic response and adaptation to pregnancy varies considerably, and the placenta plays a profound role in the nutritional experience of the fetus. A well-nourished mother with a poorly functioning placenta can produce a growth restricted fetus. Further, maternal weight may be influenced by fluid balance, which we have not measured as part of this study. Subsequently there are limitations to using maternal weight change and pre-pregnancy weight, particularly in isolation from placental weight at birth and pregnancy nutrient intake data, for broadly characterising the nutritional exposure of the fetus.

Statistically the analysis of change-with-change in the maternal and fetal data presented a significant challenge, although it was one which mandated attention because of its application to much of the longitudinal data collected during this research. Repeated

measures ANOVA is commonly employed to test whether changes within an individual are seen at different intervals (as used in section 7.3)[463]. However, repeated measures ANOVA cannot handle missing data and if any of the observations for a subject are missing, the entire subject will be omitted from the analysis[463]. In studies where observations are dependent, that is, a relationship exists because serial measurements are collected for the same individual, mixed-models may be used[464]. Linear mixed-models are able to account for both the within-individual and between-individual or 'random' variation, which results from changes in the experimental condition[464] (for example seen with advancing gestation). Linear mixed-models are well suited to unbalanced datasets where some data are missing[464]. This is common to human studies because of variations in the number of participants attending each visit, the timing of the study visits, and human error in data collection and recording. Unbalanced datasets and both between- and within-individual variation are not amenable to general multivariate modelling[464].

There are several factors that warrant further investigation as an extension of the current research. Adjustment for confounding factors may help to elucidate differences which we have not detected. Within the population studied, fetal sex, parity, smoking and socioeconomic status warrant adjustments. Participants presenting with gestational diabetes have been excluded from the analysis as this pathology can interfere with normal fetal fat deposition. Pre-eclampsia, IUGR, and/or preterm delivery should also be investigated separately.

While this analysis of maternal prepregnancy weight and weight changes during pregnancy was underway it came to the author's attention that some of the maternal biochemical indices that were being measured in pregnancy and postpartum were fluctuating considerably both between individuals and longitudinally within an individual. Pathology readings of vitamin B12 in particular were regularly reported as low, relative to the normal adult reference range, even from the first pregnancy study visit at 20 weeks' gestation. This led to an investigation of the current literature which revealed that while this was a common occurrence, the reasons for it and consequences of it, particularly for the infant, were largely underinvestigated. Until recently, the causes of this decline were largely speculative, with hemodilution, increased requirements and diversion to the fetus regularly cited as contributing factors[188, 465, 466].

Our study of the changes in vitamin B12, folate and homocysteine is the first to report longitudinal data collected during pregnancy and postpartum, up to six months after birth for a sample of over 100 Australian women. It shows the commonly reported decline in pB12 during pregnancy, with an increase up to three months after birth, and a small correction between three and six months after birth. Folate (both red cell and plasma) also declined during pregnancy, but unlike vitamin B12, it continued to do so up to six months after birth. A common criticism of nutritional research is that the period of follow-up is not long enough to capture an accurate representation of what is happening, and this study has addressed this gap.

Concurrent to our own interest in this matter, a large Danish study has made considerable headway in understanding the biochemical changes that occur in pregnancy and postpartum, with more detailed measurements of cobalamins and other haematological markers[261, 262, 449, 450]. It seems that the significant decline in pB12 results from a decline in holohaptocorrin, the partly saturated vitamin B12 binding-protein which accounts for about 80 to 90% of the endogenous pB12[263]. Importantly the holoTC, which is the metabolically active fraction accounting for up to 20% of circulating vitamin B12[263], remains unchanged during pregnancy[261]. This suggests that the delivery of vitamin B12 to metabolically active cells for use as cofactors in the two vitamin B12-dependent enzymes, methionine synthase and methylmalonyl mutase[263], is not impaired in pregnancy.

Further to the maternal biochemical characterisation of vitamin B12 and folate, this analysis included a small sub-sample of infants who also had blood collected at six months postpartum. Sixteen mother and infant pairs were included in the sub-analysis, which reported the same haematological measurements but additionally, total pHcy. Maternal levels in pregnancy and postpartum were tested as a potential predictor of homocysteine levels in the offspring, adjusted for the offspring's current vitamin B12 and folate status. This investigation was undertaken to determine whether maternal folate (a methyl donor) in pregnancy could influence the offspring's homocysteine metabolism after birth. This is in the context that these nutrients play an important role in DNA methylation (via methionine/homocysteine metabolism – refer to Figure 2.6), which is an epigenetic or developmental programming mechanism, with the potential to alter phenotypic expression and later health outcomes.

Lillycrop *et al.* (2005) have reported rat data suggesting a causal link between the disruption of methyl metabolism in the mother during pregnancy, via protein restriction, and epigenetic modification of hepatic gene expression in the offspring[224]. Hypomethylation of these genes was avoided in the mothers supplemented with folate during pregnancy[224]. Similarly, Sinclair *et al.* (2007) reported that modest restriction of periconceptional vitamin B12 and folate in ewes led to lambs that were heavier and fatter, with insulin resistance and elevated blood pressure[122]

In this study, the averaged level of maternal pFol during pregnancy was significantly predictive of infant pHcy at 26 weeks' postpartum (refer to section 7.3.4), after multivariate adjustment. The strength of the association and statistical significance suggests that the effects may be rather profound. This may have important implications for the developmental programming of the adult phenotype and the propensity towards significant diseases such as CHD. However, it is important to note that a sample of 16 is not likely to be representative of the population and an extension of the study is needed. Furthermore, direct markers of methylation will need to be measured to confirm the hypothesised link with epigenetic imprinting. Conceivably, the maternal levels of important nutrients transferred across the placenta may alter the genetic expression in the offspring.

8.1 FUTURE DIRECTIONS

This thesis has made several contributions to our understanding of some of the nutritional influences in pregnancy and postpartum for women and their children. However, it has also drawn attention to areas of need which require further investigation. Where possible, data collected as part of the WATCH Study will continued to be analysed in response to these identified research questions and needs.

Firstly, the dietary data collected for both the mothers and infants within the WATCH cohort requires validation, ideally using the triangulation method described in section 3.3.4.4. Confidence in this data will lend itself to further investigations of nutritional influences in pregnancy and postpartum for women and their children.

All future investigations for the WATCH data will need to include adjustments for the confounding factors we have measured but not yet analysed. These include, but are not limited to, smoking, parity, age, and other socioeconomic factors.

From the vitamin B12, folate, and homocysteine study there is a recognised need to move towards directly measuring DNA methylation in both mother and offspring. At the infant's two year follow-up we will request a blood sample that will contain enough DNA (from the white blood cells) and a buccal swab to perform methylation studies.

While this thesis has described the accumulation of intrauterine adipose tissue, there is a need to continue to monitor postnatal growth and adiposity. The trajectory of growth from conception to one years, with up to 10 data points available for each individual (refer to Figure 3.1) is a major strength of this project. Within this context it may be possible to separate out 'early-life' exposures considering the pre- and postnatal environments separately, without compromising a high level of detail. In this way the relative contributions of the maternal-fetal environment versus the postnatal home environment may be better quantified.

8.1.1 Clinical relevance

From this thesis it is also possible to forecast the need to update the nutritional guidelines for pregnancy, though change in practise at this stage would be premature.

A review of nutrient supplementation in pregnancy is soon to commence with the aim of generating evidence-based best-practice guidelines. This has stemmed from an observed discrepancy between the lack of current evidence supporting the use of multivitamin and mineral supplements and the frequency of women reporting their use in pregnancy, sometimes commencing after the first trimester when the window of opportunity for reducing the risk of NTDs has closed.

While the evidence mounts for the direction of specific interventions for optimising the outcomes for the mother and offspring, taking a life-course perspective, the promotion of a 'healthy, balanced diet' appears to need a revival amongst Australian women of childbearing age. This is in light of the widespread failure of women to meet the NRVs recently adopted as the best available evidence to support the health of the population[239]. Given that up to 50% of pregnancies are unplanned[240] a blanket approach to advocating for optimising maternal nutrition for pregnancy seems commendatory. This strategy has been adopted with the mandatory fortification of breads and cereals with folic acid in Australia[250].

While it is too early to claim to understand how pregnancy weight gain influences later outcomes for the offspring, the longitudinal monitoring of intrauterine growth may eventually provide evidence to support the implementation of guidelines on weight management in pregnancy. One study in the USA from the 1980s has shown that women who were given a weight gain limit during pregnancy by their doctors were more likely to keep to this limit compared to women not given any advice[467]. There is already evidence to suggest that weight gain in pregnancy affects multiple outcomes at birth, with the obese obstetric patient at particularly high risk[162]. Hence reinstating guidance on an appropriate amount of weight to gain during pregnancy may be needed as part of routine antenatal care, although the life-course perspective must be considered when establishing new guidelines for what constitutes an 'appropriate' gain.

8.2 FINAL CONCLUSION

In conclusion, this thesis has focused on understanding and advancing some of the important concepts within the DOHaD field. Data on maternal nutrition at both the individual and population level is of paramount importance. Within this thesis the quality and nutritional adequacy of the dietary intakes of young Australian women were assessed in reference to their pregnancy status. This original research shows that diet quality does not differ according to pregnancy status in a large, nationally representative, contemporary cohort of Australian women. Further to this, it appears that most young Australian women are not currently meeting key nutrient targets for dietary folate and fibre, and many are also falling short of the recommendations for calcium, iron and potassium.

Detailed prospective longitudinal data, including anthropometry, biochemistry, dietary and other health-related information, were collected for a modest cohort of women and their children during pregnancy and postpartum. This was despite the difficulties encountered during the first recruiting attempt. A retrospective analysis of the failure to recruit women to this nutrition-based research resulted in a major restructure and design of the recruitment protocol, and a dramatically improved response rate.

With the cohort established, the serial ultrasound data were analysed in reference to maternal body mass and weight change measurements to assess the potential for intrauterine origins of excess adiposity. Interestingly fetal growth was positively predicted by higher maternal pre-pregnancy BMI and gestational weight gain, but no difference in intrauterine fat towards the end of pregnancy was observed.

Finally, maternal levels of vitamin B12, and folate in pregnancy and postpartum have been characterised up to six months after birth. A small sub-analysis of paired maternal and infant data has shown that infant vitamin B12 and folate levels are not predicted by the maternal nutrient status either in pregnancy or postpartum. However, average maternal pregnancy folate was significantly predictive of pHcy in the six month old infant. This may be very important and future research should seek to understand the link between maternal folate and epigenetic imprinting of health outcomes for the offspring.

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Appendices

Appendix 1

All Cochrane systematic reviews of nutrient supplementation in pregnancy (up to June 25, 2008)

FIRST AUTHOR YEAR TITLE TRIALS PARTICIPANTS	STUDY PURPOSE	STUDY DESIGN (INCLUSION/ EXCLUSION CRITERIA)	NTERVENTION DESCRIPTION	OUTCOME MEASURES (ASSESSED)	FINDINGS	LIMITATIONS
Energy and Protein (Reference 266)						
Kramer MS 2003 ***Energy and protein intake in pregnancy*** 23 trials 6,712 pregnant women	To assess the effects of advice to increase or reduce energy or protein intake, or of actual energy or protein supplementation or restriction, during pregnancy on energy and protein intakes, gestational weight gain, and the outcome of pregnancy.	RCTs and quasi-randomised trials of dietary advice to increase or reduce energy or protein intake, or of actual energy or protein supplementation or restriction, during pregnancy.	Advice to increase dietary energy and protein intakes, energy and/or protein supplementation, or prescription of a low-energy diet. For supplementation, 'balanced' energy/ protein supplements (< 25% of total energy from protein), high-protein supplements (25% energy from protein), and isocaloric protein supplements (an equal quantity energy from protein and nonprotein) were included.	For Mother: (1) Dietary intake; (2) Gestational weight gain; (3) Complications of pregnancy (pre-eclampsia, PIH); (4) Duration of labour; (5) Postpartum weight retention; (6) Breast-milk output. For Baby: (1) Stillbirth; (2) Neonatal death; (3) Fetal growth; (4) Birthweight; (5) SGA (6) Gestational duration; (7) Child growth and development.	Dietary advice appears effective in increasing energy and protein intakes, but is unlikely to confer major benefits on infant or maternal health. Balanced energy/protein supplementation improves fetal growth and may reduce the risk of fetal and neonatal death. High-protein or balanced-protein supplementation alone may be harmful to the fetus. Protein/energy restriction in over-weight, or high weight gain, may be harmful to the fetus.	Heterogeneity in the results. Large losses to follow-up. Methodological limitations (e.g. failure to describe the allocation procedure). Some clinical outcomes of interest not reported.
Long-chain Polyunsaturated Fatty Acids (267)						
Makrides M 2006 ***Marine oil, and other prosta-glandin precursor, supplementation for pregnancy uncomplicated by***	To estimate the effects of marine oil, and other prostaglandin precursor, supplementation during pregnancy on the risk of	Randomised trials with adequate concealment of the allocation comparing oral marine oil, or other prostaglandin precursor, supplementation during pregnancy with either	Marine oil (fish or algal), orally administered, compared with placebo or no marine oil treatment. Trials using foods supplemented with marine oil were included. Trials with	For Mother: (1) Hypertension; (2) Pre-eclampsia; (3) Eclampsia; (4) Other complications of eclampsia; (4) Caesarean section; (5) Haemorrhage; (6) Serious morbidity (such as renal failure, liver failure, death); (7) Length of gestation; (8) Side-effects; (9) Prolonged gestation; (10) Length of	There is not enough evidence to support the routine use of marine oil, or other prostaglandin precursor, supplements during pregnancy to reduce the risk of pre-eclampsia, PTB, LBW or	Doses ranged from 133 mg/day to 3g /day, although most assessed a dose of 2.7 g/day. Most trials commenced

pre-eclampsia or intrauterine growth restriction 6 trials 2783 pregnant women	preeclampsia, PTB, LBW and SGA, and on other substantive measures of maternal morbidity, and of morbidity and mortality for the child.	placebo or no treatment. Pregnant women with pre-eclampsia or suspected IUGR at trial entry were excluded.	evening primrose or borage oils were also included. Trials with a single-dose treatment, or those combined with other nutrients of drugs, were excluded.	hospital stay. For Baby: (1) Stillbirths; (2) Neonatal deaths; (3) PTB; (4) Neonatal morbidity (such as intraventricular haemorrhage, RDS); (5) Birthweight; (6) LBW; (7) SGA; (8) Admission to a neonatal ICU.	SGA. The review suggests that marine oil supplementation may be of most benefit in prolonging gestation in women with high-risk pregnancies.	supplementation after 16 weeks gestation. Data regarding neonatal and child outcomes are scarce.
Multiple (≥ 3) Micronutrients (268)						
Haider BA 2006 ***Multiple-micronutrient supplementation for women during pregnancy*** 9 trials 15,378 pregnant women	To evaluate the maternal and infant benefits of multiple-micronutrient supplements in pregnancy. To assess the risk of excess supple-mentation and potential adverse interactions between micronutrients.	Prospective RCTs. HIV-positive women were excluded. No limit on gestational age at enrolment. No limits on the duration of supplementation.	Supplementing pregnant women with ≥ 3 micro-nutrients compared with placebo, or no supplementation, or supplementation with ≤ 2 micro-nutrients.	For Mother: (1) Anaemia. For Baby: (1) PTB; (2) SGA; (3) LBW; (4) Perinatal mortality.	Multiple-micronutrient supplementation led to a reduction in the number of LBW and SGA babies, and maternal anaemia, although no added benefit when compared with iron and folic acid supplementation.	Few trials with heterogeneity across supplementing regimens. Insufficient evidence to identify adverse effects. Few outcome measures reported.
Multiple (Vitamin A, Beta-carotene, Vitamin C, Vitamin E, Folic acid, Iron, Zinc, and Multivitamins) (269)						
Rumbold A 2005 ***Vitamin supplementation for preventing miscarriage*** 17 trials 35,812 women; 37,353 pregnancies	To determine the effectiveness and safety of any vitamin supplementation, on the risk of spontaneous miscarriage, maternal adverse outcomes and fetal and infant adverse outcomes.	RCTs and quasi-randomised trials comparing = 1 vitamin(s) with either placebo, other vitamins, no vitamins or other interventions, prior to conception, periconcep-tionally or in early pregnancy. Pregnant women (< 20 weeks gestation) or women planning on becoming pregnant in the near future were included.	Comparisons of any vitamin(s) alone or in combination with other agents with either placebo, other vitamin(s), no vitamin(s) or other interventions for the prevention of miscarriage, either in areas where there is inadequate dietary intake or where there is presumed adequate intake of that vitamin(s).	For Mother: (1) Fetal loss, early and late miscarriage; (2) Placental abruption; (3) Pre-eclampsia; (4) Multiple pregnancy; (5) Placental weight; (6) Method of infant feeding. For Baby: (1) Stillbirth, perinatal or neonatal death; (2) PTB and VPTB; (3) Birthweight; (4) SGA; (5) Congenital malformations; (6) Anaemia; (7) Poor childhood growth; (8) Admission to neonatal ICU.	Vitamins, alone or in combination, do not prevent miscarriage or stillbirth, but may reduce the risk of pre-eclampsia. Multivitamin supplements (with or without folic acid), may increase the risk of a multiple birth. For vitamin A, multivitamins and folic acid, modest increases in birth-weight and infant growth were seen.	Few high quality trials, due to poor allocation concealment or large loss to follow-up. Differing definitions, or no definition, of miscarriage. The timing of the onset of vitamin supplementation varied greatly, and included mid-pregnancy.

Other (Carnitine, Protein-free Calf Blood Extract, Amino Acids, Glucose) (270)						
Say L 2003 ***Maternal nutrient supplementation for suspected impaired fetal growth*** 4 trials 165 women	To assess the effects of nutrient administration for suspected fetal growth impairment, on fetal growth and perinatal outcome.	Acceptably controlled trials of nutrient administration for suspected impaired fetal growth. Women with suspected impaired fetal growth were included.	Any micro- and/or macro-nutrient administered to the mother (oral, parenteral or amnioinfusion into the amniotic cavity) for the purpose of promoting fetal growth.	For Mother: (1) Adverse effects For Baby: (1) Fetal growth; (2) Perinatal mortality; (3) Neonatal morbidity; (4) Adverse effects on the neonate.	The available evidence is highly inadequate to evaluate the effectiveness of nutrient therapy for suspected impaired fetal growth.	Underpowered trials. Methodological limitations including short supplementation periods. Poorly reported clinical endpoints.
Antioxidants (Vitamin A, Beta-carotene, Vitamin C, Vitamin E, Lycopene, Selenium and Multivitamins) (271)						
Rumbold A 2008 ***Antioxidants for preventing pre-eclampsia*** 10 trials 6533 pregnant women	To determine the effectiveness and safety of any antioxidant supplementation during pregnancy and the risk of developing pre-eclampsia and its related complications.	Randomised trials comparing = 1 antioxidants with either placebo or no antioxidants during pregnancy for the prevention of pre-eclampsia, and trials comparing = 1 antioxidants with another, or with other interventions. Quasi-randomised trials were excluded.	Comparisons of (i) Any anti-oxidant/s (any dosage regimen) with either placebo or no antioxidant/s; (ii) = 1 anti-oxidant with other anti-oxidant/s; (iii) Anti-oxidant/s with other interventions; (iv) = 1 anti-oxidants with other agents compared with placebo or no antioxidant/s, other anti-oxidants or other interventions. Subgroups were based on: type of antioxidant(s), dose of anti-oxidant/s, antioxidant intake before trial entry.	For Mother: (1) Pre-eclampsia; (2) Severe pre-eclampsia; (3) Death < 6 weeks postpartum; (4) PIH; (5) Severe hypertension; (6) Use of anti-hypertensives; (7) Elective delivery; (8) Caesarean section; (9) Bleeding episodes (placental abruption, antepartum haemorrhage, postpartum haemorrhage, need for transfusion); (10) Serious maternal morbidity (eclampsia, liver failure, renal failure, disseminated intravascular coagulation, stroke); (11) Side-effects; (12) Use of health service resources; (13) Hospital admission. For Baby: (1) PTB, VPTB; (2) SGA; (3) Miscarriage, stillbirth, neonatal or infant death; (4) Gestational duration; (5) Birthweight; (6) Apgar score; (7) RDS; (8) Chronic lung disease; (9) Bleeding episode (intraventricular haemorrhage, periventricular leukomalacia); (10) Necrotising enterocolitis; (11) Retinopathy of prematurity.	The evidence does not support routine antioxidant supplementation during pregnancy to reduce the risk of pre-eclampsia and other serious complications in pregnancy. The magnitude of effect of antioxidants on the relative risk of pre-eclampsia was much greater for women allocated lycopene (52% reduction) and for women allocated vitamin C and E combined with aspirin and fish oil (93% reduction) compared with women allocated vitamin C and E alone (8% reduction).	Many inconsistent findings between trials, and significant statistical heterogeneity possibly due to differences in the type of antioxidant

Vitamin A and Beta-Carotene (272)						
Van den Broek N 2002 ***Vitamin A supplementation during pregnancy*** 5 trials 23,426 pregnant women	To review the effectiveness of vitamin A supplementation during pregnancy, alone or in combination with other supplements.	RCTs or quasi-randomised trials evaluating the effect of vitamin A supplementation in pregnant women. The outcome HIV transmission was not considered.	Vitamin A supplementation, alone or in combination with other supplements compared with a control group (no treatment or another intervention).	For Mother: (1) Maternal death; (2) Anaemia and iron deficiency; (3) Night-blindness; (4) Infection; (5) Hb. For Baby: (1) Birthweight; (2) Neonatal anthropometry; (3) Neonatal infection; (4) Mortality at 6 months.	The evidence is not strong enough to justify antenatal vitamin A supplementation as a strategy to reduce adverse maternal outcomes (anaemia, sepsis and death).	Heterogeneity in study populations, designs, interventions and primary outcomes.
Vitamin D (273)						
Mahomed K 1999 ***Vitamin D supplementation in pregnancy*** 2 trials 232 pregnant women	To assess the effects of Vitamin D supplementation on pregnancy outcome.	Any controlled trial of Vitamin D supplementation in pregnancy with clinical endpoints was considered. Women at risk of Vitamin D deficiency were included.	Vitamin D supplementation during pregnancy.	For Baby: (1) LBW; (2) Neonatal hypocalcaemia; (3) Craniotabes (softening of the skull); (4) Perinatal mortality.	There is not enough evidence to evaluate the effects of vitamin D supplementation during pregnancy.	Few trials. Small sample sizes. Some heterogeneity in the findings. Inadequate information about adverse effects reported.
Vitamin E (274)						
Rumbold A 2005 ***Vitamin E supplementation in pregnancy*** 4 trials 566 pregnant women	To assess the effects of vitamin E supplementation, alone or in combination with other separate supplements, on pregnancy outcomes, adverse events, side-effects and use of health services.	RCTs or quasi-randomised trials evaluating the effect of vitamin E supplementation in pregnant women.	Vitamin E supplementation, alone or in combination with other separate supplements compared with placebo, no placebo or other supplements. Interventions using a multivitamin supplement (> 2 vitamins or minerals combined in the 1 tablet) that contained vitamin E were excluded.	For Mother: (1) Pre-eclampsia; (2) Death ≤ 6 weeks postpartum; (3) Elective delivery; (4) Caesarean section; (5) Bleeding episodes (such as placental abruption); (6) Serious maternal morbidity (such as eclampsia, liver failure, renal failure, disseminated intravascular coagulation, pulmonary oedema); (7) Side-effects. For Baby: (1) Stillbirth, neonatal death, or perinatal death; (2) PTB; (3) IUGR; (4) Birthweight;; (5) Gestational duration; (6) Apgar score; (7) Admission to ICU; (8) Use of mechanical ventilation.	From the limited trials reviewed, the data do not support routine vitamin E supplementation either alone or in combination with other supplements in pregnancy, for all women or women at high risk of pregnancy complications. The data are too few to produce any reliable conclusions about any benefits or harms of supplementation.	Few trials and small sample size. Half the trials were of poor quality. Subjects were either at high risk, or had established severe early onset, pre-eclampsia. No information available to assess whether vitamin E alone (rather than in combination) may be beneficial.

Folate (Folic Acid) (275)						
Lumley J 2001 ***Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects*** 4 trials 6425 pregnant women	To identify whether the prevalence of NTD can be reduced by increased consumption of multivitamins or folate before pregnancy and in the first 2 months of pregnancy (peri-conceptionally).	RCTs or quasi-randomised trials were considered.	Trials comparing periconceptional supplementation by multivitamins with placebo, folate with placebo, or multivitamins with folate; different dosages of multivitamins or folate; pre-pregnancy dietary advice and counselling in primary care settings to increase the consumption of folate-rich foods, or folate-fortified foods, with standard care; increased intensity of information provision with standard public health dissemination.	For Mother: (1) Conception; (2) Spontaneous abortion; (3) Ectopic pregnancy; (4) Multiple pregnancy; (5) Vertigo, nausea, or vomiting. For Baby: (1) NTD; (2) Facial clefts, limb reduction defects, conotruncal heart defects; (3) All other birth defects; (4) Stillbirth.	Periconceptional folate supplementation has a strong protective effect against NTD. Information about folate should be made more widely available throughout the health and education systems. Women whose fetuses or babies have NTD should be advised of the risk of recurrence in a subsequent pregnancy and offered continuing folate supplementation. The benefits and risks of fortifying basic food stuffs, such as flour, with added folate remain unresolved.	Uncertainty regarding the minimum dose required. Not all outcome measures were reported on (e.g. PTB).
Vitamin B6 (Pyridoxine) (276)						
Thaver D 2006 ***Pyridoxine (vitamin B6) supplementation in pregnancy*** 5 trials 1646 pregnant women	To evaluate clinical outcomes after vitamin B6 supplementation during pregnancy and/or labour.	RCTs administering vitamin B6 during pregnancy and/or labour, for purposes other than treatment of nausea and vomiting of pregnancy.	Pyridoxine (vitamin B6) alone compared with a placebo, or no supplementation. Trials comparing vitamin B6 containing supplements versus the same supplement, but not containing vitamin B6, were also included.	For Mother: (1) Eclampsia; (2) Pre-eclampsia; (3) Dental decay; (4) Breastmilk production; (5) Adverse events (sensory neuropathy). For Baby: (1) Birthweight; (2) Apgar score.	There is not enough evidence to detect clinical benefits of vitamin B6 supplementation in pregnancy and/or labour other than 1 trial suggesting protection against dental decay.	Few trials reporting on limited clinical outcomes. Methodological limitations (poor allocation concealment and method of randomisation) in all but 1 trial. High rates of loss to follow-up.

Vitamin C (277)						
Rumbold A 2005 ***Vitamin C supplementation in pregnancy*** 5 trials 766 pregnant women	To evaluate the effects of vitamin C supplementation, alone or in combination with other separate supplements on pregnancy outcomes, adverse events, side-effects and use of health resources.	RCTs or quasi-randomised trials evaluating the effect of vitamin C supplementation in pregnant women.	Vitamin C supplementation, alone or in combination with other supplements compared with placebo, no placebo or other supplements. Interventions using a multivitamin (> 2 vitamins or minerals in the 1 tablet that contains vitamin C were excluded, as were Interventions using iron as the primary supplement with added vitamin C.	For Mother: (1) Pre-eclampsia; (2) Death ≤ 6 weeks postpartum; (3) Bleeding episodes (such as placental abruption, antepartum haemorrhage); (4) Serious maternal morbidity (such as eclampsia, renal failure, disseminated intravascular coagulation, pulmonary oedema); (5) Elective delivery; (6) Caesarean section; (7) Side-effects. For Baby: (1) Stillbirth, neonatal death, or perinatal death; (2) Birthweight; (3) IUGR; (4) PTB; (5) Gestational duration; (6) Apgar score; (7) Admission to ICU; (8) Use of mechanical ventilation.	The limited data do not support routine vitamin C supplementation, either alone or in combination with other supplements, during pregnancy. Preterm birth may have been increased with vitamin C supplementation.	Few trials and small sample size. Half the trials were of poor quality. Some heterogeneity in the results. All of the women involved in the trials were either at high risk of pre-eclampsia or preterm birth, or the women had established severe early onset pre-eclampsia.
Iron (278)						
Reveiz L 2007 ***Treatments for iron-deficiency anaemia in pregnancy*** 17 trials 2578 pregnant women	To determine the overall effects of iron therapy given to women diagnosed with iron-deficiency anaemia in pregnancy, measuring neonatal and maternal morbidity and mortality, haematological parameters and side-effects, especially adverse effects of treatment.	RCTs assessing the effects of treatments for iron-deficiency anaemia in pregnancy. Quasi-random trials were not included.	(1) Oral iron; (2) Different regimens of oral iron treatment; (3) IM iron; (4) IV iron; (5) Parenteral route (IM or IV) versus oral route; (6) IV iron versus IM iron with different regimens of parenteral iron treatment; (7) IV administered iron sucrose with and without adjuvant recombinant human erythropoietin.	For Mothers: (1) Anaemia; (2) Hb; (3) Serum ferritin; (4) Adverse effects (nausea, vomiting, constipation, abdominal cramps, headaches, dyspepsia, shivering, itching, metallic taste in mouth, weakness); (5) Skin discolouration; (6) Hematocrit; (7) Non-anaemia; (8) Caesarean section; (9) Bleeding episodes (postpartum haemorrhage, blood transfusion required); (10) PIH; (11) GDM; (12) Arthralgias; (13) Severe allergic reactions; (14) Venous thrombosis. For Baby: (1) Birthweight, (2) Neonatal Hb; (3) Neonatal mortality; (4) Apgar score; (5) Neonatal jaundice	Daily oral iron improves haematological indices but causes frequent gastrointestinal adverse effects. Parenteral iron enhances haematological response, compared with oral iron, but there are concerns about possible important adverse effects. Treatment of mild anaemia in pregnancy remains unsupported by scientific proof. It is also unclear what treatments work better for severe anaemia in pregnancy.	Various definitions of iron-deficiency anaemia, doses, and regimens were used. Poor or no stratification according to anaemia severity. Underpowered studies. Most results were provided by 1 or 2 small trials with methodological limitations. Poor reporting of clinical outcomes.

Iron with or without Folic Acid (279)						
Pena-Rosas JP 2006 ***Effects of routine oral iron supplementation with or without folic acid for women during pregnancy*** 40 trials 12,706 pregnant women	To assess the efficacy, effectiveness and safety of routine antenatal daily or intermittent iron supplementation with or without folic acid during pregnancy on the health of mothers and newborns.	RCTs and quasi-randomised trials were included. Trails of any form of routine oral iron with or without folic acid supplements with no treatment/ placebo, or intermittent supplementation regimens were included. Combinations with other vitamins and minerals, and intervention studies for anaemic women as a medical treatment were excluded.	(a) Daily iron alone compared to no intervention/ placebo; (b) Intermittent iron alone compared to daily iron alone; (c) Daily iron-folic acid compared to no intervention/ placebo; (d) Intermittent iron-folic acid compared to daily iron-folic acid.	For Mother: (1) Hb; (2) Anaemia; (3) Haemoconcentration; (4) Iron deficiency; (5) Side-effects; (6) Severe anaemia; (7) Moderate anaemia; (8) Infection during pregnancy; (9) Puerperal infection; (10) Antepartum haemorrhage; (11) Postpartum haemorrhage; (12) Vomiting; (13) Placental abruption; (14) Pre-eclampsia. For Baby: (1) PTB, VPTB; (2) LBW, VLBW; (3) Birthweight; (4) Perinatal mortality; (5) Hb; (6) Ferritin; (7) Admission to special care unit.	Daily antenatal iron supplementation increases maternal Hb and is associated with less iron deficiency and anaemia. Side-effects and haemo-concentration are more common in women who receive daily supplementation. Further studies which assess clinically important outcomes are needed.	The results showed significant heterogeneity across most outcomes. Very limited information related to clinical maternal and infant outcomes was available.
Calcium (280)						
Hofmeyr GJ 2006 ***Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems*** 12 trials 15,206 pregnant women	To assess the effects of calcium supplementation during pregnancy on hypertensive disorders of pregnancy and related maternal and child outcomes.	All published, unpublished, and ongoing trials with random allocation to calcium supplementation during pregnancy versus placebo. Quasi-randomised trials and those with no placebo were excluded. Women with existing hypertensive disorders of pregnancy were excluded.	Supplementation with calcium from ≤ 34 weeks of pregnancy, compared with placebo treatment. Intended supplementation with calcium needed to be ≥ 1 gram/ day.	For Mother: (1) High blood pressure, with or without proteinuria; (2) High blood pressure with significant proteinuria; (3) Serious morbidity (eclampsia; renal failure; HELLP syndrome); (4) Admission to an ICU; (5) Placental abruption; (6) Caesarean section; (7) Proteinuria; (8) Severe pre-eclampsia; (9) Eclampsia; (10) HELLP syndrome; (11) Death. For Baby: (1) PTB; (2) LBW; (3) SGA; (4) Admission to neonatal ICU; (5) Stillbirth or death before hospital discharge; (6) Systolic blood pressure > 95th percentile during childhood; (7) Diastolic blood pressure > 95th percentile during childhood.	Calcium supplementation appears to almost halve the risk of pre-eclampsia, and to reduce the rare occurrence of maternal death or serious morbidity. There were no other clear benefits, or harms. The reduction in pre-eclampsia, and in maternal death or severe morbidity, supports the use of calcium supplementation, particularly where dietary intake is low.	Heterogeneity in the results possibly due to differences in the study populations, sample size, and/or dose of calcium. Little information about the long- term follow-up of children, including adverse effects. Efficacy of <1g calcium /day was not assessed.

Magnesium (281)						
Makrides M 2001 ***Magnesium supplementation in pregnancy*** 7 trials 2689 pregnant women	To assess the effects of magnesium supplementation during pregnancy on maternal, neonatal and paediatric outcomes.	All published, unpublished, and ongoing randomised trials of dietary magnesium supplementation during pregnancy. Women with normal or high-risk pregnancies were included.	Magnesium orally administered and commenced before the 25th week of gestation.	For Mother: (1) Systolic and diastolic blood pressure; (2) PIH; (3) Pre-eclampsia; (4) Need for hospitalisation; (5) Antepartum haemorrhage; (6) Length of labour; (7) Side-effects (gastrointestinal). For Baby: (1) Gestational duration; (2) PTB; (3) Birthweight; (4) LBW, VLBW; (5) SGA; (6) Admission to ICU.	There is not enough high quality evidence to show that dietary magnesium supplementation during pregnancy is beneficial.	Poor methodological quality of the included trials; only 1 was of high quality.
Zinc (282)						
Mahomed K 2007 ***Zinc supplementation for improving pregnancy and infant outcome*** 17 trials 8273 pregnant women	To assess the effects of zinc supplementation in pregnancy on maternal, fetal, neonatal and infant outcomes.	Randomised trials of zinc supplementation versus no zinc supplementation or placebo administration during pregnancy (< 27 weeks gestation).	Routine zinc supplementation versus no zinc supplementation or placebo: (i) zinc supplementation compared with no zinc or placebo in women likely or shown to be zinc deficient; (ii) zinc supplementation compared with no zinc or placebo in women in whom compliance with supplementation was good (> 80%).	For Mother: (1) PIH; (2) Pre-eclampsia; (3) Prelabour rupture of membranes; (4) Antepartum haemorrhage; (5) Post-term birth; (6) Prolonged labour; (7) Retention of placenta; (8) Meconium in liquor; (9) Instrumental vaginal birth; (10) Smell/ taste dysfunction; (11) Caesarean section; (12) Postpartum haemorrhage; (13) Infections. For Baby: (1) PTB; (2)Gestational duration; (3) Stillbirth or neonatal death; (4) Birthweight; (5) SGA; (6) LBW; (7) HBW; (8) HC; (9) MUAC; (10) Apgar score; (11) Hypoxia; (12) Jaundice; (13) Fever; (14) Infant umbilical infection; (15) Neonatal sepsis; (16) RDS; (17) Neonatal intraventricular haemorrhage; (18) Necrotising enterocolitis; (19) Length of hospital stay; (20) Lack of tubercular response; (21) Fetal heart rate; (22) Episodes of disease; (23) Infant growth; (24) Mental development; (25) Psychomotor development	There was a 14% relative reduction in PTB for zinc compared with placebo, although this was in the studies involving women of low income. There was no convincing evidence that zinc supplementation during pregnancy results in other benefits.	Heterogeneity in the results possibly due to differences in the study populations. Some methodological limitations (e.g. allocation concealment).

Abbreviations: GDM, gestational diabetes mellitus; Hb, haemoglobin; HBW, high birth weight, >4500 g; HC, head circumference; HELLP, haemolysis elevated liver enzymes and low platelets; HIV, human immunodeficiency virus; ICU, intensive care unit; IM, intramuscular; IUGR, intrauterine growth restriction; IV, intravenous; LBW, low birthweight, < 2500 g; MUAC, mid upper arm circumference; NTD, neural tube defects; PIH, pregnancy induced hypertension; PTB, preterm birth, before 37 weeks of gestation; RCTs, randomised controlled trials; RDS, respiratory distress syndrome; SGA, small for gestational age; VLBW, very low birthweight, <1500 g; VPTB, very preterm birth, before 34 weeks of gestation.

Appendix 2

THE WATCH STUDY

Self-Reported Questionnaire for Fathers

Only those who provide consent to participate should complete this form.

Instructions

Please complete the following questions and return both this and your consent form to the research team. This can be done using the reply-paid self-addressed envelope that has been provided. Alternatively it can be returned at your child's next follow-up appointment.

It is important that the details you provide are as accurate as possible. If you have any questions, please do not hesitate to contact us (telephone: 49855620).

- If you have not recently been weighed, please check your current weight before writing down your response.
- You should not be wearing shoes when obtaining either your height or weight. A single layer of clothing should be worn (e.g. shorts/pants and a shirt, but **no** jacket/jumper)
- Use a measuring tape to find your Waist Circumference. Ideally someone else should measure this for you. Make sure the tape is not twisted and measure around your middle in line with most narrow point when looking from behind.

1. Study Number_____

2. Date_____
Please complete

3. Height:_____(in cm or in feet and inches)

4. Weight:_____(in kg)

5. Waist Circumference:_____(to the nearest mm)

Thank You for your co-operation.

Appendix 3

In partnership with our community

4-DAY WEIGHED FOOD RECORD INSTRUCTIONS

The information given in the instructions explains how to record everything that you eat and drink, for a period of four days (including one weekend day).

The more precise and detailed your food record the more accurate the results, and therefore analysis of your diet. Please take your time and fill it in as accurately as you can.

INSTRUCTIONS

- Write down everything you eat and drink (including water) for a four-day period. These days do not need to be consecutive, however one of the days must be on the weekend (either Saturday or Sunday). All four days must be typical of the way you normally eat.
- Start a new page for each day.
- Specify the weight and describe each food in detail (the more information the better).
  - You will have been provided with a set of digital scales to determine the weight of each food you eat. Attempt to use the scales to weigh as many food items as possible.
  - You can record weights of foods from the labels of packaged foods.
  - If you are eating out and are able to weight your food that would be preferred, however, if you cannot do this try to estimate the amount of food by applying standard household measures, such as cup, tablespoon, teaspoon. These are helpful in estimating weights/quantities.
  - Describe where you are at the time of eating. For example: café, restaurant, bistro, friends place.
  - Describe cooking methods. For example: baked, fried (include how much oil used), boiled, steamed, microwave.
  - Use brand names wherever possible
  - Include all water, vitamin and mineral supplements, snacks, and condiments (e.g. mayonnaise, tomato sauce etc).
- Try to record each item (food or drink) as it is consumed so that nothing is forgotten.
- Maintain your normal eating habits.
- An example is provided on the next page to show you how you should record all food and drinks, providing a description of the item and the amount consumed.

If you would like any further information about keeping your Weighed Food Record please contact our Dietitian and Research Student:

Alexis Hure
At: The University of Newcastle
Phone: 4921 7486

WEIGHED FOOD RECORD EXAMPLE

NAME: *Jane Average* **DAY:** *Monday* **DATE:** *00/00/00*

TIME	FOOD/DRINK	QUANTITY	DETAIL
7.00 – 8.00am	Weet-bix Milk Honey Toast Margarine Honey Tea (milk–30ml, sugar white–1 tsp) Orange Juice	45 g 190g 15g 78g 3g 12g 242g 198g	Sanitarium;2 biscuits Lite White Multigrain; Farmland Canola 35% juice
10.00 am	Tinned fruit salad Strawberry Yoghurt Milk Arrowroot biscuits Water	220g 200g 14g 240g	Goulburn Valley Ski divine; 99% fat free 2 biscuits
12.00 - 1.00pm	Bread Mayonnaise (egg) Chicken Lettuce Apple Banana Potato chips (salt and vinegar) Water	74g 4g 80g 2g 180g 202g 30g 202g	Multigrain; Farmland BBQ; no skin Red delicious These come in a 12 pack
3.00pm	Toast Margarine Vegemite Water	120g 4g 2g 418g	White bread; Tip Top Canola
6.00 - 7.00pm	Pasta (White) Chicken Tomato based sauce whole peeled tomatoes, tomato paste, mixed herbs, garlic Zucchini Carrot Green beans Cordial (raspberry made up 20%)	98g 145g 200g 21g 1tsp 1 clove 85g 78g 53g 236g	Dry Grilled Microwave Microwave Microwave (small amoun water)
7.30pm	Tinned peaches, Goulburn Valley Ice cream	220g 134g	 Savings regular vanilla

NAME:_____ **DAY:**_____ **DATE:**_____

MEAL	TIME	FOOD/DRINK	QUANTITY	DETAIL
Breakfast				
Snack				
Lunch				
Snack				
Dinner				
Snack				

Vitamin/Mineral/Herbal Supplement(s):_____

Physical Activity:_____

Appendix 4

Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY		MTH	YEAR
		○ JAN	○ 2004
		○ FEB	○ 2005
0	0	○ MAR	○ 2006
1	1	○ APR	○ 2007
2	2	○ MAY	○ 2008
3	3	○ JUN	○ 2009
	4	○ JUL	○ 2010
	5	○ AUG	○ 2011
	6	○ SEP	○ 2012
	7	○ OCT	○ 2013
	8	○ NOV	○ 2014
	9	○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○ ■ ○ ○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you usually use?

- ○ none
- ○ full cream milk
- ○ reduced fat milk
- ○ skim milk
- ○ soya milk

4. How much milk do you usually use per day? *(Include flavoured milk and milk added to tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*

- ○ less than 1 slice per day
- ○ 1 slice per day
- ○ 2 slices per day
- ○ 3 slices per day
- ○ 4 slices per day
- ○ 5-7 slices per day
- ○ 8 or more slices per day

7. Which spread do you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On average, how many teaspoons of sugar do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

10. What types of cheese do you usually eat?

- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

DO NOT WRITE IN THIS AREA.

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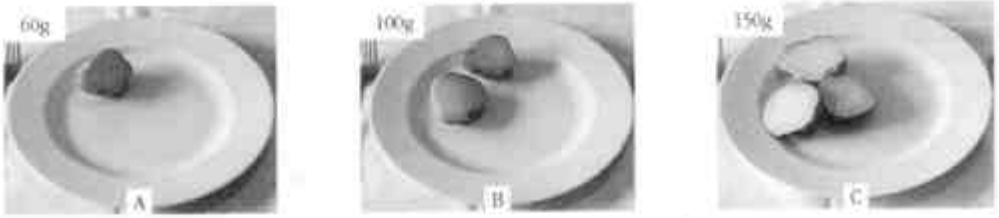
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato

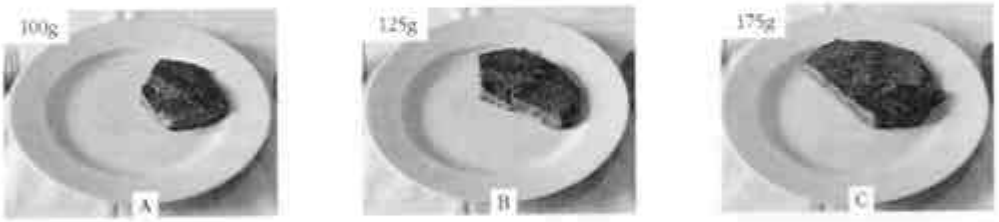


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables

○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat or vegetable casserole, did you usually eat: ○ I never ate casserole

○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

Times You Have Eaten		NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month		per week				per day		
CEREAL FOODS, SWEETS & SNACKS											
All Bran™	A1	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™	A2	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™	A3	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™	A4	○	○	○	○	○	○	○	○	○	○
Porridge	A5	○	○	○	○	○	○	○	○	○	○
Muesli	A6	○	○	○	○	○	○	○	○	○	○
Rice	A7	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne)	A8	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits	A9	○	○	○	○	○	○	○	○	○	○
Sweet biscuits	A10	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries	A11	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries	A12	○	○	○	○	○	○	○	○	○	○
Pizza	A13	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun	A14	○	○	○	○	○	○	○	○	○	○
Chocolate	A15	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™, etc.)	A16	○	○	○	○	○	○	○	○	○	○
Nuts	A17	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste	A18	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™, etc.	A19	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups	A20	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™	A21	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH											
Cheese	B1	○	○	○	○	○	○	○	○	○	○
Ice-cream	B2	○	○	○	○	○	○	○	○	○	○
Yoghurt	B3	○	○	○	○	○	○	○	○	○	○
Beef	B4	○	○	○	○	○	○	○	○	○	○
Veal	B5	○	○	○	○	○	○	○	○	○	○
Chicken	B6	○	○	○	○	○	○	○	○	○	○
Lamb	B7	○	○	○	○	○	○	○	○	○	○
Pork	B8	○	○	○	○	○	○	○	○	○	○
Bacon	B9	○	○	○	○	○	○	○	○	○	○
Ham	B10	○	○	○	○	○	○	○	○	○	○
Corned beef, luncheon meats or salami	B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	B14	○	○	○	○	○	○	○	○	○	○
Fish, tinned (salmon, tuna, sardines, etc.)	B15	○	○	○	○	○	○	○	○	○	○
FRUIT											
Tinned or frozen fruit (any kind)	C1	○	○	○	○	○	○	○	○	○	○
Fruit juice	C2	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit	C3	○	○	○	○	○	○	○	○	○	○
Apples	C4	○	○	○	○	○	○	○	○	○	○
Pears	C5	○	○	○	○	○	○	○	○	○	○
Bananas	C6	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew, etc.	C7	○	○	○	○	○	○	○	○	○	○
Pineapple	C8	○	○	○	○	○	○	○	○	○	○
Strawberries	C9	○	○	○	○	○	○	○	○	○	○
Apricots	C10	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines	C11	○	○	○	○	○	○	○	○	○	○
Mango or paw paw	C12	○	○	○	○	○	○	○	○	○	○
Avocado	C13	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten CONTINUED

	NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
		per month		per week				per day		
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)										
Potatoes, roasted or fried (include hot chips)										
Potatoes cooked without fat										
Tomato sauce, tomato paste or dried tomatoes										
Fresh or tinned tomatoes										
Peppers (capsicum)										
Lettuce, endive, or other salad greens										
Cucumber										
Celery										
Beetroot										
Carrots										
Cabbage or Brussels sprouts										
Cauliflower										
Broccoli										
Silverbeet or spinach										
Peas										
Green beans										
Bean sprouts or alfalfa sprouts										
Baked beans										
Soy beans, soy bean curd or tofu										
Other beans (include chick peas, lentils, etc.)										
Pumpkin										
Onion or leeks										
Garlic (not garlic tablets)										
Mushrooms										
Zucchini										

16. Over the last 12 months, how often did you drink beer, wine and/or spirits?

Times That You Drank

	NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)										
Beer (full strength)										
Red wine										
White wine (include sparkling wines)										
Fortified wines, port, sherry, etc.										
Spirits, liqueurs, etc.										

When answering the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more

18. Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more

Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA

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Appendix 5

Pregnancy Physical Activity Questionnaire

1. Study No.: _____ *(completed by research team)*

2. Today's Date: _____

3. Number of Weeks Pregnant: _____

Example: During this trimester, when you are NOT at work, how much time do you usually spend:

E1: Taking care of an older adult

If you take care of your mum for 2 hours a day your answer should

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

It is very important you tell us about yourself honestly. There are no right or wrong answers. We just want to know about the things you are doing during the trimester.

During this trimester, when you are NOT at work, how much time do you usually spend:

4. Preparing meals (cook, set table, wash dishes)

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

5. Dressing, bathing, feeding children while you are <u>sitting</u>

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

6. Dressing, bathing, feeding children while you are <u>standing</u>

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

7. Playing with children while you are <u>sitting or standing</u>

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

8. Playing with children while you are <u>walking or running</u>

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

9. Carrying children

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

During this trimester, when you are NOT at work, how much time do you usually spend:

10. Taking care of an older adult

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

11. Sitting and using a computer or writing while <u>not</u> at work

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

12. Watching TV or a video or DVD

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

13. Sitting and reading, talking, or on the phone, while <u>not</u> at work

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

14. Playing with pets

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

15. Light cleaning (make beds, laundry, ironing, putting things away)

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

16. Shopping (for food, clothes or other items)

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

17.Heavier cleaning (vacuuming, mopping, sweeping, washing windows)

? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

18. Mowing the lawn while on a ride-on mower

? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

19. Mowing the lawn using a walking mower, raking, gardening

? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

During this trimester, when you are NOT at work, how much time do you usually spend:

20. Walking slowly to go places (such as to the bus, work, visiting)
- Not for fun or exercise
? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

21. Walking quickly to go places (such as to the bus, work, school)
- Not for fun or exercise
? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

22. Driving or riding in a car or bus
? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

For Fun or Exercise…

During this trimester, when you are NOT at work, how much time do you usually spend:

23. Walking slowly for fun or exercise
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

24. Walking more quickly for fun or exercise
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

25. Walking quickly up hills for fun or exercise
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

26. Jogging
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

27. Antenatal exercise class
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

28. Swimming
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

Doing other things for fun or exercise? Please tell us what they are.

29. Dancing
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

30. _____
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

31. _____
? None
? Less than ½ hour per week
? ½ to almost 1 hour per week
? 1 to almost 2 hours per week
? 2 to almost 3 hours per week
? 3 or more hours per week

Please complete the next section if you work (paid or voluntary) or if you are a student. If you are a homemaker, out of work, or are unable to work, you do not need to complete this last section.

At Work…

During this trimester, how much time do you usually spend:

32. Sitting at work or in class

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

33. Standing or slowly walking at work while carrying things (heavier than 3.5kg)

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

34. Standing or <u>slowly</u> walking at work not carrying anything

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

35. Walking quickly at work while carrying things (heavier than 3.5kg)

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

36. Walking quickly at work not carrying anything

? None
? Less than ½ hour per day
? ½ to almost 1 hour per day
? 1 to almost 2 hours per day
? 2 to almost 3 hours per day
? 3 or more hours per day

Thank You!

Appendix 6

Mothers Physical Activity Questionnaire

1. Study ID: _____ *(completed by research team)*

2. Today's Date: _____ *(please complete)*

3. Age of Youngest Child: _____ *(years, months)*

Example: When you are NOT at work, how much time do you usually spend:

E1: Taking care of an older adult

If you take care of your mum for 2 hours a day your answer should

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
● 2 to almost 3 hours per day
O 3 or more hours per day

It is very important you tell us about yourself honestly. There are no right or wrong answers. We just want to know about the things you do as part of your usual routine.

When you are NOT at work, how much time do you usually spend:

4. Preparing meals (cook, set table, wash dishes)

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

5. Dressing, bathing, feeding children while you are <u>sitting</u>

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

6. Dressing, bathing, feeding children while you are <u>standing</u>

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

7. Playing with children while you are <u>sitting or standing</u>

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

8. Playing with children while you are <u>walking or running</u>

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

9. Carrying children

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

When you are NOT at work, how much time do you usually spend:

10. Taking care of an older adult

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

11. Sitting and using a computer or writing while <u>not</u> at work

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

12. Watching TV or a video or DVD

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

13. Sitting and reading, talking, or on the phone, while <u>not</u> at work

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

14. Playing with pets

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

15. Light cleaning (make beds, laundry, ironing, putting things away)

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

16. Shopping (for food, clothes or other items)

O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

17.Heavier cleaning (vacuuming, mopping, sweeping, washing windows)

O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

18. Mowing the lawn while on a ride-on mower

O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

19. Mowing the lawn using a walking mower, raking, gardening

O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

When you are NOT at work, how much time do you usually spend:

20. Walking <u>slowly</u> to go places (such as to the bus, work, visiting)
- <u>Not</u> for fun or exercise
O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

21. Walking <u>quickly</u> to go places (such as to the bus, work, school)
- <u>Not</u> for fun or exercise
O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

22. Driving or riding in a car or bus
O None
O Less than ½ hour per day
O ½ to almost 1 hour per day
O 1 to almost 2 hours per day
O 2 to almost 3 hours per day
O 3 or more hours per day

For Fun or Exercise…

When you are NOT at work, how much time do you usually spend:

23. Walking <u>slowly</u> for fun or exercise
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

24. Walking more <u>quickly</u> for fun or exercise
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

25. Walking <u>quickly up hills</u> for fun or exercise
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

26. Jogging
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

27. Yoga
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

28. Swimming
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

Doing other things for fun or exercise? Please tell us what they are:

29. Dancing
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

30. _____
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

31. _____
O None
O Less than ½ hour per week
O ½ to almost 1 hour per week
O 1 to almost 2 hours per week
O 2 to almost 3 hours per week
O 3 or more hours per week

Please complete the next section if you work (paid or voluntary) or if you are a student. If you are a homemaker, out of work, or are unable to work, you do not need to complete this last section.

At Work…

How much time do you usually spend:

32. Sitting at work or in class

- O None
- O Less than ½ hour per day
- O ½ to almost 1 hour per day
- O 1 to almost 2 hours per day
- O 2 to almost 3 hours per day
- O 3 or more hours per day

33. Standing or slowly walking at work while carrying things (heavier than 3.5kg)

- O None
- O Less than ½ hour per day
- O ½ to almost 1 hour per day
- O 1 to almost 2 hours per day
- O 2 to almost 3 hours per day
- O 3 or more hours per day

34. Standing or slowly walking at work not carrying anything

- O None
- O Less than ½ hour per day
- O ½ to almost 1 hour per day
- O 1 to almost 2 hours per day
- O 2 to almost 3 hours per day
- O 3 or more hours per day

35. Walking quickly at work while carrying things (heavier than 3.5kg)

- O None
- O Less than ½ hour per day
- O ½ to almost 1 hour per day
- O 1 to almost 2 hours per day
- O 2 to almost 3 hours per day
- O 3 or more hours per day

36. Walking quickly at work not carrying anything

- O None
- O Less than ½ hour per day
- O ½ to almost 1 hour per day
- O 1 to almost 2 hours per day
- O 2 to almost 3 hours per day
- O 3 or more hours per day

Thank You!

Appendix 7

There is no need to write your name on this form. To keep your information private we have included a study number.

WOMEN'S MEDICAL DATA COLLECTION SHEET

1. Study Number: _____ **2. Date**_____

3. Have you ever been told by your doctor that you have:

a) Gestational diabetes	Yes	No
b) Insulin dependent (Type I) diabetes	Yes	No
c) Non-insulin dependent (Type II) diabetes	Yes	No
d) Heart disease	Yes	No
e) Hypertension (high blood pressure) during pregnancy	Yes	No
f) Hypertension (high blood pressure) other than during pregnancy	Yes	No
g) Low iron (iron deficiency or anaemia)	Yes	No
h) Asthma	Yes	No
i) Postnatal depression	Yes	No
j) Depression (not postnatal)	Yes	No
k) Anxiety disorder	Yes	No

4. Are you currently taking any medications prescribed by your doctor? Yes No

***If Yes*,** please list including the dose you are taking and how often.

Medication name: _____ *Medication name:* _____

Dose: _____ *Dose:* _____

Frequency: _____ *Frequency:* _____

Medication name: _____ *Medication name:* _____

Dose: _____ *Dose:* _____

Frequency: _____ *Frequency:* _____

5. Are you currently taking any medications bought without a prescription at the chemist, supermarket, or health food shop? Yes No

***If Yes*,** please list including the dose you are taking and how often.

Medication name: _____ *Medication name:* _____

Dose: _____ *Dose:* _____

Frequency: _____ *Frequency:* _____

6. Do you smoke cigarettes or any other tobacco products? Yes No

***If Yes*,** please specify how many cigarettes you smoke on average per day _____

Appendix 8

There is no need to write your name on this form. To keep your information private we have included a study number.

SOCIOECONOMIC DATA COLLECTION SHEET

1. Study Number:_____ **2. Date**_____

3. What is your current postcode? _____

4. What is the *highest* qualification you have completed? *(tick (v) one answer only)*

- O No formal qualifications
- O Year 10 or equivalent (e.g. School certificate)
- O Year 12 or equivalent (e.g. Higher School Certificate)
- O Trade/apprenticeship (e.g. Hairdresser, Chef)
- O Certificate/diploma (e.g. Child care, Technician)
- O University degree
- O Higher university degree (e.g. Grad Dip, Masters, PhD)

*Answers questions 5 and 6 by ticking (v) the appropriate circle in the **table** below.*

5. What is the average gross *(before tax)* income that YOU receive each week, including pensions, allowances, and financial support from parents?

6. What is the average gross *(before tax)* income that YOUR HOUSEHOLD (i.e. you and your partner) receive each week, including pensions, allowances, and financial support from parents?

Income	SELF	HOUSEHOLD
No income	O	O
$1-$119 ($1-$6,239 annually)	O	O
$120-$299 ($6,240-$15,999 annually)	O	O
$300-$499 ($16,000-$25,999 annually)	O	O
$500-$699 ($26,000-$36,999 annually)	O	O
$700-$999 ($37,000-$51,999 annually)	O	O
$1,000-$1,499 ($52,000-$77,999 annually)	O	O
$1,500 or more ($78,000 or more annually)	O	O
Don't know	O	O
Don't want to answer	O	O

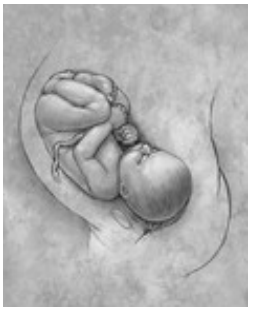
7. How many people, including yourself, are dependent on this household income?_____

8. What is your FORMAL registered marital status?
(i.e. never married, married, separated, divorced, widowed) _____

Thank you!

Appendix 9

Weight-Related Behaviours in Pregnancy



1. Study ID: _____ *(completed by research team)*

2. Today's Date: _____

3. Number of Weeks Pregnant: _____

Instructions:

Please answer all of the questions on each of the pages according to the scales provided. Circle the number that best represents how you feel. There are no right or wrong answers.

LOCUS OF CONTROL

1 Strongly agree	2 Agree	3 Neither Agree nor Disagree	4 Disagree	5 Strongly Disagree

4. Whether my weight changes is up to me.	1	2	3	4	5
5. If I eat right, and get enough exercise and rest, I can control my weight the way I want.	1	2	3	4	5
6. Being the right weight is mainly good luck.	1	2	3	4	5
7. No matter what I try to do, if I gain or lose weight, or stay the same, it is just going to happen.	1	2	3	4	5

SELF-EFFICACY

1	2	3	4	5
Very Sure	**Sure**	**Neither Sure nor Unsure**	**Unsure**	**Very Unsure**

How sure are you that you can:

8. Fit into your regular clothes.	1	2	3	4	5
9. Take off any extra weight you gain.	1	2	3	4	5
10. Get back into shape.	1	2	3	4	5
11. Eat balanced meals.	1	2	3	4	5
12. Eat foods that are good for you and avoid foods that are not.	1	2	3	4	5
13. Eat foods that are good for you even when family or social life takes a lot of your time.	1	2	3	4	5
14. Get regular exercise.	1	2	3	4	5
15. Get regular exercise even when family or social life takes a lot of your time.	1	2	3	4	5

ATTITUDES TOWARDS WEIGHT GAIN DURING PREGNANCY

1 Strongly agree	2 Agree	3 Neither Agree nor Disagree	4 Disagree	5 Strongly Disagree

16. The weight I gain during pregnancy makes me feel ugly.	1	2	3	4	5
17. I worry that I may get fat during this pregnancy.	1	2	3	4	5
18.I am embarrassed at how big I've gotten during this pregnancy.	1	2	3	4	5
19. I'm embarrassed whenever the nurse weighs me.	1	2	3	4	5
20. I am trying to keep my weight down so I don't look pregnant.	1	2	3	4	5
21. I would like to gain between 12.5 and 17.5 kilograms during this pregnancy.	1	2	3	4	5
22. I would gain 20 kilograms if it meant a healthier baby.	1	2	3	4	5
23. I will feel badly if I gain more than 20 kilograms during this pregnancy.	1	2	3	4	5
24. I like being able to gain weight for a change.	1	2	3	4	5
25. As long as I'm eating a well-balanced diet, I don't care how much I gain during this pregnancy.	1	2	3	4	5
26. I'm sure I will be able to fully control the amount of weight I will gain during this pregnancy.	1	2	3	4	5
27. You can't totally control the amount of weight you gain when you are pregnant.	1	2	3	4	5
28. I feel that women have to be very careful about getting fat during pregnancy.	1	2	3	4	5

BODY IMAGE

29. How satisfied are you with your current shape?

Very satisfied	Satisfied	Dissatisfied	Very dissatisfied

30. How satisfied are you with your current weight?

Very satisfied	Satisfied	Dissatisfied	Very dissatisfied

31. Do you consider your current weight to be…

Too heavy	About right	Too light

32. Do you consider your current body shape to be…

Too heavy	About right	Too light

MEASURES OF FEELINGS ABOUT MOTHERHOOD

1 Strongly agree	2 Agree	3 Neither Agree nor Disagree	4 Disagree	5 Strongly Disagree

33. Having a baby brings a lot of stress into a woman's life.	1	2	3	4	5
34. I'm not sure how I will manage after I have the baby.	1	2	3	4	5
35. I am afraid I will lose my identity after I have the baby.	1	2	3	4	5
36. After a woman has a baby, she is mainly just somebody's mother.	1	2	3	4	5
37. I am sure that I will be a good mother.	1	2	3	4	5
38. I felt proud when I found out I was going to have a baby.	1	2	3	4	5
39. I felt scared when I found out I was going to become a mother.	1	2	3	4	5

CAREER ORIENTATION

1 Strongly agree	2 Agree	3 Disagree	4 Strongly Disagree

40. I want a job that will help me grow.	1	2	3	4
41. Being able to express myself through a job means a great deal to me.	1	2	3	4
42. I am determined to achieve my educational and work goals.	1	2	3	4
43. Success in my work is very important to how I feel about myself.	1	2	3	4
44. I see myself as working for pay my whole adult life.	1	2	3	4
45. The responsibilities for home and family should be equally share when both partners work.	1	2	3	4
46. I need more in life than what being a wife and mother can give me.	1	2	3	4
47. Women who hope to be successful in a job must do so at the expense of home and family.	1	2	3	4
48. Women should seek work that will fit in family needs in terms of work hours, leave time, etc.	1	2	3	4
49. Women must make changes in their careers for family needs.	1	2	3	4
50. Women should not work full time when their children are young.	1	2	3	4
51. Feeling loved and needed is more important to me than having a career.	1	2	3	4
52. I would be very happy staying at home and not working at a job.	1	2	3	4

Thank you for taking the time to complete this questionnaire.

Appendix 10

Professor Roger Smith
Mothers and Babies Research Centre
Level 3 John Hunter Hospital
New Lambton NSW Australia 2305
Tel (02) 4921 4380
After hours [Ph. (02) 4921 3000] and ask for Prof. Smith
Fax (02) 49 21 4394

INFORMATION SHEET
MATHEMATICAL MODEL OF HUMAN PREGNANCY

You are invited to participate in a study being conducted by Professor Roger Smith *and others* from the Mothers and Babies Research Centre.

Why is the research being done?
The Mothers and Babies Research Centre is trying to understand the reasons why babies are born early. This study will follow women throughout their pregnancy by taking blood, urine and saliva samples to measure a number of hormones and other biochemical markers considered important in determining the start of labour. These measurements will help us to understand the relationships between the various factors controlling pregnancy and help to determine the time of onset of labour. This will allow doctors to predict more accurately those women who will deliver prematurely. We believe this method has the potential to be very valuable in the future for predicting premature birth.

Who can participate in the research?
Any pregnant woman can participate in this study. There are two parts to this study; a biochemical marker component and a genetic factor component. If you come to the clinic at any time up to 16 weeks you can join either the biochemical or the genetic component or you may be happy to join both parts of the study. If you come along to your first visit to the clinic after 17 weeks then you are able to join the genetic part of the study.

What choice do you have?
Participation in this research project is entirely your choice. You will only be included in the study if you give your informed consent. Whether or not you decide to participate, your decision will not disadvantage you and will not affect your care in any way. You may withdraw from the study at any time without affecting the care you receive at John hunter Hospital. If you decide to withdraw you have the option of withdrawing all data relating to you and having the blood, urine and saliva samples taken from you destroyed.

What will you asked to do?
If you join the **biochemical marker component** of the study we will take blood, urine and saliva samples to measure a number of hormones and other biochemical markers considered important in determining the start of labour. We will also measure indicators of infection to determine levels of these markers in normal pregnancy. You will have blood, urine and saliva samples taken every four weeks, from 16 weeks of your pregnancy and at delivery. The total blood collected will be approximately 35mls (less than two tablespoons). A blood sample will also be taken from the umbilical cord at delivery. Ultrasound measurements of your uterus will be taken at 18-20, 26, 32 and 38 weeks of pregnancy. Although ultrasound examinations during pregnancy are completely safe for both mother and baby, the more times an ultrasound is performed during pregnancy the greater the likelihood is that an existing abnormality will be discovered. If an ultrasound abnormality is discovered, the attending obstetrician will be notified and undertake whatever management is necessary. At the visits every four weeks you will also have your blood pressure measured and a urine test checked as would normally be performed in the antenatal clinics.

If you join the **genetic component** of this study, some blood will also be stored for future analysis to see if there are genetic (inherited) factors that could explain some preterm births. To join the genetic part of the study you will need to donate your placenta which we will collect and weigh after you deliver your baby. You will also need to donate one blood sample of approximately 2 tablespoons, which can be taken at any time during your pregnancy. The genetic part of the study is optional.

What are the risks and benefits of participating?

One study has reported that ultrasound may be associated with a 30% increase in left-handedness which corresponds to an extra 3 left-handed children amongst 100 births. No harm has been demonstrated from ultrasound. The placenta is delivered after the baby and our collection of it will not harm you or your baby.

How will your privacy be protected?

All information gained from the study will remain confidential and access to this information will be limited to the chief investigator and co-investigators. Personal identifying information such as name and address will be deleted from all records when the study is complete.

How will the information collected be used?

The results of the measurements taken will be used to build a model of preterm birth. The results will be presented in papers in scientific journals and at internal and national conferences. No patient will be identified in these presentations or publications.

What do you need to do to participate?

If there is anything you do not understand, or you have questions, contact a member of the research team. If you would like to participate please complete the attached consent form and return it to the research midwives. Participation, non-participation or withdrawal from the study will in no way alter the routine antenatal care each woman receives. The Mothers and Babies Research Centre will be responsible for all costs of the tests performed during the study.

Further Information

You may telephone the research team at any time. Tel (02) 4921 4380. After hours [Ph. (02) 4921 3000] and ask for Prof. Roger Smith.

Thank you for considering this invitation.

PROFESSOR ROGER SMITH
Director, Mothers and Babies Research Centre

COMPLAINTS

This project has been approved by the Hunter Area Research Ethics Committee of Hunter New England Health, Reference [990413.09] and the *University's Human Research Ethics Committee, Approval No. H-*[H-705-0699]. *Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au or Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02 49216333, email Human-Ethics@newcastle.edu.au.*

Professor Roger Smith
Mothers and Babies Research Centre
Level 3 John Hunter Hospital
New Lambton NSW Australia 2305
Tel (02) 4921 4380
After hours [Ph. (02) 4921 3000] and ask for Prof. Smith
Fax (02) 49 21 4394

CONSENT TO PARTICIPATE IN RESEARCH
FORMING A MATHEMATICAL MODEL OF HUMAN PREGNANCY

I_____(PRINT NAME) have been asked to participate in the above research project and give my free consent by signing this form. I understand that:

1. The research project will be carried out as described in the Information Sheet, a copy of which I have retained.
2. If I do not volunteer, or decide to withdraw, my decision will be accepted (and my non-participation will not affect the treatment I am receiving).
3. My consent to participation is voluntary and I may withdraw from the trial at any time. I do not have to give reason for the withdrawal of my consent.
4. I have read and understood the Information Sheet and had all my questions answered to my satisfaction.

UP TO 16 WEEKS PREGNANT ONLY

I consent to being involved in the biochemical component of this study YES NO

(if yes, please answer the questions below)

I consent to having blood samples of approximately 35mls (less than two tablespoons) taken every four weeks, from 16 weeks of pregnancy and at delivery. YES NO

I consent to having ultrasound measurements of the uterus at 18-20, 26, 32 and 38 weeks of pregnancy. YES NO

I consent to a sample of cord blood being taken from the umbilical cord at delivery. YES NO

I consent to being involved in the genetic component of this study YES NO

I consent to have my placenta weighed after it is delivered and a blood sample (less than two tablespoons) to be taken at sometime during my pregnancy.

YES NO

I consent to my blood being stored for future analysis to see if there are genetic (inherited factors) that could explain some preterm births.

YES NO

17 PLUS WEEKS PREGNANT ONLY

I consent to being involved in the genetic component of this study YES NO

I consent to have my placenta weighed after it is delivered and a blood sample (less than two tablespoons) to be taken at sometime during my pregnancy.

YES NO

I consent to my blood being stored for future analysis to see if there are genetic (inherited factors) that could explain some preterm births.

YES NO

PRINT NAME……...

SIGNATURE……. DATE……

Appendix 11

The University of Newcastle

HMRI hunter medical research institute

CHIEF INVESTIGATOR: Professor Roger Smith
Mothers and Babies Research Centre
Level 3 John Hunter Hospital
New Lambton NSW Australia 2305
Telephone: (02) 4921 4380
Facsimile: (02) 4921 4394

Letter of Invitation to Participate in Research [Version 2, 26/07/2005]

Dear _____

I am writing to inform you of a research project that will be taking place in the near future. It is called the ABCD Obesity study. ABCD Obesity stands for Assessment Before Children Develop Obesity. This study will be undertaken by a team of researchers from the Mothers and Babies Research Centre at John Hunter Hospital and also from the University of Newcastle.

At some time between 2000 and 2005 you have agreed to participate in the Mathematical Model of Pregnancy Study. In this study we were interested in trying to understand the reasons why babies are born early. Your involvement in this study included approximately 4 ultrasounds of your baby and a number of blood, urine and saliva samples over the course of your pregnancy.

We are contacting you to see if you would be interested in receiving information about the ABCD Obesity Study. This is a totally new study rather than a follow-up to the Mathematical Model of Pregnancy study. However the information that has been collected during your pregnancy is extremely valuable for the research we are going to undertake. If you decide that you would like to participate in this new study and you give your permission, we will access the data that has previously been collected in the Mathematical Model of Pregnancy Study. In addition, the ABCD Obesity study will involve gathering current information and taking measurements of both yourself and your child (the one, or more, you were pregnant with during this study).

Apart from women who have been pregnant and have participated in the Mathematical Model of Pregnancy Study we will also be inviting women who are currently pregnant to join our study. Fathers of the children in this study will be invited to provide a few details about themselves, although their involvement is only requested if you decide you and your child will participate.

It is very important to us that you do not feel pressured to participate in the current study. Your involvement in the Mathematical Model of Pregnancy Study, or any other research for that matter, does not mean you are obligated to join the current study. If you do not become involved, the care you receive at John Hunter Hospital will not be affected in any way.

If you are interested in receiving further information about the ABCD Obesity Study please contact the research team. You can do this by sending the standard response we have included in the reply paid envelope we have provided, or by contacting the research team directly either on (02) 4921 4380 or e-mail: Sheila.Duffy@hnehealth.nsw.gov.au. You can also use these channels of communication to reply that you do not want to receive further information. If you do not reply to this letter you will receive 1 follow-up letter confirming that you are indirectly electing not to participate and if you do not respond to this we will not contact you again.

Thank you for considering this invitation.

PROFESSOR ROGER SMITH, ***Director, Mothers and Babies Research Centre***

The University of Newcastle

THE RESEARCH TEAM

CHIEF INVESTIGATOR: Prof Roger Smith
Mothers and Babies Research Centre
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Dr Ian Wright
Paediatrics and Child Health
John Hunter Hospital
Phone: (02) 4921 3000
Ian.Wright@hnehealth.nsw.gov.au

Information Statement for the **ABCD Obesity Study** [Version 5, 11/10/2005]

You are invited to take part in the ABCD Obesity Study being conducted by Professor Roger Smith from the Mothers and Babies Research Centre at John Hunter Hospital and Dr Clare Collins from the University of Newcastle.

What is ABCD Obesity?
ABCD Obesity stands for **Assessment Before Children Develop Obesity.**

Why is the research being done?
Obesity is a major health problem for many adults and now even children in Australia. Being overweight or obese at any age can result in both physical and emotional harm. Unfortunately kids who are overweight do not always grow out of it and often end up as overweight adults.

Researchers from the Mothers and Babies Research Centre and the University of Newcastle are trying to understand why some children are more likely to become overweight than others. In this study we are going to collect information from mothers who have young children, and from women who are currently pregnant, in an attempt to reveal the earliest risk factors for the development of obesity. We are interested in what women and their children eat and drink, how much activity they do, and other factors that may potentially contribute to their weight.

Who can participate in the research?
- ✓ Mums who have participated in the Mathematical Model of Pregnancy Study and their child whose growth was monitored before birth as part of this study; and
- ✓ Currently pregnant women enrolled in the Mathematical Model of Pregnancy Study; and
- ✓ The child's father.

We are interested in the mothers and their children who have been involved in the Mathematical Model of Pregnancy Study that has been held at John Hunter Hospital between 2000 and 2005. While you may have more than 1 child we are specifically interested in the child(ren) you were pregnant with at the time of participating in this pregnancy study. This is because during this study the growth of the baby was closely monitored throughout pregnancy. Any woman who is currently pregnant and is participating in the Mathematical Model of Pregnancy Study can also participate. Where possible we would also like to collect a small amount of information about the child's father.

What choice do you have?
Participation in this research project is entirely your choice. Women who have participated in, or are currently enrolled in the Mathematical Model of Pregnancy Study are in no way obliged to participate in this study. Parents/guardians will be responsible for providing consent on behalf of their child. You and your child will only be included in the ABCD Obesity study if you give your informed consent. Whether or not you decide to participate, your decision will not disadvantage you or your child and will not affect the medical care either of you receive. You may withdraw your own and/or your child's involvement in the study at any time without affecting the care you receive at John Hunter Hospital in any way. If you decide to withdraw you also have the option of withdrawing all the data relating to you and/or your child, and having any blood samples taken from you destroyed.

What will you be asked to do?
If you agree to participate, you will be asked to visit the John Hunter Hospital 2-3 times during the study period. Once you have attended your first appointment, the remainder of your visits will be scheduled within the following 6 months. Appointment days and times that are appropriate for you can be negotiated with the researchers. A map of the Hospital will be provided with the building marked to show you where you need to go for your appointment and the closest available parking. A parking voucher will be provided. Those who catch public transport will receive reimbursement for the travel cost of these visits. Each visit is estimated to take approximately 1-3 hours depending on whether you would prefer to complete some of the questionnaires while you are at the hospital or if you would prefer to take them home to be completed in your own time. This can be arranged with the research team.

You will also be asked to provide consent for the research team to access your medical records. Information from these records will only be used when it directly relates to the objectives of the study. Identifying information will be removed to ensure your personal details remains confidential.

During the course of this study, you will be asked to:

- Sign a consent form.
- Deliver the 'Information Statement for Fathers' contained in this package to the child's dad.
- Attend the John Hunter Hospital on 2-3 occasions for between 1-3 hours per visit.
- Complete a medical history and a personal details questionnaire (15 minutes).
- Have your height, weight and blood pressure measured (15 minutes).
- Have your body composition measured using skinfold calipers (15 minutes).
- Weigh and record all food and drinks consumed over a 3-day period.
- Complete a food frequency and a physical activity questionnaire (1 hour).
- A small group of you will be asked to wear accelerometers for 1 week. These are a small device similar to a pedometer that measures movement.
- Donate a small blood sample (10 minutes). This will be analysed for important nutritional and hormonal indicators which relate to a person's risk of disease.
  Mothers and pregnant women will need to fast overnight. We are going to take 10mls, which is equivalent to 2 teaspoons, of blood from pregnant women and 20mls, which is equivalent to 1 tablespoon, from the mothers. Please note that this is an optional component of the study and refusal to provide this sample does not mean you cannot participate in the other aspects of the study. Those who do provide blood samples will have access to their results.
- If you are currently pregnant we will also ask you to complete a weight-related behaviours questionnaire (30 minutes).

What will you be asked to do on behalf of your child?

- Sign a consent form on their behalf.
- Bring your child to at least 1 of the appointments at John Hunter Hospital so they can be weighed, measured and have their blood pressure taken. They will also have their body composition measured using skinfold calipers (20 minutes).

- We are also requesting a small blood sample from the children in this study (10 minutes). This will also be analysed for important nutritional and hormonal indicators which relate to a child's risk of disease.
  We are going to take 5mls, which is equivalent to 1 teaspoon, from children 0-3 years old and 10mls (2 teaspoons) from children who are 3 years and older. To reduce any discomfort for the child we will use a local anaesthetic cream to numb the area of skin before inserting the needle. Again this blood sample is an optional component of the study and a desire for your child not to be involved in this component does not mean your child cannot participate in the other aspects of the study.
- The dietary information for the children will depend on what they currently eat and drink:
  - » If your child is being breastfed we would like you to weigh the baby before and after each feed for 1 day only using the electronic scales we provide you.
  - » If your child is being breastfed and is also receiving some additional food or drink (e.g. formula, juice, milk, solids etc) we would like you to weigh the baby before and after each feed, but also weigh and record any other food or drink your baby receives for a 3-day period.
  - » If your child receives no breast milk we would like you to weigh and record all food and drinks her/she consumes over a 3-day period.
- Complete a food frequency and infant or child feeding questionnaire, depending on the age of your child (1 hour).
- Complete an infant or child behaviour questionnaire (30 minutes – 1 hour).
- A small group of children who are 1 year and older who must be able to crawl or walk will be asked to wear accelerometers for 5 days.

What are the risks and benefits of participating?

In this study we are interested in gathering information about everyday dietary and lifestyle factors. As a result there is very little risk involved. We are requesting one voluntary blood sample from all participants including infants and young children. When providing blood samples, you are likely to experience a slight sting as the needle penetrates the skin, and the procedure may result in bruising. Fainting is also a possible side effect of having blood taken. Infants and children will be provided a local anaesthetic cream which will help to numb the area. Some people may also experience slight discomfort as the layer below the skin is gathered (lightly pinched) for measurement using skinfold calipers. However this will be performed by trained personnel using the correct technique to prevent or minimise any discomfort. All participants will receive feedback about the information they provide. No costs will be incurred for participating in the study.

How will your privacy be protected?

All information gathered during the study will remain confidential and access to this information will be limited to the Chief Investigator and members of the research team. Personal information such as name and address will be removed from all records so that you cannot be easily identified.

Future use of blood samples.

For those who choose to donate a blood sample, we would like your permission to store any blood that is left over from the tests we conduct. This is because new research and technology unfolds very rapidly and it may become important for us to analyse this stored blood for additional studies that relate pregnancy to the health of the baby. If you provide consent for you and/or your child's blood to be stored, it will be kept in a locked -80^{0}C freezer within one of the Mothers and Babies Research Centre laboratories, for a period of up to ten years. After this time any remaining sample will be destroyed in accordance with the State's Safe Disposal Regulations for Biological Material.

Note that it is possible for you and/or your child to participate in the research and provide a blood sample, without agreeing to have remaining blood stored for future use.

How will the information collected be used?

The results of the measurements taken will be used to build a model of risk factors for developing childhood obesity. The results will be presented through local media, medical journals, and at

national and international conferences. The combined results will be presented, therefore no individual will be identified in these presentations or publications.

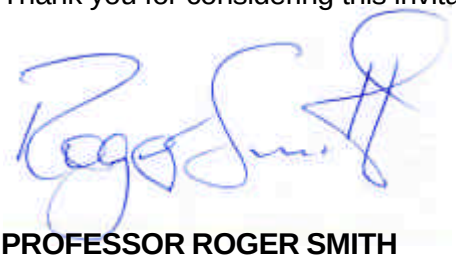
What do you need to do to participate?
If there is anything you do not understand, or you have questions, contact a member of the research team. If you would like to participate please complete the attached consent form and return it to the research team using the reply paid envelope within 4 weeks of receiving this letter.

Participation, non-participation or withdrawal from the study will in no way alter the routine medical care each woman and child receives at John Hunter Hospital. The Mothers and Babies Research Centre will be responsible for all costs of the tests performed during the study.

Further Information

You may telephone the research team at any time. Tel (02) 4921 4380.

Thank you for considering this invitation.



PROFESSOR ROGER SMITH
Director, Mothers and Babies Research Centre

COMPLAINTS

This project has been approved by the Hunter Area Research Ethics Committee of Hunter New England Health (Reference Number: 05/06/08/3.14) and the Human Research Ethics Committee of the University of Newcastle (Approval Number: H-107-0905).

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Nicole.Gerrand@hnehealth.nsw.gov.au or Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.

The UNIVERSITY *of* NEWCASTLE

THE RESEARCH TEAM

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Note: Please make sure you complete and return both pages.

Consent to Participate in the **ABCD Obesity Study** [Version 3, 11/10/2005]

I _____ (PRINT YOUR FIRST AND LAST NAME) have been asked to participate in the above research project and give my free consent by signing this form. I understand that:

5. The research project will be carried out as described in the Information Sheet, a copy of which I have retained.
6. If I do not volunteer, or decide to withdraw, my decision will be accepted and my non-participation will not affect the treatment I am receiving.
7. My consent to participate is voluntary and I may withdraw from the study at any time. I do not have to give reason for the withdrawal of my consent and I am able to request the withdrawal of the information (and blood sample if taken) that has been collected as part of this study.
8. I have read and understood the Information Sheet and have had all my questions answered to my satisfaction.

This page is to be completed by the Mothers who have previously participated in the Mathematical Model of Pregnancy Study.

(Currently Pregnant Women please complete page 3 instead of this page)

Please indicate your preferences by marking (v) either 'YES' or 'NO' below. Note that if you answer 'NO' to one or more of questions 1) to 3) you will not be included in the study.

1) a. I consent to being involved in the ABCD Obesity Study • **YES** • **NO**

1) b. I also consent to **my son/daughter** _____(PRINT YOUR CHILD'S FIRST AND LAST NAME) participating in the ABCD Obesity Study • **YES** • **NO**

2) a. I consent to the researchers linking the data that was collected for the *Mathematical Model of Pregnancy Study* with the new information collected for the *ABCD Obesity Study* • **YES** • **NO**

3) a. I consent to the researchers having access to **my** medical records when the data relates specifically to the objectives of this study. • **YES** • **NO**

3) b. I consent to the researchers having access to **my child's** medical records when the data relates specifically to the objectives of this study. • **YES** • **NO**

If you have answered 'YES' to all of the above, please answer 4) and 5) below. Note that you may choose 'NO' and will still be eligible to participate in the ABCD Obesity Study.

4) a. I consent to having one blood sample of approximately 20mls (1 tablespoon) collected during the study for analysis of nutritional and hormonal biomarkers. • **YES** • **NO**

4) b. I consent to **my child** donating 1 teaspoon (5mls) of blood if they are less than 3 years old, or 2 teaspoons (10mls) of blood if they are 3 years and older, for the analysis of nutritional and hormonal biomarkers. • **YES** • **NO**

5) I consent to a small sample of blood being stored for future studies that relate pregnancy to the health of the baby. • **YES** • **NO**

PRINT NAME.....

SIGNATURE.....

DATE.....

This page is to be completed by the Women who are Currently Pregnant and are participating in the Mathematical Model of Pregnancy Study.

(Women who have previously participated in the Mathematical Model of Pregnancy Study please complete page 2 instead of this page)

Please indicate your preferences by marking (v) either 'YES' or 'NO' below. Note that if you answer 'NO' to one or more of questions 1) to 3) you will not be included in the study.

1) I consent to being involved in the ABCD Obesity Study • **YES** • **NO**

2) I consent to the researchers linking the data that is collected for the *Mathematical Model of Pregnancy Study* with the new information collected for the *ABCD Obesity Study*
• **YES** • **NO**

3) I consent to the researchers having access to my medical records when the data relates specifically to the objectives of this study. • **YES** • **NO**

If you have answered 'YES' to all of the above, please answer 4) and 5) below. Note that you may choose 'NO' and will still be eligible to participate in the ABCD Obesity Study.

4) I consent to having one blood sample of approximately 10mls (2 teaspoons) collected during the study for analysis of nutritional and hormonal biomarkers. • **YES** • **NO**

5) I consent to a small sample of blood being stored for future studies that relate pregnancy to the health of the baby. • **YES** • **NO**

6) I consent to follow-up (of me and my baby) by this research team after my baby has been born.
• **YES** • **NO**

PRINT NAME.....

SIGNATURE.....

DATE.....

Appendix 12

How will the information collected be used?

The results will be used to work out what makes a pregnancy healthy for mothers and babies. This includes being able to predict premature birth and understanding the role of nutrition in pregnancy.

You can read about the study findings in our Mums and Bubs (biannual) Newsletter. The results will also be presented in scientific journals and at conferences. Some results will be included in theses written by Dr Sue Mei Lau and Ms Alexis Hure for their postgraduate degrees. No person will be identified in these presentations or publications.

What do you need to do to participate?

If you have any questions or would like further information please contact Alexis Hure on 49217486. You may also phone the Chief Investigator, Professor Roger Smith, any time on 49214380.

If you'd like to participate please complete the attached consent form and return it to the research midwives either in person or in the reply paid envelope we have provided. This will be taken as your informed consent to participate.

Participation, non-participation or withdrawal from the study will not alter the routine antenatal care each woman receives.

Thank you for considering this invitation.

Complaints about this research

This project has been approved by the Hunter New England Human Research Ethics Committee (HNEHREC) of Hunter New England Health, Reference Number: 06/05/24/5.06

Any concerns or complaints about the manner in which this research is conducted may be given to the researcher. If an independent person is preferred, please contact Dr Nicole Gerrand, Professional Officer (Research Ethics), HNEREC, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305, Phone (02) 49214950,
E-mail: Nicole.Gerrand@hnehealth.nsw.gov.au

The Research Team

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Biochemist & Accredited Practising Dietitian, University of Newcastle

Professor Warwick Giles
Director of Maternal & Fetal Medicine, John Hunter Hospital

Ms Alexis Hure - Tel (02) 4921 7486
PhD Candidate (Supervisors: Dr Clare Collins & Prof Roger Smith) & Accredited Practising Dietitian,University of Newcastle

Dr Mark McLean
Senior Staff Endocrinologist, Westmead Hospital

Dr Sue Mei Lau
PhD Candidate (Supervisors: Dr Mark McLean & Prof Roger Smith) & Endocrinologist, Westmead Hospital

Professor David Smith
Professor of Engineering, University of Melbourne

Dr David Somerset
Staff Specialist in Obstetrics, John Hunter Hospital

Dr Ian Wright
Staff Specialist in Neonatal Medicine, John Hunter Hospital

Information Statement for

Women And Their Children's Health

A research initiative within the Mothers and Babies Research Centre of John Hunter Hospital

Version 4, 06/07/2006

In partnership with our community

HMRI | The University of Newcastle | Hunter New England NSW Health

Congratulations on your Pregnancy!

You are invited to join a study that is running within the Mothers and Babies Research Centre of John Hunter Hospital.

It's called The ***WATCH*** Study:

Women And Their Children's Health

This research involves studying women from early in their pregnancy up until birth. After the birth we will be studying both the mother and her child during the first two to three years of life.

Why is the research being done?

The health of women and their children is our main concern. We want to know what combination of factors results in the healthiest babies. Once we know this we can give better health care to women like you, who are in the early stages of their pregnancy.

We are trying to discover why some babies are born early and hope to eventually be able to predict the chances of this happening. We also want to know why babies grow differently and how this affects your child's health and development as they get older.

We are looking specifically at things like hormones and nutritional factors. Each of these play a specific role in your own, and your child's, health. Some researchers think that the nutrients a baby receives before birth may contribute to developing obesity and other diseases later in life. We will be exploring this idea as part of this study.

Who can participate in the research?

Any pregnant woman and her:

- ❑ Child after birth
- ❑ Male partner or husband
  - He will be asked to provide details about his height, weight and waist measurement
  - His participation is optional and it will be for you to decide.

What choice do you have?

It's up to you! Participation in this research is entirely voluntary. Only those who give their informed consent will be included in the study.

Whether or not you decide to participate, your decision will not disadvantage you in any way. The care you receive at John hunter Hospital will not be affected.

If you do join the study you may decide to withdraw at any time. If you do withdraw, you can have all the data relating to you destroyed if you want. This includes any blood, urine and saliva samples you have had while in the study.

What does participation involve?

During pregnancy we will:

- Perform extra ultrasounds of your baby
  - Ultrasounds at approximately weeks 18-20, 24, 30 & 36 of pregnancy
- Take blood, urine & saliva samples every 4 to 6 weeks until birth (5 samples in total)
  - These are not part of your usual antenatal care at the John Hunter Hospital
  - The total amount of blood is about 56ml or less than 3 tablespoons altogether. Blood samples will be analysed for important indicators of health, such as hormones
- Measure blood pressure every 4 to 6 weeks
- Measure indicators of infection

And at birth we will:

- Collect blood from the umbilical cord

You will also be asked to:

- Attend 4 to 6 study visits during pregnancy at the John Hunter Hospital which we will try to match to your routine antenatal appointments
  - You will **not** have to pay for parking when you come for any of these study visits
- Complete a medical history & a personal details questionnaire (15 minutes)
- Have your body composition measured at each visit (15 minutes)
- Weigh & record all food & drinks consumed over 4-days on 2 occasions during pregnancy and 2 occasions after the birth of your child (about 15 minutes each day)
- Complete a food frequency & a physical activity questionnaire on 2 occasions during pregnancy and 2 occasions after birth (1 hour)
- Complete a weight-related behaviours questionnaire (30 minutes)

You may also be asked to wear an accelerometer for 1 week. These are a small device similar to a pedometer that measure movement.

After your baby is born we will:

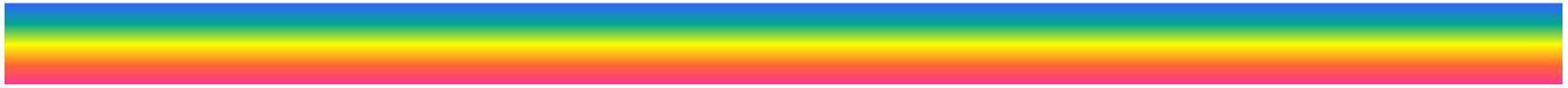
- Check on you and your child at 3, 6, 9, & 12 months of age. At these times we will look at your child's growth, body composition, and feeding patterns, and your physical measurements, blood pressure, dietary intake and physical activity
- Ask to take a small blood sample from both you (10ml or 2 teaspoons) and your child (5ml or 1 teaspoon) at 6 months of age
- Ask you to complete an Infant Behaviour and an Infant Feeding Questionnaire at 12 months (1 hour)

Are there risks & benefits of participating?

- The Mothers and Babies Research Centre pays for all of the tests you have as part of this study. Many of these you do not get to have as part of standard antenatal care.
- No harm has been demonstrated from ultrasound. Although one study suggests that it may slightly increase your child's chances of being left-handed.

How will your privacy be protected?

Any information you provide for this study will be confidential. Only the research team will have access to your information. We will allocate all participants a study code so that you are not easily identifiable. Details that identify you, such as name and address, will be removed when the study is complete.



Professor Roger Smith
Mothers and Babies Research Centre
Level 3 John Hunter Hospital
New Lambton NSW Australia 2305
Tel (02) 4921 4380 Fax (02) 4921 4394

Consent for the WATCH Study
Women and their Children's Health

I have been invited to participate in this research project and give my free consent by signing this form.

I understand that:

- The research project will be carried out as described in the Information Statement (pamphlet), a copy of which I have retained.
- If I do not volunteer, or decide to withdraw, my decision will be accepted (and my non-participation will not affect the treatment I am receiving).
- My consent to participate is voluntary and I may withdraw from the study at any time. I do not have to give a reason for the withdrawal of my consent.
- Participation in this study is intended to continue for more than two years. In signing this consent form I give permission to be followed-up over this time, unless I inform the research team that I no longer wish to be involved.

I have read and understood the Information Sheet.
All my questions have been answered to my satisfaction.

PRINT NAME……...

SIGNATURE……

DATE……